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Author	Eska Nugrahaeningtyas[1], Dong-Jun Lee[2], Jun-Ik Song[3], Jung-Kon Kim[2], Kyu-Hyun Park[1]
Affiliation	[1] Department of Animal Industry Convergence, Kangwon National University, Chuncheon 24341, Republic of Korea [2] Department of Animal Environment, National Institute of Animal Science, Wanju 55365, Republic of Korea [3] Division of Animal Husbandry, Yonam College, Cheonan 31005, Republic of Korea
ORCID (for more information, please visit https://orcid.org)	Eska Nugrahaeningtyas (0000-0002-4931-7952) Dong-Jun Lee (0000-0001-7006-4649) Jun-Ik Song (0000-0003-2815-8662) Jung-Kon Kim (0000-0001-6329-477X) Kyu-Hyun Park (0000-0002-6390-5478)
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Authors' contributions Please specify the authors' role using this form.	Eska Nugrahaeningtyas: author Dong-Jun Lee: co-author

	Jun-Ik Song: co-author
	Jung-Kon Kim: correspondence
	Kyu-Hyun Park: correspondence
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5   **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Jung-Kon Kim[2] Kyu-Hyun Park[1]
Email address – this is where your proofs will be sent	Jung-Kon Kim (kjk9207@korea.kr) Kyu-Hyun Park (kpark74@kangwon.ac.kr)
Secondary Email address	
Address	[1] Department of Animal Industry Convergence, Kangwon National University, Chuncheon 24341, Republic of Korea [2] Department of Animal Environment, National Institute of Animal Science, Wanju 55365, Republic of Korea
Cell phone number	
Office phone number	Jung-Kon Kim (+82-63-238-7407) Kyu-Hyun Park (+82-33-250-8621)
Fax number	Jung-Kon Kim (+82-63-238-7447) Kyu-Hyun Park (+82-33-259-5572)

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7   **Potential Application of Urease and Nitrification Inhibitors to Mitigate Emissions from the Livestock**

8   **Sector: A Review**

9

10   **Abstract**

11   Human activities have caused an increase in greenhouse gas emissions, resulting in climate change that affects

12   many factors of human life including its effect on water and food quality in certain areas with implications for

13   human health. CH_4 and N_2O are known as potent non-CO_2 gases. The livestock industry contributes to direct

14   emissions of CH_4 (38.24%) and N_2O (6.70%) through enteric fermentation and manure treatment, as well as

15   indirect N_2O emissions via NH_3 volatilization. NH_3 is also a secondary precursor of particulate matter. Several

16   approaches have been proposed to address this issue, including dietary management, manure treatment, and the

17   possibility of inhibitor usage. Inhibitors, including urease and nitrification inhibitors, are widely used in

18   agricultural fields. The use of urease and nitrification inhibitors is known to be effective in reducing nitrogen loss

19   from agricultural soil in the form of NH_3 and N_2O and can further reduce CH_4 as a side effect. However, the

20   effectiveness of inhibitors in livestock manure systems has not yet been explored. This review discusses the

21   potential of inhibitor usage, specifically of N-(n-butyl) thiophosphoric triamide, dicyandiamide, and 3,4-

22   dimethylpyrazole phosphate, to reduce emissions from livestock manure. This review focuses on the application

23   of inhibitors to manure, as well as the association of these inhibitors with health, toxicity, and economic benefits.

24   **Keywords**: Livestock emissions, GHG emissions, Urease inhibitor, Nitrification inhibitor, Particulate matter

25

1. 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 °C above pre-industrial levels, ranged between 0.8 °C to 1.2 °C [1]. The total amount of GHG emissions in 2018 for developed countries (Annex 1 parties) was 16,794,455.9 kt CO2 equivalent (CO_2-eq) [2]. CH_4 is an extremely potent GHG, responsible for approximately 30% of warming since pre-industrial times [3]. It has a global warming potential of 38 CO_2-eq over a 100-year time horizon, making it an important non-CO_2 GHG. Human-caused CH_4 emissions are predominantly from three sectors: fossil fuels, waste, and agriculture. N_2O is another potent GHG because of its 100-year global warming potential of 100 CO_2-eq. N_2O damages the environment and is known to contribute to the depletion of the ozone layer.

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 °C and 2 °C. The Paris Agreement in 2015 was adopted to set the long-term goals to limit the global temperature increase to 2ºC in this century while also attempting further limitation to 1.5 ºC [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.

2. Emissions from the Livestock Sector

2.1. Direct Emissions

55   Agriculture is one of the main contributing sectors of CH_4 and N_2O. Agriculture contributed

56   approximately 9.27% of the total global emissions in 2019 (Annex 1 parties) [2]. Among the emissions from

57   agriculture, 50.18% came from livestock. Emission from the livestock industry is a by-product of the digestive

58   system of ruminants, in the form of CH_4 through enteric fermentation and as CH_4 and N_2O through manure

59   handling. Livestock product demand is predicted to grow by 70% in 2050, resulting in significant increase in GHG

60   emissions from livestock [6]. Therefore, it is important to mitigate emissions from the livestock industry.

61   CH_4 production is caused by microbial fermentation that hydrolyze carbohydrates, and is an energy loss [7].

62   Methanogenesis generates CH_4 and methanogens, a group of obligate anaerobic archaebacteria that are

63   chemoautotrophs [8], are responsible for this process [9]. These methane producers are strict anaerobes and pH

64   sensitive, with an optimum pH range of 6.8 to 7.4, and function best at 95 °F [10].

65   In 2018, the contribution of GHG emissions from enteric fermentation reached 85% of the total livestock's GHG

66   emissions [11]. CH_4 from enteric fermentation is a byproduct of the digestive system in ruminants and is released

67   during eructation; approximately 87%–90% is formed in the rumen and the remaining 13%–10% in the large

68   intestine [12]. CH_4 emissions from cattle is seven times higher than that from sheep and nine times higher than

69   that from goats [12]. CH_4 production in the rumen is affected by dietary factor and genetic factor [13].

70   Nitrification is a process that converts NH_4^+ to NO_3^- through microbial action [14]. This is a two-step

71   chemolithotrophic process whereby NH_4^+ is first oxidized to NO_2^- by NH_3-oxidizing bacteria, followed by

72   oxidation to NO_3^- by nitrate-oxidizing bacteria [14]. Denitrification requires the conversion of NO_3^- to NO_2^- in the

73   absence of oxygen by the enzyme nitrate reductase, then nitric reductase converts NO_2^- to NO. Then, NO is

74   converted to N_2O by nitric oxide reductase, and finally, N_2O is converted to N_2 gas by nitrous oxide reductase.

75   Low pH inhibits reductase enzyme and compare to the other enzymes, it is even more sensitive to oxygen in the

76   denitrification pathway [9].

77   Manure handling and storage are the source of livestock CH_4 and N_2O emissions. However, on pastures or

78   rangeland, N_2O losses is more considerable than CH_4 emissions that can be very low [15]. However, CH_4

79   emissions may be highly significant in housed indoor house or on feedlots, and manure storage may be required.

80   N_2O emissions originate largely from denitrification of N soils arising from fertilizers and urinary deposits, and

81   to a lesser extent from sources of N resulting from leaching, runoff, and volatilization.

82

83   **2.2. Indirect Emissions**

84 In addition to direct emissions, livestock also contributes to indirect emissions in the form of N_2O emissions.

85 Indirect N_2O emissions account for one-third of the total global agricultural N_2O sources. In theory, indirect

86 emissions consist of five different sources, including volatilization and subsequent atmospheric deposition of NH_3

87 and NO_x [16]. Indirect N_2O emissions may arise through deposition of NH_3 volatilized from manure. The indirect

88 N_2O emissions, however, may also arise from the NH_3 deposited and NO emitted during manure management and

89 application [17]. NH_3 is generated through urea hydrolysis during manure deposition. Urea is very stable, and it

90 degrades so slowly without urease that its degradation is negligible. Urea in synthetic fertilizer does not come into

91 contact with urease until it is applied to the field [18]; therefore, the application of livestock manure as an organic

92 fertilizer is likely to accelerate NH_3 volatilization because of the urease present in feces. Urea hydrolysis also

93 occurs in the presence of urease produced by bacteria in the soil, which results in the emission of NH_3. Urea

94 hydrolysis occurs when urinary urea is catalyzed by urease in feces, resulting to the conversion of urea to NH_3

95 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].

96 The mixing of feces and urine promotes hydrolysis [20] and occurs rapidly within 1 to 2 days of excretion [21].

97 Urease concentration is known to be the highest in chicken manure, compared to that in pig and cattle manure,

98 during the initial composting process [22]. The concentration of urea N ranges between 50% and 90% of the total

99 N [21,23].

100 Ruminants excrete nitrogen in which can be loss as NH_3 for more than 50%. This significant amount of NH_3

101 emissions is attributed to the formation of PM with an aerodynamic diameter smaller than 2.5 μm ($PM_{2.5}$) [24].

102 The contribution of $PM_{2.5}$ to air pollution occurs through complex process. Primary particles interact with gaseous

103 precursors, followed by photochemical transformation pathways and lastly, transport and deposited as $PM_2.5$ by

104 meteorological process [25]. Organic carbon and sulfate control the formation of PM when NH_3 presents in

105 excessive amount [26]. Livestock operations contribute to $PM_{2.5}$, and PM_{10}. PM_{10} is a term for particles with an

106 aerodynamic diameter ≤ 10 mm. Direct PM_{10} is emitted as dust, and the reaction of NH_3 with nitrate and sulfuric

107 acids forms indirect PM_{10} [24]. In the atmosphere, NH_3 can bind to other gases, such as SO_2 and nitrogen oxides

108 (NO and NO_2) to form NH_4^+ containing fine PM [27]. This fine PM affects health when inhaled. $PM_{2.5}$ formed by

109 NH_3 can penetrate deeper into the respiratory system of humans and animals where they damage tissues[28].

110 Although the average effect on lung function is modest, peak exposures of NH_3 may cause airway symptoms in

111 vulnerable subjects [29].

112 Studies on the $PM_{2.5}$ reduction through NH_3 control have been performed. Over the eastern USA in July and

113 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]

114 showed that a reduction of 50% in NH_3 emissions from agriculture could result in a decrease in $PM_{2.5}$. Pozzer et

115 al. [25] also showed that a 50% decrease in NH_3 emissions could reduce the annual, geographical average of near-

116 surface $PM_{2.5}$ concentration by 2% to 11%. These studies confirm that the reduction in NH_3 emissions is the most

117 effective control strategy for mitigating $PM_{2.5}$.

118

119 **3. Mitigation of Emissions from Livestock**

120 **3.1. Dietary Management**

121 The single most effective way to mitigate GHG emissions is to increase animal productivity. Thus, reducing

122 animal numbers may provide the same edible product output with a reduced environmental footprint [32]. Dietary

123 management has been widely used and is the most effective method to reduce CH_4 from enteric fermentation.

124 Overall dietary manipulation by selection and utilization of high quality forages, strategic supplementation of

125 forages, changing concentrate, proportion with special emphasis on changing carbohydrate composition should

126 be considered as an immediate and sustainable methane mitigation approach of enteric CH_4 emitted from ruminant

127 livestock [33]. Methane emissions decrease in all regions when amended diets are adopted because more forage-

128 based diets are less digestible than more concentrate grain-based diets [34].

129 Haque [33] divides dietary strategies into two categories: 1) improving forage quality and changing the diet

130 proportion, and 2) dietary supplementation with feed additives. Although these strategies have been demonstrated

131 to be effective, some obstacles are encountered For instance, adding more grain in ruminant ration can be

132 profitable because this strategy increases milk production, meat production, and also reduce the environmental

133 footprint of livestock; however, the sustainability of this approach in the long term is questionable [32]. In some

134 regions, grazing management may not be the best option to improve animal productivity due to poor pasture

135 quality, in that case, improvement in productivity must come through feeding preserved forage or concentrate [32].

136 Some feed additives, known as inhibitors, are used to reduce methanogenesis by inhibiting methanogen activity.

137 This includes supplementing with anti-methanogenic agents (e.g., antibiotics that reduce the methanogen

138 population) or supplementing with electron (H^+) acceptors, such as nitrate salts [35]. Among additives, the most

139 promising results have been with nitrate and 3-nitrooxypropanol; however, more research is needed to fully

140 document the implications for environmental and animal health [36]. Although demonstrated to be effective in

7

141 reducing CH_4 emissions, these strategies may disrupt natural rumen processes, and pose potential health and other

142 welfare challenges [35]. High-concentrate supplementation increases milk production and utilization of genetic

143 potential of the animal. However, when the price of milk is lower than feed cost, this system may not be

144 economically feasible [32].

145

146 **3.2. Manure Treatment**

147 NH_3 and GHG emissions from animal facilities are influenced by several factors, i.e method of

148 collecting manure, type of manure storage, type of housing, manure separation, and manure processing [15].

149 Mitigation practice for GHG emissions from stored manure mostly includes reducing storage time, air circulation

150 (aeration), and stockpiling. These practices are intended to shorten fermentation process before land application

151 [15]. These practices are found to be effective, but the practices is unclearly economically advantageous [15].

152

153 **3.3. Inhibitors**

154 In recent years, mitigation technologies, including the use of inhibitors such as urease inhibitors and nitrification

155 inhibitors, have been explored to reduce emissions and nitrogen losses from agricultural fertilizer usage, and

156 inhibitors have already been approved and are currently in the market [37]. Livestock manure is a rich source of

157 organic compounds. Owing to this nutritional content, livestock manure is commonly used as fertilizer on

158 agricultural soil or land. However, this practice may accelerate NH_3 volatilization because of the higher urease

159 content in the manure than in the soil, which promotes the formation of indirect N_2O. The loss of nitrogen affects

160 the nitrogen content of the soil, which may result in low yield production. Due to significant nitrogen losses from

161 manure management systems, estimating the remaining amount of nitrogen in the manure is important, mainly

162 for soil application or other purposes such as feed, fuel, or construction [38]. According to IPCC [38], N_2O

163 emissions generated by manure in the pasture system, range, and paddock occur directly and indirectly from the

164 soil.

165

166 **4.   Urease and Nitrification Inhibitors**

167 **4.1. Urease Inhibitors**

168 The main principle of urease inhibitors is to deactivate urease, which hydrolyses urea into NH_4^+, so that the

169 hydrolysis of urea is delayed; hence, in the interim, several treatments can be performed to reduce the potential of

170   nitrogen loss. There are several types of urease inhibitors. N-(propyl) thiophosphoric triamide (NPPT) is known

171   to improve NH_3 volatilization; however, the application of NPPT is mostly in combination with N-(n-butyl)

172   thiophosphoric triamide (NBPT), and several studies have revealed that NPPT is suspected to be a reproductive

173   toxin [37]. N (2-nitrophenyl) phosphoric triamide (2-NPT) is a new urease inhibitor that is under development.

174   The application of NPT has been shown to lower NH_3 volatilization by 89% after 19 days of incubation [39], and

175   depending on the soil characteristics, it also has greater longevity than NBPT [40]; therefore, the inhibitory effect

176   of 2-NPT may last longer than that of NBPT. However, currently, 2-NPT is still on a laboratory-scale production;

177   therefore, it is not easily available in the market and for field purposes.

178   NBPT is currently the most widely used urease inhibitor. NBPT blocks three active sites of the urease enzyme to

179   form a tridentate bond. This bond consists of two nickel centers and one oxygen atom from the carbamate bridge

180   linking both metal ions, which reduces the probability of urea reaching the active nickel center of the urease

181   enzyme. NBPT must be converted into N-(n-butyl) phosphoric triamide (NBPTo), as it is not a direct inhibitor.

182   The factors influencing this conversion are not clear, but the reaction is rapid in soils under aerobic conditions

183   (occurring in minutes or hours) but can take days under anaerobic conditions. The direct application of NBPTo is

184   inefficient because it degrades faster than NBPT.

185   NBPT is pH labile, and chemical hydrolysis appears to be an essential function of its breakdown under acidic

186   conditions. This study concluded that under acidic conditions, chemical hydrolysis is likely the dominant pathway

187   for NBPT and NBPTo breakdown. Under alkaline conditions, the biotic breakdown of these compounds via

188   microorganisms became more significant [37].

189   NBPT mitigates NH_3 volatilization by controlling the rise in pH that occurs during urea hydrolysis, resulting in

190   the production of two units of NH_4^+ and CO_2 and reducing the soil concentration of NH_4^+ around the urea granule

191   [37]. These processes affect the equilibrium of NH_4^+ (soil), NH_3 (soil), and NH_3 (gas), which results in slow urea

192   hydrolysis and ultimately allows ample time for the fertilizer to be incorporated into the soil via rainfall or

193   irrigation, thereby protecting the applied N from volatilization [37]. Inhibition of urease by NBPT usually lasts 3

194   to 7 days as new urease enzyme production overwhelms the inhibitor [37].

195   The NBPT shows a relatively short period of protection. The ideal situation for the performance of urease

196   inhibitors is through mechanical incorporation, followed by rain or irrigation occurring within 5 to 7 days after

197   fertilization with NBPT-contained urea. In this period, depending on soil moisture or temperature, inhibitory

198 potential of NBPT is still high [41]. The results of field studies showed reductions of > 85% in NH_3 volatilization

199 as a result of NBPT application and rain event within 5 days after urea application [41].

200 A study conducted by Engel et al. [42] showed that application of urea of wet or damp soil, NH_3 loss was

201 significant. This study indicates that NH_3 loss was significantly affected by water conditions. Application of NBPT

202 delayed the peaks of NH_3 loss until 7 to 9 days, whereas without NBPT application, the highest loss occurred on

203 day 3. Not only delayed the peak of NH_3 loss, NBPT also reduced the peaks of NH_3 loss [43]. The conversion of

204 urea to NH_3 is prevented by the addition of NBPT, resulting to urea buildup in the manure. NBPT, on the other

205 hand, has limited effect as time passes, therefore, in order to hydrolyze the build-up urea, more NBPT may be

206 required [44]. Previous research has shown that high temperature affects the inhibiting ability of NBPT; Pereira

207 et al. [45] reported that at a temperature of 20 °C, NBPT was inhibited in a short time.

208

209 **4.2. Nitrification Inhibitors**

210 Nitrification inhibitors are chemical compounds that delay the bacterial oxidation of NH_4^+ to NO_2^- in the soil,

211 called nitrification. Nitrification inhibitors work by slowing down nitrifying bacteria that produce ammonia

212 monooxygenase, hydroxylamine oxidoreductase, and nitric oxide reductase [37]. Delays in nitrification result in

213 less NO_3^- formation, which is considered to be the source of nitrogen losses through leaching and denitrification

214 (N_2O); therefore, the use of nitrification inhibitors not only reduces environmental problems but also increases

215 the efficiency of nitrogen-based fertilizer. Several studies also demonstrated that CH_4 emissions could be reduced

216 as a side effect of nitrification inhibitor application (Table 1).

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

218 nitrapyrin, and thiosulfate. Thiosulfate may delay urea hydrolysis for up to 4 days and retard the conversion of

219 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

220 reduction in N_2O, high concentrations need to be applied, making it inefficient in reducing N_2O emissions.

221 Nitrapyrin is widely used in the United States. It has been shown to reduce GHG emissions by 30%–50% [46]. It

222 can also be retained in water for 7 to 10 days, whereas in soil it remains for 3 to 35 days [47], which implies that

223 it can inhibit GHG emission production over an extended period. Even so, the use of nitrapyrin should be limited

224 because of its low water solubility, and the results of nitrapyrin application differ depending on environmental

225 conditions. Nitrapyrin is categorized as a moderate oral toxin and moderate dermal irritant [47].

There are conflicting results regarding the influence of nitrification inhibitors on CH_4 emissions. Bronson and Mosier [48] and Crill et al. [49] reported that nitrification inhibitors may increase CH_4 emissions, whereas Weiske et al. [50] reported that the addition of DCD either reduced the emissions or had no effect on CH_4 emissions. Another study by Villarrasa-Nogué [51] showed that the application of DMPP tended to reduce CH_4 oxidation, resulting in high CH_4 emissions.

4.2.1. 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 [52]. The kinetics of DCD degradation are highly influenced by temperature [52]. Minet et al. [53] 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 [54].

DCD was more effective in reducing N_2O emissions and NO_3^- leaching from urine depositions during autumn than during summer or spring [55]. 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 [52]. 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 [56]. 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 [56]. 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% [56].

Theoretically, inhibiting nitrification with DCD might also inhibit CH_4 oxidation to CO_2; however, the result from Minet et al. [53] 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 [57]. Anaerobic conditions may prolong DCD persistence, and although the reasons for this are unclear, DCD degradation is unlikely to occur under anaerobic conditions [53].

4.2.2.   3-4 Dimethylpyrazole Phosphate

The DMPP with non-split application resulted in a more efficient reduction of N_2O losses than split application [58]. 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 [50]. Significant reduction due to inhibition of nitrification may take more than a week after DMPP addition [59]. DMPP could increase soil N retention, improve plant N use efficiency, and potentially stimulate the shoot yield of tea trees [59]. 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 [60]. 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 [50]. 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 [51].

4.3. Toxicity and Safety Concerns

4.3.1.   Toxicity in Plants

DMPP is safe and without any phytotoxic damage. A study conducted by Zerulla et al. [61] 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. [62] 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

12

microgram DMPP/kg soil [62]. Both DCD and DMPP may affect non-target microbial soil only at high concentrations.

4.3.2. 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) [37]. The MPI [63] 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%) [64]. 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 [65]. Thus, contamination of animal products with DCD may be avoided when the animal eats the grass after DCD is fully degraded. Cai et al. [55] 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. [66] 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/kg body weight) 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) [67].

4.3.3. 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. [66] 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 [67] 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 [68] lists DCD under the name cyanoguanidine. According to ECHA [66], 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 [68]. 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 [68].

4.4. 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% [69,70]. Chemical fertilizers and

organic manure are often applied in exceeding amount, leading to nitrogen loss, accounting for approximately 55%

of the total applied N [71]. 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 [71]. 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 120 kg/N resulted in a

7% increase in rice yield [72] and a 13% increase in wheat yield [73]. Other studies have shown that the addition

341 of NBPT increased rice yield by approximately 1%–3% [74] and increased wheat yield by as much as 1%[75].

342 The addition of DCD also increased yield. Kakabouki et al. [76] concluded that cotton yield increased by

343 approximately 364 kg/ha or 8% more than that without DCD. The addition of inhibitors is not only a feasible

344 mitigation option, but also economically beneficial if applied correctly. Laboski [77] showed that when N is

345 relatively inexpensive, if a 20% nitrogen loss occurs, the return would be maximized with additional N application;

346 however, in a situation where N is expensive, adding NBPT (AGROTAIN®) is more likely to be profitable.

347 Modern agricultural practices have been well documented to impart negative impacts on human health as well as

348 on farms, and the practice of irrational and excessive use of chemical fertilizers and pesticides has inspired the

349 search for alternatives [78]. The use of manure as fertilizer has become increasingly common in the past few years,

350 and is known to be environment-friendly because the application of manure as fertilizer can improve soil

351 composition. Manure plays an important role in regulating plant growth, potential nutrient input, and microbial

352 decomposition activity. This role can largely mediate the soil nutrient and soil micro-environment, which have a

353 strong influence on crop growth. In addition, manure could also result in increased microbial biomass and changes

354 in community structure, which provide an improved environment for crop growth [79]. Hua et al. [80] revealed

355 that the application of manure resulted in considerable beneficial income, both in terms of yield and N uptake.

356 This is owing to the increase in nutrient and organic matter availability in the soil as a result of manure as a nutrient

357 source. Moreover, with long-term applications, the use of organic fertilizer can maintain nutrient balance and soil

358 physical properties. In tomato plants, the addition of poultry manure significantly influenced tomato stem girth

359 and the mean weight of the fruit [81]. Long term application of dairy manure (> 5 years) to soil resulted in

360 significant increases in C, N, and microbial biomass, and changes in the microbial community structure. Practices

361 that enhance soil carbon and provide slowly mineralizable nutrients may result in a larger and potentially more

362 robust microbial community.

363 A laboratory study conducted by Varel [82] implied that the addition of urease inhibitor in cattle and swine waste

364 was very effective in inhibiting urease activity. The addition of phenyl phosphorodiamidate (PPDA) prevented up

365 to 70% urea hydrolysis in cattle waste and up to 92% in swine waste [82]. Prolonged inhibition can be obtained

366 by the weekly addition of inhibitors [82]. This result was validated in a field study indicating that NBPT can be

367 successfully used to inhibit urease activity in cattle feedlot manure [83], especially because the results obtained

368 in the study with the open environment of the feedlot surface were encouraging. The open environment is more

369 difficult to control due to exposed weather elements than other manure-handling systems, such as enclosed

370   environments (pits with slotted floors). For instance, NBPT application to pit slurry is less complicated than

371   application to a feedlot [83]. Application of NBPT causes urea build-up of urea in manure [83].

372   Dairy cows fed with DCD resulted in media concentrations of DCD in urine patches and were found to

373   significantly reduce NO_3-N leaching and N_2O emissions by ±45% [56]. Slurry in mixture with DCD in long period

374   of storage weaken the methanogens yet strengthen the methanothrophs [53]. Several manure treatment practices

375   tend to produce more N_2O while reducing CH_4, in particular, treatment that includes air infusion, such as aerobic

376   digestion or composting. N_2O emission mitigation by nitrification inhibitors can only be effective when the

377   nitrification activity is essential, and the control of N_2O is in favor of emissions [84]. The addition of nitrification

378   inhibitors to several manure treatment practices may be useful to reduce N_2O emissions and reduce CH_4 emissions

379   as a manure treatment function.

380

381   **5. Conclusions**

382 The use of urease and nitrification inhibitors has been recognized as a mitigation tool to reduce nitrogen loss in

383 agricultural soils. The application of inhibitors in agricultural soils decreases NH_3, N_2O, and CH_4 as a side effect;

384 and yet, increases plant yield and nitrogen use efficiency. Although several concerns related to health and toxicity,

385 either to humans, animals, or the environment, have been raised, both inhibitors have potential for long-term

386 mitigation. However, further studies are required to confirm the safety of these inhibitors. Sufficient number of

387 studies are lacking to understand the mechanisms of inhibitor application to livestock manure. In contrast, the use

388 of livestock manure as fertilizer has been shown to be as effective as chemical fertilizers; moreover, such

389 application is also known to improve soil composition and properties. However, manure application may

390 accelerate NH_3 volatilization and, as a result, promote N_2O emissions. Several studies have also shown a positive

391 effect of the application of inhibitors to manure on reducing emissions from livestock. Therefore, the use of

392 inhibitors is likely to be effective and is considered to be an alternative mitigation method to reduce emissions

393 from the livestock industry, either as an additive in organic fertilizer from manure or as an additive to manure

394 treatment.

395

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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	[42], [85], [86], [43], [87]
		Land	Cow urine	48	X	X	1.00	10.00	Once	[45]
		Land	Cow urine	X	X	45-80	3.92	85.71	Once	[56], [88], [89]
		Land	Cow slurry	X	X	47-88	21.02	99.55	Once	[90], [54]
	DCD	Land	Swine slurry	X	X	70	71.43	76.92	Once	[91]
Nitrification Inhibitor		Land	Urea	X	12	55.8	-	217.39	Monthly	[92], [93]
		Land	Urea	X	X	35	-	13.95	Monthly	[60]
		Land	Urea	X	X	30-49	-	21.74	Once	[51]
	DMPP	Land	Urea	X	X	38	-	4.65	3 times/year	[60]
		Land	Ammonium Sulfate	X	X	48.9-74.9	4.29	17.14	Monthly	[94]

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

Table 2. Hazards and ecotoxicology of nitrification and urease inhibitors

Type of Inhibitor		Hazard Risk	Ecotoxicology	Residues in Animal Products
Urease Inhibitor	NBPT	a. Causes serious eye damage[95] b. Suspected of damaging fertility or the unborn child[95]	Low acute in aquatic and terrestrial[95]	No residues were found on milk and bovine tissue from dairy cow[65]
Nitrification Inhibitor	DCD	Low hazard potential[67]	Low toxicity[67]	Minute residues in milk was found in 2013 in New Zealand[62] Administration of DCD to dairy cow at 3 or 30 g/cow/day was 1.2% recovered in milk[63]
	DMPP	a. Harmful if swallowed[94] b. Causes serious eye irritation[94] c. Suspected of damaging fertility or the unborn child[94] d. May cause damage to organs through prolonged or repeated exposure[94]	a. No hazards identified for air[94] b. No potential for bioaccumulation for predators[94]	Not available

Source:[68], [66], [64], [63], [95], [96]

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