RESEARCH ARTICLE

# Net reductions in greenhouse gas emissions from feed additive use in California dairy cattle

**Xiaoyu Feng, Ermias Kebreab** [ORCID]*

Department of Animal Science, University of California, Davis, California, United States of America

* ekebreab@ucdavis.edu

## Abstract

The livestock industry is one of the main contributors to greenhouse gas emissions and there is an increasing demand for the industry to reduce its carbon footprint. Several studies have shown that feed additives 3-nitroxypropanol and nitrate to be effective in reducing enteric methane emissions. The objective of this study was to estimate the net mitigating effect of using 3-nitroxypropanol and nitrate on total greenhouse gas emissions in California dairy industry. A life cycle assessment approach was used to conduct a cradle-to-farm gate environmental impact analysis based on dairy production system in California. Emissions associated with crop production, feed additive production, enteric methane, farm management, and manure storage were calculated and expressed as kg $CO_2$ equivalents ($CO_2$e) per kg of energy corrected milk. The total greenhouse gas emissions from baseline, 3-nitroxypropanol and nitrate offered during lactation were 1.12, 0.993, and 1.08 kg $CO_2$e/kg energy corrected milk, respectively. The average net reduction rates for 3-nitroxypropanol and nitrate were 11.7% and 3.95%, respectively. In both cases, using the feed additives on the whole herd slightly improved overall carbon footprint reduction compared to limiting its use during lactation phase. Although both 3-nitroxypropanol and nitrate had effects on decreasing the total greenhouse gas emission, the former was much more effective with no known safety issues in reducing the carbon footprint of dairy production in California.

## Introduction

The main greenhouse gases (GHG) emissions from agricultural food production include nitrous oxide ($N_2O$), carbon dioxide ($CO_2$) and methane ($CH_4$). Livestock sector contributes to approximately 14.5% of global anthropogenic GHG emissions with 80% attributed to $CH_4$ production from enteric fermentation and manure management from ruminants [1]. Dairy production is the third largest agricultural industry in the United States with total milk production increasing 13% over the past decade reaching over 215 billion pounds in 2019 [2]. California, as the top dairy production state accounted for over 20% of the total milk production with 1.73 million cows [2,3].

The dairy industry has an environmental impact including GHG emissions related to crop production, enteric and manure $CH_4$, water resource for feed production, excretion of nitrogen and phosphorus, and land management [4]. There are several mitigation strategies developed to reduce GHG emission from dairy, but most are modest in magnitude and some not applicable to California [5]. In the last few years, several feed additives have been developed to reduce enteric $CH_4$ emissions with varying results. Two of the most studied feed additives that substantially reduce enteric $CH_4$ emissions include 3-nitroxypropanol (3NOP) and nitrate (e.g., [6–9]). 3-nitroxypropanol (also known as Bovaer in Europe), is a synthetic compound that inhibit Methyl-coenzyme M reductase, the enzyme that catalyzes the methane-forming step in the rumen [10]. Dijkstra et al. [8] conducted a meta-analysis to evaluate the anti-methanogenic effects of 3NOP and concluded that on average $CH_4$ production (g day$^{-1}$) was reduced by 32.5% and $CH_4$ yield (g kg dry matter intake$^{-1}$) by 29.3%. However, 3NOP appeared to be more effective in dairy cattle by reducing $CH_4$ emission by 39.0% compared to a reduction of 22.2% in beef. The authors also reported that the effectiveness of 3NOP was positively related to dose, but impaired by increased neutral detergent fibre (NDF) content in the diet.

Nitrate as an electron acceptor has been studied as dietary feed additive for ruminants to inhibit $CH_4$ emission. Nitrate is reduced to nitrite and further reduced to ammonia, which is highly competitive with methanogens for hydrogen utilization in rumen due to a greater Gibbs energy changes than the $CO_2$ to $CH_4$ pathway [11]. The anti-methanogenic effect of nitrate has been investigated *in vivo* in various studies using beef steers, dairy cows, sheep, and goats [9,12–15]. Van Gastelen et al. [16] conducted a meta-analysis and demonstrated that $CH_4$ production consistently decreased when feeding nitrate to different ruminant animals. A recent meta-analysis by Feng et al. (unpublished) indicated that nitrate reduced $CH_4$ production by 14.4% and $CH_4$ yield by 11.4% in a dose-response manner. The main concern in using nitrate as a feed additive is the potential for nitrate toxicity. Nitrite as a result of nitrate reduction can accumulate in the animal and absorbed into blood. Increases in blood nitrite causes an increase in the concentration of methemoglobin, which can be fatal to animals. However, a denitrifying probiotic, *Paenibacillus fortis*, has been identifying as a way to enhance nitrite detoxification in nitrate treated ruminants [17]. The objective of this study was to estimate the net GHG emissions in California dairy system holistically based on supplementation of 3NOP and nitrate to the basal diet.

## Materials and methods

The study was based on a life cycle assessment (LCA) conducted for the dairy industry in California [4]. The feed ingredients used by Naranjo et al. [4] were adjusted and recalculated using NRC (2001). The main ingredients were alfalfa hay, corn silage, corn grains, canola meal, almond hulls and distillers dried grains with solubles. The impact of producing the feed additives 3NOP and nitrate was integrated in the LCA model. Energy corrected milk (ECM) was used as the functional unit and all emissions were calculated and standardized to 1 kg of ECM. The LCA conformed to Food and Agriculture Organization of the United Nations (FAO) Livestock Environmental Assessment Protocol guidelines.

The milk production supply chain in California from cradle to farm gate was considered the system boundary of the LCA including production of the feed additives. Specifically, these include: crop production, feed additives production, farm management, enteric methane, and manure storage (Fig 1). The system boundary considered emissions associated with on-farm activities, pre-farm production, and transportation of major productions up to the animal farm gate. Emissions for further activities after the products left the farm gate were not accounted in the system because they were considered to be treated in the same way for all scenarios.
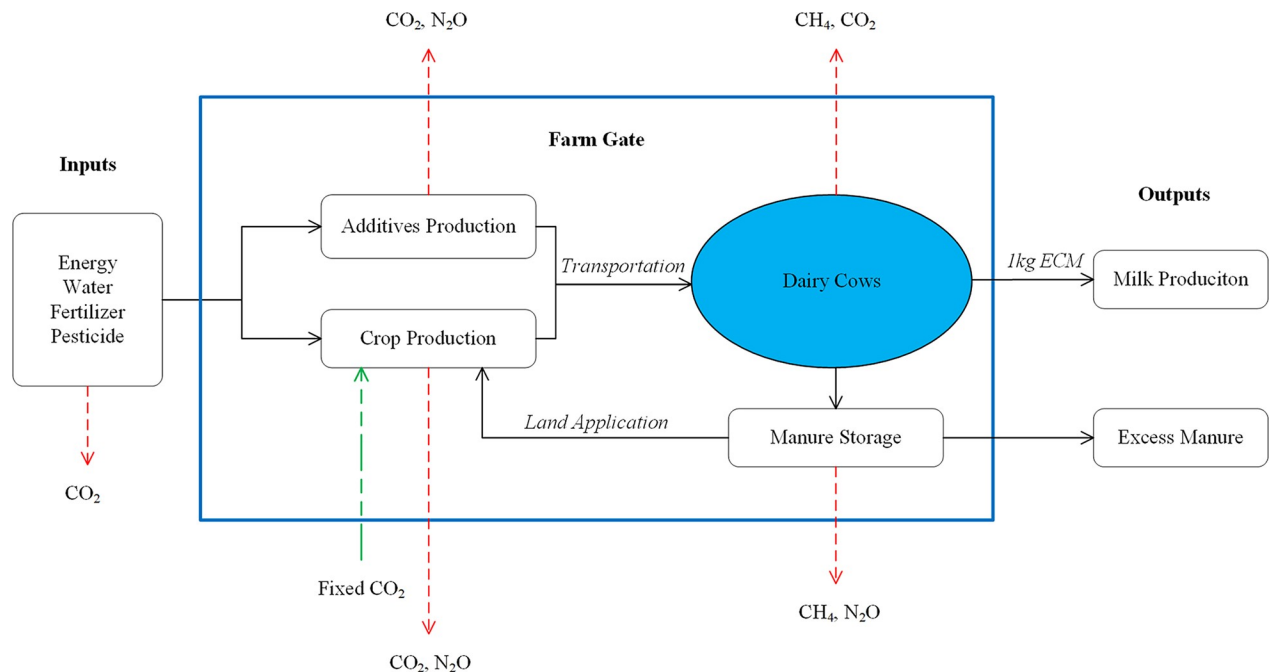
**Fig 1. System boundary of the life cycle assessment for California milk production.**

### Mitigation scenarios

Data were collected from USDA National Agricultural Statistical Service (USDA-NASS) and Economic Research Service (USDA-ERS), California Department of Food and Agriculture (CDFA), peer-reviewed literature and other published resources. The GHG emissions from each process in the LCA were estimated using the average conditions for dairy cattle in California as described by model 2 in Naranjo et al. [4]. The baseline scenario used California representative diets collected from CDFA reports. Average data from 2013 to 2015 represented the diets for year 2014 in the current analysis, which is the reference year. The diets for dairy cows at different growth stages including calf up to 1 year, heifer, pregnant heifer, close-up heifer, high lactating cow, and dry cow were weighted based on a whole production cycle. We assume 4 lactations to be the average life span of a California dairy cow. The average milk production was 36.4 kg/d with milk fat and protein percentages of 4 and 3.3%, respectively. The crop production for baseline included the activities related to producing feed, and use of land, water, fertilizers, pesticides and herbicides. Additionally, energy used for machine operation, irrigation, and transportation was included. Data from USDA-NASS Quick Stats [18], USDA farm and ranch irrigation reports [19], California specific agricultural reports [20,21], USDA-ERS reports [22], University of California crop cost and return studies [23], and values published in literatures [24] were used to estimate the emissions during the crop production. Enteric $CH_4$ emissions, farm management, energy and water used for producing crop, feeding cattle, cooling livestock facilities, animals, and milk, sanitation, cleaning, and dealing with onsite waste were according to Naranjo et al. [4]. Similarly, manure methane and nitrous oxide ($N_2O$) emissions were based on methodology described by Naranjo et al. [4].

Two scenarios were developed to estimate net mitigation effect of supplementing 3NOP to typical dairy diet in California. In scenario 1, all dairy cows were simulated to consume a diet that contains 3NOP only during lactation. In scenario 2, 3NOP was supplement to the diet at

all growing stages within a life cycle. The basal diets were the same as in the baseline and 3NOP was supplemented at a rate of 127 mg/kg dry matter (DM) in both scenarios.

Nitrate as a non-protein nitrogen source for cattle is usually used to replace other non-protein N sources such as urea [25,26]. Urea is not typically used as a nitrogen source in California representative diets, so nitrate was simulated to partially replace dietary protein in diets to keep similar N supply for all nitrate scenarios. In nitrate scenario 1, all dairy cows were simulated to consume a diet that contained nitrate only during lactation. Nitrate was supplemented to dairy cows at all stages in nitrate scenarios 2 and 3. In nitrate scenario 2, high protein meal (e.g. corn gluten, soybean meal, and distillers dried grain and solubles) was replaced by dietary nitrate on an equivalent nitrogen basis with no adjustment for dry matter intake (DMI). In nitrate scenario 3, DMI was adjusted using low protein meal (e.g. corn grain, and wheat silage) to the baseline levels after replacing high protein meal with nitrate additives. Nitrate was supplemented to dairy cattle at a rate of 16.7 g/kg of DM for all the 3 nitrate treatment scenarios.

## Emission associated with production and use of additives

**3-nitroxypropanol.** The carbon footprint of emissions associated with 3NOP production were assumed to be 52 kg $CO_2e$/kg 3NOP produced (DSM Nutritional Products, Ltd., pers. comm.). Moreover, with the improvement of process optimization in making 3NOP, the carbon footprint of 3NOP could drop to 35 kg $CO_2e$/kg 3NOP (DSM Nutritional Products, Ltd., pers. comm.). The total GHG emissions from 3NOP production were estimated using both carbon footprint values in producing 3NOP and the results were reported as mean with standard error to evaluate the effect of 3NOP emission factors on net GHG emissions. The transportation of 3NOP was calculated based on shipping from the producer (DSM Nutritional Products, Ltd., registered in Ontario, CA) and dairy farms in California by truck. The average distance used to estimate the emissions related to 3NOP transportation was weighted according to the milk production amount in California counties in 2014 [27].

The magnitude of enteric $CH_4$ emission reduction as a result of supplementing 3NOP was calculated based on an updated version of a meta-analysis conducted by Dijkstra et al. [8] on the anti-methanogenic effects of 3NOP. Four more recent references related to 3NOP effect on $CH_4$ emissions were added to the previous analysis to extend the accuracy and robustness of the meta-analytical model. The updated database included treatment means from Martinez-Fernandez et al. [28] (beef; 1 treatment), Vyas et al. [29] (beef; 2 treatments), Kim et al. [30] (beef; 4 treatments), and van Wesemael et al. [31] (dairy; 2 treatments). The final mixed-effect models for $CH_4$ production in the updated meta-analysis indicated effectiveness of 3NOP at mitigating $CH_4$ production was positively associated with 3NOP dose, and negatively associated with NDF content. Similar to the previous meta-analysis, supplementation of 3NOP had stronger anti-methanogenic effects in dairy cows compared to beef cattle, at a slightly greater magnitude of mitigation. The lifecycle of a dairy cow includes about 2 years before starting lactation and a dry period of about 60 days every year the cow is in production. During the non-lactating stages, cows are assume to produce $CH_4$ at the same rate as beef cattle. The following equations were used to calculate the mitigation effect of 3NOP (mg/kg of DM) that includes dose, NDF content (g/kg of DM) and either dairy (Eq 1) or beef (Eq 2):

$$\text{Dairy enteric methane reduction rate } (\%) = -41.5 - (0.260 \times \text{3NOP dose}) + (0.129 \times \text{NDF content}) \quad (1)$$

$$\text{Beef enteric methane reduction rate } (\%) = -22.8 - (0.260 \times \text{3NOP dose}) + (0.129 \times \text{NDF content}) \quad (2)$$

The equations were centered on the mean value of 127 mg 3NOP kg $DM^{-1}$ and 326 g NDF kg $DM^{-1}$. Therefore, the methane reduction rates were adjusted for each cattle type when the

NDF content in the 3NOP supplemented scenarios varies from the default centered value. The NDF contents for different growing stages of dairy cows in California used in this study were calculated using NRC (2001) based on ingredients supplied (Table 1). In 3NOP scenario 1, enteric $CH_4$ emitted from lactating cows was reduced by 38.8%, which includes adjustment for NDF content (Table 1). In scenario 2, if the cows were not lactating, the emission reduction rate was assumed to be similar to beef cattle so Eq 2 was applied. The enteric $CH_4$ reduction rates for various life-stages is given in Table 1.

The GHG emissions from the farm management and manure management processes in the LCA for 3NOP scenarios were the same as for the baseline scenario because we assumed no residues and by-products from the 3NOP production process. Nkemka et al. [32] confirmed that there was no residual effect on anaerobic digestion of the manure from beef cattle fed diets supplemented with 3NOP.

**Nitrate.** Nitrate was assumed to be supplemented to dairy diets as Calcium nitrate (Ca $(NO_3)_2$). Brentrup et al. [33] reported carbon footprint associated with $Ca(NO_3)_2$ production were estimated to be 1.76 kg $CO_2$e kg $Ca(NO_3)_2^{-1}$ in USA and 0.67 kg $CO_2$e kg $Ca(NO_3)_2^{-1}$ produced in Europe. Total emissions associated with $Ca(NO_3)_2$ production were calculated using both carbon footprint values for USA and Europe, and the emissions from nitrate production process are reported as the mean with standard deviation. Emissions related to transportation of $Ca(NO_3)_2$ were calculated based on the shipping distance between supplier and dairy farms in California. Several chemical companies supply $Ca(NO_3)_2$ within California and the plant with the minimum travel distance (by truck) to each county was assumed as its $Ca(NO_3)_2$ supplier. The overall average distance was weighted based on the milk production in California counties in 2014 [27] and used for emission calculations related to chemical transportations. Feed production for different nitrate treatment scenarios were recalculated based on the replacement of high protein meals by dietary nitrate to provide equivalent nitrogen compared to the diets for control scenario at each growing stage using NRC (2001).

The anti-methanogenic effects of nitrate were calculated based on equations developed by Feng et al. (unpublished). Meta-analytical results indicated nitrate effect on enteric $CH_4$ production to be significantly affected by nitrate dose (g/kg of DM), cattle type, and DMI (kg/day) and the mitigation effects of nitrate on $CH_4$ production was greater in dairy cows compared to beef cattle. The reduction rates for enteric $CH_4$ emissions estimated by the meta-analytical model were given in Eq 3 for dairy cattle and Eq 4 for beef cattle.

$$\text{Dairy enteric methane reduction rate (\%)} = -20.4 - (0.911 \times \text{nitrate dose}) + (0.691 \times \text{DMI}) \quad (3)$$

$$\text{Beef enteric methane reduction rate (\%)} = -10.1 - (0.911 \times \text{nitrate dose}) + (0.691 \times \text{DMI}) \quad (4)$$

**Table 1. Enteric methane reduction rates and total emissions per life cycle at different dairy growing stages for baseline and 3NOP treatment scenarios.**

| Cattle Stage | NDF[a] (g/kg DM) | Control | | 3NOP 1 | | 3NOP 2 | |
|---|---|---|---|---|---|---|---|
| | | Reduction (%) | CH₄ emission (kg/ lifetime) | Reduction (%) | CH₄ emission (kg/ lifetime) | Reduction (%) | CH₄ emission (kg/ lifetime) |
| Calf up to 1 year | 250 | NA | NA | NA | NA | NA | NA |
| Heifer | 419 | 0 | 10.6 | 0 | 10.6 | -11.1% | 9.4 |
| Pregnant heifer | 496 | 0 | 73.8 | 0 | 73.8 | -1.1% | 72.9 |
| Close up heifer | 425 | 0 | 9.2 | 0 | 9.2 | -10.3% | 8.2 |
| High lactating cow | 349 | 0 | 575.8 | -38.8% | 352.4 | -38.8% | 352.4 |
| Dry cow | 474 | 0 | 60.9 | 0 | 60.9 | -4.0% | 58.5 |

[a] NDF content in diets for control and 3NOP scenarios are same.

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The equations are centered on mean nitrate dose and mean DMI of the database, which were 16.7 g kg of $DM^{-1}$ and 11.1 kg $day^{-1}$, respectively. We kept the average as the dose of nitrate supplementation in the scenarios evaluated in this study. The DMI for different growing stages for baseline in this study were estimated from CDFA reports to represent the daily feed intakes of dairy cows in California (Table 2). In nitrate scenario 1, DMI for high lactating cow slightly dropped from baseline of 22.6 kg $day^{-1}$ to 22.3 kg $day^{-1}$ due to the replacement of high protein meal by concentrate nitrate and enteric $CH_4$ emitted was reduced by 13.6% when adjusted for DMI (Table 2). In nitrate scenario 2, high protein ingredients were replaced by nitrate for all growing stages which resulted in DMI differences compared to the baseline. The reduction rates of enteric $CH_4$ emissions were 14.7%, 11.4%, 10.7%, and 10.3% for heifers, pregnant heifers, close up heifers, and dry cows, respectively (Table 2), which was estimated using Eq 4. Enteric $CH_4$ emissions for high lactating cows was calculated using Eq 3, which was 13.6% (Table 2). In nitrate scenario 3, DMI were adjusted back to the baseline levels and the emission reduction rates were calculated using the same approach as nitrate scenario 2. The enteric $CH_4$ emissions for heifer, pregnant heifer, close up heifer, high lactating cow, and dry cow were reduced by 14.7%, 11.0%, 10.3%, 13.4%, and 10.0%, respectively (Table 2).

We assumed there were no residues and by-products from nitrate production and the total GHG emissions from farm management process for nitrate treatment scenarios including on-farm energy and water usage were not affected by nitrate additives. Methane emissions from manure storage were calculated as a function of volatile solids excreted [34], which was associated with NDF content, crude protein content and DMI [35]. As the dietary ingredients and DMI for nitrate scenarios varied with the adjustment of nitrate additives, the total GHG emissions from manure management were recalculated based on the different nitrate feeding scenarios.

## Results and discussion

### 3-nitroxypropanol

The GHG emissions from crop production, farm management, enteric $CH_4$ and manure storage for baseline were 0.174, 0.0608, 0.432, and 0.457 kg $CO_2$e kg of $ECM^{-1}$ produced in California, respectively (Fig 2). Total GHG emissions from crop production, farm management,

**Table 2. Enteric methane reduction rates and total emissions per life cycle at different dairy growing stages for control and nitrate treatment scenarios.**

| Cattle Stage | Control | | | Nitrate 1 | | Nitrate 2 | | Nitrate 3 | |
|---|---|---|---|---|---|---|---|---|---|
| | DMI[a] (kg/d) | Reduction (%) | $CH_4$ emission (kg/lifetime) | Reduction (%) | $CH_4$ emission (kg/lifetime) | Reduction (%) | $CH_4$ emission (kg/lifetime) | Reduction (%) | $CH_4$ emission (kg/lifetime) |
| Calf up to 1 year | 4.1 | NA | NA | NA | NA | NA | NA | NA | NA |
| Heifer | 5.8 | 0 | 10.6 | 0 | 10.6 | -14.7% | 9.7 | -14.7% | 9.8 |
| Pregnant heifer | 11.1 | 0 | 73.8 | 0 | 73.8 | -11.4% | 65.4 | -11.0% | 69.5 |
| Close up heifer | 12.1 | 0 | 9.2 | 0 | 9.2 | -10.7% | 8.0 | -10.3% | 8.2 |
| High lactating cow | 22.6 | 0 | 575.8 | -13.6% | 493.1 | -13.6% | 493.1 | -13.4% | 500.6 |
| Dry cow | 12.6 | 0 | 60.9 | 0 | 60.9 | -10.3% | 53.3 | -10.0% | 55.4 |

[a] DMI for control and nitrate scenario 3 are same for all stages. DMI for nitrate scenario 1 are same as for control except for high lactating cows (DMI = 22.3 kg/d). DMI for nitrate scenario 2 in different cattle stages are 4.1 (Calf up to 1), 5.8 (Heifer), 10.5 (Pregnant heifer), 11.6 (Close up heifer), 22.3 (High lactating cow), and 12.1 (Dry cow), respectively.

and manure storage were not affected by feeding 3NOP to dairy cows. There was no significant effect of 3NOP on DMI in cattle (e.g., [36,37]), therefore, the total amount of basal diets consumed were assumed to be similar in cows supplemented with or without 3NOP and GHG emissions from feed production remained the same. The mean GHG emissions related to production of 3NOP in scenario 1 was 3.23 g $CO_2$e kg $ECM^{-1}$ which was lower than 3.92 g $CO_2$e kg $ECM^{-1}$ in scenario 2 because 3NOP was only fed to lactating cows in scenario 1. Enteric $CH_4$ emissions were 0.298 and 0.295 kg $CO_2$e kg $ECM^{-1}$ for 3NOP scenarios 1 and 2, respectively, which were reduced by 31.0 and 31.7% compared to baseline, respectively, due to the inhibition effect of 3NOP on $CH_4$ production. Accounting for emissions from 3NOP production, the net enteric methane emission reduction was 30.3% in scenario 1 and 30.8% in scenario 2.

The total GHG emissions for baseline and 3NOP treatment scenarios 1 and 2 were 1.12, 0.993 and 0.991 kg $CO_2$e kg $ECM^{-1}$, respectively (Fig 2). Feeding 3NOP to dairy cows resulted in a net reduction of total GHG emission of 11.6% in 3NOP scenario 1 and 11.8% in 3NOP scenario 2 compared to the baseline. Using 3NOP for dairy cows at all growing stages only further reduced 0.2 percentage points more compared to limiting 3NOP supplementation during lactation. The small change in mitigation effect of GHG emissions in 3NOP scenario 2 compared to scenario 1 was due to less effectiveness of 3NOP on non-lactating cattle and relatively shorter period spent in non-productive phase (Table 1). The GHG emissions estimated from groups supplemented with 3NOP at 86 mg kg $DM^{-1}$ based on two Canadian dairy farms were 1.03 (fed 3NOP during lactations only) and 0.98 kg (fed 3NOP to entire herd) $CO_2$e kg $ECM^{-1}$ which was a reduction of 14.9% and 19.0% compared to their baseline, respectively [38]. The study also investigated the GHG emissions from two dairy farms in Australia and the reductions in GHG emissions compared to their baseline were 14.4 to 14.7%, when 3NOP was fed for 300 days of lactation (86 mg/kg DM). The net reductions of GHG emissions in California dairy farms were lower than estimated by Alvarez-Hess et al. [38] mainly because in California, $CH_4$ from manure management is greater compared to Canadian and Australian conditions, therefore, the overall effect of 3NOP was slightly diluted.
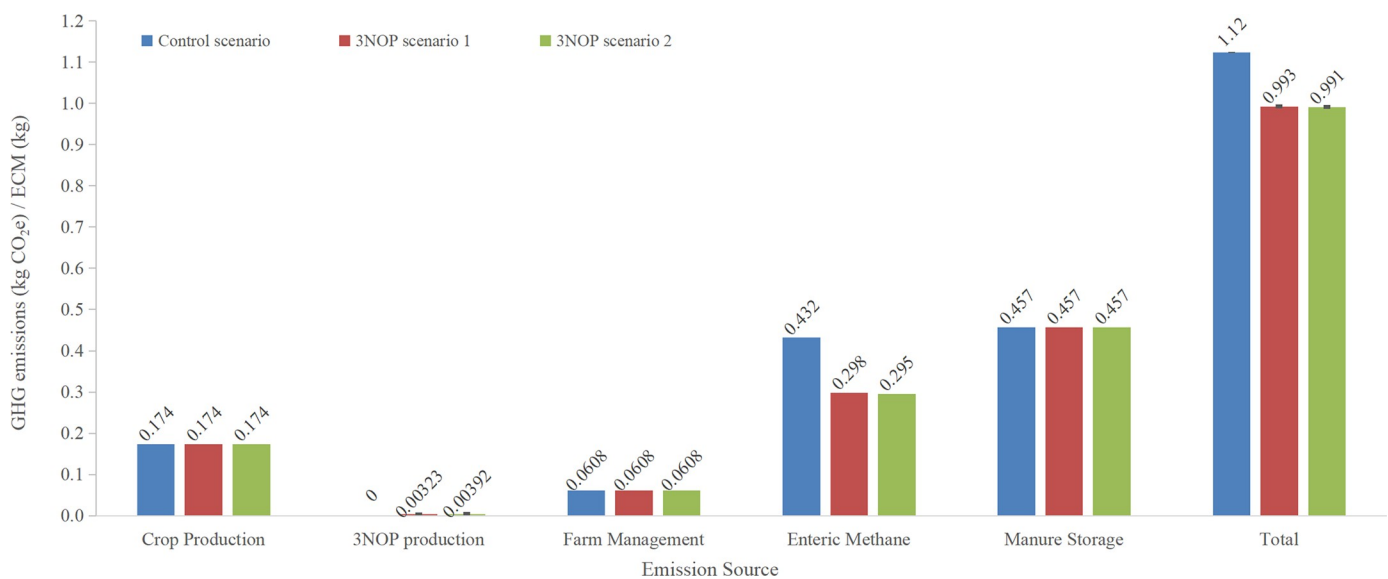


**Fig 2. Comparison of global warming potential (GWP) by emission source for control and 3-nitroxypropanol (3NOP) scenarios 1 and 2 in California dairy cows.**

The GHG emissions associated with 3NOP production for scenarios 1 and 2 were 3.86 (3NOP scenario 1) and 4.69 (3NOP scenario 2) g $CO_2$e kg $ECM^{-1}$, respectively, assuming 3NOP carbon footprint of 52 kg $CO_2$e $kg^{-1}$ and 2.60 (3NOP scenario 1) and 3.16 (3NOP scenario 2) g $CO_2$e kg $ECM^{-1}$, respectively, using manufacturer reported values of 35 kg $CO_2$e kg $3NOP^{-1}$. This indicates that with the improvement of manufacturing process, the GHG emissions from 3NOP production can be reduced by 32.6% and improve net impact of 3NOP in reducing enteric emissions.

## Nitrate

The total GHG emissions and estimates of the various components in dairy cattle supplemented with nitrate is given in Fig 3. In nitrate scenario 1, the mean GHG emissions associated with nitrate production was 0.0169 kg $CO_2$e kg $ECM^{-1}$ and 0.0203 kg $CO_2$e kg $ECM^{-1}$ in nitrate scenarios 2 and 3 due to differences in the phases of dairy production that nitrate was included. The differences in carbon footprint of $Ca(NO_3)_2$ production was mainly due to a catalyst technology developed in Europe [33]. The GHG emissions calculated with carbon footprint value for $Ca(NO_3)_2$ in USA (1.76 kg $CO_2$e kg per $Ca(NO_3)_2^{-1}$ produced) were 0.0237 kg $CO_2$e kg $ECM^{-1}$ for nitrate scenario 1, and 0.0285 kg $CO_2$e kg $ECM^{-1}$ for nitrate scenarios 2 and 3. Using the European carbon footprint (0.67 kg $CO_2$e kg $Ca(NO_3)_2^{-1}$ produced), the GHG emissions from nitrate production was 0.0101 kg CO2e kg $ECM^{-1}$ for nitrate scenario 1, and 0.0122 kg CO2e kg $ECM^{-1}$ for nitrate scenarios 2 and 3. The GHG emissions from nitrate production (averaged from three scenarios) decreased 57.3% based on European values compared to those in USA.

The GHG emissions related to crop production was 0.174 kg $CO_2$e kg $ECM^{-1}$ for the baseline, and reduced to 0.172, 0.168, and 0.172 $CO_2$e kg $ECM^{-1}$ for nitrate scenarios 1, 2, and 3, respectively, which was mainly caused by the decline in the amount of protein that was replaced by nitrate. Although replacing high protein sources such as corn gluten, and soybean meals reduced the GHG emissions of feed production, emissions from nitrate production were much greater in comparison. The DMI for scenario 3 was adjusted back to baseline level,
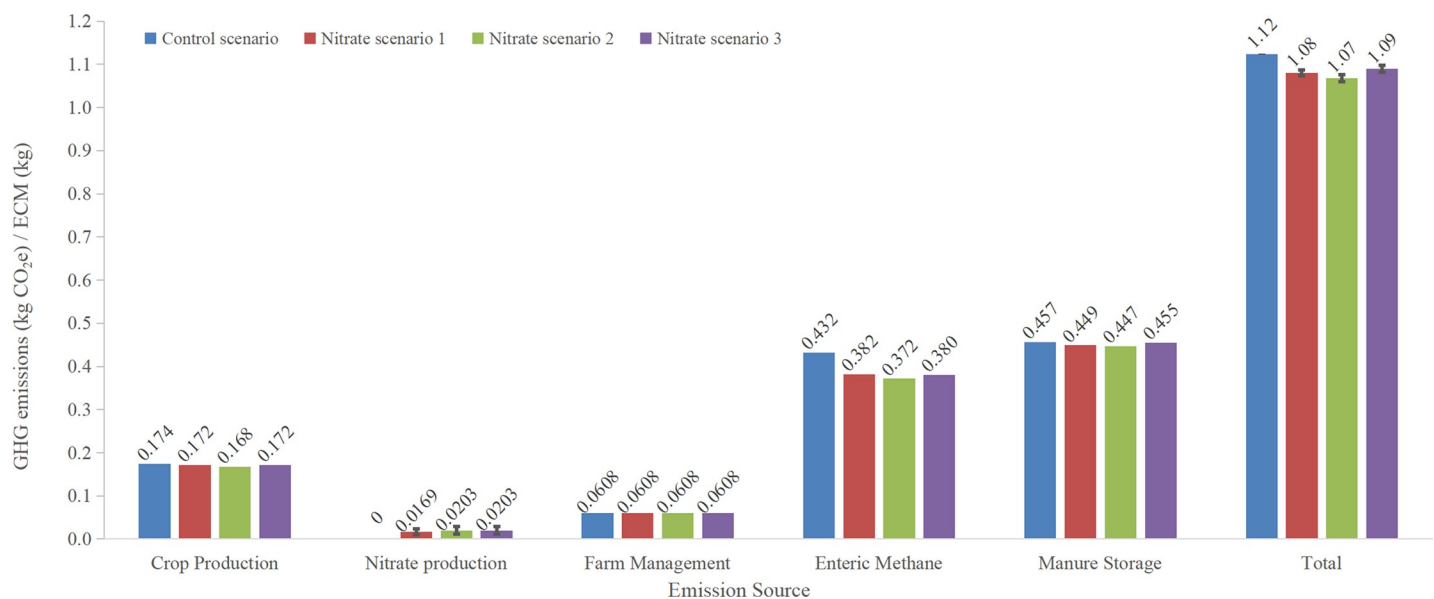


Fig 3. Comparison of global warming potential (GWP) by emission source for control and nitrate scenarios of dairy cows in California.

therefore, the GHG emissions from crop production in nitrate scenario 3 was 0.004 $CO_2$e kg $ECM^{-1}$ greater than in scenario 2. The GHG emissions from manure storage were 0.457, 0.449, 0.447, 0.455 kg CO2e kg $ECM^{-1}$ in control and three nitrate scenarios, respectively. The differences of GHG emissions from manure management among nitrate scenarios were associated with the variations in dietary NDF content, crude protein content, and DMI of adjusted diets. Enteric $CH_4$ emissions from nitrate scenarios 1 to 3 were 0.382, 0.372, and 0.380 kg $CO_2$e kg $ECM^{-1}$, respectively, which were reduced by 11.5%, 13.9%, and 12.0% respectively, compared to $CH_4$ emissions from control scenario (0.432 $CO_2$e kg $ECM^{-1}$) based on values calculated for $CH_4$-mitigating effect of dietary nitrate (Table 2). The net reduction in enteric methane emission (and GHG from nitrate production) was calculated to be 7.58, 10.4 and 8.42% for nitrate scenarios 1 to 3, respectively. The GHG emissions from farm management were the same for control and all nitrate scenarios which was 0.0608 kg $CO_2$e kg $ECM^{-1}$ (Fig 3).

The total GHG emissions for control scenario was 1.12 kg $CO_2$e kg $ECM^{-1}$, while with supplementing dietary nitrate to dairy cows in California, the total GHG emissions were 1.08, 1.07, and 1.09 kg $CO_2$e kg $ECM^{-1}$ (Fig 3), respectively, in nitrate scenarios 1, 2, and 3. The total GHG emissions for three nitrate scenarios were reduced by 3.82, 4.96, and 3.07% compared to the control scenario, respectively. In scenario 2, nitrate was fed to all growing stages with the largest amount of replaced basal diet resulting in the lowest GHG emissions from crop production and enteric $CH_4$. Therefore, scenario 2 showed the greatest net reduction of GHG emissions, which was reduced by 1.15 and 1.89% more compared to scenarios 1 and 3, respectively. These results were lower than the reduction rates estimated by Alvarez-Hess et al. [38] who reported that the GHG emissions went down from 1.21 kg $CO_2$e kg $ECM^{-1}$ (baseline) to 1.13 (a reduction of 6.61%) and 1.10 (a reduction of 9.09%) kg $CO_2$e kg $ECM^{-1}$ when nitrate was fed at a rate of 21 g/kg DM to lactating cows only and to the entire herd, respectively. Alvarez-Hess et al. [38] used an average reduction rate of 23% for $CH_4$ emissions with supplementing nitrate, while we assumed enteric $CH_4$ emissions were decreased by 20.4% for lactating cows and 10.1% for dairy cows under other growing stages without affecting milk production when the nitrate was fed at a rate of 16.7 g kg of $DM^{-1}$ and the DMI of 11.1 kg $day^{-1}$. This may explain the relatively lower net reduction of total GHG emissions in the current study.

## Comparison of 3-nitroxypropanol and nitrate additives

Total GHG emissions from baseline (1.12 kg $CO_2$e kg $ECM^{-1}$) were lower than values published in several previous studies. For example, Gerber et al. [39] reported the GHG emissions in North America to be 1.20 kg $CO_2$e kg $ECM^{-1}$ and Thoma et al. [40] reported 1.23 kg $CO_2$e kg $ECM^{-1}$. In Canada, Guyader et al. [41] reported the GHG emissions varied from 1.18 to 1.52 kg $CO_2$e kg $ECM^{-1}$ for a dairy farm and Alvarez-Hess et al. [38] reported 1.21 kg $CO_2$e kg $ECM^{-1}$, but in two Australian dairy farms the authors reported 1.09 and 0.97 kg $CO_2$e kg $ECM^{-1}$, respectively, which were lower than the value estimated in the present study. Enteric $CH_4$ and manure management are the major sources of GHG emissions in the dairy sector [40]. Emissions from manure storage accounted for 40.6% to 46.1% of the total GHG emissions, which contributed to the largest amount to total GHG emissions in all scenarios. Enteric $CH_4$ emissions from baseline scenario accounted for 38.4% of the total GHG emissions but the proportions of enteric $CH_4$ emissions dropped to between 29.8% (3NOP scenario 2) and 35.4% (nitrate scenario 1). Crop production emitted 15.5% to 17.6% of total GHG emissions and the significant decrease in enteric $CH_4$ emissions resulted in a proportional increase of GHG emissions of crop production in 3NOP scenarios. Only 0.3% to 1.9% of emissions were

attributed to feed additives production in supplemental scenarios. The GHG emissions associated with farm management were the same for all scenarios.

Although both 3NOP and nitrate additives decreased the total GHG emissions, the mitigating effect of 3NOP was greater than nitrate reaching a highest reduction rate of 11.8% (3NOP scenario 2). The average net reduction rate of GHG emissions for 3NOP scenarios was 11.7% and supplementing 3NOP to dairy cows only during lactations or to the entire growing herds had a minor difference in the total GHG emissions. The mean net reduction rate of GHG emissions in dairy cows feeding nitrate was 3.95%. The greatest net GHG emissions achieved with nitrate was 4.96% with supplementation of nitrate to dairy cows in all growing stages. In addition to differences in effectiveness, nitrate is fed at an average rate of 16.7 g kg $DM^{-1}$ compared to an average 3NOP rate of 127 mg kg $DM^{-1}$ in this study. Therefore, much higher quantities of nitrate are required for enteric $CH_4$ mitigation resulting in about 5.4 times GHG emission from production of the additive alone. Nitrate toxicity caused by the high methemoglobin levels in ruminants fed in greater quantities is a concern and currently not recommended as $CH_4$ mitigating feed additives to cattle [42,43]. Analysis of the economic impact of using the additives was not possible because the price of 3NOP is not set yet.

## Conclusions

This study was conducted based on dairy cows in California and evaluated the mitigation effect of two effective feed additives—3NOP and nitrate, on total GHG emissions. The average net reduction rate of supplementing 3NOP and nitrate were 11.7 and 3.95%, respectively. Feeding 3NOP to only lactating cows or to the entire growth stages did not make significant difference in total GHG emissions. Considering California milk production of 18 billion kg in 2017, using 3NOP on California dairy cows would reduce GHG emissions by 2.33 billion $CO_2$e and nitrate 0.90 billion $CO_2$e annually.

## Author Contributions

**Conceptualization:** Ermias Kebreab.

**Formal analysis:** Xiaoyu Feng.

**Funding acquisition:** Ermias Kebreab.

**Project administration:** Ermias Kebreab.

**Writing – original draft:** Xiaoyu Feng.

**Writing – review & editing:** Xiaoyu Feng, Ermias Kebreab.

## References

1. Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijk-man J, et al. Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO); 2013.

2. USDA National Agricultural Statistics Service [Internet], Charts and Maps: Milk Production and Milk Cows. 2019 [cited 2020 Apr 7]. Available from: https://www.nass.usda.gov/Charts_and_Maps/Milk_ Production_and_Milk_Cows/milkprod.php.

3. California Department of Food and Agriculture [Internet]. 2019 State Agriculture Overview—California. 2019 [cited 2020 Apr 7]. Available from: https://www.nass.usda.gov/Quick_Stats/Ag_Overview/ stateOverview.php?state=CALIFORNIA.

4. Naranjo A, Johnson A, Rossow H, Kebreab E. 2020. Greenhouse gas, water, and land footprint per unit of production of the California dairy industry over 50 years. Journal of dairy science. 2020; 103:3760–3773. https://doi.org/10.3168/jds.2019-16576 PMID: 32037166

5.  Hristov AN, Oh J, Firkins JL, Dijkstra J, Kebreab E, Waghorn G, et al. Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. Journal of animal science. 2013; 91:5045–5069. https://doi.org/10.2527/jas.2013-6583 PMID: 24045497.

6.  Guyader J, Eugène M, Doreau M, Morgavi DP, Gérard C, Loncke C, et al. Nitrate but not tea saponin feed additives decreased enteric methane emissions in nonlactating cows. Journal of animal science. 2015 Nov 1; 93(11):5367–5377. https://doi.org/10.2527/jas.2015-9367 PMID: 26641056

7.  Lee C, Araujo RC, Koenig KM, Beauchemin KA. Effects of encapsulated nitrate on growth performance, nitrate toxicity, and enteric methane emissions in beef steers: backgrounding phase. Journal of animal science. 2017 Aug 1; 95(8):3700–11. https://doi.org/10.2527/jas.2017.1460 PMID: 28805908

8.  Dijkstra J, Bannink A, France J, Kebreab E, van Gastelen S. Antimethanogenic effects of 3-nitrooxypro-panol depend on supplementation dose, dietary fiber content, and cattle type. Journal of dairy science. 2018 Oct 1; 101(10):9041–7. https://doi.org/10.3168/jds.2018-14456 PMID: 30055923

9.  Meller RA, Wenner BA, Ashworth J, Gehman AM, Lakritz J, Firkins JL. Potential roles of nitrate and live yeast culture in suppressing methane emission and influencing ruminal fermentation, digestibility, and milk production in lactating Jersey cows. Journal of dairy science. 2019 Jul 1; 102(7):6144–56. https://doi.org/10.3168/jds.2018-16008 PMID: 31030922

10. Duin EC, Wagner T, Shima S, Prakash D, Cronin B, Yáñez-Ruiz DR, et al. Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropa-nol. Proceedings of the National Academy of Sciences. 2016 May 31; 113(22):6172–7. https://doi.org/10.1073/pnas.1600298113 PMID: 27140643

11. Olijhoek DW, Hellwing AL, Brask M, Weisbjerg MR, Højberg O, Larsen MK, et al. Effect of dietary nitrate level on enteric methane production, hydrogen emission, rumen fermentation, and nutrient digestibility in dairy cows. Journal of dairy science. 2016 Aug 1; 99(8):6191–205. https://doi.org/10.3168/jds.2015-10691 PMID: 27236758

12. van Zijderveld SM, Gerrits WJ, Apajalahti JA, Newbold JR, Dijkstra J, Leng RA, et al. Nitrate and sulfate: effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. Journal of dairy science. 2010 Dec 1; 93(12):5856–66. https://doi.org/10.3168/jds.2010-3281 PMID: 21094759

13. Klop G, Hatew B, Bannink A, Dijkstra J. Feeding nitrate and docosahexaenoic acid affects enteric meth-ane production and milk fatty acid composition in lactating dairy cows. Journal of dairy science. 2016 Feb 1; 99(2):1161–72. https://doi.org/10.3168/jds.2015-10214 PMID: 26627858

14. Alemu AW, Romero-Pérez A, Araujo RC, Beauchemin KA. Effect of encapsulated nitrate and microen-capsulated blend of essential oils on growth performance and methane emissions from beef steers fed backgrounding diets. Animals. 2019 Jan; 9(1):21. https://doi.org/10.3390/ani9010021 PMID: 30634606

15. Zhang X, Medrano RF, Wang M, Beauchemin KA, Ma Z, Wang R, et al. Effects of urea plus nitrate pre-treated rice straw and corn oil supplementation on fiber digestibility, nitrogen balance, rumen fermenta-tion, microbiota and methane emissions in goats. Journal of animal science and biotechnology. 2019 Dec; 10(1):6. https://doi.org/10.1186/s40104-019-0312-2 PMID: 30680191

16. van Gastelen S, Dijkstra J, Bannink A. Are dietary strategies to mitigate enteric methane emission equally effective across dairy cattle, beef cattle, and sheep?. Journal of dairy science. 2019 Jul 1; 102(7):6109–30. https://doi.org/10.3168/jds.2018-15785 PMID: 31079901

17. Latham EA, Pinchak WE, Trachsel J, Allen HK, Callaway TR, Nisbet DJ, et al. Paenibacillus 79R4, a potential rumen probiotic to enhance nitrite detoxification and methane mitigation in nitrate-treated rumi-nants. Science of the total environment. 2019 Jun 25; 671:324–8. https://doi.org/10.1016/j.scitotenv.2019.03.390 PMID: 30933788

18. National Agricultural Statistics Service [Internet]. Quick Stats. 2017 [cited 2019 Jan 12]. Available from: https://quickstats.nass.usda.gov.

19. U.S. Department of Agriculture [Internet]. 2013 Farm and ranch irrigation survey. 2013 [cited 2019 Dec 25]. Available from: https://www.agcensus.usda.gov/Publications/2012/Online_Resources/FarmandRanchIrrigationSurvey/.

20. Burt CM, Howes DJ, Wilson G. California agricultural water electrical energy requirements. Bioresource and Agricultural Engineering. 2003 Dec 1:59.

21. Johnson R, Cody BA. California agricultural production and irrigated water use. Sacramento, California, USA: Congressional Research Service; 2015 Jun 30. Available from: https://fas.org/sgp/crs/misc/R44093.pdf.

22. USDA-Economic Research Service [Internet]. Data sets: Fertilizer use and price. 2011 [cited 2019 May 15]. Available from: https://www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx.

23. UC Agricultural Issues Center. Current cost and return studies. 2016 [cited 2019 Nov 9]. Available from: https://coststudies.ucdavis.edu/en/.

24. Liedke A., and Deimling S. Role of specialty feed ingredients on livestock production's environmental sustainability. Final Report. PE International AG, Leinfelden-Echterdingen, Germany; 2015.

25. Velazco JI, Cottle DJ, Hegarty RS. Methane emissions and feeding behaviour of feedlot cattle supplemented with nitrate or urea. Animal production science. 2014 Aug 28; 54(10):1737–40. https://doi.org/10.1071/AN14345

26. Rebelo LR, Luna IC, Messana JD, Araujo RC, Simioni TA, Granja-Salcedo YT, et al. Effect of replacing soybean meal with urea or encapsulated nitrate with or without elemental sulfur on nitrogen digestion and methane emissions in feedlot cattle. Animal feed science and technology. 2019 Nov 1; 257:114293. https://doi.org/10.1016/j.anifeedsci.2019.114293

27. California Department of Food and Agriculture [Internet]. 2014 Annual data: California Dairy Statistics 2014. 2014 [cited 2019 Nov 5]. Available from: https://www.cdfa.ca.gov/dairy/mp_current_cal_stats.html.

28. Martinez-Fernandez G, Duval S, Kindermann M, Schirra HJ, Denman SE, McSweeney CS. 3-NOP vs. halogenated compound: Methane production, ruminal fermentation and microbial community response in forage fed cattle. Frontiers in microbiology. 2018 Aug 7; 9:1582. https://doi.org/10.3389/fmicb.2018.01582 PMID: 30131771

29. Vyas D, Alemu AW, McGinn SM, Duval SM, Kindermann M, Beauchemin KA. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high-forage and high-grain diets. Journal of animal science. 2018 Jun 29; 96(7):2923–38. https://doi.org/10.1093/jas/sky174 PMID: 29741701

30. Kim SH, Lee C, Pechtl HA, Hettick JM, Campler MR, Pairis-Garcia MD,et al. Effects of 3-nitrooxypropanol on enteric methane production, rumen fermentation, and feeding behavior in beef cattle fed a high-forage or high-grain diet. Journal of animal science. 2019 Jul 2; 97(7):2687–99. https://doi.org/10.1093/jas/skz140 PMID: 31115441

31. van Wesemael D, Vandaele L, Ampe B, Cattrysse H, Duval S, Kindermann M, et al. Reducing enteric methane emissions from dairy cattle: Two ways to supplement 3-nitrooxypropanol. Journal of dairy science. 2019 Feb 1; 102(2):1780–7. https://doi.org/10.3168/jds.2018-14534 PMID: 30594370

32. Nkemka VN, Beauchemin KA, Hao X. Treatment of feces from beef cattle fed the enteric methane inhibitor 3-nitrooxypropanol. Water science and technology. 2019 Aug 1; 80(3):437–47. https://doi.org/10.2166/wst.2019.302 PMID: 31596255

33. Brentrup F, Hoxha A, Christensen B. Carbon footprint analysis of mineral fertilizer production in Europe and other world regions. In Conference Paper, The 10th International Conference on Life Cycle Assessment of Food (LCA Food 2016) 2016 Oct 19.

34. Nielsen NI, Volden H, Åkerlind M, Brask M, Hellwing AL, Storlien T, et al. A prediction equation for enteric methane emission from dairy cows for use in NorFor. Acta Agriculturae Scandinavica, Section A-Animal Science. 2013 Sep 1; 63(3):126–30. https://doi.org/10.1080/09064702.2013.851275

35. Appuhamy R, Moraes L, Wagner-Riddle C, Casper DP, Kebreab E. Predicting manure volatile solid output of lactating dairy cows. Journal of animal science. 2016 Oct 1; 94:567–8. https://doi.org/10.2527/jam2016-1182

36. Hristov AN, Oh J, Giallongo F, Frederick TW, Harper MT, Weeks HL, et al. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proceedings of the national academy of sciences. 2015 Aug 25; 112(34):10663–8.

37. Haisan J, Sun Y, Guan L, Beauchemin KA, Iwaasa A, Duval S, et al. The effects of feeding 3-nitrooxypropanol at two doses on milk production, rumen fermentation, plasma metabolites, nutrient digestibility, and methane emissions in lactating Holstein cows. Animal production science. 2017 Jan 30; 57 (2):282–9.

38. Alvarez-Hess PS, Little SM, Moate PJ, Jacobs JL, Beauchemin KA, Eckard RJ. A partial life cycle assessment of the greenhouse gas mitigation potential of feeding 3-nitrooxypropanol and nitrate to cattle. Agricultural systems. 2019 Feb 1; 169:14–23.

39. Gerber P, Vellinga T, Opio C, Steinfeld H. Productivity gains and greenhouse gas emissions intensity in dairy systems. Livestock science. 2011 Jul 1; 139(1–2):100–8. https://doi.org/10.1016/j.livsci.2011.03.012

40. Thoma G, Popp J, Shonnard D, Nutter D, Matlock M, Ulrich R, et al. Regional analysis of greenhouse gas emissions from USA dairy farms: A cradle to farm-gate assessment of the American dairy industry circa 2008. International Dairy Journal. 2013 Apr 1; 31:S29–40. https://doi.org/10.1016/j.idairyj.2012.09.010

41. Guyader J, Little S, Kröbel R, Benchaar C, Beauchemin KA. Comparison of greenhouse gas emissions from corn-and barley-based dairy production systems in Eastern Canada. Agricultural systems. 2017 Mar 1; 152:38–46.

42. Bruning-Fann CS, Kaneene JB. The effects of nitrate, nitrite and N-nitroso compounds on human health: a review. Veterinary and human toxicology. 1993 Dec;  35(6):521–38. PMID: 8303822

43. Lee C, Beauchemin KA. A review of feeding supplementary nitrate to ruminant animals: nitrate toxicity, methane emissions, and production performance. Canadian Journal of Animal Science. 2014 Dec;  94 (4):557–70. https://doi.org/10.4141/cjas-2014-069