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Taking action on climate change, together

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

Bovaer[®]

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DSM

BRIGHT SCIENCE. BRIGHTER LIVING.

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

Methane matters and we need to reduce methane emissions significantly to attempt to stay on a 1.5 degrees scenario. This was probably one of the key take-aways from UN COP26 climate conference in Glasgow late last year, and which was formalized in the Global Methane Pledge (reduce methane emissions by 30% by 2030) signed by over 100 countries. It is expected that this action alone could already avoid 0.2 degrees of warming.

That's huge!

This message was reiterated in the latest IPCC report which stated very clearly, that reducing methane emissions is the biggest opportunity to slow down global warming in the short term, between now and 2040.

We are therefore very excited that with our feed additive Bovaer® (scientific name 3-Nitrooxypropanol, 3-NOP) we are able to provide the farming community an effective tool to reduce methane emissions, and thereby enabling the livestock sector to play its part on fighting climate change, whilst at the same time improving food security and the livelihoods of thousands of people globally.

The feed additive Bovaer® is the result of, over a decade-long journey of research and development. It is the most extensively studied and scientifically proven solution to the challenge of enteric methane from ruminants, with over 51 scientific peer reviewed publications to date (May 2022). Over the last 12 months we were especially excited that Bovaer® became the first (and to date, only) feed additive to be successfully authorized in Europe as a zootechnical feed additive that has a positive effect on the environment. Following rigorous evaluation by EFSA FEEDAP (European Food Safety Authority Panel on Additives and Products or Substances used in Animal Feed), Bovaer® has been recognized as highly efficacious, safe for the consumer, the animal and the environment. In addition to the successful authorization in Europe, Bovaer® is available for sale today in 31 countries, including the EU, Switzerland, Australia, Chile and Brazil.

We are proud to be working with leading scientists from all around the world on Bovaer®, as our joint insights will enable farmers to take informed decisions and support adoption. Over the past 3 years, since the last GGAA meeting in 2019, an enormous breadth and depth of research was conducted, much of which is summarized in this abstract booklet and will be presented at GGAA 2022. In addition, the peer reviewed publications with Bovaer® have also been listed.

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

- Bovaer® reduces methane in all diets and ruminant breeds that have been tested.
- The impact of dose rate and diet composition needs to be considered with higher dose rates and higher starch/ lower NDF in the diet leading to greater % methane reductions in both dairy and beef cattle diets. The data from all our studies have been compiled into a new meta-analysis and resulted in the development of a new prediction model (which is submitted for publication).
- Supplementation with Bovaer® in dairy cow diets can lead to very positive changes in rumen fermentation characteristics, resulting in a shift in the fermentation profile, increasing rumen pH.
- The effect of Bovaer® on methane reduction in lactating dairy cows fed a high forage diet was investigated for 1 year across all stages of lactation, including the dry period. Overall, diet had the greatest impact on the percentage of methane yield reduction achieved (-19% reduction in methane yield), with a correlation observed between the percentage reduction and NDF digestibility of the grass silage component of the diet. Energy Corrected Milk yield was also increased in this study (+1.9 kg/d) and DMI was unaffected.
- Initial studies investigating the effect of supplementing dairy cows with Bovaer® in low and high fat diets demonstrated no interaction between the two mitigation strategies.
- When Bovaer® was combined with other methane mitigating dietary feed ingredients (fat and/or nitrate) in dairy cow diets, although methane was reduced by up to 60% when all 3 additives were fed simultaneously, the effect of each individual mitigator was not fully numerically additive. Manure was collected from these animals, and it was found there was no negative effect on biogas production.
- Additive effects in reducing methane emissions may be observed when comparing Bovaer® (-27%) and when Bovaer® is fed in combination with other essential oils, such as Crina®, leading to a 34% reduction in methane per se.

- A study in Australia showed that in finishing beef cattle diets, methane may be mitigated by up to 90% with no adverse effects on dry matter intake or average daily gain.
- Combining Bovaer® with canola oil in high forage beef backgrounding diets resulted in a significant reduction in methane emissions, with Bovaer® (-28%) reduction, oil (-24%) reduction and the effects of the two mitigating solutions being additive (-52%) in reducing methane emissions. Canola oil had a significant impact on the rumen microbiome whereas Bovaer® supplementation resulted only in minor changes, leading to a decrease in Methanobrevibacter and increase in Bacteroidetes.

We will continue our research to further expand the application of Bovaer® 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.



Does basal diet composition impact the methane mitigation potential of 3-nitrooxypropanol in dairy cattle?

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The objective was to determine whether the methane mitigation potential of 3-nitrooxypropanol (**3-NOP**) in dairy cows was affected by basal diet composition. Eight rumen-fistulated, multiparous Holstein-Friesian dairy cows were assigned to a double 4 × 4 Latin square design. The four treatments were (all DM basis) a grass silage-based diet (**GS**; 67% grass silage and 33% concentrate) and a corn silage-based diet (**CS**; 13% grass silage, 54% corn silage, and 33% concentrate) supplemented with a placebo (**NOP0**) or 80 mg 3-NOP/kg DM (**NOP80**). Each experimental period consisted of 14 d adaptation in a free-stall barn followed by 4 d of measurements in climate respiration chambers. On the last 2 d of adaptation, rumen fluid was collected 1 h before, and 1, 2, 3, 4, and 6 h after morning feeding. Rumen pH was recorded at 1-min intervals using indwelling pH loggers during the respiration chamber period. NOP80 decreased ($P = 0.038$) DM intake (**DMI**) with 1.0 kg/d, both with GS and CS. NOP80 did not affect milk yield and composition ($P > 0.100$). Ruminal pH increased ($P = 0.036$) and ruminal acetate to propionate ratio decreased ($P = 0.012$) with NOP80 both with GS and CS. Methane yield (g/kg DMI) decreased ($P = 0.002$) with NOP80 for both GS and CS, but the decrease in methane yield was numerically smaller for GS (-12.6%) compared with CS (-24.2%). A similar pattern was observed for methane intensity (g/kg energy correct milk), which decreased ($P < 0.001$) with NOP80 for both GS and CS, but the decrease was numerically smaller for GS (-14.6%) compared with CS (-24.7%). In conclusion, 3-NOP effectively decreases methane emissions in dairy cows but potentially to a greater extent with a corn silage-based diet compared with a grass silage-based diet.

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Methane emission from dairy cows receiving 3-nitrooxypropanol for one complete year

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The objective of this study was to determine the effect of 3-nitrooxypropanol on methane emission and production characteristics from dairy cows receiving 3-nitrooxypropanol in their diet for one complete year, including the dry period. Sixty-four late-lactation Holstein-Friesian dairy cows (33% primiparous) were blocked in pairs, based on expected calving date, parity, and milk yield. The experiment started with a covariate period of 3 weeks in which all cows received the same basal diet and baseline measurements were performed. Directly after, cows within a block were randomly allocated to 1 of 2 dietary treatments: a diet including on average 59 mg 3-nitrooxypropanol/kg DM (**3-NOP**) and a diet including a placebo (**CON**). The diet consisted of grass silage, corn silage, and concentrate. The ratio between grass silage and corn silage was constant throughout the experiment (70:30; DM basis). The forage to concentrate ratio depended on lactation stage (i.e., the dry period and early, mid, and late lactation) and milk yield. Diets were provided as a total mixed ration in feed bins (Hokofarm B.V.), which automatically recorded feed intake. The GreenFeed system (C-Lock Inc.) was used to measure methane emissions. Overall, 3-NOP did not affect daily milk yield (26.4 and 27.5 kg/d for CON and 3-NOP, respectively) and total DM intake (21.0 and 20.8 kg/d for CON and 3-NOP, respectively), but resulted in a higher energy-corrected milk yield (32.3 and 34.2 kg/d for CON and 3-NOP, respectively). Methane yield (g/kg DM intake) over the year was reduced by 19%, being 20.1 and 16.3 g/kg DM intake for CON and 3-NOP, respectively. Preliminary analysis show that the mitigation potential of 3-NOP depended on the quality of the grass silage (i.e., NDF digestibility in %), which appeared positively related to the mitigation potential of 3-NOP ($R^2 = 0.59$).

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Effect of 3-nitrooxypropanol alone and in combination with essential oils on methane production and dairy cow performance

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The effect of supplementing dairy cattle with 3-nitrooxypropanol (3-NOP, Bovaer 10[®]) alone or combined with a blend of essential oils (BEO, Crina[®] Ruminants) on methane emissions and milking performance compared with cows fed a placebo was investigated over 84 d. Sixty multiparous cows (BW: 606±70 kg, milk yield: 40.7±9.8 kg/d, DIM: 80±32) were divided into 3 treatments (Control; 3-NOP: 60mg 3-NOP/kg DM and COMBO: 60mg 3-NOP/kg DM + 1.5 g BEO/d). Individual intake of a diet (15% CP, 31.1% NDF, 1.70 Mcal of NEL/kg, DM basis) was monitored and cows had free access to GreenFeeds (C-Lock Inc., USA) to measure enteric emissions. Sniffers (Guardian NG Edinburgh Instruments Ltd., UK) recorded methane emissions at the exit of the milking parlor. Data were analyzed using a mixed-effects model for repeated measures. Methane emissions recorded using GreenFeeds were 27.4% and 34.2% lower (P<0.05) in 3-NOP (262.0 ±11.72 g/d) and COMBO cows (238± 11.72g/d) than Control cows (361 ± 11.72 g/d), respectively, indicating potential synergy with the combination. Methane emissions recorded with Sniffers were 15.2% and 19.5% lower (P < 0.05) from 3-NOP (422±17.6, l/d) and COMBO cows (401 ± 17.6 l/d) than Control cows (498±17.6 l/d). Inhibiting methane resulted in an increase in H₂ emissions greatest for 3-NOP (5.24 ±0.44 g/), followed by COMBO (4.85±0.44 g/d) and Control (2.56±0.44 g/d). Milk yield and quality were unaffected by treatments; however, feed intake increased (P < 0.05) in 3-NOP and COMBO cows over time, resulting in a reduction in feed efficiency (milk/intake). In conclusion, both GreenFeeds and Sniffer techniques can be used to assess potential effects of feed additives on methane emissions. Both, 3-NOP and its combination with BEO are effective substances to reduce methane emissions from dairy cattle without affecting milk yield but decreasing feed efficiency over time.

Gas exchanges and dry matter intake when lactating dairy cows are fed 3-NOP and fat

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It is well-known that fat and 3-NOP (Bovaer) reduce enteric methane production from ruminants. The aim of the study was to investigate the interaction effect between fat (33 g or 64 g crude fat kg⁻¹ DM) and 3-NOP (0 or 80 mg kg⁻¹ DM) in a 2×2 factorial arrangement. Four lactating multiparous Danish Holstein dairy cows were used in a balanced 4×4 Latin square design. Gas exchanges were measured in respiration chambers. All four cows were multi-cannulated with a rumen-, duodenum- and ileum cannula, as digestibility measures were collected as well. Whole-cracked rapeseeds were used as the fat source. Cows were fed the TMR ad libitum, and dry matter intake (DMI) was measured. The NDF content was 293 g kg⁻¹ DM and 282 g kg⁻¹ DM for TMR low and high in fat content, respectively. The DMI was reduced when cows were fed the 3-NOP diets (P=0.008), but increased fat content did not reduce the DMI (P=0.54). Increased fat content did not reduce methane production in L kg⁻¹ DMI (P=0.29), while 3-NOP reduced the methane production in L kg⁻¹ DMI by 25 % (P<0.001). No interaction between fat and 3-NOP was found on methane production in L kg⁻¹ DMI (P=0.45). Hydrogen production in L kg⁻¹ DMI increased when 3-NOP was fed (P<0.001) by 3300 %. No significant effect of fat was found on hydrogen production (P=0.98), and neither on the interaction of fat and 3-NOP (P=0.90). 3-NOP increased the carbon dioxide production (P=0.009) and oxygen consumption (P=0.005) in L kg⁻¹ DMI. In conclusion, there was no interaction of fat and 3-NOP on the methane production. The effects of fat were only numeric, while 80 mg 3-NOP kg⁻¹ DM reduced the methane production, reduced the dry matter intake and increased the hydrogen production.

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Combined Effects of Dietary Fat, Nitrate and 3-NOP on Dairy Cows' Enteric Methane Emission

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The objective was to investigate the combined effects of fat, nitrate, and 3-NOP on enteric methane production and production performance. 24 primi- and 24 multiparous lactating Holstein cows were included in an incomplete 8×8 Latin square design with six 21 d periods. Cows were blocked according to parity and days in milk. Diets were arranged in a 2×2 factorial design: 2 levels of fat (whole cracked rapeseeds) (low fat; 30 g crude fat (CF)/kg DM, or high fat; 63 g CF/kg DM), 2 levels of nitrate (calcium ammonium nitrate; Silvair) (0 g/kg DM or 10 g/kg DM; diets were isonitrogenous using urea) and 2 levels of 3-NOP (3-nitrooxypropanol; Bovaer) (0 mg/kg DM or 80 mg/kg DM). Methane emission was measured using GreenFeed. Data from the last 7 d of each period was averaged and included in the analysis, using a mixed procedure of R. For methane yield (g CH₄/kg DMI) there was no 3-way interaction between additives, however significant 2-way interactions were observed between fat, nitrate and 3-NOP. All three additives reduced methane yield, but effects were not additive and effect of combining additives did not exceed their individual effects. Methane yield varied from 16.7 for cows on low fat/no nitrate/no 3-NOP to 12.9 g/kg DMI for cows on high fat/nitrate/3-NOP. DMI was reduced by dietary supplementation and a 2-way interaction between fat and nitrate was observed. Addition of fat did not affect DMI, whereas addition of nitrate reduced DMI by 5%. However, combining fat and nitrate reduced DMI by 13%. 3-NOP reduced DMI with 13%. In conclusion, fat, nitrate, and 3-NOP are effective methane mitigation additives, but their individual effects were not additive, despite different modes of action. Potential DMI reductions may be a challenge for the implementation of otherwise effective methane mitigation additives, when feeding a Danish ration.

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Methane Potential of Manure from Dairy Cows Supplemented with Dietary Fat, Nitrate and 3-NOP

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Potent feed additives reduce enteric methane emission from dairy cows. The aim of this study was to investigate if biogas potential of manure from methane mitigated dairy cows also was affected. As part of a larger experiment, the methane potential of manure was investigated, when dairy cows were supplemented with fat, nitrate, and 3-NOP as enteric methane mitigation additives. 24 primi- and 24 multiparous lactating Danish Holstein cows were included in an incomplete 8×8 Latin square design with six 21-day periods. Every cow received six of eight diets. Diets were arranged in a 2×2 factorial design: 2 levels of fat (whole cracked rapeseeds) (low fat; 30 g crude fat (CF)/kg DM, or high fat; 63 g CF/kg DM), 2 levels of nitrate (calcium ammonium nitrate; Silvair) (0 or 10 g/kg DM; diets were isonitrogenous using urea) and 2 levels of 3-NOP (3-nitrooxypropanol; Bovaer) (0 or 80 mg/kg DM). All treatments reduced enteric methane. Manure was sampled from individual cows during the last day in period two and five. Accumulated methane production was determined in vitro in a batch assay after 90 days of incubation and methane yield was expressed at standard conditions (STP). A total of 94 observations were analyzed, using a mixed procedure of R. In cows fed low fat diets, methane yield in manure was 231, 247, 242 and 214 L CH₄/kg volatile solids (VS) when no additives, nitrate, 3-NOP and nitrate+3-NOP were fed, respectively. However, in manure from cows fed high fat diets, the yield was 286, 282, 269 and 266 L CH₄/kg VS, when no additives, nitrate, 3-NOP and nitrate+3-NOP were fed, respectively. In conclusion, dietary fat was the only factor significantly affecting the methane yield in manure, which was increased by 18% for cows fed high fat diets compared to cows fed low fat diets.

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Decreased methane production in Angus cattle fed 3-Nitrooxypropanol (Bovaer 10®)

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Abstract:

We aimed to quantify the effect of increasing diet concentration of 3-NOP (Bovaer®) on rumen fermentation and methane emissions of Angus steers fed typical Australian feedlot diets. Twenty Angus steers of initial liveweight of 356 ± 14.4 kg were allocated in a completely randomized block design. The experimental period was 112 days in which steers were housed in individual indoor pens, including the first 21 days of adaptation. Five different 3-NOP regimens in dry matter basis were compared: Control = no added 3-NOP; Low = 50 mg/kg 3-NOP from d 0 to 112; Medium A = 50 mg/kg from d 0 to 7, 75 mg/kg from d 8 to 112; Medium B = 50 mg/kg from d 0 to 7, 75 mg/kg from d 8 to 14, 100 mg/kg from d 15 to 112; and High = 50 mg/kg from d 0 to 7, 75 mg/kg from d 8 to 14, 100 mg/kg d 15 to 21, 125 mg/kg from d 22 to 112. For the 3-NOP titration regimens, the adaptation period followed a step-wise approach in terms of both increasing 3-NOP diet concentration and increasing tempered barley content in the diet. The CH₄ emission, ruminal parameters and performance traits were measured with respiratory chambers. This project demonstrated that all 3-NOP regimens significantly decreased methane of feedlot cattle from d 21 to 112 of the feeding period. The maximum CH₄ production abatement from 3-NOP was observed in day 28, averaging 99% reduction compared to control steers. For the overall feeding period, methane emissions (g/d) were 78% lower in the animals that followed the Medium B titration regimen than control animals. Although this experiment was not designed to make conclusions on an effect of 3-NOP on feedlot performance no negative effects of any 3-NOP regimen were detected on production parameters.

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Understanding the diverse impacts of methane inhibitors on the rumen microbial community

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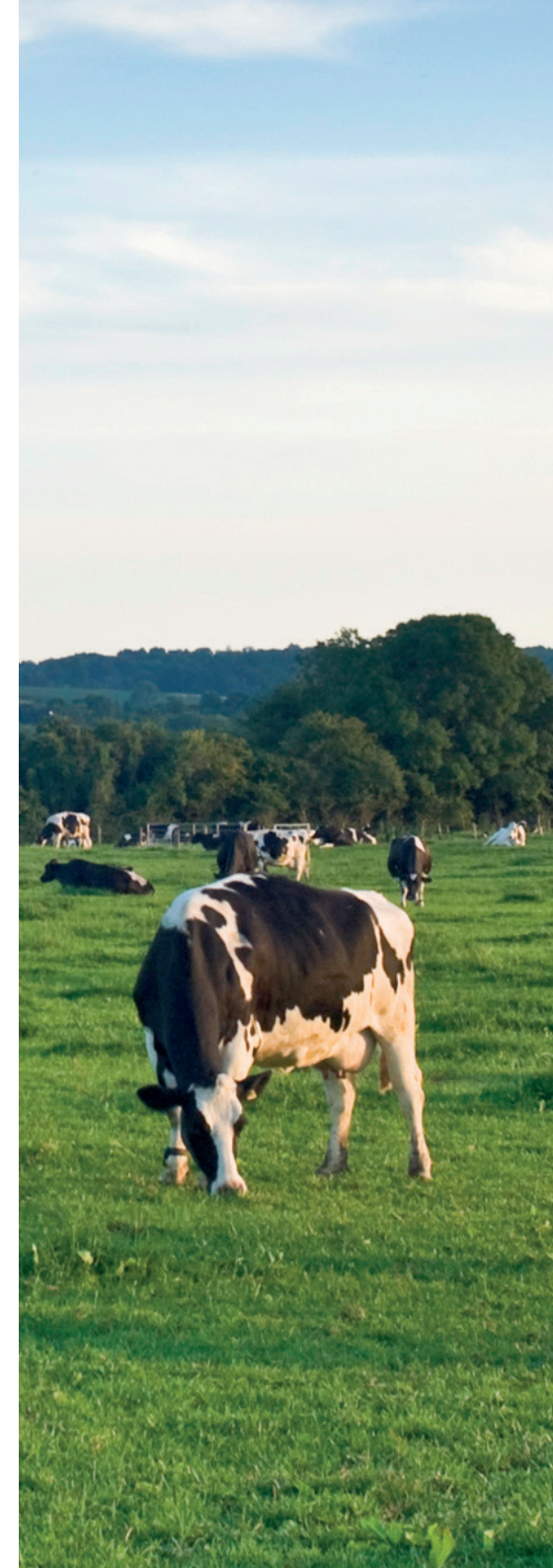
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Abstract:

It is essential to understand the impact that different methane inhibitors have on the rumen microbial community. Here we have examined the effects of supplementing a high-forage diet with the investigational methane (CH₄) inhibitor 3-nitrooxypropanol (3-NOP) and canola oil (OIL) on the rumen microbiome of beef cattle, enteric CH₄ emissions, and ruminal fermentation. 3-NOP and OIL individually reduced enteric CH₄ emission (-28.2% and -24.0%, respectively), and the effects were additive when used in combination (-51.3%). 3-NOP increased H₂ emissions 37 fold, while co-administering 3-NOP and OIL increased H₂ in the rumen 20-fold relative to the control diet. The inclusion of 3-NOP or OIL reduced the microbial diversity of the rumen microbiome. 3-NOP resulted in targeted changes in the microbiome decreasing the relative abundance of Methanobrevibacter and increasing the relative abundance of Bacteroidetes. The inclusion of OIL resulted in large scale changes to the microbial community, ruminal volatile fatty acid concentration and gas production. OIL significantly reduced the abundance of protozoa and fiber-degrading microbes in the rumen but it did not selectively alter the abundance of rumen methanogens. The addition of OIL to the diet resulted in a large decrease in the number sequences attributable to Fibrobacter (41-243 fold decrease; P < 0.001) in both rumen fluid and digesta. These data show that 3-NOP specifically targeted rumen methanogens inhibiting the hydrogenotrophic methanogenesis pathway and increased H₂ emissions and propionate production. OIL caused large scale changes in the rumen microbial community by indiscriminately altering the abundance of a range of rumen microbes, reducing the abundance of fibrolytic bacteria and altering rumen fermentation. Our data suggests that co-administering CH₄ inhibitors with distinct mechanisms of action can enhance CH₄ inhibition and provide alternative sinks to reduce the accumulation of ruminal H₂.

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