REVIEW

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Potential application of urease and nitrification inhibitors to mitigate emissions from the livestock sector: a review

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Abstract

Human activities have caused an increase in greenhouse gas emissions, resulting in climate change that affects many factors of human life including its effect on water and food quality in certain areas with implications for human health. CH_4 and N_2O are known as potent non-CO_2 GHGs. The livestock industry contributes to direct emissions of CH_4 (38.24%) and N_2O (6.70%) through enteric fermentation and manure treatment, as well as indirect N_2O emissions via NH_3 volatilization. NH_3 is also a secondary precursor of particulate matter. Several approaches have been proposed to address this issue, including dietary management, manure treatment, and the possibility of inhibitor usage. Inhibitors, including urease and nitrification inhibitors, are widely used in agricultural fields. The use of urease and nitrification inhibitors is known to be effective in reducing nitrogen loss from agricultural soil in the form of NH_3 and N_2O and can further reduce CH_4 as a side effect. However, the effectiveness of inhibitors in livestock manure systems has not yet been explored. This review discusses the potential of inhibitor usage, specifically of N-(n-butyl) thiophosphoric triamide, dicyandiamide, and 3,4-dimethylpyrazole phosphate, to reduce emissions from livestock manure. This review focuses on the application of inhibitors to manure, as well as the association of these inhibitors with health, toxicity, and economic benefits.

Keywords: Livestock emissions, Greenhouse gas (GHG) emissions, Urease inhibitor, Nitrification inhibitor, Particulate matter

INTRODUCTION

Anthropogenic activities have led to the production of large amounts of greenhouse gases (GHGs) such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), which has resulted in climate change and global warming. Human activities are estimated to have caused global warming of approximately 1℃ above pre-industrial levels, ranged between 0.8℃ to 1.2℃ [1]. The total amount of GHG emissions in 2018 for developed countries (Annex 1 parties) was 16,794,455.9 kt CO_2

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equivalent (CO_2-eq) [2]. CH_4 is an extremely potent GHG, responsible for approximately 30% of warming since pre-industrial times [3]. A global warming potential of 25 CO_2-eq over a 100-year time horizon for CH_4 was used in the report to assess pathway to zero emissions [1]. Human-caused CH_4 emissions are predominantly from three sectors: fossil fuels, waste, and agriculture. On the other hand, N_2O is another potent GHG because of its 100-year global warming potential of 298 CO_2-eq.

Global climate change affects human health, livelihoods and ecological and human systems, resulting in global monetary damage. The IPCC [1] indicated five reasons for concern that point up the risk of global warming at different level, including its impact on human, economies, and ecosystem. At present, the risk transitions of global warming range from moderate to high risk, between 1℃ and 2℃. The Paris Agreement in 2015 was adopted to set the long-term goals to limit the global average temperature increase to 2℃ in this century while also attempting further limitation to 1.5℃ above pre-industrial level [4]. To be on track toward the Paris Agreement, global GHG emissions should reduce by 7.6% each year between 2020 and 2030 [3].

According to Annex I countries' CH_4 and N_2O emissions, livestock sector (manure management and enteric fermentation) was responsible for 38.24% and 6.70% of total CH_4 and N_2O emissions, respectively, where CH_4 from livestock sector was responsible of approximately 95.4% of the total agricultural CH_4 emissions [2]. Also NH_3 emitted from livestock sector was the source of indirect N_2O emissions and NH_3 is a secondary precursor of particulate matter (PM) and contributes to the overall PM burden [5]. The sustainability of livestock production is necessary for continuity of human life and by targeting non-CO_2 for mitigation, agricultural CH_4 and N_2O emissions could be reduced; therefore, the mitigation of GHG emissions from this sector is crucial and in urgent need of being addressed.

EMISSIONS FROM THE LIVESTOCK SECTOR

Direct emissions

Agriculture is one of the main contributing sectors of CH_4 and N_2O. Agriculture contributed approximately 9.27% of the total global emissions in 2019 (Annex 1 parties) [2]. Among the emissions from agriculture, 50.18% came from livestock. Emission from the livestock industry is a by-product of the digestive system of ruminants, in the form of CH_4 through enteric fermentation and as CH_4 and N_2O through manure handling. Livestock product demand is predicted to grow by 70% in 2050, resulting in significant increase in GHG emissions from livestock [6]. Therefore, it is important to mitigate emissions from the livestock industry.

CH_4 production is caused by microbial fermentation that hydrolyze carbohydrates, and is an energy loss [7]. Methanogenesis generates CH_4 and methanogens, a group of obligate anaerobic archaebacteria that are chemoautotrophs [8], are responsible for this process [9]. These methane producers are strict anaerobes and pH sensitive, with an optimum pH range of 6.8 to 7.4, and function best at 95°F [10].

In 2018, the contribution of GHG emissions from enteric fermentation reached 85% of the total livestock's GHG emissions [11]. CH_4 from enteric fermentation is a byproduct of the digestive system in ruminants and is released during eructation; approximately 87%–90% is formed in the rumen and the remaining 13%–10% in the large intestine [12]. CH_4 emissions from cattle is seven times higher than that from sheep and nine times higher than that from goats [12]. CH_4 production in the rumen is affected by dietary factor and genetic factor [13].

Nitrification is a process that converts NH_4^+ to NO_3^- through microbial action [14]. This is a two-step chemolithotrophic process whereby NH_4^+ is first oxidized to NO_2^- by NH_3-oxidizing bacteria, followed by oxidation to NO_3^- by nitrate-oxidizing bacteria [14]. Denitrification requires the conversion of NO_3^- to NO_2^- in the absence of oxygen by the enzyme nitrate reductase, then nitric reductase converts NO_2^- to NO. Then, NO is converted to N_2O by nitric oxide reductase, and finally, N_2O is converted to N_2 gas by nitrous oxide reductase. Low pH inhibits reductase enzyme and compare to the other enzymes, it is even more sensitive to oxygen in the denitrification pathway [9].

Manure handling and storage are the source of livestock CH_4 and N_2O emissions. However, on pastures or rangeland, N_2O losses is more considerable than CH_4 emissions that can be very low [15]. However, CH_4 emissions may be highly significant in housed indoor house or on feedlots, and manure storage may be required. N_2O emissions originate largely from denitrification of N soils arising from fertilizers and urinary deposits, and to a lesser extent from sources of N resulting from leaching, runoff, and volatilization.

Indirect emissions

In addition to direct emissions, livestock also contributes to indirect emissions in the form of N_2O emissions. Indirect N_2O emissions account for one-third of the total global agricultural N_2O sources. In theory, indirect emissions consist of five different sources, including volatilization and subsequent atmospheric deposition of NH_3 and NO_x [16]. Indirect N_2O emissions may arise through deposition of NH_3 volatilized from manure. The indirect N_2O emissions, however, may also arise from the NH_3 deposited and NO emitted during manure management and application [17]. NH_3 is generated through urea hydrolysis during manure deposition. Urea is very stable, and it degrades so slowly without urease that its degradation is negligible. Urea in synthetic fertilizer does not come into contact with urease until it is applied to the field [18]; therefore, the application of livestock manure as an organic fertilizer is likely to accelerate NH_3 volatilization because of the urease present in feces. Urea hydrolysis also occurs in the presence of urease produced by bacteria in the soil, which results in the emission of NH_3. Urea hydrolysis occurs when urinary urea is catalyzed by urease in feces, resulting to the conversion of urea to NH_3 and CO_2. Urinary urea N is the source of NH_3-N, and microbial urease in feces hydrolyzes it to NH_3 and CO_2 [19]. The mixing of feces and urine promotes hydrolysis [20] and occurs rapidly within 1 to 2 days of excretion [21]. Urease concentration is known to be the highest in chicken manure, compared to that in pig and cattle manure, during the initial composting process [22]. The concentration of urea N ranges between 50% and 90% of the total N [21,23].

Ruminants excrete nitrogen in which can be loss as NH_3 for more than 50%. This significant amount of NH_3 emissions is attributed to the formation of PM with an aerodynamic diameter smaller than 2.5 μm ($PM_{2.5}$) [24]. The contribution of $PM_{2.5}$ to air pollution occurs through complex process. Primary particles interact with gaseous precursors, followed by photochemical transformation pathways and lastly, transport and deposited as $PM_{2.5}$ by meteorological process [25]. Organic carbon and sulfate control the formation of PM when NH_3 presents in excessive amount [26]. Livestock operations contribute to $PM_{2.5}$ and PM_{10}. PM_{10} is a term for particles with an aerodynamic diameter ≤ 10 mm. Direct PM_{10} is emitted as dust, and the reaction of NH_3 with nitrate and sulfuric acids forms indirect PM_{10} [24]. In the atmosphere, NH_3 can bind to other gases, such as SO_2 and nitrogen oxides (NO and NO_2) to form NH_4^+ containing fine PM [27]. This fine PM affects health when inhaled. $PM_{2.5}$ formed by NH_3 can penetrate deeper into the respiratory system of humans and animals where they damage tissues [28]. Although the average effect on lung function is modest, peak exposures of NH_3 may cause airway symptoms in vulnerable subjects [29]. Studies on the $PM_{2.5}$ reduction through NH_3 control have been performed. Over the eastern USA in July and January, a 4% and 9% decrease in $PM_{2.5}$ was caused by the reduction of NH_3 by 50%

[30], whereas in Italy, [31] showed that a reduction of 50% in NH_3 emissions from agriculture could result in a decrease in $PM_{2.5}$. Pozzer et al. [25] also showed that a 50% decrease in NH_3 emissions could reduce the annual, geographical average of near-surface $PM_{2.5}$ concentration by 2% to 11%. These studies confirm that the reduction in NH_3 emissions is the most effective control strategy for mitigating $PM_{2.5}$.

MITIGATION OF EMISSIONS FROM LIVESTOCK

Dietary management

The single most effective way to mitigate GHG emissions is to increase animal productivity. Thus, reducing animal numbers may provide the same edible product output with a reduced environmental footprint [32]. Dietary management has been widely used and is the most effective method to reduce CH_4 from enteric fermentation. Overall dietary manipulation by selection and utilization of high quality forages, strategic supplementation of forages, changing concentrate, proportion with special emphasis on changing carbohydrate composition should be considered as an immediate and sustainable CH_4 mitigation approach of enteric CH_4 emitted from ruminant livestock [33]. Methane emissions decrease in all regions when amended diets are adopted because more forage-based diets are less digestible than more concentrate grain-based diets [34].

Haque [33] divides dietary strategies into two categories: 1) improving forage quality and changing the diet proportion, and 2) dietary supplementation with feed additives. Although these strategies have been demonstrated to be effective, some obstacles are encountered. For instance, adding more grain in ruminant ration can be profitable because this strategy increases milk production, meat production, and also reduce the environmental footprint of livestock; however, the sustainability of this approach in the long term is questionable [32]. In some regions, grazing management may not be the best option to improve animal productivity due to poor pasture quality, in that case, improvement in productivity must come through feeding preserved forage or concentrate [32].

Some feed additives, known as inhibitors, are used to reduce methanogenesis by inhibiting methanogen activity. This includes supplementing with anti-methanogenic agents (e.g., antibiotics that reduce the methanogen population) or supplementing with electron (H^+) acceptors, such as nitrate salts [35]. Among additives, the most promising results have been with nitrate and 3-nitrooxypropanol which has strong mitigation effects on CH_4 emissions without adverse effects on animal performance; however, more research is needed to fully document the implications for environmental and animal health [36, 37]. Although demonstrated to be effective in reducing CH_4 emissions, these strategies may disrupt natural rumen processes, and pose potential health and other welfare challenges [35]. Other additive such as ionosphores is unable to be absorbed by animals' digestive tracts, however, unabsorbed ionophores in manure might have a negative impact on land ecosystem when the ionosphores are still active on manure at fertilization [38]. High-concentrate supplementation increases milk production and utilization of genetic potential of the animal. However, when the price of milk is lower than feed cost, this system may not be economically feasible [32].

Manure treatment

NH_3 and GHG emissions from animal facilities are influenced by several factors, i.e method of collecting manure, type of manure storage, type of housing, manure separation, and manure processing [15]. Mitigation practice for GHG emissions from stored manure mostly includes reducing storage time, air circulation (aeration), and stockpiling. These practices are intended to shorten fermentation process before land application [15]. These practices are found to be effective, but the practices is unclearly economically advantageous [15].

Inhibitors

In recent years, mitigation technologies, including the use of inhibitors such as urease inhibitors and nitrification inhibitors, have been explored to reduce emissions and nitrogen losses from agricultural fertilizer usage, and inhibitors have already been approved and are currently in the market [39]. Livestock manure is a rich source of organic compounds. Owing to this nutritional content, livestock manure is commonly used as fertilizer on agricultural soil or land. However, this practice may accelerate NH_3 volatilization because of the higher urease content in the manure than in the soil, which promotes the formation of indirect N_2O. The loss of nitrogen affects the nitrogen content of the soil, which may result in low yield production. Due to significant nitrogen losses from manure management systems, estimating the remaining amount of nitrogen in the manure is important, mainly for soil application or other purposes such as feed, fuel, or construction [40]. According to IPCC [40], N_2O emissions generated by manure in the pasture system, range, and paddock occur directly and indirectly from the soil.

UREASE AND NITRIFICATION INHIBITORS

Urease inhibitors

The main principle of urease inhibitors is to deactivate urease, which hydrolyses urea into NH_4^+, so that the hydrolysis of urea is delayed; hence, in the interim, several treatments can be performed to reduce the potential of nitrogen loss. There are several types of urease inhibitors. N-(propyl) thiophosphoric triamide (NPPT) is known to improve NH_3 volatilization; however, the application of NPPT is mostly in combination with N-(n-butyl) thiophosphoric triamide (NBPT), and several studies have revealed that NPPT is suspected to be a reproductive toxin [39]. N (2-nitrophenyl) phosphoric triamide (2-NPT) is a new urease inhibitor that is under development. The application of 2-NPT has been shown to lower NH_3 volatilization by 89% after 19 days of incubation [41], and depending on the soil characteristics, it also has greater longevity than NBPT [42]; therefore, the inhibitory effect of 2-NPT may last longer than that of NBPT. However, currently, 2-NPT is still on a laboratory-scale production; therefore, it is not easily available in the market and for field purposes.

NBPT is currently the most widely used urease inhibitor. NBPT blocks three active sites of the urease enzyme to form a tridentate bond. This bond consists of two nickel centers and one oxygen atom from the carbamate bridge linking both metal ions, which reduces the probability of urea reaching the active nickel center of the urease enzyme. NBPT must be converted into N-(n-butyl) phosphoric triamide (NBPTo), as it is not a direct inhibitor. The factors influencing this conversion are not clear, but the reaction is rapid in soils under aerobic conditions (occurring in minutes or hours) but can take days under anaerobic conditions. The direct application of NBPTo is inefficient because it degrades faster than NBPT.

NBPT is pH labile, and chemical hydrolysis appears to be an essential function of its breakdown under acidic conditions. This study concluded that under acidic conditions, chemical hydrolysis is likely the dominant pathway for NBPT and NBPTo breakdown. Under alkaline conditions, the biotic breakdown of these compounds via microorganisms became more significant [39].

NBPT mitigates NH_3 volatilization by controlling the rise in pH that occurs during urea hydrolysis, resulting in the production of two units of NH_4^+ and CO_2 and reducing the soil concentration of NH_4^+ around the urea granule [39]. These processes affect the equilibrium of NH_4^+

(soil), NH_3 (soil), and NH_3 (gas), which results in slow urea hydrolysis and ultimately allows ample time for the fertilizer to be incorporated into the soil via rainfall or irrigation, thereby protecting the applied N from volatilization [39]. Inhibition of urease by NBPT usually lasts 3 to 7 days as new urease enzyme production overwhelms the inhibitor [39].

The NBPT shows a relatively short period of protection. The ideal situation for the performance of urease inhibitors is through mechanical incorporation, followed by rain or irrigation occurring within 5 to 7 days after fertilization with NBPT-contained urea. In this period, depending on soil moisture or temperature, inhibitory potential of NBPT is still high [43]. The results of field studies showed reductions of > 85% in NH_3 volatilization as a result of NBPT application and rain event within 5 days after urea application [43].

A study conducted by Engel et al. [44] showed that application of urea of wet or damp soil, NH_3 loss was significant. This study indicates that NH_3 loss was significantly affected by water conditions. Application of NBPT delayed the peaks of NH_3 loss until 7 to 9 days, whereas without NBPT application, the highest loss occurred on day 3. Not only delayed the peak of NH_3 loss, NBPT also reduced the peaks of NH_3 loss [45]. The conversion of urea to NH_3 is prevented by the addition of NBPT, resulting to urea buildup in the manure. NBPT, on the other hand, has limited effect as time passes, therefore, in order to hydrolyze the build-up urea, more NBPT may be required [46]. Previous research has shown that high temperature affects the inhibiting ability of NBPT; Pereira et al. [47] reported that at a temperature of 20℃, NBPT was inhibited in a short time.

Nitrification inhibitors

Nitrification inhibitors are chemical compounds that delay the bacterial oxidation of NH_4^+ to NO_2^- in the soil, called nitrification. Nitrification inhibitors work by slowing down nitrifying bacteria that produce ammonia monooxygenase, hydroxylamine oxidoreductase, and nitric oxide reductase [39]. Delays in nitrification result in less NO_3^- formation, which is considered to be the source of nitrogen losses through leaching and denitrification (N_2O); therefore, the use of nitrification inhibitors not only reduces environmental problems but also increases the efficiency of nitrogen-based fertilizer. Several studies also demonstrated that CH_4 emissions could be reduced as a side effect of nitrification inhibitor application (Table 1).

Some examples of nitrification inhibitors are dicyandiamide (DCD), 3-4, dimethylpyrazole

Table 1. Application of nitrification and urease inhibitors in previous studies

Inhibitor type		Target	Fertilizer form	Reduction effect (%)			Application rate (g/kg N)		Application frequency	References
				NH_3	CH_4	N_2O	Min	Max		
Urease inhibitor	NBPT	Land	Urea	50-78	X	X	0.54	3.04	Once	[44], [45], [86], [87], [88]
		Land	Cow urine	48	X	X	1.00	10.00	Once	[47]
Nitrification inhibitor	DCD	Land	Cow urine	X	X	45–80	3.92	85.71	Once	[57], [89], [90]
		Land	Cow slurry	X	X	47–88	21.02	99.55	Once	[55], [91]
		Land	Swine slurry	X	X	70	71.43	76.92	Once	[92]
		Land	Urea	X	12	55.8	-	217.39	Monthly	[93], [94]
		Land	Urea	X	X	35	-	13.95	Monthly	[61]
	DMPP	Land	Urea	X	X	30–49	-	21.74	Once	[62]
		Land	Urea	X	X	38	-	4.65	3 times/year	[61]
		Land	Ammonium sulfate	X	X	48.9–74.9	4.29	17.14	Monthly	[95]

NBPT, N-(n-butyl) thiophosphoric triamide; DCD, dicyandiamide; DMPP, 3-4 dimethylpyrazole phosphate.

phosphate (DMPP), nitrapyrin, and thiosulfate. Thiosulfate may delay urea hydrolysis for up to 4 days and retard the conversion of NO_2-N to NO_3-N, thus resulting in a substantial amount of NO_2-N in the soil. However, to achieve a significant reduction in N_2O, high concentrations need to be applied, making it inefficient in reducing N_2O emissions. Nitrapyrin is widely used in the United States. It has been shown to reduce GHG emissions by 30%–50% [48]. It can also be retained in water for 7 to 10 days, whereas in soil it remains for 3 to 35 days [49], which implies that it can inhibit GHG emission production over an extended period. Even so, the use of nitrapyrin should be limited because of its low water solubility, and the results of nitrapyrin application differ depending on environmental conditions. Nitrapyrin is categorized as a moderate oral toxin and moderate dermal irritant [49].

There are conflicting results regarding the influence of nitrification inhibitors on CH_4 emissions. Bronson and Mosier [50] and Crill et al. [51] reported that nitrification inhibitors may increase CH_4 emissions, whereas Weiske et al. [52] reported that the addition of DCD either reduced the emissions or had no effect on CH_4 emissions. Another study by Villarrasa-Nogué [53] showed that the application of DMPP tended to reduce CH_4 oxidation, resulting in high CH_4 emissions.

Dicyandiamide

The breakdown of DCD results in NH_3, NO_3, H_2O, and CO_2, which may also contribute to increased N availability for microbial growth, as indicated by augmented CO_2 [54]. The kinetics of DCD degradation are highly influenced by temperature [54]. Minet et al. [55] found that DCD was still active after 6-month post application. Moreover, DCD did not affect the composition of the slurry during the period and cumulative N_2O-N emissions from DCD treatment was 88% lower than without DCD, which implies that DCD effectively reduced N_2O emission.

The stability of the DCD (during the 6-month period) indicates that DCD does not degrade when the slurry is stored under anaerobic conditions. Mixing stored slurry with DCD could be a means to mitigate N_2O emissions at high-risk times such as in autumn, winter, and early spring, where N_2O emissions could be at their highest. DCD addition to slurry could be highly preferred, cost-effective, and efficient for widespread adoption of N_2O mitigation using nitrification inhibitors by the agricultural sector [55].

DCD was more effective in reducing N_2O emissions and NO_3^- leaching from urine depositions during autumn than during summer or spring [56]. DCD in solid form is suggested to be applied at rates of 0.44% to 0.88% of the dry matter of composting piles (swine slurry with sawdust) with reapplication within 15 to 23 days to prevent later N_2O emissions as DCD concentrations decrease during the composting process [54]. The application of DCD with urine in both autumn and winter was effective in reducing the peak N_2O fluxes and the total amount of N_2O from urine application [57]. Increased DCD application rates would be required to sustain DCD concentrations in the surface soil above the critical level for extended periods in order to achieve a significant reduction in N_2O emissions from urine patches [57]. Application of DCD through mixing with animal urine prolonged the presence of NH_4^+ in the soil by approximately 3 to 6 weeks, which led to a reduction in the concentration of NO_3^--N by approximately 70%–85% [57].

Theoretically, inhibiting nitrification with DCD might also inhibit CH_4 oxidation to CO_2; however, the result from Minet et al. [55] showed that DCD application to slurry displayed lower CH_4 cumulative net flux than slurry without DCD application. The application of NBPT, phosphoroamide (PPD), and DMPP together with pig manure resulted in significantly reduced cumulative CH_4 emissions, because the addition of inhibitors further influenced the existing forms of nitrogen, which is beneficial to the growth of methanotrophic organisms and results in increased CH_4 oxidation [58]. Anaerobic conditions may prolong DCD persistence, and although the reasons for this are unclear, DCD degradation is unlikely to occur under anaerobic conditions [55].

3-4 Dimethylpyrazole phosphate

The DMPP with non-split application resulted in a more efficient reduction of N_2O losses than split application [59]. The DMPP treatment seemed to stimulate CH_4 oxidation more than DCD treatment because the soil clearly acted as a CH_4 sink rather than as a source [52]. Significant reduction due to inhibition of nitrification may take more than a week after DMPP addition [60]. DMPP could increase soil N retention, improve plant N use efficiency, and potentially stimulate the shoot yield of tea trees [60]. Although data related to DMPP are limited, DMPP has potential as an alternative nitrification inhibitor.

The application of DMPP was found to be more efficient than that of DCD. Compared to DCD, DMPP applied at very low rates (one-third application rate) resulted in comparable or improved inhibitory effects on N_2O emission [61]. DMPP decreased the amount of N_2O released on average by 49%, whereas DCD reduced N_2O emissions by only 26%, although DMPP was applied at rates ca. 10 times lower than that of DCD [52]. At high N doses, mitigation of DMPP was not observed, possibly because nitrogen has a priming effect that if microbial activity increases sufficiently, the surplus N threshold is reached above which the effectiveness of DMPP application is lost [53].

Toxicity and safety concerns

Toxicity in plants

DMPP is safe and without any phytotoxic damage. A study conducted by Zerulla et al. [62] revealed that an overdose of DMPP (8 times higher than the recommended application rate) did not cause any symptoms, while pronounced symptoms were found in the plant with overdose application of DCD. Tindaon et al. [63] concluded that the use of DCD and DMPP is environmentally compatible and safe. In addition, the recommended application rate of DCD is 10 kg DCD per ha per application and that for DMPP is 1.84 kg active ingredient/mg urea or 0.71 microgram DMPP/kg soil [63]. Both DCD and DMPP may affect non-target microbial soil only at high concentrations.

Residues in agricultural and animal products

Despite the fact of benefits associated with the use of urease and nitrification inhibitors, safety related to their residues in agriculture and animal products is debatable. In 2013, food safety concerns were raised regarding the use of DCD, which appeared as a residual contaminant in dairy products (Table 2) [39]. The MPI [64] reported that low-level residues of DCD were found in milk powder; however, there were no other reports on residues in other animal products. A study demonstrated that administration of DCD to dairy cows at 3 or 30 g DCD/cow/day was predominantly recovered in urine (61%–82%), feces (10%–19%), and milk (1.2%) [65]. This may be because of the residence time of DCD in plants. The residence time of DCD in plants was long in tall plants and under low rainfall conditions; therefore, the consideration in plant height and rainfall should be taken when selecting DCD application time to maximize the effectiveness of DCD [66]. Thus, contamination of animal products with DCD may be avoided when the animal eats the grass after DCD is fully degraded. Cai et al. [56] recommends to apply inhibitors before urine excretion. This method would be more efficient than other application method, i.e. at other timing.

In contrast, the NBPT is safe and has no influence on animal products (Table 2). A study conducted by Van De Ligt et al. [67] showed that there was no residue found in milk and bovine tissue from dairy cows fed with 1, 3, and 10 mg/kg body weight NBPT. The dose of NBPT was assumed from the maximum tolerable amount of urea (approximately 1 g NBPT/kg body weight)

Table 2. Hazards and ecotoxicology of nitrification and urease inhibitors

Type of inhibitor		Hazard risk	Ecotoxicology	Residues in animal products
Urease inhibitor	NBPT	Causes serious eye damage [97] Suspected of damaging fertility or the unborn child [97]	Low acute in aquatic and terrestrial [97]	No residues were found on milk and bovine tissue from dairy cow [67]
Nitrification inhibitor	DCD	Low hazard potential [69]	Low toxicity [69]	Minute residues in milk was found in 2013 in New Zealand [64] Administration of DCD to dairy cow at 3 or 30 g/cow/day was 1.2% recovered in milk [65]
	DMPP	Harmful if swallowed [96] Causes serious eye irritation [96] Suspected of damaging fertility or the unborn child [96] May cause damage to organs through prolonged or repeated exposure [96]	No hazards identified for air [96] No potential for bioaccumulation for predators [96]	Not available

NBPT, N-(n-butyl) thiophosphoric triamide; DCD, dicyandiamide; DMPP, 3-4 dimethylpyrazole phosphate.

that a cow can consume on a daily basis and the maximum concentration of commercial NBPT for urea (0.1 % w/w NBPT in urea) [68].

Hazards to animal and human health

Urease and nitrification inhibitors are not considered harmful, either to animals or humans; however, several precautions are needed when handling the substance owing to its possible hazard risk (Table 2). A study by Van De Ligt et al. [67] concluded that a high dose of NBPT fed to dairy cattle did not result in any harm. The possibility of urea toxicity to occur is rare, despite the fact that consuming NBPT in high level causes urea toxicity [36].

In 2006, NICNAS [68] reported that two workers became ill after handing NBPT with the trade name AGROTAIN® with the following symptoms: nausea and nose bleed. The following investigation revealed that there was no mechanical exhaust in the room during installation and calibration of AGROTAIN®-urea spray application system. Although the workers were wearing respirators with the recommended cartridge, they reported that after several hours of work, they could smell the product. The work was continued, and the same cartridges were used for two and half days. The ensuing investigation revealed that because of the saturated cartridges, the respirator failed to perform. No exposures were reported by the employees, and no symptoms were reported by the production workers. Following the event, the company amended the current product label to read "Apply product with coarse spray only. Do not atomize."

The ECHA [69] lists DCD under the name cyanoguanidine. According to ECHA [67], DCD is relatively low-hazardous for short-term or long-term exposure. However, caution is needed because it is an eye irritant. DMPP is non-hazardous, but it is considered to be low-hazardous if swallowed (oral exposure) and an eye irritant. Therefore, increased caution is needed. However, even though NBPT is low-hazardous, it is considered safe to use.

The DCD has a log octanol-water partition coefficient of –1 and is highly water soluble; therefore, it is unlikely to be taken up by fish gills or across other biological membranes [69]. However, DCD is not regarded as readily biodegradable in water; thus, the accumulation of DCD may occur, which may harm aquatic life. Information on bioaccumulation in aquatic environments or sediments is unavailable; thus, further research is needed to meet these criteria. NBPT is not considered to have a low potential for bioaccumulation [69].

Potential use of inhibitors in the livestock sector

The global population is estimated to increase to 9 billion people by 2050, and to ensure global food security, global agricultural production is expected to increase by approximately 100% [70,71]. Chemical fertilizers and organic manure are often applied in exceeding amount, leading to nitrogen loss, accounting for approximately 55% of the total applied N [72]. A significant amount of nitrogen loss not only has major consequences on human and environmental health, but also a significant economic loss for farmers.

The use of enhanced efficiency fertilizers prepared with coatings of low-permeability materials with an inhibitor attached as an additive may be used to reduce nitrogen loss and increase N uptake by plant and soil microbial populations [72]. Several studies have shown that with the addition of urease or nitrification inhibitors, plant yield is increased more than that without the use of such inhibitors. Adding DMPP at a rate of 0.232 g/100 g urea (120 kg N/ha of urea rate) resulted in a 7% increase in rice yield [73] and a 13% increase in wheat yield [74]. Other studies have shown that the addition of NBPT increased rice yield by approximately 1%–3% [75] and increased wheat yield by as much as 1% [76]. The addition of DCD also increased yield. Kakabouki et al. [77] concluded that cotton yield increased by approximately 364 kg/ha or 8% more than that without DCD. The addition of inhibitors is not only a feasible mitigation option, but also economically beneficial if applied correctly. Laboski [78] showed that when N is relatively inexpensive, if a 20% nitrogen loss occurs, the return would be maximized with additional N application; however, in a situation where N is expensive, adding NBPT (AGROTAIN®) is more likely to be profitable.

Modern agricultural practices have been well documented to impart negative impacts on human health as well as on farms, and the practice of irrational and excessive use of chemical fertilizers and pesticides has inspired the search for alternatives [79]. The use of manure as fertilizer has become increasingly common in the past few years, and is known to be environment-friendly because the application of manure as fertilizer can improve soil composition. Manure plays an important role in regulating plant growth, potential nutrient input, and microbial decomposition activity. This role can largely mediate the soil nutrient and soil micro-environment, which have a strong influence on crop growth. In addition, manure could also result in increased microbial biomass and changes in community structure, which provide an improved environment for crop growth [80]. Hua et al. [81] revealed that the application of manure resulted in considerable beneficial income, both in terms of yield and N uptake. This is owing to the increase in nutrient and organic matter availability in the soil as a result of manure as a nutrient source. Moreover, with long-term applications, the use of organic fertilizer can maintain nutrient balance and soil physical properties. In tomato plants, the addition of poultry manure significantly influenced tomato stem girth and the mean weight of the fruit [82]. Long term application of dairy manure (> 5 years) to soil resulted in significant increases in C, N, and microbial biomass, and changes in the microbial community structure. Practices that enhance soil carbon and provide slowly mineralizable nutrients may result in a larger and potentially more robust microbial community.

A laboratory study conducted by Varel [83] implied that the addition of urease inhibitor in cattle and swine waste was very effective in inhibiting urease activity. The addition of phenyl phosphorodiamidate (PPDA) prevented up to 70% urea hydrolysis in cattle waste and up to 92% in swine waste [83]. Prolonged inhibition can be obtained by the weekly addition of inhibitors [83]. This result was validated in a field study indicating that NBPT can be successfully used to inhibit urease activity in cattle feedlot manure [84], especially because the results obtained in the study with the open environment of the feedlot surface were encouraging. The open environment is more difficult to control due to exposed weather elements than other manure-handling systems, such as enclosed environments (pits with slotted floors). For instance, NBPT application to pit slurry is less

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complicated than application to a feedlot [84]. Application of NBPT causes urea build-up of urea in manure [84].

Dairy cows fed with DCD resulted in media concentrations of DCD in urine patches and were found to significantly reduce NO_3-N leaching and N_2O emissions by ±45% [57]. Slurry in mixture with DCD in long period of storage weaken the methanogens yet strengthen the methanothrophs [55]. Several manure treatment practices tend to produce more N_2O while reducing CH_4, in particular, treatment that includes air infusion, such as aerobic digestion or composting. N_2O emission mitigation by nitrification inhibitors can only be effective when the nitrification activity is essential, and the control of N_2O is in favor of emissions [85]. The addition of nitrification inhibitors to several manure treatment practices may be useful to reduce N_2O emissions and reduce CH_4 emissions as a manure treatment function.

CONCLUSION

The use of urease and nitrification inhibitors has been recognized as a mitigation tool to reduce nitrogen loss in agricultural soils. The application of inhibitors in agricultural soils decreases NH_3, N_2O, and CH_4 as a side effect; and yet, increases plant yield and nitrogen use efficiency. Although several concerns related to health and toxicity, either to humans, animals, or the environment, have been raised, both inhibitors have potential for long-term mitigation. However, further studies are required to confirm the safety of these inhibitors. Sufficient number of studies are lacking to understand the mechanisms of inhibitor application to livestock manure. In contrast, the use of livestock manure as fertilizer has been shown to be as effective as chemical fertilizers; moreover, such application is also known to improve soil composition and properties. However, manure application may accelerate NH_3 volatilization and, as a result, promote N_2O emissions. Several studies have also shown a positive effect of the application of inhibitors to manure on reducing emissions from livestock. Therefore, the use of inhibitors is likely to be effective and is considered to be an alternative mitigation method to reduce emissions from the livestock industry, either as an additive in organic fertilizer from manure or as an additive to manure treatment.

REFERENCES

1. IPCC [Intergovernmental Panel on Climate Change]. Global warming of 1.5°C: IPCC special report on impacts of global warming of 1.5°C above pre-industrial levels in context of strengthening response to climate change, sustainable development, and efforts to eradicate poverty. Cambridge: Cambridge University Press; 2022. p. 616.
2. UNFCCC [United Nations Framework Convention on Climate Change]. Greenhouse gas inventory data - GHG profiles - Annex I [Internet]. United Nation Climate Change. 2021 [cited 2021 Sep 27]. https://di.unfccc.int/ghg_profile_annex1
3. UNFCCC [United Nations Framework Convention on Climate Change]. Global assessment: urgent steps must be taken to reduce methane emissions this decade [Internet]. United Nation Climate Change. 2019 [cited 2021 Sep 27]. https://unfccc.int/news/global-assessment-urgent-steps-must-be-taken-to-reduce-methane-emissions-this-decade
4. UNFCCC [United Nations Framework Convention on Climate Change]. Paris Agreement [Internet]. United Nations. 2015 [cited 2021 Sep 27]. https://unfccc.int/sites/default/files/english_paris_agreement.pdf
5. Koolen CD, Rothenberg G. Air pollution in Europe. ChemSusChem. 2019;12:164-72. https://doi.org/10.1002/cssc.201802292

6. Gerber PJ, Hristov AN, Henderson B, Makkar H, Oh J, Lee C, et al. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. Animal. 2013;7:220-34. https://doi.org/10.1017/S1751731113000876
7. Alemu AW, Ominski KH, Kebreab E. Estimation of enteric methane emissions trends (1990–2008) from Manitoba beef cattle using empirical and mechanistic models. Can J Anim Sci. 2011;91:305-21. https://doi.org/10.4141/cjas2010-009
8. Atlas RM. Principles of microbiology. St. Louis, MO: Mosby; 1995.
9. Maier R, Pepper IL, Gerba CP. Environmental microbiology. Oxford: Elsevier; 2009.
10. Monteny GJ, Groenestein CM, Hilhorst MA. Interactions and coupling between emissions of methane and nitrous oxide from animal husbandry. Nutr Cycl Agroecosyst. 2001;60:123-32. https://doi.org/10.1023/A:1012602911339
11. FAOSTAT. Emission totals [Internet]. Food and Agriculture Organization of the United Nations. 2021 [cited 2021 Sep 28]. http://www.fao.org/faostat/en/#data/GT
12. Broucek J. Production of methane emissions from ruminant husbandry: a review. J Environ Prot. 2014;5:1482-93. https://doi.org/10.4236/jep.2014.515141
13. Nkrumah JD, Okine EK, Mathison GW, Schmid K, Li C, Basarab JA, et al. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J Anim Sci. 2006;84:145-53. https://doi.org/10.2527/2006.841145x
14. Bitton G. Wastewater microbiology. 4th ed. Hoboken, NJ: John Wiley & Sons; 2011.
15. Hristov AN, Oh J, Lee C, Meinen R, Montes F, Ott T, et al. Nutritional and management strategies to mitigate animal greenhouse gas emissions. In: Proceedings of the 2013 24th Annual Florida Ruminantnutrition Symposium; 2013; Gainesville, FL. p. 90-7.
16. IPCC [Intergovernmental Panel on Climate Change] National Greenhouse Gas Inventories Programme. Good practice guidance and uncertainty management in national greenhouse gas inventories. Kanagawa: IPCC by the Institute for Global Environmental Strategies; 2000.
17. Grönroos J, Mattila P, Regina K, Nousiainen J, Perälä P, Saarinen K, et al. Development of the ammonia emission inventory in Finland. Revised model for agriculture. Helsinki: Finnish Environment Institute; 2009.
18. Sigurdarson JJ, Svane S, Karring H. The molecular processes of urea hydrolysis in relation to ammonia emissions from agriculture. Rev Environ Sci Biotechnol. 2018;17:241-58. https://doi.org/10.1007/s11157-018-9466-1
19. Spek JW, Dijkstra J, Van Duinkerken G, Bannink A. A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. J Agric Sci. 2013;151:407-23. https://doi.org/10.1017/S0021859612000561
20. Hao C, Pan Y, Zhang Z, Zeng Y. Kinetic determination of urease activity in fresh pig feces and slurry and the effect on ammonia production at different conditions. Sustainability. 2019;11:6396. https://doi.org/10.3390/su11226396
21. Dijkstra J, Oenema O, van Groenigen JW, Spek JW, van Vuuren AM, Bannink A. Diet effects on urine composition of cattle and N_2O emissions. Animal. 2013;7:292-302. https://doi.org/10.1017/S1751731113000578
22. Feng L, Li X, Zhen X, Dong H, Zheng J, Wang Y. Study of enzyme activity changing pattern in livestock manures composting. Appl Ecol Environ Res. 2019;17:6581-93. https://doi.org/10.15666/aeer/1703_65816593
23. Bristow AW, Whitehead DC, Cockburn JE. Nitrogenous constituents in the urine of cattle, sheep and goats. J Sci Food Agric. 1992;59:387-94. https://doi.org/10.1002/jsfa.2740590316
24. Hristov AN. Technical note: contribution of ammonia emitted from livestock to atmospheric

fine particulate matter (PM2.5) in the United States. J Dairy Sci. 2011;94:3130-6. https://doi.org/10.3168/jds.2010-3681

25. Pozzer A, Tsimpidi AP, Karydis VA, de Meij A, Lelieveld J. Impact of agricultural emission reductions on fine-particulate matter and public health. Atmos Chem Phys. 2017;17:12813-26. https://doi.org/10.5194/acp-17-12813-2017
26. Dai C, Huang S, Zhou Y, Xu B, Peng H, Qin P, et al. Concentrations and emissions of particulate matter and ammonia from extensive livestock farm in South China. Environ Sci Pollut Res. 2019;26:1871-9. https://doi.org/10.1007/s11356-018-3766-4
27. Guthrie S, Giles S, Dunkerley F, Tabaqchali H, Harshfield A, Ioppolo B,et al. The impact of ammonia emissions from agriculture on biodiversity: an evidence synthesis. Santa Monica, CA: Rand; 2018.
28. Naseem S, King AJ. Ammonia production in poultry houses can affect health of humans, birds, and the environment—techniques for its reduction during poultry production. Environ Sci Pollut Res. 2018;25:15269-93. https://doi.org/10.1007/s11356-018-2018-y
29. Smit LAM, Heederik D. Impacts of intensive livestock production on human health in densely populated regions. GeoHealth. 2017;1:272-7. https://doi.org/10.1002/2017GH000103
30. Tsimpidi AP, Karydis VA, Pandis SN. Response of inorganic fine particulate matter to emission changes of sulfur dioxide and ammonia: the eastern United States as a case study. J Air Waste Manag Assoc. 2007;57:1489-98. https://doi.org/10.3155/1047-3289.57.12.1489
31. de Meij A, Thunis P, Bessagnet B, Cuvelier C. The sensitivity of the CHIMERE model to emissions reduction scenarios on air quality in Northern Italy. Atmos Environ. 2009;43:1897-907. https://doi.org/10.1016/j.atmosenv.2008.12.036
32. Montes F, Meinen R, Dell C, Rotz A, Hristov AN, Oh J, et al. Special topics—mitigation of methane and nitrous oxide emissions from animal operations: II. a review of manure management mitigation options. J Anim Sci. 2013;91:5070-94. https://doi.org/10.2527/jas.2013-6584
33. Haque MN. Dietary manipulation: a sustainable way to mitigate methane emissions from ruminants. J Anim Sci Technol. 2018;60:15. https://doi.org/10.1186/s40781-018-0175-7
34. Caro D, Kebreab E, Mitloehner FM. Mitigation of enteric methane emissions from global livestock systems through nutrition strategies. Clim Change. 2016;137:467-80. https://doi.org/10.1007/s10584-016-1686-1
35. Llonch P, Haskell MJ, Dewhurst RJ, Turner SP. Current available strategies to mitigate greenhouse gas emissions in livestock systems: an animal welfare perspective. Animal. 2017;11:274-84. https://doi.org/10.1017/S1751731116001440
36. Petersen SO. Greenhouse gas emissions from liquid dairy manure: prediction and mitigation. J Dairy Sci. 2018;101:6642-54. https://doi.org/10.3168/jds.2017-13301
37. Kim H, Lee HG, Baek,YC, Lee S, Seo J. The effects of dietary supplementation with 3-nitrooxypropanol on enteric methane emissions, rumen fermentation, and production performance in ruminants: A meta-analysis. J Anim Sci Technol 2020;62:31–42. https://doi.org/10.5187/JAST.2020.62.1.31
38. Takahashi J, Iwasa M. Entomological approach to the impact of ionophore-feed additives on greenhouse gas emissions from pasture land in cattle. J Anim Sci Technol. 2021;63:16–24. https://doi.org/10.5187/JAST.2021.E11
39. Byrne MP, Tobin JT, Forrestal PJ, Danaher M, Nkwonta CG, Richards K, et al. Urease and nitrification inhibitors—as mitigation tools for greenhouse gas emissions in sustainable dairy systems: a review. Sustainability. 2020;12:6018. https://doi.org/10.3390/su12156018
40. IPCC [Intergovernmental Panel on Climate Change]. 2006 IPCC guidelines for national

greenhouse gas inventories. Kanagawa: Institute for Global Environmental Strategies; 2006.

41. Ni K, Kage H, Pacholski A. Effects of novel nitrification and urease inhibitors (DCD/TZ and 2-NPT) on N2O emissions from surface applied urea: an incubation study. Atmos Environ. 2018;175:75-82. https://doi.org/10.1016/j.atmosenv.2017.12.002
42. Adhikari KP, Saggar S, Hanly JA, Guinto DF. Comparing the effectiveness and longevity of the urease inhibitor N-(2-nitrophenyl) phosphoric triamide (2-NPT) with N-(n-butyl) thiophosphoric triamide (nBTPT) in reducing ammonia emissions from cattle urine applied to dairy-grazed pasture soils. Soil Res. 2019;57:719-28. https://doi.org/10.1071/SR18337
43. Pan B, Lam SK, Mosier A, Luo Y, Chen D. Ammonia volatilization from synthetic fertilizers and its mitigation strategies: a global synthesis. Agric Ecosyst Environ. 2016;232:283-9. https://doi.org/10.1016/j.agee.2016.08.019
44. Engel R, Jones C, Wallander R. Ammonia volatilization from urea and mitigation by NBPT following surface application to cold soils. Soil Sci Soc Am J. 2011;75:2348-57. https://doi.org/10.2136/sssaj2011.0229
45. Soares JR, Cantarella H, Menegale MLC. Ammonia volatilization losses from surface-applied urea with urease and nitrification inhibitors. Soil Biol Biochem. 2012;52:82-9. https://doi.org/10.1016/j.soilbio.2012.04.019
46. Parker DB, Pandrangi S, Greene LW, Almas LK, Cole NA, Rhoades MB, et al. Rate and frequency of urease inhibitor application for minimizing ammonia emissions from beef cattle feedyards. Trans Am Soc Agric Eng. 2005;48:787-93. https://doi.org/10.13031/2013.18321
47. Pereira J, Barneze AS, Misselbrook TH, Coutinho J, Moreira N, Trindade H. Effects of a urease inhibitor and aluminium chloride alone or combined with a nitrification inhibitor on gaseous N emissions following soil application of cattle urine. Biosyst Eng. 2013;115:396-407. https://doi.org/10.1016/j.biosystemseng.2013.05.002
48. Akiyama H, Yan X, Yagi K. Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: meta-analysis. Glob Change Biol. 2010;16:1837-46. https://doi.org/10.1111/j.1365-2486.2009.02031.x
49. EPA [United States Environmental Protection Agency]. Perticide fact sheet: nitrapyrin [Internet]. United States Environmental Protection Agency. 1985 [cited 2021 Feb 28]. https://nepis.epa.gov/Exe/ZyNET.exe/91024KR8.TXT?ZyActionD=ZyDocument&Client=EPA&Index=1981+Thru+1985&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=
50. Bronson KF, Mosier AR. Suppression of methane oxidation in aerobic soil by nitrogen fertilizers, nitrification inhibitors, and urease inhibitors. Biol Fertil Soils. 1994;17:263-8. https://doi.org/10.1007/BF00383979
51. Crill PM, Martikainen PJ, Nyka̋nen H, Silvola J. Temperature and N fertilization effects on methane oxidation in a drained peatland soil. Soil Biol Biochem. 1994;26:1331-9. https://doi.org/10.1016/0038-0717(94)90214-3
52. Weiske A, Benckiser G, Herbert T, Ottow J. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. Biol Fertil Soils. 2001;34:109-17. https://doi.org/10.1007/s003740100386
53. Vilarrasa-Nogué M, Teira-Esmatges MR, Pascual M, Villar JM, Rufat J. Effect of N dose, fertilisation duration and application of a nitrification inhibitor on GHG emissions from a peach orchard. Sci Total Environ. 2020;699:134042. https://doi.org/10.1016/

j.scitotenv.2019.134042

54. Oliveira LV, Higarashi MM, Nicoloso RS, Coldebella A. Use of dicyandiamide to reduce nitrogen loss and nitrous oxide emission during mechanically turned co-composting of swine slurry with sawdust. Waste Biomass Valorization. 2020;11:2567-79. https://doi.org/10.1007/s12649-019-00616-x
55. Minet EP, Jahangir MMR, Krol DJ, Rochford N, Fenton O, Rooney D, et al. Amendment of cattle slurry with the nitrification inhibitor dicyandiamide during storage: a new effective and practical N2O mitigation measure for landspreading. Agric Ecosyst Environ. 2016;215:68-75. https://doi.org/10.1016/j.agee.2015.09.014
56. Cai Y, Akiyama H. Effects of inhibitors and biochar on nitrous oxide emissions, nitrate leaching, and plant nitrogen uptake from urine patches of grazing animals on grasslands: a meta-analysis. Soil Sci Plant Nutr. 2017;63:405-14. https://doi.org/10.1080/00380768.2017.1367627
57. Luo J, Ledgard S, Wise B, Welten B, Lindsey S, Judge A, et al. Effect of dicyandiamide (DCD) delivery method, application rate, and season on pasture urine patch nitrous oxide emissions. Biol Fertil Soils. 2015;51:453-64. https://doi.org/10.1007/s00374-015-0993-4
58. Wu K, Gong P, Zhang L, Wu Z, Xie X, Yang H, et al. Yield-scaled N2O and CH4 emissions as affected by combined application of stabilized nitrogen fertilizer and pig manure in rice fields. Plant Soil Environ. 2019;65:497-502. https://doi.org/10.17221/286/2019-PSE
59. Huérfano X, Fuertes-Mendizábal T, Duñabeitia MK, González-Murua C, Estavillo JM, Menéndez S. Splitting the application of 3,4-dimethylpyrazole phosphate (DMPP): influence on greenhouse gases emissions and wheat yield and quality under humid Mediterranean conditions. Eur J Agron. 2015;64:47-57. https://doi.org/10.1016/j.eja.2014.11.008
60. Qiao C, Mia S, Wang Y, Hou J, Xu B. Assessing the effects of nitrification inhibitor DMPP on acidification and inorganic N leaching loss from tea (Camellia sinensis L.) cultivated soils with increasing urea–N rates. Sustainability. 2021;13:994. https://doi.org/10.3390/su13020994
61. Liu C, Wang K, Zheng X. Effects of nitrification inhibitors (DCD and DMPP) on nitrous oxide emission, crop yield and nitrogen uptake in a wheat–maize cropping system. Biogeosciences. 2013;10:2427-37. https://doi.org/10.5194/bg-10-2427-2013
62. Zerulla W, Barth T, Dressel J, Erhardt K, von Locquenghien KH, Pasda G, et al. 3,4-Dimethylpyrazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture. Biol Fertil Soils. 2001;34:79-84. https://doi.org/10.1007/s003740100380
63. Tindaon F, Benckiser G, Ottow JCG. Evaluation of ecological doses of the nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) and 4-chloromethylpyrazole (ClMP) in comparison to dicyandiamide (DCD) in their effects on dehydrogenase and dimethyl sulfoxide reductase activity in soils. Biol Fertil Soils. 2012;48:643-50. https://doi.org/10.1007/s00374-011-0655-0
64. MPI [Ministry of Primary Industries]. New Zealand government assures safety of country's dairy products [Internet]. Ministry of Primary Industries. 2013 [cited 2021 Sep 27]. https://www.mpi.govt.nz/news/media-releases/new-zealand-government-assures-safety-of-countrys-dairy-products/
65. Welten BG, Ledgard SF, Balvert SF, Kear MJ, Dexter MM. Effects of oral administration of dicyandiamide to lactating dairy cows on residues in milk and the efficacy of delivery via a supplementary feed source. Agric Ecosyst Environ. 2016;217:111-8. https://doi.org/10.1016/j.agee.2015.10.013
66. Kim DG, Giltrap D, Saggar S, Palmada T, Berben P, Drysdale D. Fate of the nitrification inhibitor dicyandiamide (DCD) sprayed on a grazed pasture: effect of rate and time of

application. Soil Res. 2012;50:337-47. https://doi.org/10.1071/SR12069

67. van de Ligt J, Borghoff SJ, Yoon M, Ferguson LJ, DeMaio W, McClanahan RH. Nondetectable or minimal detectable residue levels of N-(n-butyl) thiophosphoric triamide in bovine tissues and milk from a 28-d NBPT dosing study. Transl Anim Sci. 2019;3:1606-16. https://doi.org/10.1093/tas/txz153
68. NICNAS [National Industrial Chemicals Notification and Assessment Scheme]. N-(n-butyl) thiophosphoric triamide (NBPT). Sydney: The Australian Government Department of Health and Ageing; 2011.
69. ECHA [European Chemical Agency]. Cyanoganidine [Internet]. European Chemical Agency. 2021 [cited 2021 Sep 27]. https://echa.europa.eu/el/registration-dossier/-/registered-dossier/15751/7/1
70. Grafton RQ, Daugbjerg C, Qureshi ME. Towards food security by 2050. Food Secur. 2015;7:179-83. https://doi.org/10.1007/s12571-015-0445-x
71. Rodriguez A, Sanders IR. The role of community and population ecology in applying mycorrhizal fungi for improved food security. ISME J. 2015;9:1053-61. https://doi.org/10.1038/ismej.2014.207
72. Mahmud K, Panday D, Mergoum A, Missaoui A. Nitrogen losses and potential mitigation strategies for a sustainable agroecosystem. Sustainability. 2021;13:2400. https://doi.org/10.3390/su13042400
73. Hashim MM, Yusop MK, Othman R, Wahid SA. Field evaluation of newly-developed controlled release fertilizer on rice production and nitrogen uptake. Sains Malays. 2017;46:925-32. https://doi.org/10.17576/jsm-2017-4606-12
74. Mousavi Shalmani MA, Lakzian A, Khorassani R, Khavazi K, Zaman M. Interaction of different wheat genotypes and nitrification inhibitor 3,4-dimethylpyrazole phosphate using 15N isotope tracing techniques. Commun Soil Sci Plant Anal. 2017;48:1247-58. https://doi.org/10.1080/00103624.2016.1261888
75. Yang G, Ji H, Sheng J, Zhang Y, Feng Y, Guo Z, et al. Combining Azolla and urease inhibitor to reduce ammonia volatilization and increase nitrogen use efficiency and grain yield of rice. Sci Total Environ. 2020;743:140799. https://doi.org/10.1016/j.scitotenv.2020.140799
76. Guardia G, Sanz-Cobena A, Sanchez-Martín L, Fuertes-Mendizábal T, González-Murua C, Álvarez JM, et al. Urea-based fertilization strategies to reduce yield-scaled N oxides and enhance bread-making quality in a rainfed Mediterranean wheat crop. Agric Ecosyst Environ. 2018;265:421-31. https://doi.org/10.1016/j.agee.2018.06.033
77. Kakabouki IP, Karydogianni S, Zisi C, Folina A. Effect of fertilization with N-inhibitors on root and crop development of flaxseed crop (Linum usitatissimum L.). Agrivita J Agric Sci. 2020;42:411-24. https://doi.org/10.17503/agrivita.v42i3.2650
78. Laboski C. Does it pay to use nitrification and urease inhibitors. In: Proceedings of the 2006 Wisconsin Fertilizer, Aglime & Pest Management Conference; 2006; Madison, WI. p. 44–50. http://www.soils.wisc.edu/extension/wcmc/2006/pap/Laboski1.pdf
79. Saffeullah P, Nabi N, Liaqat S, Anjum NA, Siddiqi TO, Umar S. Organic agriculture: principles, current status, and significance. In: Hakeem KR, Dar GH, Mehmood MA, Bhat RA, editors. Microbiota and biofertilizers. Cham: Springer; 2020. p. 17-37.
80. Peacock AD, Mullen MD, Ringelberg DB, Tyler DD, Hedrick DB, Gale PM, et al. Soil microbial community responses to dairy manure or ammonium nitrate applications. Soil Biol Biochem. 2001;33:1011-9. https://doi.org/10.1016/S0038-0717(01)00004-9
81. Hua W, Luo P, An N, Cai F, Zhang S, Chen K, et al. Manure application increased crop yields by promoting nitrogen use efficiency in the soils of 40-year soybean-maize rotation. Sci Rep.

2020;10:14882. https://doi.org/10.1038/s41598-020-71932-9

82. Ewulo BS, Sanni KO, Eleduma AF. Effects of urea and poultry manure on growth and yield attributes of tomatoes (Lycopersicon esculentum Mill) and soil chemical composition. Int J Innov Res Adv Stud. 2016;3:5-9.
83. Varel VH. Use of urease inhibitors to control nitrogen loss from livestock waste. Bioresour Technol. 1997;62:11-7. https://doi.org/10.1016/S0960-8524(97)00130-2
84. Varel VH, Nienaber JA, Freetly HC. Conservation of nitrogen in cattle feedlot waste with urease inhibitors. J Anim Sci. 1999;77:1162-8. https://doi.org/10.2527/1999.7751162x
85. Kong X, Eriksen J, Petersen SO. Evaluation of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) for mitigating soil N2O emissions after grassland cultivation. Agric Ecosyst Environ. 2018;259:174-83. https://doi.org/10.1016/j.agee.2018.02.029
86. Adotey N, Kongchum M, Li J, Whitehurst GB, Sucre E, Harrell DL. Ammonia volatilization of zinc sulfate-coated and NBPT-treated urea fertilizers. Agron J. 2017;109:2918-26. https://doi.org/10.2134/agronj2017.03.0153
87. Sanz-Cobena A, Misselbrook T, Camp V, Vallejo A. Effect of water addition and the urease inhibitor NBPT on the abatement of ammonia emission from surface applied urea. Atmos Environ. 2011;45:1517-24. https://doi.org/10.1016/j.atmosenv.2010.12.051
88. Watson CJ, Akhonzada NA, Hamilton JTG, Matthews DI. Rate and mode of application of the urease inhibitor N-(n-butyl) thiophosphoric triamide on ammonia volatilization from surface-applied urea. Soil Use Manage. 2008;24:246-53. https://doi.org/10.1111/j.1475-2743.2008.00157.x
89. Simon PL, Dieckow J, Zanatta JA, Ramalho B, Ribeiro RH, van der Weerden T, et al. Does Brachiaria humidicola and dicyandiamide reduce nitrous oxide and ammonia emissions from cattle urine patches in the subtropics? Sci Total Environ. 2020;720:137692. https://doi.org/10.1016/j.scitotenv.2020.137692
90. Simon PL, Dieckow J, de Klein CAM, Zanatta JA, van der Weerden TJ, Ramalho B, et al. Nitrous oxide emission factors from cattle urine and dung, and dicyandiamide (DCD) as a mitigation strategy in subtropical pastures. Agric Ecosyst Environ. 2018;267:74-82. https://doi.org/10.1016/j.agee.2018.08.013
91. Cahalan E, Ernfors M, Müller C, Devaney D, Laughlin RJ, Watson CJ, et al. The effect of the nitrification inhibitor dicyandiamide (DCD) on nitrous oxide and methane emissions after cattle slurry application to Irish grassland. Agric Ecosyst Environ. 2015;199:339-49. https://doi.org/10.1016/j.agee.2014.09.008
92. Suleiman AKA, Gonzatto R, Aita C, Lupatini M, Jacques RJS, Kuramae EE, et al. Temporal variability of soil microbial communities after application of dicyandiamide-treated swine slurry and mineral fertilizers. Soil Biol Biochem. 2016;97:71-82. https://doi.org/10.1016/j.soilbio.2016.03.002
93. Jumadi O, Hala Y, Muis A, Ali A, Palennari M, Yagi K, et al. Influences of chemical fertilizers and a nitrification inhibitor on greenhouse gas fluxes in a corn (Zea mays L.) field in Indonesia. Microbes Environ. 2008;23:29-34. https://doi.org/10.1264/jsme2.23.29
94. Malla G, Bhatia A, Pathak H, Prasad S, Jain N, Singh J. Mitigating nitrous oxide and methane emissions from soil in rice–wheat system of the Indo-Gangetic plain with nitrification and urease inhibitors. Chemosphere. 2005;58:141-7. https://doi.org/10.1016/j.chemosphere.2004.09.003
95. Li Y, Xu J, Liu X, Qi Z, Wang H, Li Y, et al. Nitrification inhibitor DMPP offsets the increase in N2O emission induced by soil salinity. Biol Fertil Soils. 2020;56:1211-7. https://doi.org/10.1007/s00374-020-01490-9

96. ECHA [European Chemical Agency]. 1H-Pyrazole, 3,4-dimethyl-, phosphate (1:1) [Internet]. European Chemical Agency. 2021 [cited 2021 Sep 27]. https://echa.europa.eu/el/registration-dossier/-/registered-dossier/23160
97. ECHA [European Chemical Agency]. N-butylphosphorothioic triamide [Internet]. European Chemical Agency. 2021 [cited 2021 Sep 27]. https://echa.europa.eu/el/registration-dossier/-/registered-dossier/16245