

# Taking action on climate change, together

Summary of  
scientific research how  
3-NOP effectively reduces  
enteric methane  
emissions from cows



This scientific abstract booklet is the result of excellent interdisciplinary scientific teamwork from across the globe. A big thank you goes to all the contributors for their effort and outstanding research work which allowed this abstract booklet to be made.



## Introduction

As population and income levels grow, so do the demands humans place on our planet. Adapting to more sustainable ways is a responsibility we all share. If future generations are to enjoy the foods we know and love, we need to move forward in ways that are farm wise and climate friendly. Cows provide nutritious dairy and beef products that we thrive on today – full of the high-quality protein, micronutrients and essential fatty acids our bodies need. Dairy cows alone support the livelihood and food security of more than a billion people around the world. A lot has already been done to make our farms more sustainable and our foods more climate-friendly.

However, cows generate methane, a greenhouse gas with consequences for our planet. Nearly 60% of emissions created globally during milk production come in the form of enteric methane, released into the atmosphere by burping and breathing. This equates to three tons of CO<sub>2</sub> equivalents every year for a single dairy cow. As greenhouse gas, methane has a high global warming potential and a short lifetime. Given this, reducing methane emission could play an important role to mitigate further warming and do so immediately – and therewith could contribute to finding near term warming mitigation actions to avoid we surpass the 1.5–2.0°C tipping points.

With the feed additive as developed by DSM (scientific name 3-NOP, tradename to be announced soon), we are able to provide the farming community with an effective tool to reduce methane emissions, and thereby contribute significantly to reduce the global warming of our planet, whilst at the same time improving food security and livelihoods of thousands of people globally. The feed additive is a result of DSM's Project Clean Cow, a decade-long journey of research and development. It is the most extensively studied and scientifically proven solution to the challenge of burped methane to date. We have commenced registration of 3-NOP in Europe, with other markets to follow.

We are extremely proud to be working with leading scientists from all around the world on 3-NOP, as our joint insights will enable farmers to take informed decisions and support adoption.

Over the past 2 years, an enormous breadth and depth of research was conducted, much of which is summarized in this abstract booklet.

For us, the key take-home messages from these studies are:

- 3-NOP reduces methane in all diets and ruminant breeds that have been tested.
- The dose and application strategy will need to be tailored for different types of (feed) management systems.
- The lowest proposed commercial dose of 3-NOP (60 mg/kg DM of the total daily ration) when applied to TMR can reduce methane emissions from dairy cows by 22–35%. Further work is ongoing with the higher ranges of the commercial dose.
- In most trials, feed efficiency was significantly improved by at least 4% through the increased production of milk fat or milk protein. The effect was consistent and long lasting (>100 days) with no sign of adaptation occurring. These results were observed across all stages of lactation and parity.
- The scaling up from university trials to a commercial setting was proven, with methane reductions seen at high levels in both beef finishing trials (80%) and dairy trials consistently delivered CO<sub>2</sub>e savings of ~1ton CO<sub>2</sub>e (GWP100) per cow and year, and a substantial gain in feed efficiency (>5%).
- Detailed microbial community analyses demonstrated that 3-NOP was able to shift activity away from methanogenic bacterial species found in the digestive tract towards Prevotella and Succinivibrio species, which are able to benefit from the excess hydrogen.
- In order to make 3-NOP useful in a broad set of farming systems, alternative forms are being developed with a sustained release form for grazing systems. Initial prototypes were able to extend methane reduction from feeding time to 6-8hrs with 1 small dose delivered in a supplemental feed.
- Early indications show 3-NOP may be an effective solution in calf feeding programs. When calves were supplemented with 3-NOP there was a persistent decrease in methane emissions throughout the post weaning period. Further research is required to demonstrate this on a larger scale.

We will continue our research to further expand the application of 3-NOP as we believe that this novel product can contribute to help the society to transition to a low carbon footprint economy. We therefore welcome any research and collaboration proposals as we continue this journey.

*Maik Kindermann, Nicola Walker and Mark van Nieuwland*



# 3-nitrooxypropanol mitigates enteric methane with a minimal impact on the feedprint

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## Abstract

The investigational product 3-nitrooxypropanol (3-NOP) showed clear reductions in enteric methane emissions in cattle in studies worldwide. However, a mitigation strategy will only be effective in abating the global warming problem if there is a net reduction in greenhouse gas emission. In this study the net abatement potential of 3-NOP in dairy cattle was assessed by performing a life cycle assessment. There were three treatments: REF (no 3-NOP), NOPbas (3-NOP mixed in with the basal diet, the roughage) and NOPconc (3-NOP incorporated in a pelleted balanced compound feed). The system boundaries were as follows: the life cycle of the feed and the feed additive from the “cradle”, i.e. resource extraction from the natural environment, to the production of milk at the farm. The measured enteric CH<sub>4</sub> emissions in CO<sub>2</sub> equivalents were added to the carbon footprint of the consumed feed to calculate a combined carbon footprint. The functional unit was defined as 1 kg fat-and-protein-corrected milk (FPCM). The combined carbon footprint of REF, NOPbas and NOPconc was 1.14, 1.06 and 1.04 kg of CO<sub>2</sub>-eq./kg of FPCM, respectively. The enteric methane production was 23 and 21% lower for NOPbas and NOPconc, respectively whereas the contribution of 3-NOP in the combined carbon footprint of NOPbas and NOPconc was only 0.32% and 0.34%, respectively. Feed supplementation with 3-NOP is definitely a successful strategy for lowering enteric CH<sub>4</sub> emissions of dairy cattle, even when pre-farm emissions for feed and 3-NOP are taken into account.

Keywords: **Bos taurus** **Feed additive** **Methane mitigation** **Carbon footprint**

# Enteric gas emissions and lactational performance of dairy cows fed 3-nitrooxypropanol: A meta-analysis

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## Abstract

This study evaluated effects of 3-nitrooxypropanol (3-NOP), an investigational product, on enteric methane emission and lactational performance of Holstein cows. Forty multi- and primiparous cows ( $118 \pm 28$  days in milk) were blocked and fed a control diet or a diet containing 60 mg 3-NOP/kg feed dry matter for 15 consecutive weeks. Enteric gaseous emissions were measured using the GreenFeed system. Data were analyzed using PROC MIXED of SAS as repeated measures. Compared with control, 3-NOP decreased ( $P < 0.001$ ) daily methane emission (411 vs. 302 g/d; SEM = 6.16), yield (16.4 vs. 11.9 g/kg dry matter intake; SEM = 0.25), and intensity (11.5 vs. 8.2 g/kg energy corrected milk; SEM = 0.28). Treatment increased ( $P < 0.001$ ) hydrogen emission (0.44 vs. 2.55 g/cow/d; SEM = 0.119) and had no effect on carbon dioxide emission ( $P = 0.27$ ). Dry matter intake, milk yield, and cow body weight change were not affected ( $P \geq 0.45$ ) by 3-NOP (mean  $\pm$  SEM:  $25.6 \pm 0.38$  kg/d,  $38.4 \pm 0.63$  kg/d, and  $458 \pm 50.9$  g/d, respectively). Treatment increased ( $P = 0.01$ ) milk fat concentration (3.83 vs. 4.08%; SEM = 0.064), tended to increase ( $P = 0.07$ ) fat yield, increased ( $P = 0.04$ ) milk urea nitrogen (11.6 vs. 12.1 mg/dL; SEM = 0.175) and had no other effects on milk components. In this experiment, 3-NOP decreased daily enteric methane emission by 26% and methane yield (27%) and intensity (29%) without affecting feed intake and milk yield and increased milk fat in lactating dairy cows.

Keywords: Dairy cattle 3-nitrooxypropanol Enteric methane Milk fat

# Enteric gas emissions and lactational performance of dairy cows fed 3-nitrooxypropanol: A meta-analysis

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## Abstract

A meta-analysis was performed to examine the effect of the investigational product 3-nitrooxypropanol (3-NOP) on enteric gaseous emissions (methane, carbon dioxide, and hydrogen) and lactational performance of dairy cows. Data from four randomized complete block design experiments (a total of 185 cows; duration of 31 to 105 days) conducted at The Pennsylvania State University were used in the analysis. Cows received a control diet or a diet containing 3-NOP at 40 to 200 mg/kg feed dry matter. Enteric gas emissions were measured using the GreenFeed system. Addition of 3-NOP decreased ( $P < 0.001$ ) daily methane emission [effect size (ES)  $\pm$  SE:  $-101 \pm 8.05$  g/d], emission yield ( $-1.95 \pm 0.198$  g/kg dry matter intake), and emission intensity ( $-1.30 \pm 0.179$  g/kg energy corrected milk). Treatment increased hydrogen emission ( $+2.23 \pm 0.211$  g/d;  $P < 0.001$ ) and had no effect on carbon dioxide emission ( $P = 0.34$ ). Dry matter intake, milk yield, and cow body weight were not affected ( $P \geq 0.72$ ) by 3-NOP (mean  $\pm$  SE:  $25.4 \pm 1.01$  kg/d,  $42.8 \pm 1.69$  kg/d, and  $631 \pm 16.3$  kg, respectively). Treatment increased milk fat and milk urea nitrogen concentrations (ES  $\pm$  SE:  $+0.33 \pm 0.167$  % and  $+0.38 \pm 0.166$  mg/dL, respectively;  $P \leq 0.05$ ) but had no other effects on milk components. In this meta-analysis, 3-NOP decreased daily enteric methane emission by 24% and both methane yield and intensity by 25% without affecting dry matter intake and milk yield and increased milk fat concentration in lactating dairy cows.

Keywords: Dairy cattle Enteric methane Milk production Milk fat



# Towards the application of 3-nitrooxypropanol in pastoral farming systems

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## Abstract

The investigational product 3-nitrooxypropanol (3-NOP) is an effective methane inhibitor that has been extensively evaluated mixed into rations in non-pastoral dairy and beef systems. The effects of 3-NOP when used in fresh-pasture rations were evaluated in three studies with cattle in respiration chambers (n=4 per treatment). In trial 1, compared to controls, there was a 52% reduction ( $P < 0.01$ ) in methane yield when 3-NOP (2.5 g/d per cow) was mixed into fresh cut pasture fed in two daily allocations. Methane yield was decreased by 39% ( $P = 0.03$ ) when 3-NOP was mixed in a supplement offered twice daily immediately before allowing access to fresh-cut pasture meals. In trial 2, no significant effect of 3-NOP was detected when 3-NOP was dosed at 2.5 g/d per cow in a supplement during milking but access to pasture was withheld for 1 h to simulate the delay in time from milking to grazing ( $P = 0.20$ ). Feed intake was not affected by 3-NOP In both trials and 3-NOP had no effect on milk production in Trial 2. These results indicate that the time between 3-NOP administration and access to feed needs to be shortened or its longevity in the rumen extended. In trial 3, new slow release formulations of 3-NOP were tested and showed potential to extend the time that 3-NOP is active in the rumen, based on gas emission profiles from cows. Further studies are planned to refine promising formulations and to establish their methane reduction potential for pasture fed cattle.

Keywords: Methane mitigation Dairy cattle Temperate pastures Pastoral systems

# Rumen fermentation and microbial changes in grazing heifers treated with 3- nitrooxypropanol under tropical conditions

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## Abstract

The aim of this trial was to study the effect of the methane inhibiting compound, 3-Nitrooxypropanol (3-NOP), an investigational product, on rumen metabolites and microbial community in cattle grazing tropical forage. Approximately 2 months prior to calving forty pregnant heifers were allocated to two groups and maintained in replicate paddocks at Lansdown Research Station (Australia). Each group received a treatment for 6 months: 3-NOP (2.5 g / animal/ day (DSM Nutritional Products, Switzerland; application form contains 10% 3-NOP mixed with silicon dioxide and propyleneglycol) or as a placebo control silicon dioxide/propyleneglycol (50/50) (23 g placebo / animal/ day). The treatments were first mixed with steam flaked barley (88.8%) and molasses (7.4%) and offered daily at 8:00 and 14:00 h (675 g/head) to the respective groups. Rumen fluid samples were collected by stomach intubation at 3 h post feeding the morning treatment at 1.5, 4 and 6 months to determine the effect on rumen fermentation parameters and rumen microbial communities. No significant differences were observed on live weight between 3-NOP and control groups. Overall, increases in rumen formate, ammonia and all VFAs concentrations (except acetate) were observed in 3-NOP supplemented animals. Significant differences ( $P < 0.05$ ) in rumen bacterial composition between 3-NOP and control animals were observed with increases in *Prevotella* and *Succiniclasticum* and decreases in methanogenic species in the 3-NOP supplemented group. The response to 3-NOP feeding appears consistent with the methane inhibiting mode of action of the compound and elevated hydrogen levels observed in previous studies.

Keywords: Methane inhibitor Microbiota Hydrogen sinks Volatile fatty acids

# Persistency of the 3-nitrooxypropanol effect on methane and hydrogen emissions in postpartum Holstein-Friesian dairy cows

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## Abstract

The aim of this study was to determine the methane mitigation potential of 3-nitrooxypropanol (3-NOP) and its persistency when fed to dairy cows in early lactation. Sixteen Holstein-Friesian cows (parity  $2.3 \pm 0.48$ , average  $\pm$  SD) were blocked in pairs and randomly allocated to 1 of 2 dietary treatments; a diet including 52 mg 3-NOP/kg DM (3-NOP) and a diet including a placebo at the same concentration (control). Diets consisted of 35% grass silage, 25% corn silage, and 40% concentrates (on DM basis). Cows received the diets from 3d post-calving up to 115 days in milk. Every 4 weeks, the cows were housed in climate respiration chambers for 5 consecutive days to measure production characteristics and gas emissions. Data were subjected to repeated measures ANOVA using the MIXED procedure in SAS with dietary treatment, days in lactation, and their interaction as fixed effect and block and cow as random effects. The results indicate that dietary 3-NOP supplementation did not affect DM intake, milk yield, and fat-and protein-corrected milk. These characteristics, as well as hydrogen and methane emission, changed over time ( $P < 0.038$ ) following the expected pattern of advancing days in lactation. On average, feeding 3-NOP persistently increased ( $P < 0.001$ ) hydrogen emissions 11-fold and decreased ( $P < 0.014$ ) methane emission by 16% (e.g., 21.0 vs. 17.7 g methane /kg DM intake for 3-NOP and control diet, respectively). In conclusion, feeding 3-NOP persistently decreases methane emission in early lactation Holstein-Friesian cows without affecting animal production characteristics.

Keywords: Methane mitigation Feed additive Gas emissions Production performance

# An early life methane inhibitor treatment reduced methane emissions in dairy calves

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## Abstract

Recent evidence suggests that changes in colonization of the rumen prior to weaning may influence the rumen microbiome and modify phenotypes later in life. The influence of dietary manipulation from birth on growth, methane production, and gastrointestinal microbial ecology in dairy calves was investigated. At birth, 18 female Holstein and Montbéliarde calves were randomly assigned to either the investigational product, 3-Nitrooxypropanol (3-NOP; 3 mg/ kg BW, n=10) or control (CONT, n=8). Treatment was administered daily from birth until three weeks post-weaning (week 14). Samples of rumen fluid were collected at weeks 11, 14, 23, and 60 of life. Enteric methane emissions from the calves were measured using GreenFeed systems (week 11-23 and week 56-60 of life). BW, ADG and individual VFA were similar across groups throughout the trial. Treated calves showed a persistent reduction (11.6%,  $P = .03$ ) in methane emissions (g CH<sub>4</sub>/d) throughout the post-weaning period, despite treatment ceasing three weeks post-weaning. CONT calves aged 60 weeks showed greater archaeal richness than 3-NOP calves. Correspondingly, beta-diversity differed ( $P < 0.036$ ) between CONT and 3-NOP calves in week 60. No clear trends emerged in microbes' relative abundance as a result of 3-NOP treatment. However, patterns of co-occurrence of ruminal archaea, bacteria and anaerobic fungi indicated a more inter-dependent relationship in 3-NOP calves. Despite only minimal modifications to the microbiota composition, the reductive effect of the treatment on methane production persisted following cessation of the treatment period, perhaps indicating that microbial activity or a host response was programmed by the treatment.

Keywords: Bovine Microbial Rumen 3-nitrooxypropanol

# Variability in the relationship between enteric methane emission and dry matter intake in dairy cows

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## Abstract

We examined the relationship of enteric methane emission, measured using the GreenFeed system (GF), and dry matter intake (DMI) in a 15-week experiment with lactating dairy cows receiving a control diet or a diet containing the investigational product 3-nitrooxypropanol (3-NOP at 60 mg/kg feed DM). Daily methane emission and DMI were clustered into 12 time-slots of 2 h each. Methane emission and DMI were the lowest 2-h before feeding and highest within 6 h after feeding. The overall (24 h) relationship between methane emission and DMI was poor ( $R^2 = 0.01$ ). The relationship for the control (but not 3-NOP) cows was improved ( $R^2 = 0.31$ ;  $P < 0.001$ ) when DMI was allocated to time-slots and was strongest ( $R^2 = 0.53$ ;  $P < 0.001$ ) 8-10 h after feeding. Analysis of the 3-NOP emission data showed marked differences in the mitigation effect of this proven methane inhibitor with time. There was a lack of effect in the 2-h time-slot before feeding, the mitigation effect was highest (45%) immediately after feeding, persisted at around 32-39% within 10 h after feeding, and decreased to 13%, 4 h before feeding. These trends were clearly related to DMI (i.e., 3-NOP intake). This analysis showed that the relationship of enteric methane emission, measured using GF, and DMI in dairy cows depends on the time of measurement relative to time of feeding and can be as high as determined in respiration chambers. The methane mitigation effect of 3-NOP is highest immediately after feeding and lowest before feeding.

Keywords: **Cattle** **Measurement technique** **3-nitrooxypropanol**

# Commercial-scale evaluation of fed 3-NOP in reducing methane from cattle feedlots using micrometeorological methods

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## Abstract

It is highly desirable to use agricultural emission mitigation strategies on a whole-farm scale to ensure all aspects of management and production operations are included in the treatment response. Working at a commercial beef cattle feedlot in southern Alberta, Canada, we compared the cattle enteric methane emissions using the Concentration Ratio Method and an Inverse Dispersion Method for a control and treatment block of pens containing a total of 1500 cattle. There was clear reduction in methane emission reduction of 70% for cattle fed the compound 3-nitrooxypropanol (3-NOP) compared to cattle fed just the basal diet. Both micrometeorological methods measured an increase in the concentration ratio (a decrease in the emission rate) during study. There was also a diel fluctuation in methane emission that coincide with feeding in the early morning. The simplicity and sensitively of the Concentration Ratio Method is expected to have applications for evaluating other mitigation strategies at large commercial scales, such as the application of manure additives to pen floors to reduce odours and ammonia emissions.

Keywords: **Emissions** **Concentration** **Ruminant** **Techniques** **Inhibitor**

# 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

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## Abstract

Effects of the investigational inhibitor 3-nitrooxypropanol (3-NOP) on dry matter intake (DMI) and enteric methane (CH<sub>4</sub>) production of beef cattle fed a backgrounding grower diet were evaluated in a commercial feedlot. Fifty crossbred steers (body weight = 328 ± 29 kg) were assigned in a completely randomized design to one of two treatment pens (n = 25/treatment): control and 3-NOP. The treatment pen received increasing doses of 3-NOP to adapt to the final level: 150 (low), 175 (medium) and 200 (high, final dose) mg/kg dry matter (DM), with each dose fed for 28 days. 3-NOP was mixed daily into the high-forage diet (70% corn or barley silage, DM basis). DMI of individual animals was monitored with the GrowSafe system and CH<sub>4</sub> was measured using the GreenFeed system. Over the entire 84 d study, compared with control (11.4 kg/d), DMI was decreased by 6.3% (P = 0.008) with 3-NOP supplementation, and this reduction was similar among the 3-NOP doses (5.8 to 6.4% decrease). On average, addition of 3-NOP lowered (P ≤ 0.002) CH<sub>4</sub> emissions (g/d) by 26% and yield (g CH<sub>4</sub>/kg DMI) by 19%. Compared with control, the greatest decrease in CH<sub>4</sub> yield occurred with the medium dose (-23%), followed by high (-19%) and low (-15%) doses. In conclusion, regardless of dose, supplementation of 3-NOP lowered enteric CH<sub>4</sub> production from commercial feedlot cattle fed a forage-based diet, with the decrease maintained over the entire study. A concurrent large pen study at the same feedlot will evaluate the effects of 3-NOP on cattle performance.

Keywords: Beef steers Commercial feedlot GreenFeed Methane

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