IMPLEMENTATION OF THE USE OF THE ENTERIC METHANE MITIGATING FEED ADDITIVE BOVAER® IN THE NATIONAL DANISH EMISSION INVENTORIES FOR DAIRY COWS

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Implementation of the Use of the Enteric Methane Mitigating Feed Additive Bovaer® in the National Danish Emission Inventories for Dairy Cows

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Background

This report is based on a commission from the Danish Veterinary and Food Administration dated 17.04.24. This report constitutes of part 2 of predicted 3 tasks aimed at ensuring that the use of Bovaer in Denmark can be reflected in the national emissions inventory. The first methane-reducing feed additive, Bovaer, has been approved for use in the EU, but its application is not yet reflected in the national emissions inventory. This requires documentation of methane emissions from cattle fed Bovaer, the number of cattle in Denmark annually administered Bovaer, and documentation of the dosage used (mg/kg feed dry matter). The purpose of this task is to incorporate Bovaer into the Danish model for calculating methane emissions as a prerequisite for Aarhus University's (AU) calculation of the proportion of gross energy in feed lost as methane (a new Ym factor) and to ensure that this is approved by the UN/IPCC.

The first task was to ensure sufficient data on the effect of Bovaer under Danish conditions. AU submitted 2 reports on this in 2023 based on experimental results. Johansen, M., Maigaard, M. & P. Lund (2023a and 2023b) This second task, which concerns this assignment, is to define a new Ym factor that incorporates data on the effect of Bovaer under Danish conditions, includes results from the first task. The new Ym factor must be approved by the UN/IPCC. The expected third task is to establish a process for the annual collection and submission of activity data for the use of Bovaer.

This task will be carried out in accordance with the general conditions and the conditions in Article 21 of Commission Regulation (EU) No. 702/2014. The aim is not only to establish documentation for the use of Bovaer but also for the use of other substances with similar effects. However, since Bovaer is currently the only relevant product in this context, the focus of this assignment is on Bovaer.

Following the government-commissioned feeding trials that investigated the effect of Bovaer under Danish conditions, a Ym factor that incorporates the use of Bovaer will be calculated. The task includes ensuring that the calculated Ym factor, via DCE, is forwarded to and approved by the UN/IPCC. A timeline for this process is requested to be included in the task. The calculation is intended to apply to all relevant types of cattle (dairy cows of all relevant dairy breeds, not heifers) and any other relevant factors, ensuring that an IPCC approval covers all legal uses of Bovaer in Denmark.

Introduction

The use of feed additives that can mitigate enteric methane (CH4) from ruminants is a promising way to reduce, not only the carbon footprint of the products, but also the significant contribution of enteric methane in National Emission Inventories for Agriculture, especially in countries like Denmark, where dairy production is of quantitative importance. However, incentive schemes must be in place before such strategies will be implemented and used in practical settings. This includes incorporation in models for calculating the carbon footprint of the products if incentive schemes are led by the industry as well as National Emission Inventories if such incentive schemes are on a national level.

3-nitrooxypropanol (3-NOP) is the active component in Bovaer®, a commercially available product on the European market and produced by dsm-firmenich. The product has been shown to reduce enteric methane yield (g CH_4/kg dry matter (DM) intake) from dairy cows by on average 30.8 %, but with quite pronounced differences in reduction potential between doses and nutrient composition of rations (Kebreab et al., 2023). The mode of action is that 3-NOP binds to the active site of coenzyme M reductase, an enzyme system unique for methanogens, and thereby temporarily inactivates the enzyme system (Duin et al., 2016).

The aim of this reviewed report from Aarhus University is to provide the scientific evidence for including the use of Bovaer® in the Danish National Emission Inventories for Agriculture and subsequently to provide guidelines for how to include the use of Bovaer® in the Danish model system and more specifically in the calculation of the emission factor for dairy cows.

Description of the current model for calculating methane emission from dairy cows

The emission factor for enteric methane from dairy cows (EF_{CH4}) is in the current version of the Danish National Emission Inventories (Albrektsen et al., 2021) calculated using feed intake, gross energy (GE) concentration of the ration, and the CH_4 conversion factor (Y_m), expressing the proportion of ration GE lost as CH_4 (Equation 1). Data on feed intake are available from farm activity data (Lund et al., 2023) and GE concentration in the ration is a default value (18.9 MJ/kg DM) based on farm activity data (Albrektsen et al., 2021).

Equation 1: EF_{CH4} (kg CH_4/cow/year) = *DM intake* (kg/d) x *GE concentration in ration* (MJ/kg DM) x 0.01 x Y_m (% GE lost as CH_4) x 365 (days/year) / 55.65 (MJ/kg CH_4)

The Y_m factor is therefore an essential component in protocols for estimating CH_4 emission from livestock and is for dairy cows estimated based on the model presented by Hellwing et al. (2016). In brief, an experimental dataset was compiled including 183 observations, 41 rations, and 10 experiments, where methane emissions were measured by means of indirect calorimetry (respiration chambers) using the same experimental equipment. Nutrient content of the rations was determined using standard feed analysis procedures. Feed intake and milk yield were also included in the dataset.

The equation for prediction of Y_m based on nutrient composition of the diet combined with dry matter intake is shown below (Equation 2).

Equation 2: Y_m (%) = 7.55 – 0.0343 x *DM intake* (kg/day) – 0.0199 x *Crude fat* (g/kg DM) – 0.0014 x *Ash* (g/kg DM) + 0.0028 x *NDF* (g/kg DM) – 0.0045 x *Starch* (g/kg DM)

The Y_m factor can subsequently be calculated based on activity data for DM intake and nutrient composition of the diet (Table 1). These data are provided from the normative system (Lund et al., 2023) and from farm survey data compiled by SEGES Innovation (Martinussen & Kjeldsen, 2023).

Table 1: *Example of how to calculate the CH_4 conversion factor (Y_m, %) and the emission factor for CH_4 from dairy cows (EF_{CH4}, kg CH_4/cow/year) based on activity data for DM intake (Lund et al., 2023) and nutrient composition of the ration (Martinussen & Kjeldsen, 2023).*

	Large breed[a]	**Jersey**
DM intake, kg/d	23.2	18.7
Crude fat, g/kg DM	45.7	49.0
Ash, g/kg DM	73.1	74.0
NDF, g/kg DM	320	316
Starch, g/kg DM	190	194
Y_m, %	5.79	5.84
Y_m, %[b]	5.79	
EF_{CH4}, kg CH_4/cow/year	166	135
EF_{CH4}, kg CH_4/cow/year[b]	162	

[a] Large breed includes all dairy cows with less than 87.5 % Jersey genetics.

[b] Average value, based on the Danish dairy cow population consisting of 86.3 % Large breed and 13.7 % Jersey fed average rations (Lund et al., 2023).

Prediction of the CH_4 mitigating effect of Bovaer based on dose of 3-NOP and nutrient composition of the ration

Based on data from 14 experiments and 48 treatment means, Kebreab et al. (2023) have developed an equation (Equation 3) for the prediction of the effect of dose of 3-NOP (3-nitrooxypropanol; the active component in Bovaer) on CH_4 yield (g CH_4/kg DM intake) dependent on nutrient composition of the diet.

Equation 3: Change (%) in CH_4 yield with dietary addition of Bovaer = –30.8 – 0.226 x (*3-NOP* (mg/kg DM) – 70.5) + 0.906 x (*NDF* (% of DM) – 32.9) + 3.871 x (*Crude fat* (% of DM) – 4.2) – 0.337 x (*Starch* (% of DM) – 21.1)

A dose of 60 mg 3-NOP/kg DM is expected to be the recommended level in a Danish setting (Lund, 2024) based on the potential risk for reductions in feed intake if dose is greater (Kjeldsen et al., 2024; Maigaard et al., 2024a, Maigaard et al., 2024b).

Using the activity data for chemical composition of the ration (Martinussen & Kjeldsen, 2023, Table 1) this will be equivalent to a 27 % reduction in CH_4 yield for Large breed, 26 % for Jersey, and 27 % for the average population using Equation 3 (Kebreab et al., 2023).

A reduction of 27 % in CH_4 yield (g CH_4/kg DMI) is therefore suggested to be implemented in the Danish National Inventory when calculating the emission factor for dairy cows and a dose of Bovaer, equivalent to 60 mg 3-NOP/kg DM, is used.

Danish data on the CH4 mitigating effect of Bovaer when fed to dairy cows

Data from Aarhus University

At Aarhus University several experiments (Johansen et al., 2023a, Johansen et al., 2023b, Kjeldsen et al., 2024, Maigaard et al., 2024a; Maigaard et al., 2024b) have been completed using different dosages of Bovaer (60 to 80 mg 3-NOP/kg DM) and with rations differing in composition of the diet (fat level, forage proportion, and forage type). The experimental designs and outcome are summarised below and observed and predicted reductions in methane yield are shown in Figure 1.

Johansen et al. (2023a) examined how addition of Bovaer in feed rations with high proportion of grass-clover silage (42 % of total ration) affected dry matter intake (DMI), milk production, and gas production in lactating dairy cows. Forty-eight Holstein cows (half primiparous and half multiparous; half in early lactation and half in mid-late lactation) were included in a balanced 4 x 4 Latin square design, with a 2 x 2 factorial arrangement of dietary treatments. The first factor was 2 different sources of grass-clover silage (spring growth and first regrowth), and the other factor was 2 levels of Bovaer addition (0 or 60 mg 3-NOP/kg DM) provided in partial mixed rations (62 % forage proportion) fed ad libitum. Additional concentrate (max 0.81 kg DM/d) was offered as bait in the GreenFeed units used for measurement of gas emission. Dry matter intake was reduced by 1.1 kg/d by supplementation of Bovaer. Likewise, energy corrected milk (ECM) yield decreased with 0.8 kg/d when Bovaer was fed, and there was a tendency, that this reduction was larger in early lactation than in mid-late lactation (-1.1 vs. -0.3 kg/d). Independent of source of grass-clover silage, addition of Bovaer reduced CH_4 production (g/d), yield (g/kg DMI), and intensity (g/kg ECM) with 33.0 %, 28.1 % and 31.3 %, respectively. The experiment concluded that Bovaer was effective in reducing CH_4 production but did also affect DMI and ECM yield negatively. The experiment indicated that reduction in milk production by addition of Bovaer is larger for cows in early lactation than for cows in mid-late lactation.

Johansen et al. (2023b) examined how addition of Bovaer in feed rations with high proportion of maize silage (42 % of total ration) affected gas production, dry matter intake (DMI), and milk production in lactating dairy cows. Forty-eight Holstein cows (half primiparous and half multiparous; half in early and mid-lactation and half in late lactation) were included in a balanced 4 x 4 Latin square design, with a 2 x 2 factorial arrangement of dietary treatments. The first factor was 2 different sources of maize silage (high (0.93) or low (0.83) in starch-to-NDF ratio) and the other factor was 2 levels of Bovaer addition (0 or 60 mg 3-NOP/kg DM) provided in partial mixed rations (62 % forage proportion) fed ad libitum. Additional concentrate (max 0.81 kg DM/d) was offered as bait in the GreenFeed units used for measurement of gas emission. Dry matter intake was reduced by 3.2 kg/d with supplementation of Bovaer, and ECM yield decreased with 1.2 kg/d with supplementation of Bovaer. Independent of source of maize silage, addition of Bovaer reduced CH_4 production (g/d), yield (g/kg of DMI), and intensity (g/kg ECM) with 34.6 %, 22.9 % and 33.0 %, respectively. The experiment concluded that Bovaer was effective in reducing CH_4 production, but reduced DMI and ECM yield as well.

The study by Kjeldsen et al. (2024) was a 4 × 4 Latin square design with 2 levels of fat supplementation; 33 g crude fat/kg DM or 64 g crude fat/kg DM and two levels of 3-NOP; 0 mg/kg DM or 80 mg/kg DM. In total, 4 diets were formulated: low fat (LF), high fat (HF), 3-NOP and low fat (3LF), and 3-NOP and high fat (3HF). Four rumen and intestinally cannulated cows were used in the experiment. After adaptation to the treatments, gas exchange was measured for 5 days in respiration chambers. Methane yield (g/kg DM) was decreased by 24 % when cows were fed 3-NOP. In addition, 3-NOP increased hydrogen yield (g/kg DM) by 3,500 %. A decrease (11 %) in DMI was observed, when cows were fed 3-NOP. Supplementation of 3-NOP tended to increase rumen and total tract NDF digestibility. Total VFA concentrations in the rumen were negatively affected by 3-NOP supplementation. Furthermore, 3-NOP caused a shift in the VFA fermentation profile, with decreased acetate proportion and increased butyrate proportion, whereas propionate proportion was unaffected. Increased concentrations of alcohols (methanol, ethanol, propanol, butanol, and 2-butanol) were observed in the ruminal fluid when cows were fed 3-NOP. These changes in rumen metabolites indicate partial re-direction of hydrogen into other hydrogen sinks, when methanogenesis is inhibited by 3-NOP. In conclusion 3-NOP reduced methane yield, irrespective of fat level and decreased DMI.

Maigaard et al. (2024a) investigated the individual and combined effects of using dietary fat, nitrate, and 3-NOP as CH_4 mitigating additives on dairy cows' enteric CH_4 emission and production performance (feed intake and milk production). Twenty-four primiparous and 24 multiparous Danish Holstein cows (111 ± 44.6 d in milk; mean ± standard deviation) were included in an incomplete 8 × 8 Latin square design with six 21-d periods. Dietary treatments were organized in a 2 × 2 × 2 factorial arrangement, aiming for 2 levels of FAT (30 or 63 g of crude fat/kg DM; LF or HF, respectively), 2 levels of NITRATE (0 or 10 g of nitrate/kg DM; UREA or NIT, respectively), and 2 levels of 3-NOP (0 or 80 mg/kg DM; BLANK or NOP, respectively). Treatments were included in ad libitum fed partial mixed rations. Additional concentrate was offered as bait in GreenFeed units used for measurement of gas emission. Inclusion of 3-NOP decreased DMI by 13.4 % and the combination of 3-NOP with fat and nitrate decreased DMI in an additive way (no significant 3-way interaction). Cows supplemented with 3-NOP had 9.0 % lower ECM yield than cows fed no 3-NOP. Based on three 2-way interactions including FAT, NITRATE, and 3-NOP, the combined use of the additives resulted in antagonistic effects on CH_4 reduction, hence no combinations of additives resulted in CH_4 yield-reductions that were greater than what was obtained by separate supplementation of the most potent additive within the combination. Thus, an 18 % to 23 % reduction in CH_4 yield (g CH_4/kg DM) could be ascribed to the effect of 3-NOP. In conclusion, 3-NOP reduced CH_4 emission, but negatively affected production performance.

The study by Maigaard et al. (2024b) investigated the effect of combining different doses of 3-NOP with varying forage composition on gas emission and production performance of dairy cows. Seventy-two lactating Danish Holstein cows were enrolled in a continuous randomized block design with an initial two-week covariate period followed by application of treatments for 12 consecutive weeks. Initial DMI and ECM yield were 23.8 ± 3.34 and 38.3 ± 7.12 kg, respectively. Cows were blocked according to parity and DIM. Treatments were organized in a 2 × 3 factorial arrangement. The first factor was diet type, reflecting two different forage compositions of the diet. Ratio of grass-clover silage to corn silage was 60 %:40 % of total forage DM in grass-based diets and 40 %:60 % in corn-based diets. In total, forage constituted 56 % of the DM in the partial mixed rations. The second factor reflected three doses of 3-NOP: 0, 60, or 80 mg 3-NOP/kg DM. Gas emission were measured using GreenFeed. Methane yield (g/kg DM) was reduced by 34 and 31 % at 60 and 80 mg 3-NOP/kg DM, respectively, and there was no difference in CH_4 yield between the two doses. The reduction in CH_4 yield was numerically greater in corn-based diets (38 %) compared with grass-based diets (30 %), despite similar NDF and starch levels, but there was no interaction between dose of 3-NOP and diet type. Total DMI was reduced by 9 % in cows fed 80 mg 3-NOP/kg DM across diet types, whereas there was no effect at 60 mg 3-NOP/kg DM. Similarly, ECM yield was reduced by 5% in cows fed 80 mg 3-NOP/kg DM compared with no 3-NOP supplementation. In conclusion, 3-NOP at 60 mg/kg DM is efficient in reducing CH_4 without compromising production performance, while 80 mg 3-NOP/kg DM negatively affected production performance.

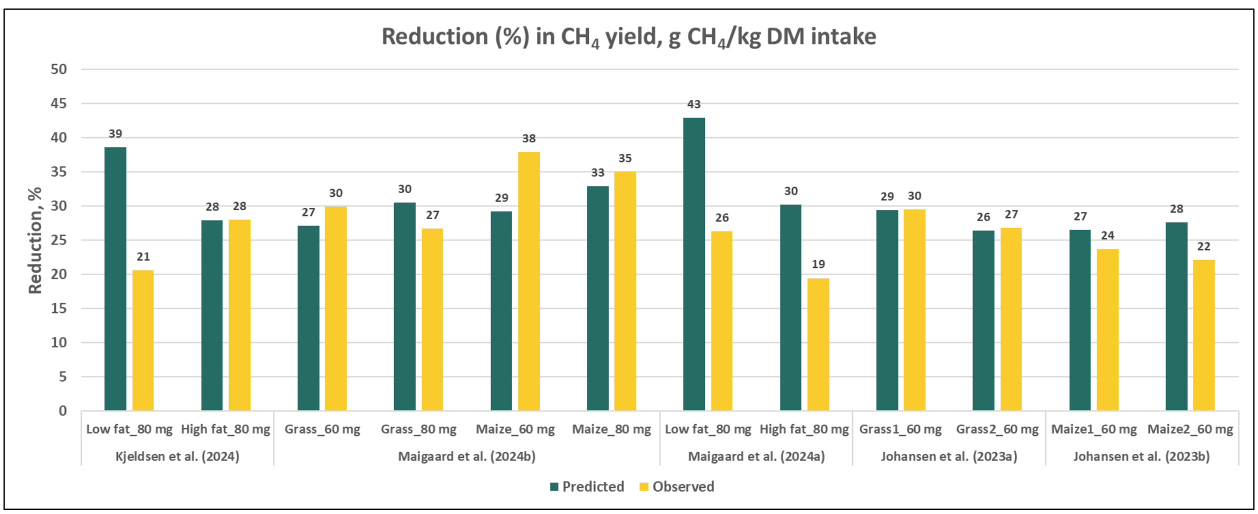

Figure 1: *Observed and predicted (Kebreab et al., 2023) reductions (%) in CH_4 yield (g CH_4/kg DM) in experiments conducted at Aarhus University using 60 or 80 mg 3-NOP/kg DM in different diet compositions. Modified from Lund (2024).*

Based on experiments conducted at Aarhus University, using a dose of 60 mg 3-NOP/kg DM resulted in an average observed reduction in methane yield of 28 % which is very well aligned with the average predicted reduction in methane yield of 28 % using the Kebreab equation, despite that differences between observed and predicted reduction were seen within rations. These values are also very similar to the reduction obtained above (27 %) using the Kebreab equation applied on activity data for average chemical composition of Danish rations for dairy cows.

Data from SEGES Innovation

The use of Bovaer at a dose of 60 mg 3-NOP/kg DM has also been evaluated on 5 commercial farms by SEGES Innovation and an average reduction in CH_4 yield of 33 % was obtained (Nielsen, 2024). This is slightly higher than the reduction predicted using the Kebreab equation and activity data as well as the average predicted, and average observed reductions obtained in Aarhus University experiments (27-28 %).

Research in Northwestern Europe on the methane mitigation potential of Bovaer

Despite that the CH_4 mitigating effect of Bovaer seems to be highly dependent upon management strategies and especially strategies related to feeding, the CH_4 mitigation potential of Bovaer obtained in experiments conducted in Northwestern Europe (The Netherlands (NED), Finland (FIN), Germany (GER)), where the production systems generally are comparable to a Danish setting, will most likely be somewhat similar to what can be expected in a Danish context. The Danish CH_4 mitigation potentials are in general in terms within the range found in similar research projects based on supplying 3-NOP in total mixed feed rations from Northwestern Europe (23 % on average), although the variation in CH_4 mitigation potential is noteworthy (16 % (51 mg 3-NOP; van Gastelen et al., 2020; NED), 17 % (70 mg 3-NOP; Vattulainen et al., 2024; FIN), 20 % (70 mg 3-NOP; van Gastelen et al., 2024; NED), 28 % (50 mg 3-NOP; Schilde et al., 2021; GER), 34 % (60-80 mg 3-NOP; van Gastelen et al., 2022; NED)).

In Flanders in Belgium, ILVO has provided a list for strategies to reduce enteric methane, and these strategies are expected to be included in the national inventory. For Bovaer (3-NOP) a daily reduction potential of 26 % is listed (ILVO, 2024).

Calculating a corrected Y_m factor taking the methane mitigation potential and activity data for the use of Bovaer into account

A corrected Y_m factor ($Y_{m,corr.}$, %) can be calculated (Equation 4) using the Y_m factor derived from Equation 2, the methane mitigation potential of Bovaer ($Red.Pot._{Bovaer}$, %) with respect to methane yield (g CH_4/kg DM intake) derived from Equation 3, and activity data on the proportion of cows using Bovaer ($Proportion_{Bovaer}$, %) into account.

Equation 4: $Y_{m,corr.}$ (%) = 0.01 x $Proportion_{Bovaer}$ (%) x Y_m (%) x 0.01 x [100 - $Red.Pot._{Bovaer}$ (%)] + 0.01 x [100 - $Proportion_{Bovaer}$ (%)] x Y_m (%) = 0.01 x Y_m(%) x [[100 - $Red.Pot_{Bovaer}$(%)] x 0.01 x $Proportion_{Bovaer}$(%) + 100 - $Proportion_{Bovaer}$(%)]

Assuming that the Y_m factor is 5.79 % (Table 1), that the reduction potential of Bovaer with respect to methane yield is 27 % (Equation 3), and the proportion of dairy cows fed Bovaer is 60 %, then the corrected Y_m factor ($Y_{m,corr}$) can be calculated as (Equation 5):

Equation 5: $Y_{m,corr.}$ (%) = 0.01 x 5.79 x [[100 – 27] x 0.01 x 60 + 100 - 60] = 4.84 %

If the above equations are merged, this combined overall equation can be used to calculate the emission factor for enteric CH_4 from dairy cows based on activity data for 1) DM intake, 2) Nutrient composition of the ration, 3) Dose of Bovaer, and 4) Proportion of cows fed Bovaer.

Incorporation in the Danish emissions inventory

The Danish emissions inventory follows the guidelines established by the United Nations Framework Convention on Climate Change (UNFCCC). The rules for reporting under the Paris Agreement are established through Decision 18/CMA.1 (UNFCCC, 2019) and Decision 5/CMA.3 (UNFCCC, 2022). The IPCC Guidelines (IPCC, 2006) requires thorough documentation for the effect of mitigation measures and that this should be supported by peer-reviewed publications. The robust documentation included in this paper and the associated references will form the documentation for estimating the mitigating effect of Bovaer on the methane emission from enteric fermentation from dairy cattle.

However, equally important is the information on the activity data necessary to estimate the impact of emissions, i.e. documentation for the share of cattle divided into different subgroups that are fed with Bovaer and documentation that the recommended dosage has been applied. At this stage the system for documenting the use and dosage of Bovaer has not been established, so the final implementation in the national inventory awaits the collection of the required data by the relevant authorities. When the information has been collected and documented, the methane emissions from enteric fermentation from dairy cattle will be estimated using Equation 5.

Conclusion and recommendation

Based on Danish experiments, the published Kebreab equation and national activity data a reduction potential of 27 % in methane yield (g CH_4/kg of DMI) is recommended, when a dose of 60 mg 3-NOP/kg DM is used in Danish diets for dairy cows. This reduction potential is aligned with the outcome of experiments in other parts of Northwestern Europe and with the average predicted and average observed reductions in methane yield from experiments with dairy cows fed a dose of 60 mg 3-NOP/kg DM performed at Aarhus University, but slightly lower than the average reduction potential observed on commercial farms.

This reduction potential can subsequently be incorporated in the equation for calculating the methane conversion factor (Y_m) and thereby the emission factor for enteric methane from dairy cows (EF_{CH4}) if reliable activity data for the use of Bovaer can be provided.

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About DCA

DCA - Danish Centre for Food and Agriculture is the entrance to research in food and agriculture at Aarhus University (AU).

The Centre comprises AU departments with food and agricultural science activities. These are primarily Department of Agroecology, Department of Animal Science, Department of Food Science, Centre for Quantitative Genetics and Genomics, and parts of Department of Engineering.

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SUMMARY

Use of feed additives that can mitigate enteric methane from ruminants is a promising technology. This calls for the inclusion of the effect of such feed additives in National Emission Inventories. 3-nitrooxypropanol (3-NOP) is the active component in Bovaer®. The product has been shown to reduce enteric methane yield from dairy cows, but with quite pronounced differences in reduction potential between doses and nutrient composition of rations. The aim of this peer-reviewed report is to provide the scientific evidence for including the use of Bovaer® in the calculation of the emission factor for dairy cows in the Danish National Emission Inventories for Agriculture. An average reduction potential of 27 % in methane yield (g methane/kg of feed dry matter) is expected based on inclusion of national activity data in a prediction model based on a recently published meta-analysis, when a dose of 60 mg 3-NOP/kg feed dry matter is used in Danish diets for dairy cows. This reduction potential is aligned with the average predicted and average observed reductions in methane yield from experiments performed at Aarhus University with dairy cows fed a dose of 60 mg 3-NOP/kg feed dry matter and with the outcome of experiments in other parts of Northwestern Europe, but is slightly lower than the average reduction potential observed on commercial farms. This reduction potential can subsequently be incorporated when calculating the emission factor for enteric methane from dairy cows, if reliable activity data for the use of Bovaer can be provided.