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Research Article

Effect of Dicyandiamide and Hydroquinone on Ammonia and Nitrous Oxide Emission from Pig Slurry Applied to Timothy (*Phleum pretense* L.) Sward

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ABSTRACT

The objective of this study was to determine the effect of nitrification inhibitor dicyandiamide (DCD) and urease inhibitor hydroquinone (HQ) on ammonia (NH_3) and nitrous oxide (N_2O) emission from pig slurry applied to Timothy (*Phleum pretense* L.) sward. The daily emission of ammonia (NH_3) and nitrous oxide (N_2O) was monitored for 9 days in three different treatments; 1) control (only pig slurry application), 2) DCD treatment (pig slurry + DCD), and 3) HQ treatment (pig slurry + HQ). Most NH_3 emission occurred after 4~5 days in three treatments. Total NH_3 emission, expressed as a cumulative amount throughout the measurement time, was 1.33 kg N ha^{-1} in the control. The DCD and HQ treatment decreased total NH_3 emission by 16.3% and 25.1%, respectively, compared to the control. Total N_2O emission in the control was 47.1 g N ha^{-1}. The DCD and HQ treatment resulted in a reduction of 67.9% and 41.8% in total N_2O emission, respectively, compared to the control. The present study clearly indicated that nitrification and urease inhibitor exhibited positive roles in reducing N losses through NH_3 and N_2O emission.

(**Key words :** Ammonia, Dicyandiamide, Hydroquinone, Nitrous oxide, Timothy)

Ⅰ. INTRODUCTION

Livestock production is one of the most important bases for supplying the high-grade, protein-rich foods. It does however, also bear an ecological risk by emitting an excessive amount of manure produced by domestic animals. The management of nitrogen (N) in animal manure often remains a challenge. N is an essential nutrient element and a key limiting factor for the growth of forage crops. The N in crops, primarily in the form of protein, is then used for animal growth and development. However, surplus amount of dietary protein relative to animal demand cannot be utilized by the metabolism caused by the limited capacity for producing food-protein: for example, in the dairy farms, 20 to 30% of the N consumed by the herd used for production of milk and meat, and the remnant is excreted in manure (Dou et al., 1996; Kohn et al., 1997; Oenema et al., 2001).

When animal manure is applied to the cropland, the majority of N is bound in organic compounds. The organic N should be mineralized by the soil microbes to be a plant available N form. Soil N mineralization is the process of microbial transforming organic N into inorganic N (e.g., NH_4^+ and NO_3^-). The soil N mineralization is affected by manure composition, physical and chemical properties of the soil, soil management and soil microorganisms (Eckard et al., 2003; Boeckx et al., 2005; Li and Li, 2014). Ammonia (NH_3) is produced from livestock operations when urea (presents in urine) is degraded by the enzyme urease to form NH_3 gas and carbamine acid, which further decomposes to release another molecule of NH_3 gas and CO_2 (Laboski, 2006). A proportion of volatilized and deposited ammonia produces nitrous oxide (N_2O) through nitrification and denitrification (Eckard et al., 2003; Liu et al., 2013). NH_3 volatilization has been known as the largest pathway of N loss in agro-ecosystem (USEPA, 2005). N_2O has become the third most important greenhouse gas after CO_2 and CH_4, with a global warming potential around 300

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times that of CO_2 (USEPA, 2005), and is also the major source of ozone depletion (Forster et al., 2007).

Many strategies have been applied to reduce the N losses via gaseous emission. One of approaches is the use of nitrification or urease inhibitors. The application of urease inhibitors hydroquinone (HQ), phenyl phosphorodiamidate (PPDA) and N-(n-butyl) thiophosphoric triamide (NBTPT) reduce the rapid hydrolysis of urea in soils, which results in N losses through NH_3 volatilization (Wang et al., 1991). NBTPT can form a tridentate ligand to slacken urea hydrolysis, resulting in reduced ammonia volatilization (Zaman et al., 2008). As nitrification inhibitors, nitrapyrin (NP) and 3,4-dimethylpyrazole phosphate (DMPP), can delay the microbial oxidation of NH_4^+ to nitrite (NO_2^-) for several weeks of months (Weiske et al., 2001) Also, the nitrification inhibitors have increased the N use efficiency by reducing nitrogen loss such as leaching and nitrification / denitrification losses (Di and Cameron, 2002). Dicyandiamide (DCD) is also useful in increasing the N use efficiency by retarding the rate at which NH_4^+-N is converted to NO_3^--N, thus reducing nitrous oxide emissions (Prasad and Power 1995). Liu et al. (2013) reported that nitrification inhibitors (DCD and DMPP) decreased the cumulative emissions by 35% and 38% of the annual emission of the urea treatment. Also, HQ effectively regulates the mineral N status in soil and decrease the nitrous oxide emission from urea fertilizer application (Xu et al., 2000).

This study aimed to evaluate the effects of urease and nitrification inhibitor on NH_3 and N_2O emission from the Timothy sward applied with pig slurry.

Ⅱ. MATERIALS AND METHODS

1. Experiment design

The present study is based on a field experiment conducted at the east-northern upland area of South Korea (Pyeongchang, Kangwon, E128°23′, S37°22′) in May 2015. The experiment was carried out on a grass sward, consisting mainly of Timothy (*Phleum pretense* L.). The experiment site is located on a sandy loamy soil with the chemical properties presented in Table 1. The local climate is semi-continental with mean temperature of 19.4°C and rainfall of 48.6 mm during experiment period. The experiment was allocated with three treatments; control (only pig slurry application), DCD treatment [pig slurry + nitrification inhibitor (Dicyandiamide, DCD, NH_4F, DAEJUNG)] and HQ treatment [pig slurry + urease inhibitor (Hydroquinone, HQ, $C_6H_6O_2$, DAEJUNG)]. DCD and HQ were mixed with pig slurry and applied at a rate 5% and 0.3% of the total-N in pig slurry (w/w), respectively. The pig slurry used for this experiment was obtained from the organic manure processing center of Crop-Animal Farming Cooperatives (Damyang-gun, Korea) at May 2015. The pig slurry was filtered and fully fermented before application. The N property of pig slurry used in this study is presented in Table 2.

2. Measurements of ammonia and nitrous oxide emissions

To collect ammonia emission, we used ammonia trap

Table 1. Chemical properties of the soil of Timothy sward before pig slurry application

pH_{water} (1:5)	EC (dS m^{-1})	OM (%)	Total N (%)	P_2O_5 (mg kg^{-1})	Exchangeable cation ($cmol^+$ kg^{-1})		
					K	Ca	Mg
5.6	0.76	2.45	0.15	256.2	0.23	3.06	1.98

EC: electrical conductivity.

Table 2. Nitrogen compounds of the pig slurry applied to Timothy sward

	Total N (g N kg^{-1})	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})	Organic N (g N kg^{-1})
Pig slurry	1.57 ± 0.03	206.7 ± 1.87	207.2 ± 14.16	1.16 ± 0.03

Values are mean ± SE of three replicates.

system which was modified Dräger Tube method described by Ndegwa et al. (2009). The chamber (20 cm diameter × 30 cm deep) to collect ammonia (NH_3) emissions, attached to NH_3-N trapping bottles containing 20 mL of 50 mM sulfuric acid and a vacuum system to pull air through the chambers. The NH_3-N traps flew a rate of approximately 1 L per minute. The collected NH_3 in trapping bottle was reacted with sulfuric acid (H_2SO_4) and converted into ammonium sulfate ($(NH_4)_2SO_4$), which is used to analyze the ammonia emission. Ammonia emissions were determined for a period of 9 days. Sulfuric acid, which trapped the ammonia gas, was changed every day after pig slurry and inhibitors treatment.

The acryl chamber was used to determine N_2O emissions. The N_2O emissions were determined for a period of 9 days from the Timothy sward applied with pig slurry. Sampling was done in the morning 09:00 in order to minimize diurnal variation in flux patterns. Each time, samples were injected into 10 mL BD vacutainer tube. N_2O concentration was analyzed on a gas chromatograph (Agilent technologies 7890A) equipped with a thermal conductivity detector (TCD). The columns were HP-Plot 5A 30 m, 0.53 mm, 25 μm. The temperature of column oven, injector and detector were 40, 100 and 300°C, respectively. The flow rate of carrier gas (Helium, 99%) was 2 mL min^{-1}. The N_2O fluxes were calculated using the following equation (Guo et al., 2012a)

$$F = \rho \times (P / 760) \times (V / A) \times \Delta C / \Delta t) \times [273 / (273 + T)]$$

where F is the N_2O flux (μg N_2O-N m^{-2} h^{-1}), ρ is the density of N_2O at 0°C and 760 mm Hg (kg m^{-3}), V is the chamber volume (m^3), A is the area from which N_2O was emitted into the chamber (m^2), $\Delta C / \Delta t$ is the rate of N_2O accumulation in the chamber (ppmv N_2O-N h^{-1}), T is the chamber air temperature in Celsius and P is the air pressure of the experimental site (mm Hg). The altitude of the experimental site for this study is very close to sea level, so $P / 760 \approx 1$.

3. Chemical analysis

N property of pig slurry used for this study was determined according to the method of Bremner (1996). The pH measurement was regularly done after shaking a 1:5 (sample : water, w/v) solution for 1 h on a rotary shaker. Total nitrogen was determined by digestion using the Kjeldahl procedure. Inorganic nitrogen was extracted with 2 M KCl and the NH_4^+-N was determined by distillation in an alkaline medium (MgO). The same procedure was used for NO_3^--N after reduction with Devarda's alloy (Lu, 2000). The pig slurry contained on average (kg m^{-3}): 1.42 total N, 0.31 NH_4^+-N, 0.20 NO_3-N, 0.68 P, 1.11 K with pH_{water} (1:5) of 8.0. For the determination of NH_3 volatilization, the N concentration in collected samples of acid traps was measured. The solution in the form of $(NH_4)_2SO_4$ was quantified by a colorimetric determination with ammonium color reagent (Nessler's reagent, Sigma, 72190) as described by Kim and Kim (1996).

4. Statistical analysis

Statistical analyses were conducted using the SAS 9.1.3 package. One-way analysis of variance (ANOVA) was used with inhibitor and sampling time as the main effect to test differences in the NH_3 and N_2O emission. Statistical significance was set at $p < 0.05$ unless otherwise stated.

Ⅲ. RESULTS AND DISCUSSION

The rate, source and timing of N fertilizer application are important management factors affecting the efficiency of crop and pasture growth responses, and thus potential N losses (Laboski, 2006). However, there is likely to be increasing pressure to further reduce N losses via gaseous emission. In the present study, we attempted to estimate the effectiveness of urease and nitrification inhibitor in mitigating NH_3 and N_2O emission from the pig slurry-applied sward. The most important NH_3 emission occurred at earlier 4~5 days after pig slurry application in three all treatments; control, DCD treatment and HQ treatment. The amount of NH_3 emitted during this period accounted 69%, 77% and 76% of total amount of NH_3 emission, respectively (Table 3). This result was consistent with previous reports (Rochette et al. 2001; Sommer and Hutching 2001), which showed a large portion of NH_3 loss at the earlier days after

Table 3. Changes in daily emission of ammonia and nitrous oxide in the control (pig slurry only), DCD-(pig slurry + nitrification inhibitor) and HQ-treated (pig slurry + urease inhibitor) Timothy sward

Days after treatment	Ammonia (g NH_3-N kg^{-1} d^{-1})			Nitrous Oxide (g N ha^{-1} day^{-1})		
	Control	DCD	HQ	Control	DCD	HQ
1d	0.54 ± 0.01[a]	0.45 ± 0.00[b]	0.36 ± 0.04[c]	1.91 ± 0.44[a]	1.45 ± 0.68[a]	2.02 ± 0.19[a]
2d	0.17 ± 0.02[a]	0.18 ± 0.01[a]	0.13 ± 0.01[a]	4.79 ± 0.39[a]	1.59 ± 0.17[c]	3.59 ± 0.13[b]
3d	0.10 ± 0.00[a]	0.14 ± 0.01[a]	0.14 ± 0.03[a]	2.85 ± 0.11[a]	1.10 ± 0.27[b]	2.21 ± 0.21[a]
4d	0.12 ± 0.01[a]	0.10 ± 0.02[a]	0.15 ± 0.03[a]	0.84 ± 0.27[a]	0.52 ± 0.02[a]	0.63 ± 0.14[a]
5d	0.08 ± 0.01[a]	0.06 ± 0.01[ab]	0.05 ± 0.00[b]	1.43 ± 0.19[a]	0.34 ± 0.00[b]	1.01 ± 0.25[a]
6d	0.10 ± 0.02[a]	0.05 ± 0.01[b]	0.04 ± 0.01[b]	2.73 ± 0.23[a]	0.82 ± 0.02[c]	1.47 ± 0.18[b]
7d	0.10 ± 0.02[a]	0.04 ± 0.00[b]	0.04 ± 0.00[b]	5.75 ± 1.60[a]	0.78 ± 0.08[c]	1.37 ± 0.25[b]
8d	0.06 ± 0.01[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	9.10 ± 0.24[a]	2.43 ± 0.17[c]	4.40 ± 0.56[b]
9d	0.08 ± 0.03[a]	0.07 ± 0.00[a]	0.06 ± 0.01[a]	17.71 ± 1.62[a]	6.02 ± 0.33[c]	10.67 ± 0.56[b]

Values are mean ± SE of three replicates. Different letters in horizontal row indicate significantly different at $p < 0.05$ according to the Duncan's multiple range test.

slurry application. In addition, in laboratory aerobic incubation, most of the NH_3 production from chicken, pig and cattle manure occurred within 7 days (Li and Li, 2014).

The patterns of the cumulative amount of NH_3 emission from 3 treatments are shown Fig. 1. The emission rate was relatively less in the plots treated with nitrification or urease inhibitor throughout the experimental period, resulting in a reduction of 16.3% and 25.1% in total NH_3 emission, respectively, in DCD- and HQ-treated plot as compared to control. Similar results have been observed in the previous study which reported that DCD treatment decreased the NH_3 volatilization by 11.5% in rice (Banerjee et al., 2002). Zhengping et al. (1991) reported ammonia volatilization was delayed by urease inhibitors such as HQ and phenyl phosphorodiamidate. Therefore, the present study confirms that urease inhibitor (HQ) delays or halts the breakdown of the urea in pig slurry, and this possibly results in an increase in N use efficiency of pig slurry by reducing the N loss through NH_3 volatilization (Laboski, 2006).

The N_2O emission was decreased in all treatments for

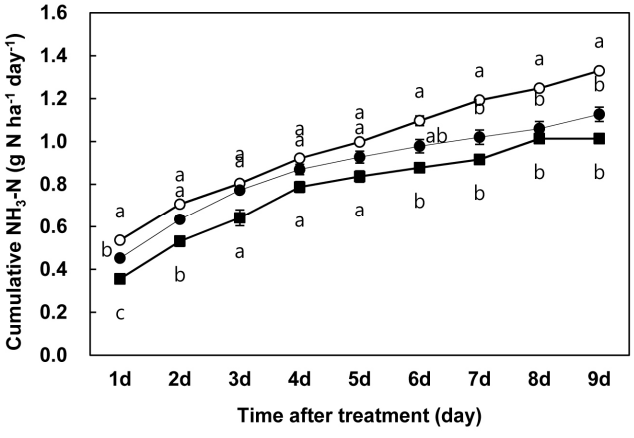

Fig. 1. Cumulative ammonia emission in the control (○, pig slurry only), DCD-(●, pig slurry + nitrification inhibitor) and HQ-treated (■, pig slurry + urease inhibitor) Timothy sward. Data are mean ± SE (n=3). Different letters indicate significantly different at $p < 0.05$ according to the Turkey test.

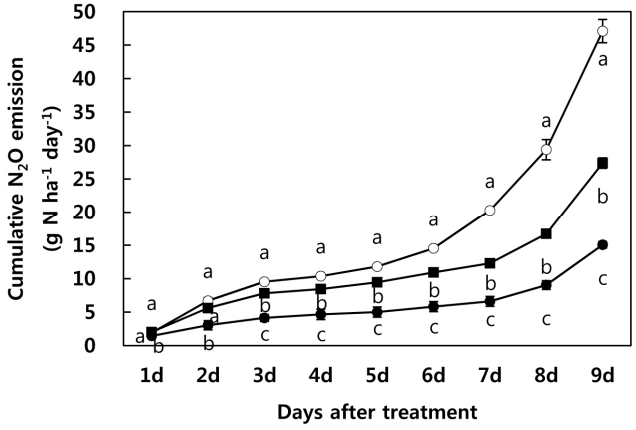

Fig. 2. Cumulative nitrous oxide emission in the control (○, pig slurry only), DCD-(●, pig slurry + nitrification inhibitor) and HQ-treated (■, pig slurry + urease inhibitor) Timothy sward. Data are mean ± SE (n=3). Different letters indicate significantly different at $p < 0.05$ according to the Turkey test.

4~5 days after an increased within 2 days, and then continuously increased up to the end of measurement (Table 3). Guo et al. (2012b) reported that most of the N_2O was emitted within 1 week of the fertilizer application and the flux peaks appeared within 3 days. In this study, the highest the daily fluctuation in N_2O emission was observed in the control, as shown the highest level on day 2 and day 9. In the control, the highest total N_2O emission (47.1 g N ha^{-1}), and followed by HQ- and DCD-treated plot (27.4 and 15.1 g N ha^{-1}, respectively). This study indicated that nitrification inhibitor more efficiently reduced N_2O emission than urease inhibitor.

These results are agreed with the finding of Boeckx et al. (2005), who reported that HQ and DCD treatment reduced the N_2O emission from urea by 11.4% and 22.3%, respectively. Under greenhouse condition, DCD plus optimum managed irrigation decreased total NH_3 volatilization by 20.6~41.5% and N_2O emission factors by 84.1~96.2% (Guo et al., 2012b). In the present study, we found that urease inhibitor more efficiently reduced NH_3 emission, while nitrification inhibitor decreased N_2O emission. These results confirmed that nitrification inhibitor has a positive role in reducing denitrification losses of N in accordance with previous reports (Aulakh et al., 2001; Banerjee et al., 2002). In addition, Zaman et al. (2008) reported that the mixture of urease and nitrification inhibitor reduced both NH_3 and N_2O emissions more effectively by preserving the N as NH_4^+ form.

In conclusion, the present study indicated that utilization of nitrification and urease inhibitors is a useful strategy to reduce the NH_3 and N_2O emission to the environment, but failed to explain a significant corresponding increase in N use efficiency. Thus, further study needs to determine the long-term fate of N, the mineralization dynamics, and N use efficiency for herbage yield in relation to the effectiveness of the inhibitors examined in this study.

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