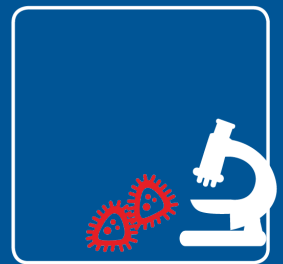


Nina Aagaard Poulsen:
ClimateMilk – Effekt af metan reducerende
tilsætningsstoffer i foderet på mælkenes
kvalitet og funktionalitet

ClimateMilk – Effect of feed additives on milk
quality and functionality



Final report

for collaborative projects funded via the Danish Dairy Research Foundation (DDRF)

1. Title of the project

Danish: ClimateMilk - Effekt af metan reducerende tilsætningsstoffer i foderet på mælkenes kvalitet og funktionalitet

English: ClimateMilk – Effect of feed additives on milk quality and functionality

2. Project manager

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4. Sources of funding

Apart from the Danish Dairy Research Foundation, the co-financing comes from the project “Fodring og fænotype af den klimaeffektive malkeko” (PI Peter Lund, AU-ANIVET, 2020-2022), which is funded from Landbrugsstyrelsen, as in-kind from Arla Innovation Centre and from Graduate School of Technical Science (GSTS).

5. Project period

Project period with DDRF funding:

October 2020 – December 2023

6. Project summary

Danish:

I et stort fodringsforsøg blev effekten på køernes metanudledning af 3-NOP (3-nitro-oxy-propanol), nitrat samt fedttildeling (behandlede rapsfrø) testet i foderrationer med typiske danske fodermidler. I ClimateMilk er formålet at undersøge effekten af disse tilsætningsstoffer på mælkens sammensætning og forarbejdningskvalitet, samt en eventuel overførsel af disse stoffer, enten direkte eller i modificeret form til mælken. Dette relaterer sig både til foderforsøget samt forsøg med 3-NOP alene.

Resultaterne viste at et øget fedtindhold i foderet uden samtidig 3-NOP tildeling forbedrede mælkens ernæringsmæssige værdi på grund af reduceret andel af SFA og øget andel af MUFA og PUFA, men disse effekter blev reduceret, når fedt blev fodret i kombination med 3-NOP. Omvendt ændrede tilskud af nitrat alene, eller sammen med fedt og/eller 3-NOP, ikke på andelen af SFA, MUFA og PUFA i mælken. Proteinsammensætning og makromineraler i mælken blev kun i mindre grad påvirket. Fodring med nitrat gav en stigning i indholdet af vitamin B2 og nitratniveauet i mælk. Indhold af nitrit i mælken var under målemetodens detektionsniveau. Tilskud af 3-NOP førte til en stigning på 21,1% i vitamin B12. Ved fodring med 3-NOP kunne en overførsel til mælk ikke observeres. Mindre ændringer i krystallisations- og smelteegenskaber af 3-NOP mælkefedt blev observeret, men ingen ændringer i fastheden af 3-NOP smør, sammenlignet med kontrolsmør. Sensoriske tests detekterede også kun en mindre forskel, mens der blev påvist en forbedret oxidativ stabilitet af 3-NOP smør under lagring. Samlet set giver ændringerne i mælken ikke anledning til bekymring, men de underliggende mekanismer for de ændringer, der observeres bør undersøges nærmere og i kombination med andre produktionsforhold og fodringer.

English:

A large feeding trial tested the effect of including 3-NOP (3-nitro-oxypropanol), nitrate, and fat supplementation (cracked rapeseed) in feed rations with typical Danish feed materials on cow's methane emissions. In ClimateMilk, the aim is to investigate the effect of these additives on the composition and processing quality of the milk as well as a possible transfer of these substances either directly or in modified form to milk. This relates both to the feeding trial with a combination of feed additives and in trials with 3-NOP alone.

The results showed that an increased fat content in the feed without 3-NOP improved the nutritional value of milk due to a reduced amount of SFA and an increased amount of MUFA and PUFA, but these effects were reduced, when fat was fed in combination with 3-NOP. Conversely, supplementation of nitrate alone, or with fat and/or 3-NOP, did not change the proportions of SFA, MUFA and PUFA in milk. Protein composition and macrominerals in the milk were only slightly affected. Feeding added nitrate resulted in an increase in vitamin B2 content and nitrate levels in milk. The content of nitrite in the milk was below the detection level of the measurement method. Supplementation of 3-NOP led to a 21.1% increase in vitamin B12 in milk. When feeding 3-NOP, a transfer of 3-NOP to the milk could not be observed. When feeding with 3-NOP, minor changes in milk fat's crystallization and melting properties were observed, but no changes in the firmness of 3-NOP butter compared to control butter. In addition, only a minor difference in sensory attributes was observed, while an improved oxidative stability of 3-NOP butter was demonstrated during storage over 12 weeks. Overall, the changes in milk are not a cause for concern, but the underlying mechanisms of the changes observed should be investigated more closely and in combination with other management systems and feedings.

7. Project aim

Danish:

Mælks klimaaftryk er især relateret til udledning af klimagasser fra gården, som udgør ca. 75% af den totale udledning. Derfor skal denne del reduceres betydeligt for at sikre et lavere klimaaftryk på kort sigt og opnå det ambitiøse mål om klimaneutral mælk i 2050 som er målsætningen for nogle mejerier. Tilsætningsstoffer såsom 3-NOP (3-nitrooxypropanol) og nitrat er dokumenteret til at reducere koens metan produktionen med 30% ved henholdsvis at manipulere de tilstedeværende metanogene mikroorganismer i vommen og ændre fermentationen. I 2020 startede et stort fodringsforsøg, som tester brugen af disse fodertilsætningsstoffer i kombination med typiske danske fodermidler som en del af projektet "Fodring og fænotype af den klimaeffektive malkeko" (projektleder Peter Lund, AU-ANIVET, støttet af Landbrugsstyrelsen). **Dette giver en unik og betimelig mulighed for at undersøge effekten af disse tilsætningsstoffer på mælkens sammensætning og forarbejdningskvalitet samt en eventuel overførsel af disse enten direkte eller i modificeret form til mælken, hvilket samlet er dette projekts formål.** Især fraktioner i mælken som er direkte relateret til vommens mikroorganismer (f.eks. fedtsyrer og B vitaminer) kan potentielt være påvirkelige, men er endnu ikke undersøgt. Tidligere studier har dokumenteret en uændret mælkeydelsen men øget proteinindhold. Ændringer i mælkens proteinprofil vil kunne påvirke stabiliteten af kasein miceller og mineralbalancen i mælk. Tilsammen kan sådanne ændringer manifestere sig i mælkens forarbejdningssevne, lagringsstabilitet og sensoriske egenskaber. Dette projekt består til dels af et stort fodringsforsøg på Danmarks Kvægforskningscenter i Foulum som undersøger effekten af 3-NOP og nitrat på dyrenes metanudledning og vommens mikrobiota samt enkelte besætningsforsøg som tester mulig implementering af disse virkemidler så vel som dette studie som undersøger effekten af disse fodertilsætningsstoffer på mælkens sammensætning, funktionalitet og smag.

English:

Farm level emissions, which account for around 75% of the GHG emissions from dairy must be reduced dramatically in order to reduce the climate footprint of milk and to achieve the ambitious goals of climate-neutral dairy production in 2050 put forward by some dairies. This calls for the implementation of new management strategies including feed additives, like nitrate or 3-NOP (3-nitrooxypropanol). Both feed additives are promising and have been documented to reduce enteric methane by as much as 30%, achieved by rumen manipulation. In 2020, a large-scale feeding-trial was started testing the effect of these feed additives in combination with Danish feeding conditions as part of the project "Fodring og fænotype af den klimaeffektive malkeko" (lead by Peter Lund, AU-ANIVET), which is granted by Landbrugsstyrelsen. **This offers a unique and timely possibility to explore the effect of these feed additives on milk composition and functionality and to check how/if these additives manifest themselves in original or modified forms in the milk, which is the aim of this project.** Especially the specific fractions directly related to rumen microorganisms (fatty acids and B vitamins) are expected to be the main factors potentially affected. Milk protein composition is another important fraction, which may be altered and thereby potentially affect the casein micelle stability and mineral balance in the milk. Taken together, these impacts may also manifest into processing abilities, functionality, storage stability and sensory properties. The current project consists partly of the already granted feeding trials at the Danish Cattle Research Centre, Foulum together with farm studies examining the effect of nitrate and 3-NOP feed additives on methane emission and rumen microbiome, and was interested in investigating the dairy part relating to the quality of the produced milk in terms of composition, functionality, processability and sensory attributes.

8. Background for the project

Current methane-reducing dietary strategies include changing feed composition and quality, increasing the fat content in the diet, and adding feed additives, such as nitrate and 3-NOP to the diet. These strategies can lower the methane emissions from dairy cows up to 30%. However, the effects of these strategies on the ruminal environment, including the microbiota, pH, and physiological processes, such as synthesis and degradation of nutrients, may alter milk composition. Previous studies have shown the impact of these strategies on milk yield, gross composition, and milk FA profile (Almeida et al., 2022; Givens et al., 2003; Melgar et al., 2021; Van Gastelen et al., 2015). However, no studies have

examined the potential alterations in protein and mineral compositions and vitamins in milk caused by these strategies. Additionally, some feed additives can transfer residues to milk, raising possible health issues for consumers. Only a few studies have conducted a residual analysis of nitrate and 3-NOP in milk (Olijhoek et al., 2016; Meller et al., 2019; Almeida et al., 2022; Thiel et al., 2019); thus, additional studies are needed. Changes in the FA profile in milk affect the physical and functional properties of milk fat, such as solid fat content (SFC), melting, and crystallization (Smet et al., 2010; Pacheco-Pappenheim et al., 2022). These modifications in FA composition and functional properties of milk fat subsequently impact quality aspects such as sensory attributes and storage stability of fat-based dairy products (Bobé et al., 2003; Couvreur et al., 2006; Barros et al., 2014; O'Callaghan et al., 2016). Therefore, it is crucial to determine the impact of these feeding interventions on the functional properties of milk and the quality of dairy products.

9. Sub-activities in the entire project period

In 2020/2021, the first experimental animal trial (WP1 - Animal trials and farm experiments, funded by Landbrugsstyrelsen), determining the effects of supplementing feed with fat, nitrate and 3-NOP (Bovaer) on milk composition and functionality, was conducted at the Danish Cattle Research Centre at Foulum. Forty-eight lactating Holstein cows were blocked according to parity and days in milk and allocated to 8 different rations (2 levels of fat (cracked rapeseed), +/- nitrate, +/- 3-NOP) over 6 periods of 21 days each, according to the incomplete Latin square design testing both the effect of each treatment individually as well as possible synergy between treatments.

In WP2 (Micro- and macronutrients of milk), 288 milk samples from the animal trial at the Danish Cattle Research Centre were aliquoted and skimmed to obtain skim milk and fat fractions. At each sampling, fresh milk samples were analysed for overall milk composition, including citrate and urea using Milkoscan, as well as pH and conductivity. Furthermore, milk fat globule size was measured by Mastersizer. Using samples, which had been frozen, the fatty acid and protein compositions were determined by GC-FID and LC-MS, respectively. Casein micelle size was also determined in all samples using Zetasizer. Vitamin E (HPLC) was analysed in all samples. In 2023, analysis of vitamin B2 (HPLC) and minerals (Ca, Mg, P, K, by ICP-MS) at AU-FOOD was finalized. Furthermore, after pretreatment at AU-FOOD, milk samples were sent for analysis of vitamin B12 to Arla Foods Arinco. This analysis was finalized in 2023. Full milk samples were sent to DSM in Switzerland and used for analysis of 3-NOP in milk by HPLC (analysed by Katrien Schaefer, DSM). In total, 24 samples were analysed (only 3-NOP treatments, pooling milk from individual cows (n=4) for each treatment (n=4) in each period (n=6). In 2023, nitrate and nitrite contents were also analysed using a colorimetric method.

As part of WP3 (Milk functionality and processing quality), solid fat content (SFC), measured by Nuclear Magnetic Resonance (NMR), and changes in the crystallization and melting behavior, measured by Differential Scanning Calorimetry (DSC) were evaluated in fat from the experimental animal trial (Master project Cecilie Kaysen, MEF). In November 2021, a 3-NOP implementation study was initiated at the Assendrup farm holding 280 Danish red cows. Samples from all the cows were sent to Eurofins for the analysis of gross milk composition. A subset of samples (40) from control and 3-NOP groups were skimmed. The coagulation properties (Rheolaser), protein composition (LC-MS), casein micelle size (Zetasizer) of skim milk, and fatty acids of milk fat (GC-FID) have been analysed (Master project, Julia Prangchat Stub Thomsen). In the second part of this experiment, Arla Foods Innovation Center (AIC) produced butter of milk from control and 3-NOP groups. Butter received by AU-FOOD was used to take the color parameters, crystallization and melting properties. Furthermore, 10 cubes of butter from each treatment were stored at 5 and 20 °C for 12 weeks, samples were collected every 2 weeks, and peroxide values were measured.

In June 2022, a new trial was conducted at AU campus, Viborg, to test effect of 3-NOP at 60 mg/kg DM with Danish Holstein cows. At the latter part of the experiment, Arla Food Innovation Center collected milk and produced butter. Full milk received by AU-FOOD was used to measure overall milk composition (Milkoscan). Casein micelle size, mineral composition and protein composition were analysed using skim milk samples. Butter samples have been used to measure color parameters (Minolta), and crystallization and melting characteristics. Similar to the above trial, a storage experiment was done, and peroxide value was measured.

these effects became smaller in combination with 3-NOP (Figure 1). Supplementation of nitrate alone or with fat and/or 3-NOP did not change the proportions of SFA, MUFA and PUFA in milk (Lokuge et al., 2024a).

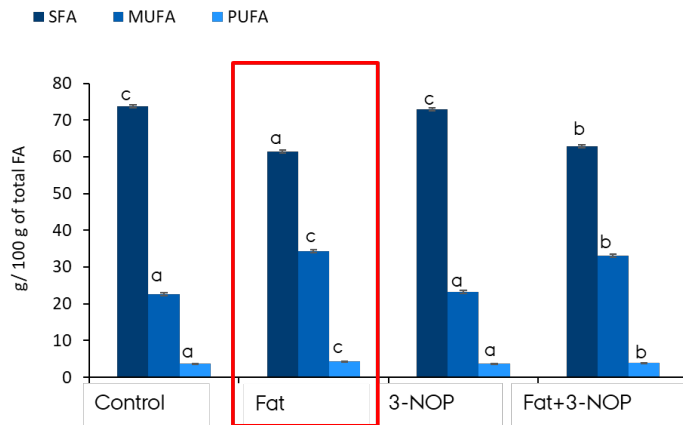


Figure 1. Effect of fat x 3-NOP interaction on milk fatty acids (Lokuge et al., 2024a)

Feeding nitrate at the dose of 10 g/kg DM resulted in a 5.4% increase in riboflavin (vitamin B2) in milk (Figure 2). Furthermore, supplementation of 3-NOP at the concentration of 80 mg/kg DM led to a 21.1% increase in cobalamin (vitamin B12) content in milk (Figure 3) (Lokuge et al., 2024b). From a nutritional point of view, these changes are beneficial for the dairy consumers.

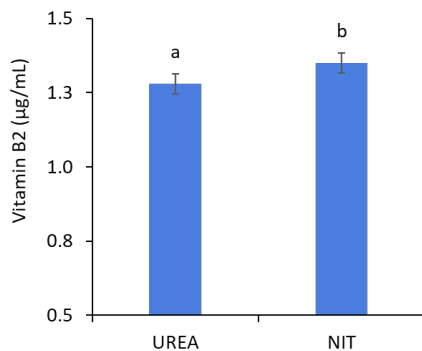


Figure 2. Effect of nitrate supplementation on vitamin B2 in milk (Lokuge et al., 2024b)

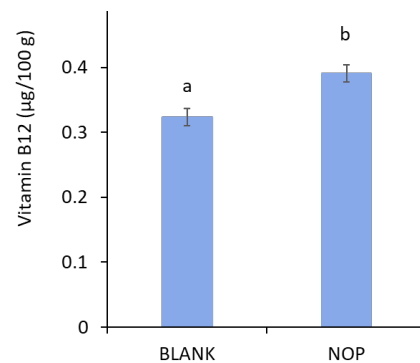


Figure 3. Effect of 3-NOP supplementation on vitamin B12 in milk (Lokuge et al., 2024b)

2. Milk functionality and quality of dairy products:

Due to the observed effects of fat supplementation and 3-NOP on FA composition, analyses were extended to test how these treatments affected the functionality of milk fat. According to the results, the increased UFA content resulting from fat supplementation led to a reduction in the solid fat content of milk fat (Figure 4), while the changes in crystallization and melting properties of milk fat by fat, nitrate and 3-NOP were small, and may not be of practical importance for dairy product quality (Lokuge et al., 2024a).

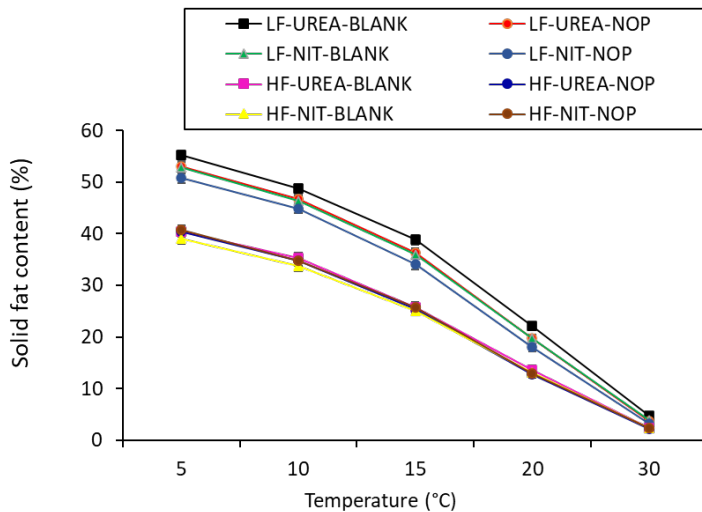


Figure 3. Effect of fat supplementation (HF-High fat diets and LF-Low fat diets) on solid fat content of milk fat (Lokuge et al., 2024a).

An additional animal trial with Holstein cows was performed testing the effect of feeding 3-NOP at the dose of 60 mg/kg DM on butter quality. From the collected milk, a pilot-scale butter production was conducted at Arla Innovation Centre including sensory quality of butter. According to the results, the minor changes observed in SFC, crystallization and melting characteristics of milk fat induced by 3-NOP did not result in any discernible changes in firmness of the resultant butter when compared to the control butter (Lokuge et al., 2024c). Furthermore, the sensory analysis found only minor differences between the 3-NOP and control butters as seen in Figure 5. This suggests that the addition of 3-NOP at the dose of 60 mg/kg DM to the diets of dairy cows do not modify the organoleptic properties of butter.

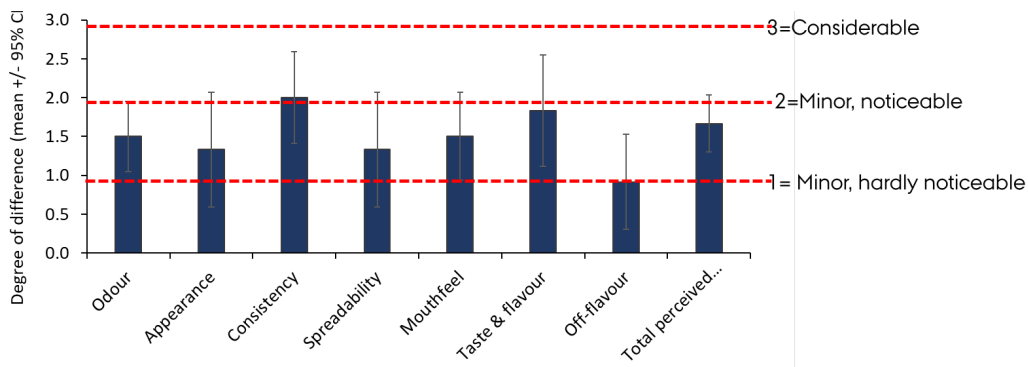


Figure 5. The degree of difference in sensory properties of butter made of milk from cows fed with 3-NOP supplemented diets compared to control diets

The butter was stored over 12 weeks in a storage experiment. During storage, the peroxide value was measured as an indicator of primary oxidation of fat. The results revealed improved oxidative stability of 3-NOP butter compared to control butter (Lokuge et al., 2024c).

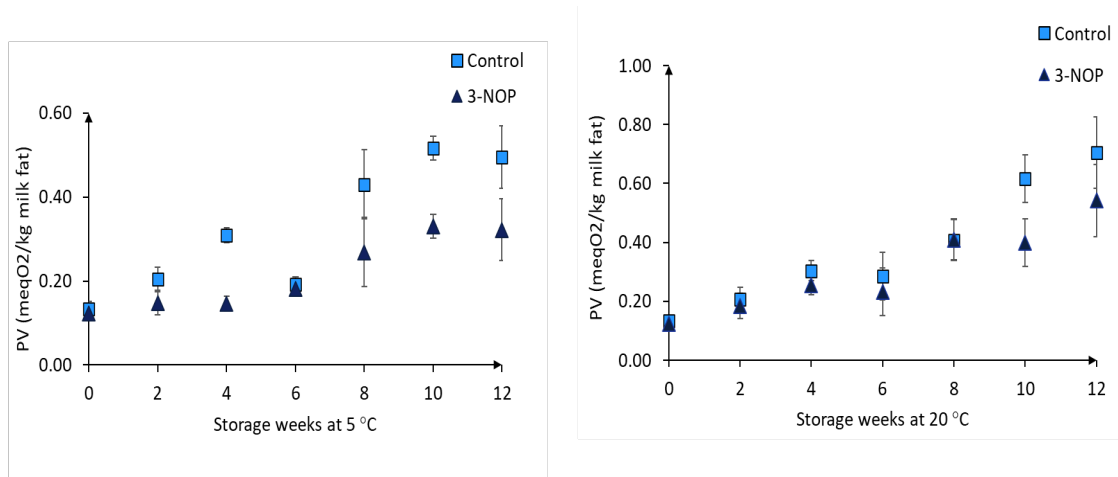


Figure 6. The peroxide value (PV) of butter made of milk from cows fed with 3-NOP supplemented diets and control diets, over 12 weeks of storage at 5 °C and 20 °C

3. Manifestation of feed additives in original or modified forms in the milk:

The residues of nitrate and 3-NOP in milk were analyzed. According to the results, the concentration of 3-NOP in milk was below the limit of detection (0.86 mg 3-NOP/L milk). Increases in nitrate levels in milk were observed, when supplementing diets with nitrate at the dose of 10 g/kg DM (Figure 7). However, this increased level of nitrate in milk was well below the maximum permissible level (50 mg/L). Therefore, the observed increment of nitrate in milk was deemed safe for human consumption. Nitrite in milk was below the limit of detection (30 µg/L), further confirming the safety of liquid milk for human consumption (Lokuge et al., 2024b).

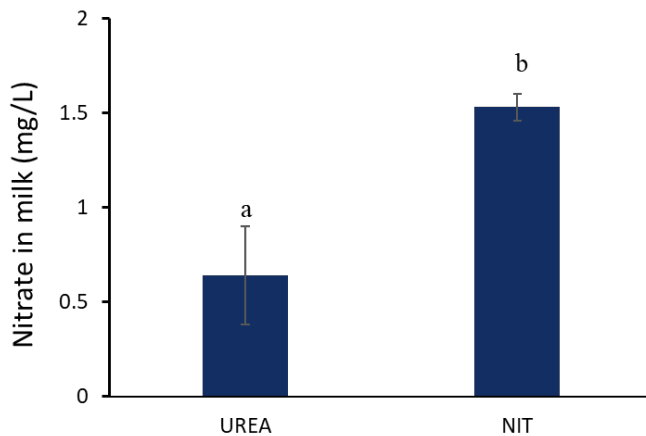


Figure 7. Effect of nitrate supplementation on nitrate level in milk (Lokuge et al., 2024b)

4. Protein composition and macro-minerals in milk

The supplementation of fat by rapeseed, feeding nitrate and 3-NOP did only result in marginal changes in protein and mineral composition in milk (Lokuge et al., 2024b).

12. The relevance of the results, including relevance for the dairy industry

Implementation of 3-NOP in European dairy production is ongoing, e.g. with the large-scale on-farm pilot program. 3-NOP is, however, a synthetic compound and is not approved for organic production. Fat supplementation may be a solution in organic production. Nitrate is currently tested in a small number of Danish herds as part of the project ME-TAKS (GUDP). Here additional tests of milk nitrate and nitrite concentrations will be analysed. Depending on the use of milk, the effects of feed additives on milk quality and functionality may be unwanted in some dairy streams or production types.

The experimental animal trial has confirmed the effect on methane reduction of the tested feed additives and with the current 3-NOP implementation study also practical considerations for implementation are evaluated. 3-NOP was approved by EFSA in November 2021 and Arla Foods and DSM are currently conducting a large-scale on-farm pilot program on 3-NOP. Both nitrate, 3-NOP and fat supplementation provide significant effect on milk quality, and further evaluation and additional analyses on e.g. vitamins are still needed in order to provide a comprehensive evaluation and understanding of underlying mechanisms. The butter production performed at Arla Innovation Centre in spring 2022, has provided novel insight into the effect of 3-NOP on functional and sensory properties of butter. The results do not lead to any major concerns regarding the use of 3-NOP.

13. Communication and knowledge sharing about the project

Papers in international journals:

Lokuge, GMS, N Nielsen, M Maigaard, P Lund, LB Larsen, L Wiking, NA Poulsen. Differential effects of 3-nitrooxypropanol supplementation on milk fatty acids profiles in three different dairy breeds. Submitted to JDS Communications.

Lokuge GMS, C Kaysen, P Lund, M. Maigaard, LB Larsen, L Wiking and NA Poulsen. 2024a. Effects of feeding whole-cracked rapeseeds, nitrate and 3-nitrooxypropanol on composition and functional properties of milk fat fraction from Danish Holstein cows. *Journal of Dairy Science* 107:5330-5342

Lokuge, G.M.S., Larsen, M.K., Maigaard, M., Wiking, L., Larsen, L. B., Lund, P. & Poulsen, N.A. 2024b. Effects of feeding whole-cracked rapeseeds, nitrate, and 3-nitrooxypropanol on protein composition, minerals, and vitamin B in milk from Danish Holstein cows. *Journal of Dairy Science* 107: 5353-5365.

Lokuge, GMS, M Maigaard, P Lund, TAM Rovers, LB Larsen, NA Poulsen, L Wiking. 2024c. Physico-chemical, sensory and oxidative quality of butter from cows fed 3-nitrooxypropanol. *International Dairy Journal* 152:105885

Easily read papers:

Bæredygtig mælkeproduktion – optimal mælke kvalitet. Mælkeritidende nr 6, 2021, s. 2-3.

Metanreducerende foderadditiver: Hvad sker der med mælke kvaliteten? DCA nyheder. <https://dca.au.dk/aktuelt/nyheder/vis/artikel/metan-reducerende-foderadditiver-hvad-sker-der-med-maelkekvaliteten>

Mælke kvalitet i lyset af klimavenligt foder. Mælkeritidende *in press*

Student theses:

Cecilie Kaysen. 2021. Master Thesis: Climate-friendly feed solutions influence on the functionality of milk fat, Master of Science (MSc) in Engineering (Biotechnology), Aarhus University

Julia Prangchat Stub Thomsen, 2022. Master Thesis: Quality and functionality of milk from cows with low climate footprint, Msc in Molecular Nutrition and Food Technology, Aarhus University

Nanna Juhl Rasmussen. 2023. Master Thesis: Effect of climate-friendly feeding strategies on milk quality and composition. Master of Science (MSc) in Chemical Engineering, Aarhus University

Gayani Madushani Sirinayake Lokuge. 2023. PhD Thesis: Impact of climate-friendly dietary interventions on the composition and quality of milk and dairy products.

Oral presentations at scientific conferences, symposiums etc.:

Poulsen NA. Sustainable milk production and the impact on milk quality and functionality. Keynote speaker. Dairy Science and Technology Symposium – virtual, Aarhus University, June 21-25, 2021.

Lokuge, GMS, Sørensen MM, Wiking L, Larsen, LB, Poulsen NA. The effects of feeding fat, nitrate and 3-NOP on methane emission and milk composition of dairy cows. Abstract and flash presentation. Dairy Science and Technology Symposium – virtual, Aarhus University, June 21-25, 2021.

Poulsen NA, GMS Lokuge, M Maigaard, B Buitenhuis, P Lund, M Johansen, L Wiking, LB Larsen. Sustainable milk production: Effect of feeding and feed additives on milk quality and functionality. Abstract and oral presentation. Nordic Dairy Congress, Malmö, Sweden. May 18-20, 2022

Lokuge GMS, LB Larsen, M Maigaard, L Wiking, P Lund, NA Poulsen. Combined effect of anti-methanogenic compounds (rapeseeds, nitrate and 3-NOP) on fatty acids in milk. Abstract and oral presentation EAAP, Porto, Portugal, September 5-9, 2022

Lokuge, Gayani M. S., Morten Maigaard, Nicolaj Ingemann Nielsen, Lotte Bach Larsen, Peter Lund, Lars Wiking, Nina Aagaard Poulsen. Sustainable dairy: Effect of feed additives on milk composition and functionality. Abstract and Oral Presentation, *ADSA International Partnership Program Symposium - Dairy Research in Denmark, ADSA Annual Meeting*, West Palm Beach, Florida, June 15-19, 2024

Oral presentations at meetings:

Poulsen, NA Bovaer og mælke kvalitet – hvad ved vi? Webinar: Bovaer - Hvad er op og ned i debatten om tilsætning af Bovaer til køernes foder? Mejeriteknisk Selskab. March 4, 2025

Poulsen, NA. Sustainable dairy production; determining milk quality, international webinar. Invited speaker “Importance of milk quality in sustainable milk production”, May 17, 2021, National Dairy Research Institute, ICAR, Karnal, India

Poulsen NA, Wiking L, Larsen LB. Sustainable milk production and the impact on milk quality and functionality. Abstract and presentation. Sustainable Dairy Symposium, Mejeriteknisk Selskab, June 2, 2021.

Milk quality relative to sustainable dairy production, Presentation at Sustainable Livestock Production Seminar, Nestle, virtual, March 4, 2022

Poulsen, NA, *Status reporting: Climate Milk*, DDRF Coordination group Technology & Safety, October 11, 2022, Aarhus University

Poulsen, NA. Climate mitigation strategies – effect on milk composition. Annual Meeting at the Finnish Society of Dairy Science, “A food chain that takes into account climate change”, invited speaker, Helsinki, Finland, October 25, 2023

Lokuge, G.M.S., Larsen, L.B., Maigaard, M., Lund, P., Wiking, L. & Poulsen, N.A. (2023). Effect of anti-methanogenic compounds on milk composition and quality of dairy products, Dairy Research Day 2023, Herning, Denmark, (abstract and poster presentation).

14. Contribution to master and PhD education

Cecilie Kaysen. 2021. Master Thesis: Climate-friendly feed solutions influence on the functionality of milk fat, Master of Science (MSc) in Engineering (Biotechnology), Aarhus University

Julia Prangchat Stub Thomsen, 2022. Master Thesis: Quality and functionality of milk from cows with low climate footprint, MSc in Molecular Nutrition and Food Technology, Aarhus University

Nanna Juhl Rasmussen. 2023. Master Thesis: Effect of climate-friendly feeding strategies on milk quality and composition. Master of Science (MSc) in Chemical Engineering, Aarhus University

Gayani Madushani Sirinayake Lokuge. 2023. PhD Thesis: Impact of climate-friendly dietary interventions on the composition and quality of milk and dairy products.

15. New contacts/projects

Measurement and reduction of methane in practice (METAKS). GUDP. 2022-2025. DKK 13.384.700,00. AU-FOOD, AU-ANIVET, ARLA amba, Danish Crown, Vilomix, SEGES, DLG amba. Principal investigator: Nicolaj Ingemann Nielsen (SEGES)