Dietary Crude Protein and Tannin Impact Dairy Manure Chemistry and Ammonia Emissions from Incubated Soils

J. M. Powell,* M. J. Aguerre, and M. A. Wattiaux

Excess crude protein (CP) in dairy cow diets is excreted mostly as urea nitrogen (N), which increases ammonia (NH₃) emissions from dairy farms and heights human health and environmental concerns. Feeding less CP and more tannin to dairy cows may enhance feed N use and milk production, abate NH₃ emissions, and conserve the fertilizer N value of manure. Lab-scale ventilated chambers were used to evaluate the impacts of CP and tannin feeding on slurry chemistry, NH₃ emissions, and soil inorganic N levels after slurry application to a sandy loam soil and a silt loam soil. Slurry from lactating Holstein dairy cows (Bos taurus) fed two levels of dietary CP (low CP [LCP], 155 g kg⁻¹; high CP [HCP], 168 g kg⁻¹) each fed at four levels of dietary tannin extract, a mixture from red quebracho (Schinopsis lorentzii) and chestnut (Castanea sativa) trees (0 tannin [0T]; low tannin [LT], 4.5 g kg⁻¹; medium tannin [MT], 9.0 g kg⁻¹; and high tannin [HT], 18.0 g kg⁻¹) were applied to soil-containing lab-scale chambers, and NH₃ emissions were measured 1, 3, 6, 12, 24, 36, and 48 h after slurry application. Emissions from the HCP slurry were 1.53 to 2.57 times greater (P < 0.05) than from the LCP slurry. At trial’s end (48 h), concentrations of inorganic N in soils were greater (P < 0.05) in HCP slurry–amended soils than in LCP slurry–amended soils. Emissions from HT slurry were 28 to 49% lower (P < 0.05) than emissions from 0T slurry, yet these differences did not affect soil inorganic N levels. Emissions from the sandy loam soil were 1.07 to 1.15 times greater (P < 0.05) than from silt loam soil, a result that decreased soil inorganic N in the sandy loam compared with the silt loam soil. Larger-scale and longer-term field trials are needed to ascertain the effectiveness of feeding tannin extracts to dairy cows in abating NH₃ loss from land-applied slurry and the impact of tannin-containing slurry on soil N cycles.

Human activities have increased the Earth’s reactive N pool, resulting in significant improvements in crop and animal production but also in ecosystem disruption and environmental degradation in some areas (Galloway et al., 2003, 2008). Animal agriculture is a significant source of reactive N release to the environment. Many N pools, including the N contained in feed and manure, organic and inorganic soil N, N fixed by legume crops, and inorganic fertilizer N, are subject to cycling through synthetic or degradation pathways in plants, animals, and soils. All biological systems (including livestock) are limited in their ability to incorporate N into products. For example, on a global basis, of the total fertilizer N applied to agricultural land, only 5 to 15% is incorporated into human food, with most of the remainder lost to the environment (Erisman et al., 2007).

Major pathways of agricultural N loss are ammonia (NH₃) volatilization, gaseous emission of nitrous oxide (N₂O), and the leaching of nitrate (NO₃⁻) through soil. Ammonia volatilization is an environmental concern because it is the primary gas that neutralizes acids in the atmosphere produced from the combustion of fossil fuels, a reaction that produces aerosols that are a component of atmospheric haze and implicated as a potential human health hazard. Recent research summaries highlight how the deposition of NH₃ can also adversely affect natural and seminatural ecosystems through direct foliar toxicity and damage, N enrichment, acidification, and susceptibility to secondary stress, such as disease and pests (Bobbink, 2010; Dise et al., 2010).

Nitrogen passes through a continuous cycle on dairy farms. On typical dairy farms, cows are fed conserved forages, grain, and protein and mineral supplements in barns, and manure is collected, stored, and applied to cropland. Of the total N consumed by a dairy cow fed a diet typical to the Midwestern United States, 25 to 35% is secreted in milk, and the remaining N is excreted approximately equally in feces and urine, yet this proportion can be highly influenced by the amount of CP fed (Olmos Colmenero and Broderick, 2006). Well balanced rations containing 164 g kg⁻¹ of dietary CP (26.4 g N kg⁻¹) maximize milk production and minimize urinary N excretion by dairy cows (Broderick, 2003, 2009). As consumption of feed N increases above this requirement, milk N secretion does not increase, slightly more N is excreted in feces,
and most excess N is excreted as urea N in urine (Broderick, 2003). Urease enzymes present in feces, plants, and soil rapidly convert urea to NH₄⁺, which is in equilibrium with ammonium (NH₄⁺). Ammoniacal-N (NH₄⁺ + NH₃) is strongly affected by pH, with higher pH values driving the equilibrium to the NH₃ gas form and thus loss to the atmosphere.

Excessive intake of feed N increases urea N excretions and NH₃ emissions from dairy manure and storage and after land application of manure (Rotz, 2004; Powell et al., 2008; Arriaga et al., 2010; Aguerre et al., 2010a). Ammonia N loss from dairy farms typically ranges from 20 to 55% of total manure N excreted (National Research Council, 2003; Pinder et al., 2003; Steinfield et al., 2006). It was estimated that NH₃ emissions from dairy farms contribute to annual N deposition rates from manure of 23 to 40 kg N ha⁻¹ in the Upper Midwest (Burtkard and James, 1999). Reductions in NH₃ emissions through diet manipulation, improved manure storage, and land application techniques would not only save feed costs and enhance the fertilizer N value of manure but would also reduce the environmental impacts of dairy production. Reductions in dietary CP on confinement dairy farms could reduce NH₃ N losses by 17 to 31% (13–34 kg ha⁻¹) and increase annual net return by 46 to 569 per cow without sacrificing milk production (Rotz et al., 1999).

A promising approach to enhance feed N utilization and to reduce urea N excretion and NH₃ loss from dairy farms is to decrease the concentration of CP and increase the concentration of tannin in cow diets (Misselbrook et al., 2005; Aguerre et al., 2010b, 2010c; Powell et al., 2011). Tannins extracted from quebracho and chestnut trees have been used as feed additives to improve feed utilization by ruminants (Hagerman et al., 1992; Dawson et al., 1999; Komolong et al., 2001; Benchaar et al., 2008) and to reduce urinary N excretion (Dawson et al., 1999; Komolong et al., 2001). Preliminary results of a recent study revealed that the addition of a mixture of quebracho and chestnut tannin extracts to the diets of dairy cows did not significantly affect animal performance (Aguerre et al., 2010b) but increased feed N use efficiency (more feed N secreted in milk) and decreased N excretion in urine (Aguerre et al., 2010c). The excreta collected from those studies was applied to lab-scale ventilated chambers with concrete floors to determine that tannins decreased NH₃ emissions by 30% at the low CP (LCP) diet and by 16% at the high CP (HCP) diet, and that the application of tannin directly to the barn floor inhibited urease activity and decreased ammonia emission by 20% (Powell et al., 2011). The same excreta and lab-scale chambers were used in the present study to determine the impacts of dietary CP and tannin extracts on NH₃ emission from a sandy loam soil and a silt loam soil after slurry application. An additional study objective was to determine dietary CP and tannin impacts on concentrations of inorganic N in soil after NH₃ emissions subsided.

Materials and Methods

Soils Description

Representative soil samples were taken with a stainless steel shovel from the surface (0–20 cm) horizons of a Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argudolls) and a Symco sandy loam (fine-loamy, mixed, mesic Aquollc Hapludalfs). The soils were reported to have received no manure for at least 3 yr (to reduce possible variability from previous manure applications). Soil samples were air dried, sieved to pass a 2-mm screen, and stored in plastic containers until use in emission trials. Average soil pH (1:1 water) and concentrations (g kg⁻¹) of sand, total C, and total N (SPALS, 2004) from composite soils samples were 7.4, 260, 35.5, and 2.2, respectively, for the Plano silt loam soil and 6.3, 730, 8.1, and 0.87, respectively, for the Symco sandy loam soil.

Cow Diets and Collection of Feces and Urine

Feces and urine were collected from eight lactating Holstein cows fed eight diets: four tannin extract levels: 0, 4.5, 9.0, and 18.0 g kg⁻¹ (corresponding to zero tannin [0T], low tannin [LT], medium tannin [MT], and high tannin [HT] diets, respectively), each fed at two dietary CP levels: 155 and 168 g kg⁻¹ (corresponding to LCP and HCP, respectively). The feeding trial (Aguerre et al., 2010b) was a split-plot within a 4×4 Latin Square design in which four cows were assigned to each of the four tannin levels within the LCP diet and four other cows were assigned to the four tannin levels within the HCP diet. The experimental diets fed to lactating dairy cows were total mixed rations containing, on a dry matter (DM) basis, approximately 21% alfalfa silage, 29% corn silage, and 50% concentrate (comprised of ground corn, solvent soybean meal, expeller soybean meal, roasted soybeans, soy hulls, and cottonseed). The tannin extract (Byprow; Silvateam, Indunor S.A., Argentina) was comprised (by weight) of approximately one third chestnut tannin extract and two thirds quebracho tannin extract having the following chemical properties (polyvinylpyrrolidone powder method; Tempel, 1982): tannin concentration of 792 g kg⁻¹ and pH of 3.72. Additional information on the experimental design of the lactation trial, diets, and animal responses to diets can be found in Aguerre et al. (2010b). Feces and urine for the present trial were collected quantitatively (Aguerre et al., 2010c) during the last day of a 21-d period, just before reassigning diets to the eight cows for the next 21-d period. All feces produced during the 24-h collection periods were combined within a diet, and the same procedure was followed for urine. Samples of composited feces and urine from each diet were frozen until just before chemical analyses and application to the laboratory chambers described below.

Laboratory Chambers for Ammonia Emission Measurement

The construction and operation of the laboratory chambers have been described previously (Misselbrook et al., 2005; Misselbrook and Powell, 2005). Briefly, the chambers were constructed from plastic drainage pipe (10 cm diameter, 19 cm height) with an end-cap glued to the base and a lid fitted to the top with silicone grease to provide an airtight seal. The internal surfaces of the lid were sprayed with Teflon (DuPont, Wilmington, DE) to minimize adsorption of NH₃. Each chamber lid had four horizontally positioned inlet and outlet ports to ensure good mixing of air within the chamber. Chambers were packed with sieved (to 2 mm) soil to a natural bulk density of approximately 1.2 g cm⁻³ for the silt loam soil and approximately 1.4 g cm⁻³ for the sandy loam soil, leaving approximately 0.35 L headspace in each chamber. Sufficient water was added to soils in each chamber to achieve approximately 60% water-

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Urine § Wet basis. Average fecal dry matter content (153.5 g kg⁻¹) was similar among all dietary CP and tannin extract levels. As excreted (wet basis). A total of 16 g of feces+urine was added to each chamber.

† Crude protein.

¶ Amount of total N (feces+urine) applied to each chamber to trap all applied N in outflow air (Misselbrook et al., 2005).

Conducted to determine that a single acid trap was sufficient conducted at the same temperature (15°C). Preliminary tests were conducted to determine that a single acid trap was sufficient to trap all applied N in outflow air (Misselbrook et al., 2005).

# pH

## Chemical Analyses

Samples of feces were analyzed in triplicate for DM (100°C, 48 h) content, pH (a water/feces mixture [2:1 ratio], using a portable pH meter [Accumet AP61; Fisher Scientific, Pittsburgh, PA]), and fecal total N (FN) by combustion assay (Elementar VarioMax CN analyzer; Elementar, Hanau, Germany). Samples of urine were analyzed in triplicate for pH and total N and urea N content. After pH determination (undiluted urine, using the same Accumet AP61 portable pH meter used for feces), urine samples were acidified (60 mL of 3.43 g H₂SO₄ L⁻¹ and 15 mL urine) before subsequent analyses. Total urinary N (UN) was measured by combustion assay (Vario MAX CN analyzer; Elementar), with 200 mg sucrose being added to the 2.5-mL urine sample to aid combustion. Urea N was determined using an automated colorimetric assay (Broderick and Clayton, 1997) adapted to a flow-injection analyzer. The soils removed from chambers at the completion of NH₃ measurements (48 h) were thawed overnight, extracted with 2 mol L⁻¹ KCl (5 g of soil in 50 mL of 2 mol L⁻¹ KCl), shaken for 2 h, filtered, and analyzed for ammonium N (NH₄⁺) and nitrate N (NO₃⁻ and NO₂⁻) using QuickChem Methods 12–107–06–2–A (ammonium) and 12–107–04–1–B (nitrate) on a Lachat automated N analyzer (Lachat Instruments, 1996).

The DM amount of feces applied to each chamber was multiplied by its respective concentrations (mg g⁻¹) of FN to determine the amount (mg) of FN applied to each chamber. Similarly, volumes of urine applied to each chamber were multiplied by their respective concentrations (mg mL⁻¹) of UN and urea N to determine the amount (mg) of UN and urea N applied to each chamber. Total slurry N (TN) added to each chamber was the sum of FN and UN (Table 1).

### Slurry Applications to Emission Chambers

A block of four chambers was used. The first block (replication 1) was used to determine NH₃ emissions over a 48-h period (as described below) from the silt loam soil amended with the four tannin extract slurries within the LCP level. At the end of replication 1 determinations, soil was removed from chambers, stored in a plastic container, and frozen immediately. Chambers were cleaned with distilled water, dried, and repacked with soil. Slurries from the same diets were applied to the silt loam soil, and emissions were recorded for 48 h (replication 2). The same procedure was followed for replications 3 and 4 of the silt loam amended with the four tannin extract slurries within the LCP level and for all other treatment–soil combinations. Each soil type therefore had a total of eight blocks: four replicates of slurries from 0T, LT, MT, and HT at LCP and four replicates of slurries from 0T, LT, MT, and HT at HCP.

Feces and urine were combined (to total 16 g) in the mass ratio (g g⁻¹) that they were excreted (Table 1). To assure uniform soil moisture conditions, distilled water was added to achieve a uniform slurry DM content of 70 g kg⁻¹. Field application rate equivalents were between 30 and 35 m³ ha⁻¹ containing 90 to 120 kg total N ha⁻¹. Slurries were applied evenly on the soil surface. Immediately after an application, chamber lids were closed and sealed with silicon grease, and the air flow through the system was started. Acid traps were changed after 1, 3, 6, 12, 24, and 36 h, and measurement was stopped at 48 h. At each sampling time, acid from the outlet acid traps was made up to 0.1 L with deionized water and then analyzed for NH₄⁺ by flow injection analysis (QuickChem Methods 12–107–06–2–A; Lachat Instruments, 1996). The total emission (mg N) for a sampling time (1, 3, 6, 12, 24, 36, or 48 h) was calculated as the product of ammoniacal-N concentration of the acid trap solution (mg L⁻¹) and the volume of acid trap solution (0.01 L).

Cumulative emission for each 48-h period was derived by summing emissions determined at each sampling time.

### Table 1. Dietary crude protein and tannin extract impacts on relative mass of feces and urine applied, total nitrogen (TN) applied, fecal pH, fecal total nitrogen (FN), urine pH, urine total nitrogen (UN), and urea nitrogen applied to simulated barn floors in lab-scale ventilated chambers.

<table>
<thead>
<tr>
<th>Diet Information</th>
<th>CP†</th>
<th>Tannin</th>
<th>Feces/urine application ratio‡</th>
<th>TN¶</th>
<th>pH</th>
<th>FN#</th>
<th>pH</th>
<th>UN</th>
<th>Urea N</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>g kg⁻¹</td>
<td>g g⁻¹</td>
<td>mg</td>
<td></td>
<td></td>
<td>g kg⁻¹</td>
<td></td>
<td></td>
<td>g L⁻¹</td>
</tr>
<tr>
<td>155</td>
<td>0</td>
<td>2.01</td>
<td>75</td>
<td>5.60</td>
<td>4.12</td>
<td>8.86</td>
<td>5.77</td>
<td>1.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>2.20</td>
<td>70</td>
<td>5.47</td>
<td>3.98</td>
<td>8.91</td>
<td>5.21</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>2.26</td>
<td>69</td>
<td>6.09</td>
<td>3.83</td>
<td>8.82</td>
<td>5.35</td>
<td>2.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.0</td>
<td>2.41</td>
<td>71</td>
<td>5.77</td>
<td>4.42</td>
<td>8.76</td>
<td>4.55</td>
<td>2.00</td>
<td></td>
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<tr>
<td>168</td>
<td>0</td>
<td>1.64</td>
<td>95</td>
<td>6.75</td>
<td>4.24</td>
<td>9.22</td>
<td>8.65</td>
<td>5.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>1.56</td>
<td>88</td>
<td>6.50</td>
<td>4.41</td>
<td>8.92</td>
<td>7.20</td>
<td>4.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>1.70</td>
<td>87</td>
<td>6.63</td>
<td>4.42</td>
<td>9.15</td>
<td>7.26</td>
<td>4.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.0</td>
<td>2.13</td>
<td>86</td>
<td>6.70</td>
<td>4.54</td>
<td>8.87</td>
<td>7.07</td>
<td>3.56</td>
<td></td>
</tr>
</tbody>
</table>

*CP† Crude protein.

† As excreted (wet basis). A total of 16 g of feces+urate was added to each chamber.

‡ Wet basis. Average fecal dry matter content (153.5 g kg⁻¹) was similar among all dietary CP and tannin extract levels.

§ Amount of total N (feces+urine) applied to each chamber.
Statistical Analyses
Statistical analyses were performed using the SAS statistical package (SAS Institute, 2007). For the emission data, a repeated measures model within PROC MIXED was used to account for auto-correlated errors, and the Kenward-Roger method was used to compute the denominator degrees of freedom for the tests of the fixed effects of soil, CP, and tannin extract. A spatial power error structure was included to account for the uneven time intervals between NH₃ emission measurements. The model included all interactions between soils, CP, tannin extract, and time of NH₃ emission measurement. Dietary CP and tannin extract effects on concentrations of inorganic N in soils were analyzed by generalized least squares ANOVA. A macro developed by Saxton (1998) was used to generate LSD ($P < 0.05$) values to separate treatment means among soil, CP, and tannin extract levels.

Results and Discussion
The composite samples of feces and urine used to make slurries for application to soils had different chemical properties (Table 1). Average fecal pH (5.73) and urine pH (8.84) from cows fed LCP were lower than average fecal pH (6.64) and urine pH (9.04) from cows fed HCP. Also, average concentrations (g kg⁻¹) of FN (4.09), UN (5.22), and urea N (1.82) in the excreta of cows fed LCP were lower than average FN (4.40), UN (7.54), and urea N (4.50) in the excreta of cows fed HCP. Urea N comprised from 23 to 44% of UN excreted by cows fed LCP and from 50 to 67% of UN excreted by cows fed HCP. The range of urea N concentrations within the LCP diet was lower than what would be expected from cows fed similar diets without tannin extract (Broderick, 2003, 2009). For the HCP diet, UN and urea N concentrations tended to decrease as the level of tannin extract increased (Table 1).

Treatment Impacts on the Time Pattern
of Ammonia Emissions
The time patterns of NH₃ emissions from dairy slurry applied to soil (Fig. 1a, 2a, and 3a) correspond to patterns typically observed in similar lab chamber experiments (Misselbrook et al., 2005; Powell et al., 2011) and under field conditions (Jokela and Meisinger, 2008). Initial NH₃ emission rates are rapid, with peak emissions occurring from 24 to 36 h after slurry application and declining precipitously thereafter.

The present study results revealed that the impacts of dietary CP (Fig. 1a) and tannin extracts (Fig. 2a) on NH₃ emission were variable across time (i.e., the significant interactions CP × time and tannin × time; Table 2). These variable impacts may offer new ways to evaluate how the timing of slurry incorporation may affect the fertilizer N value of land-applied slurry. For example, NH₃ loss from the LCP slurry is much less than from the HCP slurry during the 12-, 24-, and 36-h sampling times (Fig. 1a), and most tannin impacts on NH₃ loss occur during the 24-, 36-, and 48-h sampling times (Fig. 2a). In Wisconsin, most plant-available N in dairy manure is considered lost within 72 h, and it is recommended to incorporate manure as soon as possible after application (Madison et al., 1995; Laboski et al., 2006). The results of the present study suggest that most NH₃ is emitted within 24 to 36 h and that N loss during this period varies according to the level of CP (Fig. 1a) and tannin (Fig. 2a) fed.

A principal objective of the present study was to make preliminary, small-scale determinations of NH₃ emissions from soils after application of tannin-containing slurries. Slurries were applied immediately after mixing fresh feces and urine (Table 1). On commercial dairy farms, however, feces and urine are mixed, and NH₃ emissions persist during manure removal from barns and during manure storage and transport to fields. Although NH₃ loss during manure handling and storage would reduce the magnitude of NH₃ loss after slurry application to soil, tannins likely would be effective in abating NH₃ loss from various components of a dairy farm. For example, in the previous lab-chamber experiment using the same slurries as used in the present study (Powell et al., 2011), NH₃ emissions from simulated barn floors were lower from tannin-containing slurries than from non–tannin-containing slurry. Tannins also abate NH₃ emissions from soil after slurry has been stored for 2 wk: NH₃ emissions from soil were lower after application of stored slurry that contained tannin than from stored slurry that did not contain tannin (Misselbrook et al., 2005).

Impacts of Dietary Crude Protein on Ammonia Emission
Ammonia emissions from applied slurries differed significantly between the two dietary CP levels. During the 3- to 48-h measurement times, NH₃ emissions from the HCP slurry were 1.53
Fig. 2. Impact of dietary tannin extract levels on (a) pattern of NH$_3$–N emissions and (b) cumulative NH$_3$–N emissions from dairy excreta applied to soils (0T, LT, MT, and HT refer to dietary tannin extract levels of 0, 4.5, 9.0 and 18.0 g kg$^{-1}$ of dry matter intake, respectively). *Significant differences at $P < 0.05$. Numbers in parentheses are standard errors.

Fig. 3. Soil type differences in (a) pattern of NH$_3$–N emissions and (b) cumulative NH$_3$–N emissions from dairy excreta applied to a sandy loam soil and a silt loam soil. *Significant differences at $P < 0.05$. Numbers in parentheses are standard errors.

Table 2. Summary of fixed effects on rates of ammonia emissions and cumulative ammonia emissions from soil.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Rates of NH$_3$ emissions</th>
<th>Cumulative NH$_3$ emissions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean squares</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Time§</td>
<td>6</td>
<td>92.2</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>CP level¶</td>
<td>1</td>
<td>132.8</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Time × CP</td>
<td>6</td>
<td>14.4</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Soil type#</td>
<td>1</td>
<td>9.0</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Time × soil type</td>
<td>6</td>
<td>3.9</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Tannin extract level††</td>
<td>3</td>
<td>4.3</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Time × tannin</td>
<td>18</td>
<td>1.1</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>CP × soil</td>
<td>1</td>
<td>0.1</td>
<td>0.552</td>
</tr>
<tr>
<td>CP × tannin</td>
<td>3</td>
<td>0.2</td>
<td>0.441</td>
</tr>
<tr>
<td>Soil × tannin</td>
<td>3</td>
<td>0.1</td>
<td>0.812</td>
</tr>
<tr>
<td>CP × soil × tannin</td>
<td>6</td>
<td>0.1</td>
<td>0.638</td>
</tr>
<tr>
<td>Error</td>
<td>394</td>
<td>0.3</td>
<td>–</td>
</tr>
</tbody>
</table>

† Emission during the time interval between two consecutive time points, as depicted in Fig. 1a, 2a, and 3a.
‡ Cumulative emission over a 48-h period, as depicted in Fig. 1b, 2b, and 3b.
§ 1, 3, 6, 12, 24, 36, and 48 h after manure application to soil.
¶ Crude protein level (155 vs. 168 g kg$^{-1}$).
# Sandy loam vs. silt loam.
†† 0, 4.5, 9.0, and 18.0 g kg$^{-1}$. 
to 2.57 times greater than emissions from the LCP slurry (Fig. 1a), and cumulative NH$_3$ emissions from the HCP slurry were 2.14 to 2.75 times greater than from the LCP slurry (Fig. 1b). At trial’s end (48 h), cumulative NH$_3$ emissions from the HCP slurry was on average 2.16 times greater than from the LCP slurry.

Probable reasons for the much greater NH$_3$ emission from the HCP slurry than the LCP slurry are (i) the greater amounts of excreta N (especially urea N) applied with the HCP treatment and (ii) the higher pH of applied feces and urine from cows fed the HCP diet (Table 1). Similar to the present study, Paul et al. (1998) and Misselbrook et al. (2005) compared NH$_3$ emissions from manure of cows fed a HCP diet with emissions from manure of cows fed a LCP diet. In those studies, the greater NH$_3$ emissions from the HCP diet were attributed to higher manure pH, which was hypothesized to increase urease activity and NH$_3$ formation and loss. Maximum urease activity occurs between pH 6.8 and 7.6, and activity decreases linearly with pH outside this range (Muck, 1982). In the present study, the average pH values of feces (6.6) and urine (9.0) from cows fed the HCP diets were higher than the pH values of feces (5.7) and urine (8.8) from cows fed the LCP diet (Table 1). The higher pH of feces and urine from HCP may have contributed somewhat to more NH$_3$ being emitted from the HCP slurries compared with the LCP slurries.

The relative amount of applied urea N, urine N (UN), and total excreta N (TN) emitted as NH$_3$ was influenced by dietary CP level (Table 3). The HCP slurry lost approximately 53% of applied urea N, which was significantly less than the 72% loss of applied urea N from the LCP slurry. This lower relative NH$_3$ loss from the HCP slurry can be attributed to the much greater amount (average, 26 mg) of urea N applied to the HCP treatment chambers compared with the amount (average, 9 mg) of urea N applied to the LCP treatment chambers (the total NH$_3$ loss from the HCP slurry was more than twice the NH$_3$ loss [13.8 mg] from the HCP slurry). Of the total UN and TN applied with the HCP slurry, approximately 31 and 15%, respectively, was emitted as NH$_3$; these values are significantly greater than the 24 and 9% of UN and TN, respectively, emitted from the LCP slurry.

The Impact of Excessive CP Feeding on urine N Excretions is well established in the dairy nutrition literature (c.f., Castillo et al., 2000; Kebreab et al., 2001). Greater NH$_3$ emissions after application of slurry to soil from HCP diets have been documented under similar laboratory conditions (Paul et al., 1998; Misselbrook et al., 2005). Although NH$_3$ losses were greater in soils amended with HCP slurry (Fig. 1b), at trial’s end there were significantly greater concentrations of NH$_3^+$ and inorganic N in soils amended with the HCP slurry than in soils amended with the LCP slurry (Table 4). This result tends to corroborate other findings under laboratory conditions, indicating that, although LCP diets reduce urine N excretion and therefore NH$_3$ emissions, the application of LCP dairy manures to soil provides less plant-available N than application of HCP manures (Paul et al., 1998; Powell et al., 2006).

### Impacts of Tannin Extract Levels on Ammonia Emission

The slurries from the four tannin extract levels had different impacts on NH$_3$ emissions from soil. Ammonia emissions from the HT slurry were 28, 46, and 49% lower ($P < 0.05$) than emissions from the 0T treatments at the 24-, 36-, and 48-h measurement times, respectively (Fig. 2a). Ammonia emissions from the LT and MT diets were similar and 16, 18, and 25% lower ($P < 0.05$) than emissions from the 0T treatment during the 24-, 36-, and 48-h measurement times, respectively. Cumulative NH$_3$ emissions from the LT and HT slurries were similar and 16 and 21% lower than from the 0T slurry at the 24- and 36-h measurement times, respectively (Fig. 2b). At trial’s end (48 h), cumulative NH$_3$ emissions from the MT, LT, and HT slurries were 11, 19, and 27% lower, respectively, than from the 0T slurry. It is uncertain why the MT slurries did not provide NH$_3$ emissions that were intermediate to the LT and HT slurries.

Tannin extract level also had differential impacts on the relative amounts of applied urea N and TN emitted as NH$_3$ (Table 3). Of the urea N applied, approximately 54% was emitted as NH$_3$ from the MT slurries, which was similar to the 58% emitted from the HT slurries and significantly lower than the 66% emissions from the 0T slurry (which was similar to the 72% emission from the LT slurries). The lower NH$_3$ emission from the tannin extract slurries may be attributed to tannins’ ability to reduce (i) urea N excretion (especially by cows fed HCP; Aguerre et al., 2010c), which reduces the amount of urea N applied (Table 1), and (ii) urease activity in feces. In a preceding trial where the same excreta was applied to cement-filled lab chambers to simulate dairy barn floors, a 11.5%
reduction in NH₃ emissions was attributed to tannins’ ability to reduce ureaase activity in dairy feces (Powell et al., 2011). Tannins’ inhibitory effects on enzymatic activity have been linked to (i) the formation of substrate-tannin complexes, which prevents conversion of urea by enzymes, and (ii) the strong inhibitory effects of tannins alone (Goldstein and Swain, 1965). Tannins are known to bind to ruminal bacterial surfaces and to forage proteins to reduce enzyme access and activity (Waghorn, 2008). Similar factors appear to influence tannins’ inhibitory effects on enzymatic activity in soils (Joanisse et al., 2007).

Although slurries from cows fed tannin extracts had lower NH₃ emissions than slurries from cows fed no tannin (Fig. 2a and 2b), the apparent conservation of applied slurry N did not significantly affect concentrations of inorganic N in soils (Table 4). After repeated slurry applications over the long term, however, tannins may accumulate in soils, which would affect soil organic matter formation and N cycling (Halverson et al., 2009).

### Soil Type Differences in Ammonia Emissions

Ammonia emissions from the sandy loam soil were 1.31 and 1.44 times greater than emissions from the silt loam soil 24 and 36 h after slurry application (Fig. 3a). Cumulative NH₃ emissions from the sandy loam soil were 1.07, 1.15, and 1.15 times greater than from silty loam soils 24, 36, and 48 h, respectively, after slurry application (Fig. 3b). Relative NH₃ losses of applied urea N (70%), UN (30%), and TN (13%) from the sandy loam soil were significantly greater than relative losses of urea N (56%), UN (25%), and TN (11%) from the silt loam soil (Table 3). Greater urea N loss and NH₃ emission from the sandy loam soil may be attributed to the lower clay content, which lowers H⁺ buffering capacity resulting in pH rise (and increased NH₃ emission) during urea hydrolysis (Ferguson et al., 1984) and lowers the soil’s capacity to absorb NH₃ upon urea hydrolysis (Buresh, 1987). The relatively high DM content (~70 g kg⁻¹) of the applied slurry likely resulted in its similar infiltration into the sandy loam and the silt loam soil and therefore its similar physical exposure to NH₃ loss.

The greater NH₃ loss from the sandy loam soil resulted in significantly lower concentrations of NO₃−N and inorganic N compared with the silt loam soil (Table 4). This result indicates that after slurry application to the surface of the sandy loam soil, the greater NH₃ loss reduces the fertilizer value of slurry, as indicated by the lower levels of inorganic available N after NH₃ loss subsides.

### Conclusions

Concentrations of CP and tannin extracts in lactating dairy cow diets have significant impacts on excretions of urea N, urine N, and fecal N (Aguerre et al., 2010c), which affects the pattern of NH₃ emissions and cumulative NH₃ loss after excreta has been applied to soils incubated in laboratory chambers (Fig. 1–3). Over the 48-h measurement period, slurries from HCP diets emitted 2.16 times more NH₃ from soils than slurries from LCP diets. Greater NH₃ emissions from the HCP slurries were due to the much greater amounts of total N (especially urea) applied than with the LCP slurries. The greater amounts of N applied with the HCP slurries increased inorganic N in soils. Slurries from 0T diets emitted 1.23 times more NH₃ than slurries from LT, MT, and HT diets. Reductions in NH₃ emissions due to feeding tannins were likely due to tannin impacts on reducing urea N excretion and therefore urea N application to soil and to tannin’s inhibitory effect on urease activity in feces (and perhaps in soil). Ammonia emissions were greater from the sandy loam soil than from the silt loam soil. The consequences of this effect were lower concentrations of inorganic N in the sandy loam soil compared with the silt loam soil. The next step in this research is to ascertain the impact of dietary CP and tannin extracts on NH₃ emissions and soil N cycles in larger-scale, longer-term trials that simulate more closely manure management on commercial dairy farms.

### Acknowledgments

We thank Mr. Peter Crump, Senior Info Processing Consultant, College of Agricultural and Life Sciences, University of Wisconsin-Madison for assistance with statistical analyses.

### References

Aguerre, M.J., M.A. Wattiaux, T. Hunt, and B.R. Larget. 2010a. Effect of dietary crude protein level, soil type, and tannin extract level on soil nitrogen at the completion (48 h) of ammonia emission measurements.

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† Ammonium N plus nitrate N.
§ Low crude protein (155 g kg⁻¹).
¶ High crude protein (168 g kg⁻¹).
# 0T, 0 tannin; LT, low tannin (4.5 g kg⁻¹); MT, medium tannin (9.0 g kg⁻¹); HT, high tannin (18.0 g kg⁻¹).