

Acidification of pig slurry effects on ammonia and nitrous oxide emissions, nitrate leaching, and perennial ryegrass regrowth as estimated by ^{15}N -urea flux

Sang Hyun Park¹, Bok Rye Lee^{1,2}, Kwang Hwa Jung³, and Tae Hwan Kim^{1,*}

* Corresponding Author: Tae Hwan Kim
Tel: +82-62-530-2126, Fax: +82-62-530-2129,
E-mail: grassl@chonnam.ac.kr

¹ Department of Animal Science, Institute of Agricultural Science and Technology, College of Agriculture & Life Science, Chonnam National University, Gwangju 61186, Korea

² Biotechnology Research Institute, Chonnam National University, Gwangju 61186, Korea

³ National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea

ORCID

Sang Hyun Park
<https://orcid.org/0000-0002-8766-5077>
Bok Rye Lee
<https://orcid.org/0000-0002-1912-0390>
Kwang Hwa Jung
<https://orcid.org/0000-0001-9721-6323>
Tae Hwan Kim
<https://orcid.org/0000-0002-9202-1655>

Submitted Jul 25, 2017; Revised Oct 2, 2017;
Accepted Dec 2, 2017

Objective: The present study aimed to assess the nitrogen (N) use efficiency of acidified pig slurry for regrowth yield and its environmental impacts on perennial ryegrass swards.

Methods: The pH of digested pig slurry was adjusted to 5.0 or 7.0 by the addition of sulfuric acid and untreated as a control. The pig slurry urea of each treatment was labeled with ^{15}N urea and applied at a rate of 200 kg N/ha immediately after cutting. Soil and herbage samples were collected at 7, 14, and 56 d of regrowth. The flux of pig slurry-N to regrowth yield and soil N mineralization were analyzed, and N losses via NH_3 , N_2O emission and NO_3^- leaching were also estimated.

Results: The pH level of the applied slurry did not have a significant effect on herbage yield or N content of herbage at the end of regrowth, whereas the amount of N derived from pig slurry urea (NdfSU) was higher in both herbage and soils in pH-controlled plots. The NH_4^+ -N content and the amount of N derived from slurry urea into soil NH_4^+ fraction (NdfSU- NH_4^+) was significantly higher in the pH 5 plot, whereas NO_3^- and NdfSU- NO_3^- were lower than in control plots over the entire regrowth period. Nitrification of NH_4^+ -N was delayed in soil amended with acidified slurry. Compared to non-pH-controlled pig slurry (i.e. control plots), application of acidified slurry reduced NH_3 emissions by 78.1%, N_2O emissions by 78.9% and NO_3^- leaching by 17.81% over the course of the experiment.

Conclusion: Our results suggest that pig slurry acidification may represent an effective means of minimizing hazardous environmental impacts without depressing regrowth yield.

Keywords: Acidification; Gas Emission; *Lolium perenne*; Nitrate Leaching; Pig Slurry; Regrowth

INTRODUCTION

Manure emission from pig production accounts for 38.2% of the total quantity of manure issued from livestock production (46 million tons per year) in Korea. Since pig farms usually have little or no arable surface for forage production in Korea, utilization of pig slurry as an alternative organic fertilizer has become the most viable recycling option and composes more than 80% of all recycled animal manure [1]. However, mismanagement and application of nitrogen (N) to pig slurry can increase the risk of environmental pollution via nutrient losses to air, soil, water, and the biosphere [2,3]. Animal manure is a major contributor of odorous gases (ammonia [NH_3] and hydrogen sulfide [H_2S]) and greenhouse gases (GHGs; nitrous oxide [N_2O], methane [CH_4], and carbon dioxide [CO_2]) that strongly impact the environment. The largest pathway of N loss is ammonia volatilization, which is not only of public concern for its adverse impacts on health and the environment [4], but also represents a loss of N from organic fertilizers [5,6]. Thus, improving the use efficiency of manure-derived

N is essential [7]. Diet management (e.g. acidifying pig diets) [8], management during storage (e.g. covering of the slurry tank) [9], and slurry application methods (e.g. injection or banding) [6,10] have all been studied as ways for agricultural operations to minimize NH_3 volatilization of animal manures and improve their N use efficiency. However, most results have often limited to evaluate the whole slurry management including gases emission, soil mineralization, and plant N use efficiency.

The NH_3 in manure is generated from the hydrolysis of urinary urea, which is mediated by the microbial urease that is present in feces. The NH_3 volatilization occurs when ammonium (NH_4^+) is converted to a gas under alkaline conditions, accounting typically for 40% to 50% in housing (and pasture), 5% to 15% from storage, and 40% to 55% during land application [4]. Both aerobic nitrification, in which NH_4^+ is oxidized to NO_2^- and further NO_3^- , and anaerobic microbial denitrification, in which NO_3^- is reduced to gaseous nitrogen compounds, can generate N_2O , which is then released into the atmosphere [11,12]. The simple way to minimize NH_3 and additional N_2O emission is to create conditions that minimize the concentration of NH_3 relative to those of NH_4^+ , specifically by lowering slurry pH, a process that has been developed, widely tested and recently reviewed [3]. The positive effects of slurry acidification in reducing NH_3 and N_2O emissions [13] as well as on field crop yields [14-16] have been widely reported. However, acids are highly corrosive and hazardous to use, unless diluted with water. Furthermore, spreading of acidified slurry on soil will increase soil acidification, and chemical scorching of crops following application of slurries containing nitric acid have been reported. A recent review by Fanguerio et al [13] revealed that the effects of slurry acidification on the mineral fertilizer equivalent (MFE) varied significantly in several studies. As such, there is a need to improve our understanding of the implications of increasing the use efficiencies of manure N, and mitigating NH_3 and N_2O emissions and nitrate leaching, in order to achieve global targets.

The primary objective of this study was to determine the fate of pig slurry N to four major sinks: i) plant uptake, ii) soil N pool, iii) nitrogenous gases emission, and iv) nitrate leaching during regrowth of pasture sward. We emphasized the N flux derived from pig slurry urea because about 50% to 60% of N is excreted in the urine by pigs and cows, and 65% to 90% of N in urine is present as urea [17]. In this study, the turnover of pig slurry ^{15}N -urea was directly quantified under the assumption that rates of microbial mineralization, nitrifica-

tion, and denitrification were the same between labeled ^{15}N -urea and unlabeled ^{14}N -urea in pig slurry.

MATERIALS AND METHODS

Site, weather condition and experimental design

Field experiments were conducted on a permanent grass sward consisting mainly of perennial ryegrass (*Lolium perenne*), which was used for grass silage in the year preceding the treatment application. The experimental site was located in the uplands of southwestern South Korea (E126°90', S35°18') and featured a sandy loamy soil, the properties of which are presented in Table 1. The prevailing climate over the experimental period was humid temperate, with a mean temperature of 22.5°C and a total precipitation of 420 mm. Three treatments were applied: i) untreated pig slurry, as a control; ii) slurry adjusted to neutral pH 7.0; and iii) acidified pig slurry adjusted to pH 5.0. The experimental design consisted of a randomized complete block design with four replications; each treatment block measured 2.5 m×10 m. Adjacent blocks were separated by a 2-m margin and bordered with 45-cm metal retainers inserted 30-cm deep into the soil to prevent surface runoff and cross-plot contamination. Each treatment block contained 12 micro-plots (0.5 m×0.5 m) for monitoring the fate of ^{15}N -labeled pig slurry, four bottomless acrylic glass tubes (20-cm diameter and 30-cm length) for collecting gas samples, and four suction cups (P80, eco Tech, Bonn, Germany) for collecting leachate samples.

Pig slurry treatments and ^{15}N labeling

Slurry was collected from a local pig farm, where it was stored for approximately 1 week in a concrete tank at ambient temperatures, then placed into three different 400 L plastic containers. One container of the untreated slurry (control) was preserved at its original pH (7.9), whereas the slurries in the two other containers were acidified by slowly (to avoid foaming) adding 1.5 M H_2SO_4 until pH 7.0 and pH 5.0 were reached. The slurry urea fraction of the three treatments was labeled by thoroughly mixing ^{15}N urea immediately prior to field application. Highly enriched ^{15}N urea (98% ^{15}N atom excess) was used to minimize the portion of chemical urea for a target ^{15}N -urea enrichment of approximately 5.0 atom %. Subsamples of the applied slurry were analyzed at the time of application (Table 1). The pig slurry contained on average (kg/m^3) 1.63±0.11 total N, 0.197±0.012 NH_4^+ -N, 0.148±0.015 NO_3^- -N, 0.78±0.02 P, and 1.04±0.01 K with pH_{water} (1:5) of 7.9±0.02.

Treated pig slurry was applied at a rate of 200 kg N/ha. For

Table 1. Nitrogenous composition and ^{15}N atom % of the pig slurry used for experiment

N fraction	Total N (g N/kg)	Urea (g/kg)	NH_4^+ -N (mg N/kg)	NO_3^- -N (mg N/kg)	^{15}N atom excess (%)
Pig slurry	1.55±0.32	0.96±0.12	196.9±12	148.4±9.0	5.00122±0.00217

The values are mean±standard deviation of four replicates.

the application of pig slurry, 307 L of pig slurry, which contained 95.8 kg P/ha and 127 kg K/ha, was applied to the 25-m² plots after herbage was cut at 5-cm above ground level in the early spring (February 12).

Herbage, soil, gases, and leachate sampling

Soil and herbage samples were collected from the micro-plots bounded 0.5 m×0.5 m in a depth of 30 cm for soils) at 7, 14, and 56 d after cutting (e.g. application of pig slurry), respectively. Two earlier (7 and 14 d) and one final (56 d after regrowth) sampling times were also considered based on previous reports, which showed that endogenous N stored in roots or stubble is a main source of N for the earliest 2 weeks of regrowth, whereas exogenously applied N is important for later periods of regrowth [18,19]. For herbage sampling, herbage mass was cut manually at each sampling day, leaving stubble of approximately 5-cm at four randomly placed micro-plots in each treatment block. About 500 g of the harvested herbage tissue was sliced into 2-cm long segments, which were then lyophilized, ground, and stored in a vacuum desiccator for further analysis. Soil sampling was conducted in the same micro-plots where herbage samples were collected, with soil cores (0 to 30 cm depth) taken randomly using a 3-cm diameter tube auger. Soil samples were air-dried and finely milled to <0.15 mm.

Airtight acrylic glass chambers (20-cm diameter×30-cm depth) were inserted to a depth of 5 cm in the soil for gas sampling. To collect NH₃ emission, acid trap system method described by Ndegwa [20] was adopted, with minor modifications. Each chamber was connected (via a septum located in the lid of the chamber) to NH₃-N trapping bottles containing 150 mL of 0.2 mol/L H₂SO₄ (equivalent to 0.03 moles of acid). The other glass tube was connected to the vacuum system that created an airflow through the chambers at a constant rate of 1.5 L per min to exhaust the NH₃-scrubbed air. Each chamber was closed and clamped with attached with silicon sealing for 24 h. Sampling of N₂O gas from the acrylic glass chamber was conducted from each closed chamber using a syringe just before the sampling of NH₃ emission and then stored in 10 mL vacutainer tube prior to analysis. For the sampling NH₃ emission, the vacuum was connected to pull the sweep-air in the chamber into the acid trap bottle. A pair of four parallel ammonia trapping systems in each treatment block was run concurrently over 1 h to avoid extraneous effects during gas collection. Potential NH₃ emissions were determined daily for the first 15 d, then at 1 to 2 week intervals afterward. The gas samples were taken. Four ceramic suction cup samplers (P80, eco Tech, Germany) were randomly positioned at depths of 50 cm for collecting soil water leachate for NO₃⁻-N analysis in each treatment block, with soil water samples obtained weekly under a tension of -250 hPa and stored at -20°C.

Measurements and chemical analysis

The biomass harvested from each micro-plot was converted to kg/ha. This estimate was multiplied by the N concentration determined in the subsamples in order to calculate the N recovery in herbage (kg N/ha) at a given regrowth time. The total N content and ¹⁵N atom % of herbage, soil, and pig slurry samples were measured with a stable isotope ratio mass spectrometer (IRMS, IsoPrime, GV Instrument, Manchester, UK). For determination of soil NH₄⁺-N and NO₃⁻-N, about 20 g of finely sieved soil was extracted with 250 mL of 2 M KCl; extracts were then placed in distillation flasks and steam-distilled with MgO to ascertain the NH₄⁺ fraction. Samples in the flasks were then distilled again after addition of Devarda's alloy for NO₃⁻ determination. The N liberated from each distillation was collected in the form of H₂SO₄ to be a more stable form (NH₄)₂SO₄ for the evaporation procedure. These soluble fractions were acidified to pH 2 to 3 to avoid possible loss through ammonia volatilization, and then evaporated to dryness. The dried powder, in the form of (NH₄)₂SO₄, was analyzed for the determination of N content and ¹⁵N atom % excess of each N fraction. Concentrations of total N, NH₄⁺-N, and NO₃⁻-N in soil samples were converted to kg N/ha using the soil bulk density determined from the soil cores. The concentration of NH₃-N in the acid trap solution (e.g. ammonium sulfate) was colorimetrically determined with Nessler's ammonium color reagent after microdiffusion in a Conway dish [21] and expressed as the content of NH₃-N emitted per hectare. The N₂O concentration were analyzed by a gas chromatograph (7890A, Agilent technologies, Santa Clara, CA, USA) equipped with a thermal conductivity detector. Separation was achieved with a HP-Plot 5A column (30 m×0.53 mm×25 μm) using helium as the carrier gas, at a flow rate of 2 mL/min. The N₂O fluxes were calculated as described by Guo et al [22]. Cumulative NH₃ and N₂O emissions over the entire experimental period were calculated by summing all daily measurements and period estimations (number of day×mean flux between sampling dates). Concentrations of NO₃⁻-N in leachates were determined via ion chromatography (Dionex, DX-120, Sunnyvale, CA, USA), as previously described [23].

Calculation

The measured ¹⁵N atom % excess abundances in the total N, NH₄⁺, and NO₃⁻ fractions in herbage and soil samples were converted to relative specific activity (RSA, i.e. % of recently incorporated atoms relative to the total number of atoms in the sample) using equation (1):

$$\text{RSA} = \left(\begin{array}{l} \text{¹⁵N atom \% in herbage sample of labeled plot} \\ - \text{natural abundance of herbage} \\ / (\text{atom \% ¹⁵N-urea of applied pig slurry} \\ - \text{natural abundance of pig slurry}) \\ \times 100 \end{array} \right) \quad (\text{Equ. 1})$$

In which the natural abundance of herbage and pig slurry was adopted from the ^{15}N atom % of non- ^{15}N -fed herbage and that of unlabeled pig slurry. The amount of N derived from the urea of applied pig slurry (NdfsU) in herbage samples was calculated via equation (2):

$$\text{NdfsU} = \frac{(\text{RSA} \times \text{N content measured in a herbage sample})}{100} \quad (\text{Equ. 2})$$

Similar equations were applied for the total N, NH_4^+ , and NO_3^- fractions in the soil samples. The percentage of slurry urea-N recovered in the total N, NH_4^+ , and NO_3^- fractions in herbage and soil samples was calculated by the ratio between the NdfsU and the quantity of applied N. The percentage of loss therefore refers to the portion that was not recovered in herbage and soil.

Statistical analysis

Analysis of variation was conducted to assess the effects of acidification of pig slurry at each sampling time on herbage yield, N uptake, and fate of slurry urea-N. Significant differences among acidification treatments were tested using Duncan's multiple range test at the 5% level of probability using SAS 9.1.3 software [24].

RESULTS AND DISCUSSION

Soil pH, herbage dry matter, and N uptake

The initial soil pH of the experimental plots averaged 5.54 ± 0.41 . The pH in the non-pH-controlled (control) plots and those in the pH-controlled pig slurry plots set to pH 7 slightly

increased during the initial 7 to 14 d and then decreased to 6.31 and 5.90 at the end of the measurement period (56 d after pig slurry application), respectively, whereas pH remained within the 5.51 to 5.72 range in the plots to which acidified pig slurry was applied (pH 5) (Table 2). This implies that the increase in pH over the initial days following pig slurry application was offset by the acidifying effect of N sources and CO_2 formed from mineralization of organic material, thus, ensuring that soil pH would be relatively stable for the latter experimental period.

A previous study revealed that N fertilization with pig slurry significantly enhanced the annual dry matter (DM) yield by 135% in a four-cut regime of perennial ryegrass sward compared to the non-fertilized control [25]. Positive N use efficiencies of animal slurry have been widely reported for herbage yields of pasture [6] and field crop productivity [26]. However, slurry application to soil promotes NH_3 volatilization, which represents not only an N fertilizing value, but is also of concern for its adverse environmental impacts [7]. Although slurry acidification has been widely adopted to minimize NH_3 emission, plant growth responses to acidified slurry application to soil has been poorly documented. In the present study, at 56 d of regrowth, mean herbage yield over the three plots receiving pig slurry with or without pH control was 3,345 kg DM/ha, or 34.4% higher than that obtained from the first regrowth in the non-fertilized perennial ryegrass sward [25]. The impact of acidification on herbage regrowth was low throughout the regrowth period, as shown a significant reduction of herbage dry weight only in the pH 5 plots at 14 d (Table 3).

Consistent with regrowth yield, the total amount of N taken up by herbage regrowth for 56 d of regrowth was not signifi-

Table 2. Changes in soil pH in the plot applied with untreated pig slurry (control), acidified (pH 5) or mutualized slurry (pH 7) during regrowth of perennial ryegrass sward

	Day 0	Day 7	Day 14	Day 56
Control	5.71 ± 0.05^a	6.24 ± 0.04^a	6.31 ± 0.06^a	5.92 ± 0.04^a
pH 7	5.61 ± 0.04^a	6.21 ± 0.06^a	6.18 ± 0.06^a	5.99 ± 0.02^a
pH 5	5.72 ± 0.06^a	5.64 ± 0.03^b	5.91 ± 0.05^b	5.51 ± 0.06^b

The values are mean \pm standard deviation of four replicates.

^{a,b} Different letters in horizontal row indicate significantly different at $p < 0.05$ according to the Duncan's multiple range test.

Table 3. Total nitrogen content and the amount of N derived from slurry urea (NdfsU) in herbage as affected by the pH of pig slurry during regrowth of perennial ryegrass sward

Treatment	Day 7			Day 14			Day 56		
	DM (kg/ha)	Total N (kg N/ha)	NdfsU (kg N/ha)	DM (kg/ha)	Total N (kg N/ha)	NdfsU (kg N/ha)	DM (kg/ha)	Total N (kg N/ha)	NdfsU (kg N/ha)
Control	268 ± 6^a	11.7 ± 0.4^a	8.0 ± 0.7^a	942 ± 115^a	39.9 ± 3.7^a	15.2 ± 1.3^a	$3,382 \pm 90^a$	103 ± 9.8^a	40.8 ± 2.2^b
pH 7	258 ± 9^a	10.4 ± 0.4^b	8.8 ± 0.6^a	895 ± 57^a	37.1 ± 2.3^a	16.2 ± 1.1^{ab}	$3,434 \pm 140^a$	114 ± 8.8^a	54.8 ± 3.1^a
pH 5	254 ± 3^a	10.1 ± 0.1^b	8.6 ± 0.5^a	746 ± 29^a	32.2 ± 1.6^b	13.8 ± 0.8^b	$3,220 \pm 61^a$	110 ± 8.4^a	57.4 ± 3.7^a

DM, dry matter.

The values are mean \pm standard deviation of four replicates.

^{a,b} Different letters in horizontal row indicate significantly different at $p < 0.05$ according to the Duncan's multiple range test.

cantly different ($p > 0.05$), with the exception of the pH 5 plots at 14 d (Table 3). The amount of N derived from slurry urea (NdfsU) in herbage showed similar pattern with that of total herbage N up to 14 d (9.2% decrease in pH 5 plot compared to control). The mean NdfsU across the three treatments was 8.5 kg N/ha, a rather low value due to the high concentrations of inorganic N available in the soil. This may reflect a common physiological pattern characterized by low N uptake for the earlier regrowth period [18,19]. Nitrogen uptake was previously shown to gradually increase with progressing regrowth in perennial ryegrass swards to which cattle slurry was applied through three cycles of regrowth [6]. Likewise, it was demonstrated, via ^{15}N tracing, that N release from applied organic amendments and N uptake by Chinese cabbage were more distinct in latter growth periods than during the earliest 30 d [27]. The final NdfsU in herbage at 56 d was significantly higher in both the pH 7 (+34.3%) and pH 5 (+40.7%) plots than in the controls (Table 3), suggesting that acidified pig slurry may release plant available N more slowly.

Soil N dynamics

Common finding that the enhanced growth and N uptake by plants in organic manure N fertilized soil than in non-fertilized soils [5,6,25,27] could be attributed to the inorganic N released from organic N rather than the N pool size. Indeed, pH control of pig slurry did not affect the N pool size in the soil, as shown no significant differences among the three treatments throughout the entire regrowth period (Table 4). In the present study, a gradual decrease in ^{15}N atom % in the soils treated with pig slurry (data not shown) suggested that N released from the applied ^{15}N -urea in pig slurry diluted the soil inorganic N pool, and thus more N was available for plant uptake. Thus, the relatively higher NdfsU in herbage at 56 d in the pH-controlled

plots (pH 7 and pH 5, Table 2) may be due to the higher availability of N to plants resulting from the mineralization of the organic N in pig slurry, as evidenced by the higher levels of NdfsU in the soil (Table 4).

Concentrations of soil $\text{NH}_4^+\text{-N}$ in the pH 7 and pH 5 plots significantly increased, by 9.5% and 23.2%, respectively, at 7 d after pig slurry application, with the highest content found in the pH 5 plots by 56 d (Table 4). The amount of N derived from slurry urea into the soil NH_4^+ fraction (NdfsU- NH_4^+) was significantly higher in the pH 5 plot during the whole period of regrowth, while no significant difference was found between the control plots and pH 7 plot until 14 d. Levels of NdfsU- NH_4^+ at 56 d were higher in both pH 7 (+44.4%) and pH 5 (+61.1%) plots than in the controls (Table 4). Concentrations of both $\text{NH}_4^+\text{-N}$ and NdfsU- NH_4^+ decreased in the soil with progressing regrowth as nitrification occurred, but the decline was slower in the pH 5 plots. Our results were similar to those of previous laboratory incubation studies [13,28] and field experiments [29] that examined the effects of acidified pig and cattle slurry applied to different types of soils.

The initial concentration of $\text{NO}_3^-\text{-N}$ in the soil was 36.2 ± 2.1 kg/ha and then increased during 56 days of regrowth in all treatment. Acidification led to significant reductions in the soil $\text{NO}_3^-\text{-N}$ pool by 14 d. The amount of N derived from slurry urea into the soil NO_3^- fraction (NdfsU- NO_3^-) responded more quickly to pig slurry acidification, as shown by the significant decrease from 7 d (Table 3). Retention of higher levels of NH_4^+ and NdfsU- NH_4^+ in soils amended with acidified slurry, which are adversely lower NO_3^- and NdfsU- NO_3^- until 14 d, suggested that acidification may delay or inhibit nitrification of $\text{NH}_4^+\text{-N}$ in the soil, in accordance with previous findings [13,14,28]. In the present study, no significant differences in NO_3^- and NdfsU- NO_3^- were detected between control plots

Table 4. The content of nitrogen fraction and the amount of N derived from slurry urea in each fraction as affected by the pH of pig slurry during regrowth of perennial ryegrass sward

Treatment	Day 7		Day 14		Day 56	
	Nitrogen (kg N/ha)	NdfsU (kg N/ha)	Nitrogen (kg N/ha)	NdfsU (kg N/ha)	Nitrogen (kg N/ha)	NdfsU (kg N/ha)
Total N						
Control	1,958 \pm 60 ^a	129.2 \pm 7.8 ^a	1,894 \pm 145 ^a	1,16.8 \pm 8.8 ^a	1,755 \pm 124 ^a	80.2 \pm 5.6 ^b
pH 7	1,984 \pm 98 ^a	134.4 \pm 6.8 ^a	1,849 \pm 136 ^a	122.8 \pm 8.6 ^a	1,705 \pm 112 ^a	82.4 \pm 7.9 ^{ab}
pH 5	1,974 \pm 48 ^a	137.4 \pm 8.3 ^a	1,907 \pm 105 ^a	129.6 \pm 9.5 ^a	1,717 \pm 108 ^a	85.0 \pm 6.2 ^a
$\text{NH}_4^+\text{-N}$						
Control	158.1 \pm 9.5 ^c	54.8 \pm 3.7 ^b	100.6 \pm 9.2 ^b	26.4 \pm 0.9 ^b	26.6 \pm 2.1 ^b	1.8 \pm 0.1 ^c
pH 7	173.8 \pm 8.9 ^b	57.6 \pm 3.8 ^b	109.1 \pm 9.8 ^{ab}	27.6 \pm 1.2 ^{ab}	34.7 \pm 0.7 ^{ab}	2.6 \pm 0.1 ^{ab}
pH 5	194.8 \pm 9.3 ^a	67.2 \pm 4.1 ^a	124.7 \pm 8.4 ^a	30.2 \pm 1.4 ^a	38.8 \pm 1.7 ^a	2.9 \pm 0.2 ^a
$\text{NO}_3^-\text{-N}$						
Control	52.7 \pm 2.7 ^a	8.8 \pm 0.5 ^{ab}	100.4 \pm 3.2 ^a	34.2 \pm 0.8 ^a	158.9 \pm 9.6 ^a	59.2 \pm 3.9 ^a
pH 7	52.0 \pm 3.6 ^a	9.8 \pm 0.6 ^a	103.2 \pm 3.3 ^a	32.4 \pm 1.2 ^a	145.4 \pm 8.8 ^{ab}	53.8 \pm 2.9 ^{ab}
pH 5	46.3 \pm 1.6 ^a	8.2 \pm 0.4 ^b	85.1 \pm 2.2 ^b	25.0 \pm 0.8 ^b	133.6 \pm 7.2 ^b	51.2 \pm 2.1 ^b

The values are mean \pm standard deviation of four replicates.

^{a-c} Different letters in horizontal row indicate significantly different at $p < 0.05$ according to the Duncan's multiple range test.

and pH 7 plots. Given that the activity of nitrifying bacteria is strongly inhibited when soil pH is lower than 6 [30], application of pH 7 slurry had little effect on microbial nitrification rates because soil pH in these plots remained above 5.8.

Recovery of pig slurry urea-¹⁵N

At the end of regrowth (56 d after pig slurry application), the percentage slurry urea-¹⁵N recovered in herbage and soil averaged over all treatments was 25.5% and 41.3%, respectively; thus, 33.2% was unaccounted for and designated as loss (Table 5). The percentage of slurry urea-¹⁵N by herbage in the pH 5 plots was significantly lower at 14 d, after which it increased at the highest rate. This may reflect the increased N availability released from slurry urea relative to the non-acidified treatments (Table 3). The positive effects of slurry acidification on plant nutrient availability through estimations of the MFE have been previously reported [14,29], as higher levels of MFE in soils amended with acidified slurry was shown to significantly increase annual crop yields [14-16]; in addition, acidified slurry application led to higher herbage yields in permanent grasslands [31].

The percentage of slurry urea-¹⁵N recovery in total soil N gradually decreased, from 66.8% (mean of three treatments) at 7 d to 41.3% at 56 d, without significant influence of slurry pH treatment. However, the effect of acidification on soil inorganic N recovery varied with time after application, as shown by the higher soil NH₄⁺ recovery throughout the whole period while lower soil NO₃⁻ recovery for the earlier 14 d relative to non-acidified treatments. The percentage slurry urea-¹⁵N recovered in soil inorganic N (NH₄⁺ plus NO₃⁻) at 56 d was 25.5%, 28.2%, and 27.1%, respectively, in the control, pH 7, and pH 5 plots. Urea-N has often been reported to have lower N use efficiency for DM production per kg of applied N compared to other ammonium- and nitrate-based fertilizers [32,33]. In this study, the percentage of slurry urea-¹⁵N not recovered

in herbage and soil (designated as N loss) was within the range of 27.0% to 39.5% over the three treatments throughout the experimental period. Several ¹⁵N-urea tracing studies estimated N losses of 20% to 37% in wheat treated with ureases and/or nitrification inhibitors [32], 20% to 35% in maize [34], and 24% to 40% in Chinese cabbage [27] grown in soils amended with different levels of organic N. Taking the full N cycle in agroecosystems into consideration, N losses include gaseous N emissions in the form of ammonia (NH₃), nitric oxide (NO), nitrous oxide (N₂O), and molecular N (N₂), as well as nitrate leaching and immobilization to organic N by microorganisms. Our results clearly show that acidification significantly reduced N loss from pig slurry N applied to perennial ryegrass sward.

NH₃, N₂O emission, and NO₃⁻ leaching

NH₃ emissions following animal manure application to soil derive from the decomposition of N present in the feces and hydrolysis of urea, mainly in form of urine. Urea hydrolysis is mediated by the enzyme urease and produces ammonium and carbonate. In soils amended with animal manure, significant increases in the daily NH₃ flux have been shown to occur in various cropping systems [13,14,28] and laboratory incubation assays [35,36]. One primary goal of acidification of animal slurries is to reduce NH₃ emissions, because of its negative impacts on both the environment and animal health [14,37]. Gaseous NH₃ may be formed depending in the pH value of the slurry-soil system. Hydrolysis of urea can take place both under aerobic and anaerobic conditions, with optimum pH levels for urease activity between 6.5 and 7.0 [38]. In the present study, more than 50% of total NH₃ emissions occurred within the first 14 d after application to the soil in all treatments. Daily NH₃ emissions for this period decreased significantly in the pH 5 plots relative to the control and pH 7 plots (Figure 1A), confirming that the final pH of slurry is directly associated with NH₃ emissions following slurry appli-

Table 5. Recovery percentage of pig slurry urea-¹⁵N in herbage and soil, and calculated loss (not recover in herbage or soil) as affected by pH control of pig slurry during regrowth of perennial ryegrass sward

Date	Treatment	Herbage	Soil			Loss
			Total N	(NH ₄ ⁺ -N)	(NO ₃ ⁻ -N) ¹⁾	
Day 7	Control	4.0 ± 0.2 ^a	64.6 ± 4.1 ^a	(27.4 ± 1.4 ^b)	(4.4 ± 1.4 ^{ab})	31.4 ± 2.8 ^a
	pH 7	4.4 ± 0.2 ^a	67.2 ± 5.2 ^a	(28.8 ± 1.6 ^b)	(4.9 ± 1.4 ^a)	28.4 ± 3.6 ^a
	pH 5	4.3 ± 0.1 ^a	68.7 ± 4.7 ^a	(33.6 ± 1.9 ^a)	(4.1 ± 1.4 ^b)	27.0 ± 3.3 ^{ab}
Day 14	Control	7.6 ± 0.5 ^a	58.4 ± 4.2 ^a	(13.2 ± 0.7 ^b)	(17.1 ± 1.1 ^a)	34.0 ± 2.9 ^a
	pH 7	8.1 ± 0.5 ^{ab}	61.4 ± 3.9 ^a	(13.8 ± 0.9 ^{ab})	(16.2 ± 1.0 ^a)	30.5 ± 2.5 ^{ab}
	pH 5	6.9 ± 0.4 ^b	64.8 ± 4.7 ^a	(15.1 ± 1.0 ^a)	(12.5 ± 0.8 ^b)	28.3 ± 2.4 ^b
Day 56	Control	20.4 ± 1.5 ^b	40.1 ± 2.4 ^a	(0.9 ± 0.1 ^c)	(24.6 ± 1.6 ^b)	39.5 ± 2.9 ^a
	pH 7	27.4 ± 1.4 ^a	41.2 ± 1.9 ^a	(1.3 ± 0.1 ^{ab})	(26.9 ± 1.4 ^a)	31.4 ± 2.9 ^b
	pH 5	28.7 ± 1.7 ^a	42.5 ± 2.1 ^a	(1.5 ± 0.2 ^a)	(25.6 ± 1.2 ^{ab})	28.8 ± 2.9 ^b

¹⁾ Percentage of pig slurry urea-¹⁵N in soil inorganic N (NH₄⁺-N or NO₃⁻-N).

The values are mean ± standard deviation of four replicates.

^{a-c} Different letters in horizontal row indicate significantly different at p < 0.05 according to the Duncan's multiple range test.

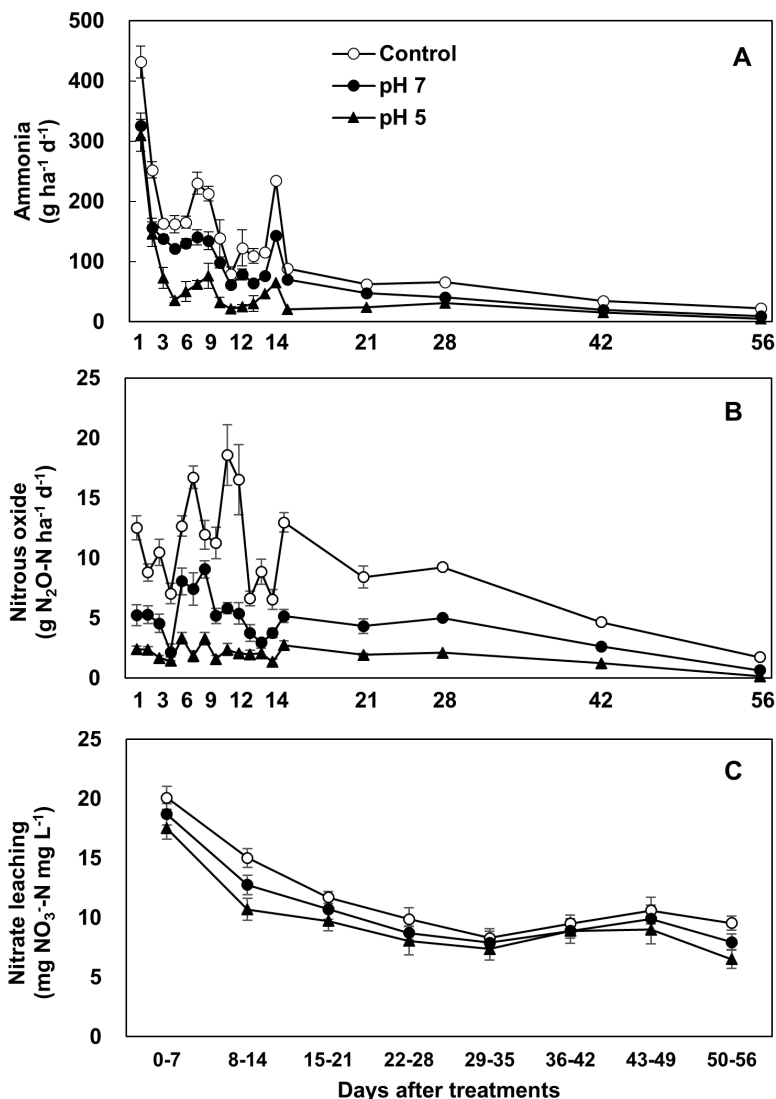


Figure 1. Daily emission of ammonia (A) and nitrous oxide (B), and weekly accumulative nitrate concentration in leachate (mg NO₃⁻-N/L, C) from the soil amended with untreated (control, ○), mutualized (pH 7, ●) and acidified pig slurry (pH 5, ▲) during regrowth of perennial ryegrass sward. The values are mean±standard deviation of four replicates.

cation to soil [13,29,39]. Cumulative NH₃ emissions throughout the period of measurement decreased by 60.9% in the pH 5 plots but by only 33.7% in the pH 7 plots compared to the control plots (Figure 2A). Previous work has shown that the efficacy of slurry acidification in reducing NH₃ emissions after field application varied within the range of 40% to 80% for pig slurry [40,41] and 15% to 80% for cattle slurry [28,42,43] applications, depending on the target pH and/or the types of acids used.

Nitrous oxide emissions from soils amended with animal slurry result from the nitrification and denitrification processes. The main determinants of N₂O emissions are thus the amount of NH₄⁺ available for nitrification to NO₃⁻ and the amount of soluble organic C available for denitrification. Consistent with NH₃ emissions, daily N₂O emissions were lower over the first

14 d in the pH 5 plots, and remained lower than in the control and pH 7 plots up to 56 d (Figure 1B). Cumulative N₂O emissions throughout the period of measurement decreased by 71.8% in the pH 5 plots but by only 48.7% in the pH 7 plots compared to the controls (Figure 2B). The results of the limited number of studies of the effects of slurry acidification on N₂O emissions relative to NH₃ emissions are inconsistent. Velthof and Oenema [44], for instance, reported that N₂O emissions increased following acidified slurry application compared with HNO₃ emissions in grasslands, which they attributed to the addition of NO₃⁻ rather than to the pH of slurry. In contrast, other studies have shown that slurry acidification reduced N₂O emissions during storage [45] and after soil application of acidified pig slurry [35]. In laboratory incubation assays, N₂O emissions from acidified cattle slurry were

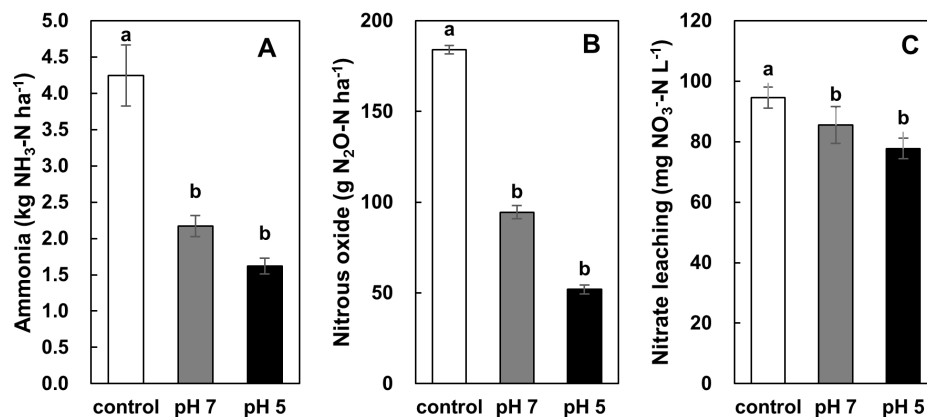


Figure 2. Total emission of ammonia (A) and nitrous oxide (B) and nitrate leaching (C), as estimated by cumulative amount for 56 days of regrowth. The values are mean±standard deviation of four replicates. Different letters in horizontal row indicate significantly different at $p < 0.05$ according to the Duncan's multiple range test.

significantly decreased relative to non-acidified slurry [13]. Fanguero et al [35] attributed a decrease in N₂O emissions following slurry application to a delay in or inhibition of the nitrification process. This interpretation is well consistent with our results, which show lower NO₃⁻ concentrations and NdfSU-NO₃⁻ in soils amended with acidified pig slurry for the first 14 d (Table 4). The lower N₂O emission from acidified slurry may be due to the reduced amount of organic C available for denitrification resulting from CO₂ losses during the acidification process [13].

The NH₄⁺ in mineral N and organic manure is rapidly nitrified in soil after application [5,46]. The surplus N, after converting to NO₃⁻, is prone to leaching when there is drainage. This process depends on the mineralization dynamics of soil N, as well as soil moisture and temperature [24,47]. We found that the weekly cumulative leaching of NO₃⁻ was lower in the pH 5 plots than in the other treatment plots, especially prior to 21 d after pig slurry application (Figure 1C). The lower rates of NO₃⁻ leaching from soils amended with acidified slurry for this earlier period may be due to the priming effect of acidification on delaying nitrification, as shown by the higher levels of NH₄⁺ and lower soil NO₃⁻ content (Table 4). Cumulative NO₃⁻ leaching declined throughout the experimental period, by 17.8% in the pH 5 plots and by 9.6% in the pH 7 plots, compared to the controls (Figure 2C). Thus, we conclude that only a small proportion of the total amount of N is lost via NO₃⁻ leaching following application of acidified pig slurry. However, the apparent effects of pig slurry acidification on the overall environmental impact have significance.

IMPLICATIONS

Appropriate acidification of animal slurry represents an efficient means of improving the use efficiency of pig slurry-N with significant reductions in NH₃ and N₂O emission, as well as in NO₃⁻ leaching. The acid in the present study was added

just before application of slurry in the field; however the slurry is often stored for a variable time before or after acidification. The stored time may influence the turnover of manure organic matter during storage and thereby also the N availability. Further studies would be useful to determine the mineralization dynamics and N loss of ammonia volatilization in relation to the storage time after acidification.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

This study was carried out with the support of "Investigation for the use efficiency of different organic manures in upland grassland (Project No. PJ010099)" Rural Development Administration, Republic of Korea.

REFERENCES

1. Ministry of Agriculture, Food and Rural Affairs. Outcome of animal waste generation and recycling (2006-2012). Sejong, Korea: Ministry of Agriculture, Food and Rural Affairs; 2013.
2. Schröder J. Revisiting the agronomic benefits of manure: a correct assessment and exploitation of its fertilizer values spares the environment. *Bioresour Technol* 2005;96:253-61.
3. Fanguero D, Maibritt H, Gioelli F. Acidification of animal slurry - a review. *J Environ Manage* 2015;149:46-56.
4. Bittman S, Mikkelsen R. Ammonia emissions from agricultural operations: livestock. *Better Crops* 2009;93:28-31.
5. Chantigny MH, Angers DA, Morvan T, Pomar C. Dynamics of pig slurry nitrogen in soil and plant as determined with ¹⁵N. *Soil Sci Soc Am J* 2004;68:637-43.

6. Hoekstra NJ, Lalor STJ, Richards KG, et al. Slurry $^{15}\text{NH}_4\text{-N}$ recovery in herbage and soil: effects of application method and timing. *Plant soil* 2010;330:357-68.
7. EEA. Ammonia (NH_3) emissions (APE 003) [Internet]. Copenhagen, Denmark: 2014 [cited 2014 Jan 29]. Available from: <http://www.eea.europa.eu/data-and-maps/indicators/eea-32-ammonia-nh3-emissions-1/assessment-4>
8. Eriksen J, Nørgaard JV, Poulsen HD, et al. Effects of acidifying pig diets on emissions of ammonia, methane, and sulfur from slurry during storage. *J Environ Qual* 2014;43:2086-95.
9. Balsari P, Dinuccio E, Gioelli F. A low cost solution for ammonia emission abatement from slurry storage. *Int Congr Ser* 2006; 1293:323-6.
10. Webb J, Pain B, Bittman S, Morgan J. The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response - a review. *Agric Ecosyst Environ* 2010; 137:39-46.
11. Gilsanz C, Bàez D, Misselbrook TH, Dhanoa MS, Càrdenas LM. Development of emission factors and efficiency of two nitrification inhibitors, DCD and DMPP. *Agric Ecosyst Environ* 2016;216:1-8.
12. Moeletsi ME, Tongwane MI. 2004 Methane and nitrous oxide emission from manure management in South Africa. *Animals* 2015;5:193-205.
13. Fangueiro D, Pereira J, Bichana A, et al. Effects of cattle-slurry treatment by acidification and separation on nitrogen dynamics and global warming potential after surface application to an acidic soil. *J Environ Manage* 2015;162:1-8.
14. Kai P, Pedersen P, Jensen JE, Hansen MN, Sommer SG. A whole-farm assessment of the efficacy of slurry acidification in reducing ammonia emission. *Eur J Agron* 2008;28:148-54.
15. Birkmose T, Vestergaard A. Acidification of slurry in barns, stores and during application: review of Danish research, trials and experience. In: Proceedings from the 15th RAMIRAN Conference; 2013 Jun 3-5: Versailles, France.
16. Petersen J, Lemming C, Rubæk GH. Side-band injection of acidified cattle slurry as starter P-fertilization for maize seedlings. In: Proceedings from the 15th RAMIRAN Conference; 2013 Jun 3-5: Versailles, France.
17. Gustafsson G, Jeppsson KH. Ammonia reduction from dairy houses. In: Geers R, Madec F, editors. *Livestock production and society*. The Netherlands: Wageningen Academic Publishers; 2006. p. 167-79.
18. Ourry A, Bourcaud J, Salette J. Partitioning and remobilization of nitrogen during regrowth of ryegrass subjected to nitrogen deficiency. *Crop Sci* 1990;30:1251-4.
19. Kim TH, Ourry A, Boucaud J, Lemaire G. Changes in source-sink relationship for nitrogen during regrowth of Lucerne (*Medicago sativa* L.) following removal of shoots. *Aust J Plant Physiol* 1991;18:593-602.
20. Ndegwa PM, Vaddella VK, Hristov AN, Joo HS. Measuring concentration of ammonia in ambient air or exhaust air steam using acid traps. *J Environ Qual* 2009;38:647-53.
21. Kim TH, Kim BH. Ammonia microdiffusion and colorimetric method for determining nitrogen in plant tissues. *J Korean Soc Grassl Sci* 1996;16:253-9.
22. Guo Y, Li B, Di H, Zhang L, Gao Z. Effects of dicyandiamide (DCD) on nitrate leaching, gaseous emissions of ammonia and nitrous oxide in a greenhouse vegetable production system in northern China. *Soil Sci Plant Nutr* 2012;58:647-58.
23. Kelly H, Annemie R, Hauke S, Dirk S, Winnie D. Determinants of the microbial community structure of eutrophic, hyporheic river sediments polluted with chlorinated aliphatic hydrocarbons. *FEMS Microbiol Ecol* 2014;87:715-32.
24. SAS Institute. *SAS User's Guide: Statistics* (fourth ed.). Cary, NC, USA: SAS Institute; 1982.
25. Park SH, Lee BR, Cho WM, Kim TH. Comparative nitrogen use efficiency of urea and pig slurry for regrowth yield and nutritive value in perennial ryegrass sward. *Asian-Australas J Anim Sci* 2017;30:514-22.
26. Burger M, Jackson LE. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. *Soil Biol Biochem* 2003;35:29-36.
27. Choi WJ, Ro HM, Chang SX. Recovery of fertilizer-derived inorganic- ^{15}N in a vegetable field soil as affected by application of an organic amendment. *Plant Soil* 2004;263:191-201.
28. Fangueiro D, Surgy S, Coutinho J, Vasconcelos E. Impact of cattle slurry acidification on carbon and nitrogen dynamics during storage and after soil incorporation. *J Plant Nutr Soil Sci* 2013;176:540-50.
29. Sørensen P, Eriksen J. Effect of slurry acidification with sulphuric acid combined with aeration on the turnover and plant availability of nitrogen. *Agric Ecosyst Environ* 2009;131:240-46.
30. Gandhapudi SK, Coyne MS, Angelo EMD, Matocha C. Potential nitrification in alum treated soil slurries amended with poultry manure. *Biores Technol* 2006;97:664-70.
31. Frost JP, Stevens RJ, Laughlin RJ. Effect of separation and acidification of cattle slurry on ammonia volatilization and on the efficiency of slurry nitrogen for herbage production. *J Agric Sci* 1990;115:49-56.
32. Xu X, Zhou L, Van Cleemput O, Wang Z. Fate of Urea- ^{15}N in a soil-wheat system as influenced by urease inhibitor hydroquinone and nitrification inhibitor dicyandiamide. *Plant Soil* 2000;220:261-70.
33. Zaman M, Zaman S, Adhinarayanan C, et al. Effects of urease and nitrification inhibitors on the efficient use of urea for pastoral systems. *Soil Sci Plant Nutr* 2013;59:649-59.
34. Choi WJ, Jin SA, Lee SM, RO HM, Yoo SH. Corn uptake and microbial immobilization of ^{15}N -labeled urea-N in soil as affected by composted pig manure. *Plant Soil* 2001;235:1-9.
35. Fangueiro D, Ribeiro H, Coutinho J, et al. Nitrogen mineralization and CO_2 and N_2O emissions in a sandy soil amended with original or acidified pig slurries or with the relative frac-

- tions. *Biol Fertil Soil* 2010;46:383-91.
36. Wang K, Huang D, Ying H, Luo H. Effects of acidification during storage on emissions of methane, ammonia, and hydrogen sulfide from digested pig slurry. *Biosyst Eng* 2014;122: 23-30.
 37. Erisman JW, Schaap M. The need for ammonia abatement with respect to secondary PM reductions in Europe. *Environ Pollut* 2004;129:159-63.
 38. Dai X, Karring H. A determination and comparison of urease activity in feces and fresh manure from pig and cattle in relation to ammonia production and pH changes. *PLoS One* 2014;9: e110402.
 39. Petersen SO, Andersen AJ, Eriksen J. Effects of cattle slurry acidification on ammonia and methane evolution during storage. *J Environ Qual* 2012;41:88-94.
 40. Stevens RJ, Laughlin RJ, Frost JP. Effect of acidification with sulphuric acid on the volatilization of ammonia from cow and pig slurries. *J Agric Sci* 1989;113:389-95.
 41. Nyord T, Liu D, Eriksen J, Adamsen APS. Effect of acidification and soil injection of animal slurry on ammonia and odour emission In: *Proceedings from the 15th RAMIRAN Conference*; 2013 Jun 3-5: Versailles, France.
 42. Bussink DW, Huijsmans JFM, Ketelaars JJMH. Ammonia volatilization from nitric-acid-treated cattle slurry. *Neth J Agric Sci* 1994;42:4.
 43. Pain BF, Misselbrook TH, Rees YJ. Effects of nitrification inhibitor and acid addition to cattle slurry on nitrogen losses and herbage. *Grass Forage Sci* 1994;49:209-15.
 44. Velthof G, Oenema O. Nitrous oxide from nitric-acid-treated cattle slurry applied to grassland under semi-controlled conditions. *Neth J Agric Sci* 1993;41:81-93.
 45. Berg W, Türk M, Hellebrand HJ. Effects of acidifying liquid cattle manure with nitric or lactic acid on gaseous emissions. *Workshop on Agricultural Air Quality*. Washington D.C USA: State of the Science, Department of Communication Service, North Carolina State University, Potomac, Maryland, USA; 2006. p. 492-8.
 46. Morvan T, Leterme P, Arsène GG, Mary B. Nitrogen transformations after the spreading of pig slurry on bare soil and ryegrass using ¹⁵N-labelled ammonium. *Eur J Agron* 1997;7: 181-8.
 47. Cookson WR, Rowarth JS, Cameron KC. The effect of autumn applied ¹⁵N-labelled fertilizer on nitrate leaching in a cultivated soil during winter. *Nutr Cycl Agroecosyst* 2000;56:99-107.