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Urease Inhibitor for Reducing Ammonia Emissions from an Open-Lot Beef Cattle Feedyard in the Texas High Plains

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Urease Inhibitor for Reducing Ammonia Emissions from an Open-Lot Beef Cattle Feedyard in the Texas High Plains

Abstract

Reducing ammonia (NH₃) emissions from animal feeding operations is important from the perspective of environmental policy and its impact on agriculture. In laboratory studies, urease inhibitors have been effective in reducing NH₃ emissions from beef cattle manure, however there has been little testing under field conditions. An experiment was conducted to evaluate the effectiveness of the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) for reducing NH₃ flux from the pen surface at a beef cattle feedyard. NBPT was applied to six feedyard pens for 42 d (April-June) at rates of 0, 1, 2, 4, 8, and 40 kg ha⁻¹. NH₃ flux was quantified using a flow-through flux chamber and chemiluminescence NH₃ analyzer. Nutrient concentrations in the manure were monitored over the 42 d period. There was high spatial variability in NH₃ emissions within individual pen, and NH₃ flux was lognormally distributed (geo. mean=1,618 $\frac{1}{4}$ g m⁻² min⁻¹; geo. std. dev. = 2.52 $\frac{1}{4}$ g m⁻² min⁻¹; range 512 to 14,993 $\frac{1}{4}$ g m⁻² min⁻¹). No statistical differences were observed in NH₃ flux among the different NBPT application rates due to the high spatial variability in NH₃ flux within pens. A post-experiment power analysis revealed that more than 500 individual flux chamber measurements would be needed per pen in order to detect a 10% difference in treatment means, an impractical task given the time and labor required for flux chamber measurements. At the completion of the six-week experiment, the 2 and 40 kg ha⁻¹ NBPT treatments retained 9% and 20% more total Kjeldahl nitrogen (TKN) in the manure than the control, respectively. In future research evaluating the long-term effectiveness of urease inhibitors for reducing NH₃ emissions from open-lot beef cattle feeding operations, we recommend that micrometeorological methods would be more appropriate than small flux.

Keywords

Air quality, Ammonia, Cattle, Feedyard, Manure, Thiophosphoric triamide, Urease, Nitrogen, Soil

Disciplines

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UREASE INHIBITOR FOR REDUCING AMMONIA EMISSIONS FROM AN OPEN-LOT BEEF CATTLE FEEDYARD IN THE TEXAS HIGH PLAINS

D. B. Parker, M. B. Rhoades, B. H. Baek, J. A. Koziel, H. M. Waldrip, R. W. Todd

ABSTRACT. Reducing ammonia (NH_3) emissions from animal feeding operations is important from the perspective of environmental policy and its impact on agriculture. In laboratory studies, urease inhibitors have been effective in reducing NH_3 emissions from beef cattle manure, however there has been little testing under field conditions. An experiment was conducted to evaluate the effectiveness of the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) for reducing NH_3 flux from the pen surface at a beef cattle feedyard. NBPT was applied to six feedyard pens for 42 d (April–June) at rates of 0, 1, 2, 4, 8, and 40 kg ha^{-1} . NH_3 flux was quantified using a flow-through flux chamber and chemiluminescence NH_3 analyzer. Nutrient concentrations in the manure were monitored over the 42 d period. There was high spatial variability in NH_3 emissions within individual pen, and NH_3 flux was lognormally distributed (geo. mean = 1,618 $\mu\text{g m}^{-2} \text{min}^{-1}$; geo. std. dev. = 2.52 $\mu\text{g m}^{-2} \text{min}^{-1}$; range 512 to 14,993 $\mu\text{g m}^{-2} \text{min}^{-1}$). No statistical differences were observed in NH_3 flux among the different NBPT application rates due to the high spatial variability in NH_3 flux within pens. A post-experiment power analysis revealed that more than 500 individual flux chamber measurements would be needed per pen in order to detect a 10% difference in treatment means, an impractical task given the time and labor required for flux chamber measurements. At the completion of the six-week experiment, the 2 and 40 kg ha^{-1} NBPT treatments retained 9% and 20% more total Kjeldahl nitrogen (TKN) in the manure than the control, respectively. In future research evaluating the long-term effectiveness of urease inhibitors for reducing NH_3 emissions from open-lot beef cattle feeding operations, we recommend that micrometeorological methods would be more appropriate than small flux chambers.

Keywords. Air quality, Ammonia, Cattle, Feedyard, Manure, Thiophosphoric triamide, Urease, Nitrogen, Soil.

Beef cattle producers face many challenges as the result of increased public concerns regarding effects of agricultural practices on the environment. In the Southern High Plains area, which includes parts of Texas, Kansas, Oklahoma, Colorado, and New Mexico, about 7 million beef cattle are produced annually in open-lot feedyards (TCFA, 2015). Cattle consume nitrogen (N) in the form of crude protein (CP), and convert it into body tissue (e.g., meat), milk, and byproduct waste (urine and feces). In the Southern High Plains, beef cattle excrete

more than 31.5 million metric tons of wet manure annually which contains about 88% water and 0.6% N (ASABE Standards, 2005). Nutrients such as N and phosphorus (P) accumulate on the pen surface during the finishing period (approximately 185 d). Considerable amounts of N can be volatilized from manure on the feedlot surface during this time. Scientists have estimated that 40% to 60% of the N that is fed to animals is ultimately lost as ammonia gas (NH_3) from the feedyard surface (Bierman et al., 1999; Rhoades et al., 2010; Todd et al., 2011). This loss of N should be minimized because it is a valuable resource that would otherwise be used as fertilizer on cropped fields and pasture. Mitigation of N emissions to the atmosphere as NH_3 , volatile amines and N_2O (a greenhouse gas) is part of a wider goal of reducing odor, volatile organic compounds, hydrogen sulfide, particulate matter (PM), and greenhouse gases from livestock production. Comprehensive control of emissions for all important constituents is not always feasible, and at times, is associated with tradeoffs. Relatively large gaps in field performance data on mitigation technologies and their effectiveness in animal production systems (particularly in beef cattle production) were identified in a review of nearly 270 papers (Koziel et al., 2015; Maurer et al., 2016). Thus, there is still a need for field-proven mitigation technologies for gaseous and PM emissions.

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A number of NH_3 emission control measures have been identified for animal feeding operations, and these are generally classified as either pre-excretion or post-excretion strategies (Parker et al., 2011). Pre-excretion strategies are measures that can be implemented before the manure is excreted from the animal. Modification of animal diet is the main pre-excretion strategy. Scientists have shown that overall NH_3 emissions can be reduced by reducing dietary CP concentrations (Cole et al., 2005; Todd et al., 2006; Vasconcelos et al., 2009). However, decreasing CP to less than 11.5% of dry matter (DM) intake has been shown to adversely affect animal performance (Cole et al., 2006). Phase-feeding, where CP was decreased from 13.0% to 11.5% of DM intake to align with nutrient requirements of finishing cattle at open-lot beef cattle feedyards reduced annual NH_3 flux by 28% (Todd et al., 2006).

Post-excretion strategies include various manure management techniques, such as chemical amendments and additives that are applied to the manure after it has been excreted. A number of manure additives have been studied including acids, calcium sulfate, aluminum sulfate (alum), calcium chloride, and aluminum chloride (Stevens et al., 1989; Moore et al., 1995; et al., 1999; Shi et al., 2001; Pereira et al., 2013).

One of the most promising methods for reducing NH_3 gas production is to slow or stop the conversion of urea $\text{CO}(\text{NH}_2)_2$ to ammonium (NH_4^+). Compounds that inhibit the enzymatic breakdown of nitrogenous compounds present in feces and urine, such as urease inhibitors, have been shown to reduce NH_3 production. Urease inhibitors work by slowing the conversion of urinary urea to NH_4^+ and thereby decreasing NH_3 production (VanderZaag et al., 2011; Montes et al., 2013). The most common commercially-available urease inhibitors are N-(n-butyl) thiophosphoric triamide (NBPT) and cyclohexylphosphoric triamide (CHPT).

Urease inhibitors have been shown to be useful for reducing N losses, reducing NH_3 emissions, and increasing crop yields in numerous commercially-fertilized field crop studies (Subbarao et al., 2006; Soares et al., 2012; Saggar et al., 2013; Abalos et al., 2014). Sanz-Cobena et al. (2011) reported 77% to 88% reduction in soil-derived NH_3 emissions when NBPT was used with surface applied urea.

Urease inhibitors have been used to reduce NH_3 emissions from manure in numerous laboratory-scale studies (Shi et al., 2001; Pereira et al., 2013; Hagenkamp-Korth et al., 2015a). Shi et al. (2001) reported 64% to 66% reduction in NH_3 emissions for manure treated with 1 to 2 kg ha^{-1} NBPT, while Parker et al. (2005) reported 49% to 69% reductions at the same treatment rates. At an NBPT application rate of 5 kg ha^{-1} , there were 67% to 74% reductions in NH_3 emissions (Parker et al., 2011).

Urease inhibitors have also been used successfully in a number of field studies of land-applied manure and effluent. In New Zealand, Li et al. (2014a, b) showed a 27% to 58% reduction in NH_3 emissions when NBPT was used during land application of dairy effluent and manure. In Germany, Hagenkamp-Korth et al. (2015b) evaluated application of a urease inhibitor to the floors of dairy confinement housing systems, and reported an 80% reduction in average urease activity. While NH_3 emissions were not directly measured,

the authors inferred that increased urea accumulation in the manure indicated a potential reduction in NH_3 emissions.

Varel et al. (1999) evaluated CHPT and NBPT applied to open-lot beef cattle feedyard pens in Nebraska, and reported short-term increases in urea accumulation at the manure surface. NH_3 emissions were not directly measured, but the authors inferred from the urea accumulation that urease inhibitors could be used to control NH_3 emissions from the pen surface.

To date, the effectiveness of NBPT for reducing NH_3 emissions from manure has been demonstrated in laboratory experiments. However, there has been little research on the effectiveness or practicality of these products in reducing NH_3 emissions from manure surfaces at animal feeding operations under field conditions, where temporal and spatial variability is known to be greater than in laboratory conditions (Sherlock and Goh, 1984; Haynes and Williams, 1992; Baek et al., 2003; Woodbury et al., 2005; Cole et al., 2007; Gilley et al., 2008). The objectives of this research were to 1) quantify the spatial variability in NH_3 flux from the beef cattle pen surface, and 2) evaluate the effectiveness of NBPT application for reducing NH_3 emissions and increasing manure N at a beef cattle feedyard under field conditions. In addition to these objectives, the suitability of using flux chambers for comparing treatment emissions on a field-scale was evaluated.

MATERIALS AND METHODS

EXPERIMENTAL LAYOUT

The research was conducted at the West Texas A&M University Research Feedyard near Canyon, Texas. The NBPT was applied over a 42 d study period (April-June) to six pens (6 × 26 m) containing 10 beef cattle each (fig. 1). The 60 steers (avg. live weight = 485 kg) were randomly assigned to each pen. The cattle were placed in the pens 20 days prior to the first NBPT application.

NBPT was applied to pens A, B, and C on d 0 (DOY=117, late April), then every seven days for 42 d (DOY=159, early June) at treatment rates of 0, 1, and 2 kg ha^{-1} (table 1). Pens D, E, and F received NBPT at rates of 0, 1, and 2 kg ha^{-1} on d 0 and d 7. On d 14, pen E and pen F received a one-time application at rates of 4 and 8 kg ha^{-1} NBPT, respectively. Pen F received no further NBPT application after the d 14 treatment. Pen E received a final one-time application at a rate of 40 kg ha^{-1} NBPT on d 18.

The NBPT was mixed with water, then immediately applied to the feedyard pen surface using a tractor-mounted commercial weed sprayer equipped with nine nozzles spaced 0.5 m apart (Wylie Mfg. Co, Petersburg, Tex.). The cattle were removed from the pens for the short time required to apply the NBPT. The width of the sprayer allowed for a single spray pass to cover the entire pen surface to within 0.7 m of the fence lines (fig. 2).

AMMONIA FLUX MEASUREMENT

For the 0, 1, and 2 kg ha^{-1} treatments, NH_3 flux was measured on Pens A, B, and C on d 10, 11, 14 (prior to the 14 d NBPT application), and 38. NH_3 flux was measured on Pens

Figure 1. Layout of the feedyard pens and treatment assignments for the N-(n-butyl) thiophosphoric triamide (NBPT) study.

Table 1. Rates and timing of N-(n-butyl) thiophosphoric triamide (NBPT) application to the feedyard pens.

Day	Action
0	Applied NBPT to Pens B, C at rates of 2 and 1 kg ha ⁻¹ , respectively
	Applied NBPT to Pens E, F at rates of 1 and 2 kg ha ⁻¹ , respectively
7	Applied NBPT to Pens B, C at rates of 2 and 1 kg ha ⁻¹ , respectively
	Applied NBPT to Pens E, F at rates of 1 and 2 kg ha ⁻¹ , respectively
14	Applied NBPT to Pens B, C at rates of 2 and 1 kg ha ⁻¹ , respectively
	Applied NBPT to Pens E, F at rates of 4 and 8 kg ha ⁻¹ , respectively
18	Applied NBPT to Pen E at rate of 40 kg ha ⁻¹
21	Applied NBPT to Pens B, C at rates of 2 and 1 kg ha ⁻¹ , respectively
28	Applied NBPT to Pens B, C at rates of 2 and 1 kg ha ⁻¹ , respectively
35	Applied NBPT to Pens B, C at rates of 2 and 1 kg ha ⁻¹ , respectively
42	End of Experiment
0-42	Pens A and D served as control pens (0 kg ha ⁻¹)

D, E, and F on d 15, 16, 17, and 18, which corresponded to 1, 2, 3, and 4 d after NBPT application of the 4 and 8 kg ha⁻¹ treatments. NH₃ flux was measured on Pens D and E on d 21, 22, and 23, which corresponded to 3, 4, and 6 d after NBPT application of the 40 kg ha⁻¹ treatment.

A dynamic flow-through chamber was used to measure NH₃ flux from the pen surface. Six flux measurements were

measured in each pen daily. The six sampling locations were systematically selected by dividing the pen into six blocks, on a grid of three rows (8.7 m) and two columns (3.0 m). The flux chamber was placed in the center of each of the six grid blocks. The flux measurements were conducted with the cattle in the pen. Technicians guarded the flux chamber during the measurements to keep cattle from disturbing the instruments.

The flux chamber was constructed of translucent LexanTM with a 265 mm inside diameter and 472 mm height (26.0 L volume) (fig. 3) (Baek et al., 2003; Koziel et al., 2004). Compressed zero-grade sweep air was directed into the chamber at 24 L min⁻¹ using 6 mm polytetrafluoroethylene (PTFE, Teflon[®]) tubing. At this sweep air flow rate, the exchange rate was 0.92 exchange volumes min⁻¹. Sweep air flow rate was controlled by a mass flow controller (Aalborg, Orangeburg, NY). A stirring paddle operating at 50 rpm provided additional mixing. A small fraction of the total exhaust flow (1.0 L min⁻¹) was directed to the continuous chemiluminescence NH₃ analyzer (Thermo Scientific, Franklin,

Figure 2. The urease inhibitor (NBPT) was mixed with water, then applied to the feedyard pen surface using a commercial weed sprayer.

Figure 3. A portable dynamic flow-through chamber was used for measuring ammonia flux from the beef feedyard pen surface.

Mass.) located in the lab trailer. The NH₃ analyzer was calibrated using UHP-grade air, certified standard span NH₃ gas in air (50 and 10 ppmv) (Matheson TriGas, Kerrville, Tex.).

The instrument shelter was a modified 1.5 × 2.1 m box trailer with air-conditioning unit. A data acquisition system (FieldPoint™ and LabVIEW™, National Instruments, Austin, Tex.) was used to collect all measurements.

MANURE SAMPLES

Six samples of loose, unconsolidated surface manure were collected from Pens A, B, C, and E on d 0, 28, and 42. The six sampling locations were systematically selected by dividing the pen into a grid of three rows (8.7 m) and two columns (3.0 m). Each manure sample was collected by scraping the surface manure from an area near the center of the grid into a pile, and a representative composite sample was placed into a polyethylene bag and frozen for later analyses. At the completion of the experiment, manure samples were analyzed for total Kjeldahl nitrogen (TKN), NH₄-N and nitrate+nitrite (NO_x-N) by Servi-Tech Laboratories, Dodge City, Kansas. Total N was calculated by summing the TKN and NO_x-N values.

EVALUATION OF SPATIAL VARIABILITY IN NH₃ FLUX

The spatial variability in NH₃ emissions was evaluated by sampling at 27 individual locations within Pen D on d 18. The sampling locations were systematically selected by dividing the pen into 27 blocks, on a grid of nine rows (2.9 m) and three columns (2.0 m). The flux chamber was placed in the center of each of the 27 grid blocks. Immediately following measurement of NH₃ emissions, individual manure samples were collected from the footprint of the flux chamber and analyzed for TKN, organic N, NH₄-N, NO_x-N, moisture content and volatile solids.

STATISTICAL ANALYSIS – NORMALITY

The NH₃ flux data and the manure N data were tested for normality using the Shapiro-Wilk test within SAS 9.4 (SAS Institute, Cary, N.C.). The raw NH₃ flux data was not normally distributed ($P < 0.0001$), however, log-transformed NH₃ flux data was normal ($P = 0.78$), indicating that the NH₃ flux data was lognormally distributed. Therefore, the log-transformed NH₃ flux data were used in the subsequent statistical analysis. The manure total N and NH₄⁺ data were both normally distributed ($P > 0.6$); therefore, the untransformed manure N data were used in the subsequent statistical analysis.

STATISTICAL ANALYSIS – COMPARISON OF MEANS

The NH₃ flux data and the manure N data were both analyzed as a completely randomized design with repeated measures over time using the SAS MIXED Procedure. Fixed effects were treatment, location, time, and the treatment × time interaction. Location × treatment was the subject of the repeated measures, and a variety of covariance structures were evaluated. The covariance structure resulting in the smallest Akaike and Schwarz Bayesian criteria, which was the autoregressive heterogeneous ARH(1) structure, was used for the statistical analysis (SAS, 2005). When significant ($P \leq 0.05$) treatment × time interactions were detected

using single-degree-of-freedom F-tests, pairwise comparisons of the simple-effects means were conducted within the PDIF option of SAS.

STATISTICAL ANALYSIS – PRECISION, POWER, AND SAMPLE SIZE

Based on the results of the spatial variability study, post-hoc precision and power analyses were conducted to determine the number of individual flux chamber measurements required to be within a predetermined fraction (D) of the true geometric mean. The precision analyses was conducted using the methods of Hale (1972) and Velthof et al. (1996). The method of Hale (1972), which was derived for use with lognormally distributed data, takes the form in equation 1:

$$n = \frac{Z^2 S^2 N}{N \ln^2(D+1) + Z^2 S^2} \quad (1)$$

where n = sample size; Z = normal deviate corresponding to the upper percentage point for a specified level of confidence ($Z = 1.96$ for 95% level); S = standard deviation of the logarithms of the original observations; N = population size; D = degree of precision, expressed as a fraction, by which the observed geometric mean can differ from the true geometric mean. For the case of a feedyard pen of known width and length, N is equal to the number of individual flux chamber measurements that can be conducted on the pen surface. For our 6 × 26 m pens and the flux chamber diameter of 265 mm, approximately 2,156 individual flux chamber measurements could be theoretically conducted on a 22 × 98 grid of the pen surface if the flux chamber areas were not overlapped.

The method proposed by Velthof et al. (1996), which was used to quantify spatial variability of lognormally distributed N₂O flux in pastures, takes the form in equation 2:

$$n = \frac{t^2 S^2}{D^2} \quad (2)$$

where t = value of Student's t for a chosen probability (t varies with n); and n , S , and D are as described for equation 1.

Post-hoc power analyses were also performed using the SAS POWER procedure with the 'twosamplemeans' statement, where the hypothesis with lognormal data was defined as the ratio of the geometric means of the two treatments. The method was used to calculate the number of samples required per treatment for given values of power and geometric mean ratios.

RESULTS AND DISCUSSION

STATISTICAL COMPARISON OF AMMONIA FLUX

Since the treatment × time interactions were significant ($P \leq 0.05$) for NH₃ flux, only the treatment × time means were statistically compared. Because of the significant treatment × time interactions ($P \leq 0.05$), statistical comparisons between time periods were not appropriate and therefore were not conducted.

Overall, there was little evidence of statistical differences in geometric mean NH_3 flux values across the NBPT treatments (table 2). The only statistically significant differences was between the 0 and 1 kg ha^{-1} treatments on d 10, where the treated pen had a 34% lower geometric mean NH_3 flux than the untreated pen ($P=0.027$).

At the highest application rate of 40 kg ha^{-1} , the geometric mean NH_3 flux was between 4% and 23% lower for the treated pens as compared to the control pen. However, none of the geometric means were statistically different than the control, primarily because of the large variation in NH_3 flux within each pen. There was a marginal, but not statistically significant difference between 0 and 40 kg ha^{-1} of 17.6% on d 4 ($P=0.058$). Even at the highest application rate of 40 kg ha^{-1} , the NH_3 flux in Pen E ranged from 963 to 3,095 $\mu\text{g m}^{-2} \text{min}^{-1}$.

While geometric means are more appropriate for making statistical comparisons, arithmetic means are used for calculation of emission factors. The arithmetic mean NH_3 flux across the control pens (A and D) ranged from 474 to 2,258 $\mu\text{g m}^{-2} \text{min}^{-1}$, with an overall arithmetic mean NH_3 flux of 1,326 $\mu\text{g m}^{-2} \text{min}^{-1}$ (fig. 4). This was consistent with previous measurements of NH_3 flux from cattle pens using the same flux chamber method. Baek et al. (2003) and Koziel et al. (2004) reported mean summertime NH_3 flux of 1,670 and 1,680 $\mu\text{g m}^{-2} \text{min}^{-1}$ using the flux chamber method. For comparison, Baek et al. (2006) used micrometeorological methods and a chemiluminescence NH_3 analyzer and reported a summertime mean flux of 3,620 $\mu\text{g m}^{-2} \text{min}^{-1}$. Similarly, Rhoades et al. (2010) used micrometeorological methods and a chemiluminescence NH_3 analyzer, and reported a mean NH_3 flux of 5,160 $\mu\text{g m}^{-2} \text{min}^{-1}$ in May, while Todd et al. (2008) reported a mean NH_3 flux of 4,200 $\mu\text{g m}^{-2} \text{min}^{-1}$ during summer at commercial Texas feedyards. The arithmetic mean NH_3 flux values from our research, which were measured using a flux chamber, are considerably lower than those measured using the micrometeorological methods

Figure 4. Arithmetic mean ammonia (NH_3) flux ($\mu\text{g m}^{-2} \text{min}^{-1}$) for the control pens over the 42 d study period. The arithmetic mean NH_3 flux was 1,326 $\mu\text{g m}^{-2} \text{min}^{-1}$ (dashed line).

of Baek et al. (2006), Rhoades et al. (2010), and Todd et al. (2008). This underestimation of NH_3 emission values has been reported previously for flux chamber methods used on beef feedyard pen surfaces (Rhoades et al., 2005a; Baek et al., 2006; Cole et al., 2007; Parker et al., 2013) and is likely due to artificial conditions created within the chamber (Waldrip et al., 2015). Despite potential underestimation, flux chambers are still one of the primary methods available for comparison of treatments on pen surfaces. However, flux chamber data should only be used for treatment comparisons and not for estimation of NH_3 emission factors from beef cattle feedyard pens without applying appropriate correction factors (Parker et al., 2013).

MANURE NUTRIENT CONCENTRATIONS

Since the treatment $\times$ time interactions were significant ($P \leq 0.05$) for total N in the manure, only the treatment $\times$ time means were statistically compared. Mean total N concentrations are presented in table 3. Because of the significant treatment $\times$ time interactions ($P \leq 0.05$), comparisons between time periods were not statistically appropriate and therefore were not conducted.

At the start of the experiment (d 0), there were no significant differences in mean manure N concentrations among the 0, 1, 2, and 40 kg ha^{-1} treatments (table 3). At the completion of the six-week experiment (d 42), the 40 kg ha^{-1} treatment had a 20% higher mean total N concentration than the control ($P=0.0013$). While the 2 kg ha^{-1} retained 9% more total N than the control, the difference was not significant ($P=0.117$). There were no significant differences in manure N concentrations among the 0, 1, and 2 kg ha^{-1} NBPT treatments (table 3).

On d 28, the 0, 1, and 2 kg ha^{-1} treatments had similar mean N concentrations ($P \geq 0.16$). However, contrary to the d

Table 2. Least squares geometric mean NH_3 emission rates ($\mu\text{g m}^{-2} \text{min}^{-1}$) for the different NBPT application rates^[a].

Days after First Application of 1 or 2 kg ha^{-1} ^[b]	Pen A (control) 0 kg ha^{-1}	Pen C 1 kg ha^{-1}	Pen B 2 kg ha^{-1}
10	1261 a ^[c]	834 b	969 ab
11	683 a	776 a	885 a
14	1027 a	859 a	1036 a
38	1539 a	1969 a	1487 a
Days after Applying Single Application of 4 or 8 kg ha^{-1}	Pen D (control) 0 kg ha^{-1}	Pen E 4 kg ha^{-1}	Pen F 8 kg ha^{-1}
1	1630 a	1478 a	1527 a
2	1464 a	1788 a	1439 a
3	439 a	459 a	432 a
4	1036 a	705 a	802 a
Days after Applying Single Application of 40 kg ha^{-1}	Pen D (control) 0 kg ha^{-1}	Pen E 40 kg ha^{-1}	
3	1296 a	1000 a	
4	829 a	683 a	
6	1870 a	1801 a	

^[a] All raw NH_3 flux data were log-transformed prior to statistical analysis. Geometric means calculated as the inverse logarithm of the average of the logarithms of six individual flux measurements per pen.

^[b] 1 and 2 kg ha^{-1} treatments were applied on d 7, 14, 28, and 35.

^[c] Means in a row without a common letter are significantly different ($P < 0.05$).

Table 3. Mean total nitrogen (N) concentrations of manure samples at times of 0, 28, and 42 days.^[a]

Day	Pen A (control) ^[b] 0 kg ha^{-1}	Pen C 1 kg ha^{-1}	Pen B 2 kg ha^{-1}	Pen E 40 kg ha^{-1}
0	0.87 a	0.78 a	0.79 a	0.86 a
28	1.92 a	1.88 a	2.01 a	1.75 b
42	1.61 b	1.59 b	1.75 b	1.94 a

^[a] Each mean was calculated from six individual manure samples from each pen.

^[b] Means in a row without a common letter are significantly different ($P < 0.05$).

42 results, on d 28 the manure in the 40 kg ha⁻¹ treatment had 9% less total N than the control (P=0.013). This inconsistency makes it difficult to make definitive conclusions regarding manure N content, and further research will be required.

SPATIAL VARIABILITY IN NH₃ FLUX

For the 27 individual NH₃ flux measurements in Pen D (control) on d 18, there was no statistical difference in mean NH₃ flux across the nine rows within the pen (P=0.44), but there was a statistical difference in mean NH₃ flux across the three columns (P=0.009). Three of the four elevated NH₃ flux values were located near the feed bunk or water trough (fig. 5). Cattle spend a considerable amount of time near the feed bunk and water trough, thus it is reasonable that NH₃ flux would be higher in these locations. Gilley et al. (2008) reported similar findings in commercial-sized beef feedyard pens in Nebraska, where manure N concentration were 30% to 62% higher in the upper third of the pen near the feed bunk than in the middle and lower thirds of the pen.

The geometric mean NH₃ flux from the 27 locations was 1,618 µg m⁻² min⁻¹ with geometric standard deviation of 2.52 µg m⁻² min⁻¹ and range of 512 to 14,993 µg m⁻² min⁻¹ (fig. 5). The arithmetic mean was 2,726 µg m⁻² min⁻¹ and arithmetic standard deviation was 3,655 µg m⁻² min⁻¹.

Figure 5. Spatial variability of NH₃ flux (µg m⁻² min⁻¹) as measured on 27 individual locations across the control pen surface. Sample locations with visual occurrence of feces and/or urine are noted.

For comparison, the geometric mean NH₃ flux for the N=66 observations within the control pens across the experimental period was 1,104 µg m⁻² min⁻¹ with geometric standard deviation of 1.83 µg m⁻² min⁻¹ and range of 247 to 5,662 µg m⁻² min⁻¹. The arithmetic mean was 1,326 µg m⁻² min⁻¹ and arithmetic standard deviation was 925 µg m⁻² min⁻¹ for these 66 observations.

The four locations with elevated flux values ranged from 7,870 to 14,993 µg m⁻² min⁻¹, as compared to the arithmetic mean of the remaining 23 locations of 1,321 µg m⁻² min⁻¹. One of these spots had fresh feces scattered across the surface. When the surface layer of manure was removed for nutrient analysis sampling, all four of these spots had a moist subsurface, indicative of recent urine deposition. Cattle often excrete several liters of urine in a single event, which saturates both the surface and subsurface of a small portion of the pen. The surface moisture usually evaporates rapidly, and surface manure visually becomes similar to the surrounding dry areas. Mielke et al. (1974) and Cole et al. (2009) discussed how the manure pack in most beef feedyard pens is composed of three layers: a top loose-surface layer, a middle dry-pack layer, and a deeper wet-pack layer. The deeper wet-pack layer stays moist continually, whereas the moisture content of the top and middle layers changes with time due to precipitation, evaporation, and feces or urine deposition. In the current study, we learned that when the middle dry-pack layer was moist with urine, these urine spots continued to emit more NH₃ than the surrounding area for a day or longer, even when the loose surface manure appeared as dry as the surrounding dry areas that did not receive urine.

RELATIONSHIP BETWEEN NH₃ FLUX AND MANURE NITROGEN/MANURE MOISTURE CONTENT

The highest NH₃ flux values were associated with recent urination spots. However, H₂O content alone was a poor predictor of NH₃ flux (R²<0.001; fig. 6a), because of wet spots associated with either fresh feces or spillage from the water trough. The coefficient of determination was much higher when those locations associated with fresh feces or the water trough were removed (R²=0.79; fig. 6b).

There was a strong exponential relationship between NH₃ flux and manure NH₄⁺ concentration (R²=0.71; fig. 6c). Urea in fresh urine is quickly converted to NH₄⁺ by the urease enzyme which is present in the feces and underlying soil of the pen surface. Given the pH of the feedyard pen surface generally ranges between 7 and 8 (Cole et al., 2009), the NH₄⁺ is rapidly converted to NH₃ gas and escapes to the atmosphere. There was a very weak relationship between NH₃ flux and total manure N (R²=0.01; fig. 6d), an indication that NH₃ flux is more dependent on urine than feces deposition. Feces contains a high concentration of organic N, which requires mineralization to NH₄⁺ before emission as NH₃.

PRECISION, POWER, AND REQUIRED SAMPLE SIZE

The methods of Hale (1972) and Velthof et al. (1996) produced similar sample requirements. As shown in figure 7, Hale's equation suggests that 462 individual flux measurements would be required to be within 5% of the true geometric mean, while Velthof's equation suggests 561 flux

Figure 6. Graphs showing the relationship between NH_3 flux and (a) moisture content, (b) moisture content with those samples containing fresh feces removed, (c) $\text{NH}_4^+\text{-N}$ concentration, and (d) total nitrogen concentration.

measurements would be required. The number of required samples decreased with increasing degree of precision (D), ranging from 142 to 144 measurements at precision of 10% and 37 to 41 measurements at precision of 20%. If six individual flux measurements were conducted per pen, as was done in the present experiment, then the precision analysis demonstrates that we would only be 95% confident that the measured geometric mean was within about 60% of the true geometric mean.

Results of the power analyses are shown in figure 8. For a ratio of geometric means between the two treatments of

1.1, the number of samples required per treatment was 541, 687, and 920 for power of 0.7, 0.8, and 0.9, respectively. Based on the 2,156 available flux chamber sampling locations for the 6×26 m pen, this is equivalent to sampling between 25 and 43% of the pen surface area. For a geometric mean ratio of 1.5, the number of samples required per treatment was reduced to 31, 39, and 52 for power of 0.7, 0.8, and 0.9, respectively. The ratio of 1.1 is analogous to detecting differences in two geometric means with values of 1,000 and 909 $\mu\text{g m}^{-2} \text{min}^{-1}$ (approx. 10% reduction in geometric

Figure 7. Relationship between the required number of samples and the degree of precision of the estimated geometric mean NH_3 flux ($\alpha=0.05$).

Figure 8. Relationship between the required number of samples per treatment, and the ratio of geometric mean NH_3 flux of the two treatments, at power levels of 0.7, 0.8, and 0.9.

mean NH_3 flux) whereas the ratio of 1.5 is analogous to detecting differences in two geometric means with values of 1,000 and $667 \mu\text{g m}^{-2} \text{min}^{-1}$ (approx. 33% reduction in geometric mean NH_3 flux).

Given the time required to set up and collect each flux chamber measurement, it is apparent that the difference between treatments must be more than 30% for flux chambers to be useful for comparing NH_3 flux at the pen scale, and even then there must be 30 to 50 flux chamber measurements collected per pen. For this reason, future research on field-scale treatment comparisons to quantify NH_3 emissions from large open-lot beef cattle feedyard pens should focus on either larger footprint flux chambers (Rhoades et al., 2005b) or micrometeorological methods such as those used by Baek et al. (2006) and Todd et al. (2008, 2011).

CONCLUSIONS

The following conclusions were drawn from this research:

1. There was a large spatial variability in NH_3 flux within pens. The NH_3 flux data were lognormally distributed. Within a single pen, the geometric mean NH_3 flux from the 27 locations was $1,618 \mu\text{g m}^{-2} \text{min}^{-1}$ with geometric standard deviation of $2.52 \mu\text{g m}^{-2} \text{min}^{-1}$ and range of 512 to $14,993 \mu\text{g m}^{-2} \text{min}^{-1}$.
2. At the completion of the six-week experiment, the 2 and 40 kg ha^{-1} NBPT treatments retained 9% and 20% more TKN in the manure than the control, respectively, indicating a possible beneficial effect from the urease inhibitor. However, because of the large spatial variability within pens, there was little evidence of statistical differences in geometric mean NH_3 flux values across the NBPT treatments.
3. Based on the precision and power analyses for the lognormally distributed NH_3 flux data, about 140 individual flux measurements would be required per pen to be 95% confident of being within 10% of the true geometric mean. For treatment comparisons, more than 500 individual flux measurements would be required per pen to detect a 10% difference in geometric mean NH_3 flux. Thus, because of the large spatial variability, it appears that small footprint flux chambers such as used in this research are an impractical method for assessing treatment differences for NH_3 flux at the field-scale. Future research should focus on either chambers with a much larger footprint or on micrometeorological methods.

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