Feeding more tannin and less crude protein (CP) to dairy cows may have synergistic impacts on reducing NH$_3$ emissions from dairy barns. Three trials using lab-scale ventilated chambers with concrete floors were conducted to determine the impacts on NH$_3$ emission of tannin and CP feeding, tannin feeding on urease activity in feces, and tannin application directly to the barn floor. For Trial 1, mixtures of feces and urine from lactating Holstein dairy cows (Bos taurus) fed four levels (g kg$^{-1}$) of dietary tannin extract [a mixture from red quebracho (Schinopsis lorentzii) and chestnut (Castanea sativa) trees]: 0 tannin (0T), 4.5 (low tannin [LT]), 9.0 (medium tannin [MT]), and 18.0 (high tannin [HT]); each fed at two levels of dietary CP: 155 low CP (LCP) and 168 high CP (HCP) were applied to chambers. For Trial 2, urea solution was added to feces obtained from cows fed 0T, MT, and HT at HCP. For Trial 3, tannin amounts equivalent to those fed at 0T, MT, and HT were applied directly to feces—urine mixtures from the same cows. For all trials, NH$_3$ emissions were measured 1, 3, 6, 12, 24, 36, and 48 h after treatment application. For Trial 1, reductions in NH$_3$ emission due to tannin feeding were greatest when fed at HCP. The LCP-LT and LCP-HT treatments emitted 30.6% less NH$_3$ than LCP-0T, and the HCP-LT and HCP-HT treatments emitted 16.3% less NH$_3$ than HCP-0T. For Trial 2, feeding tannin decreased urease activity in feces, resulting in an 11.5% reduction in cumulative NH$_3$ loss. For Trial 3, the application of tannin directly to simulated barn floors also apparently decreased urease activity, resulting in an average reduction in cumulative NH$_3$ emissions of 19.0%. Larger-scale trials are required to ascertain the effectiveness of tannin extracts in abating NH$_3$ loss from dairy barn floors.

A principal goal in dairy production is to feed least-cost rations that maximize milk production and assure good animal health and reproduction. On most commercial dairy farms, a general range of 20 to 30% of dietary crude protein (CP) fed to cows is converted into milk protein (Chase, 2004; Jonker et al., 2002; Powell et al., 2010). Feed CP not secreted into the milk is excreted about equally in urine and feces. Feeding CP in excess of dairy cow requirements (~165 g kg$^{-1}$ of ration dry matter) (Broderick, 2003) has little impact on milk production or quality, but it decreases feed nitrogen (N) use efficiency (percentage of feed N secreted in milk) and increases excretion of urea N in urine. For example, reducing dietary CP from 194 to 165 g kg$^{-1}$ had no significant impact on milk production or composition but increased feed N use efficiency from 25 to 37% and decreased daily excretion of N as urea from 208 to 63 g cow$^{-1}$ (Olmos Colmenero and Broderick, 2006).

Dairy barns are thought to emit large amounts of ammonia (NH$_3$) (NRC, 2003) because dairy cows excrete large amounts of N in urine (~50–100 kg N cow$^{-1}$ yr$^{-1}$, depending on CP concentration in the diet) (Castillo et al., 2000; Broderick, 2009; Kebreab et al., 2010). The urea N contained in cow urine is the major source of NH$_3$. The urease present in dairy feces, and therefore abundant on barn floors (Ketelaars and Rap, 1994; Muck and Steenhuis, 1981), rapidly converts urea to NH$_3$, which is in equilibrium with NH$_4$ in the aqueous phase. As pH increases, NH$_3$ in solution is converted to NH$_4$ which is lost rapidly to the atmosphere. Ammonia emissions from dairy barns can range from 10 to 55% of manure N excretions (MWPS, 2001; Webb and Moll, 2004; Pedersen, 2006; Jokela and Meininger, 2008). After release in the atmosphere, NH$_3$ is highly reactive with acids and forms compounds such as ammonium nitrate or ammonium sulfate particulate matter <2.5 μm, which can adversely affect human health and, when deposited to the surface as weak acids and nitrates in rain, can be detrimental to natural ecosystems (Bobbink et al., 2010).

Various management practices are effective in reducing NH$_3$ emissions from dairy barns, including reduction of N excretion through diet manipulation, reducing urine N in manure to abate NH$_3$ formation and loss, and segregating urine from feces to reduce contact between urine and the urease enzyme (Ndegwa
A close matching of diets to animal nutritional requirements, feeding only enough rumen-undegraded protein to meet cows’ metabolizable protein requirements, and reducing particle size to increase ruminal digestion of starch increases microbial protein formation, maximizes feed N conversion into milk, and minimizes urinary N excretion (Broderick, 2009).

On confinement dairy farms, forages comprise approximately one half of the ration dry matter (DM) consumed by lactating dairy cows. In the Midwest and Northeast regions of the United States, the primary forages are silages made from alfalfa (Medicago sativa L.) and corn (Zea mays L.). In spite of the high CP content in alfalfa, the extensive protein breakdown during ensiling often results in an excess supply of rumen-degraded protein in the diet of dairy cows. As a result, cows must be fed protein supplements rich in rumen-undegraded protein, which not only increases feed costs but also increases total dietary CP and manure N excretion. One approach to enhance protein utilization and reduce N excretion by dairy cows is to increase the concentrations of tannin in their diets. For example, compared with alfalfa, feeding tannin containing birdsfoot trefoil (Lotus corniculatus) can enhance milk production by 9 to 15% (Hymes-Fecht et al., 2006). Consumption of tannin-containing forages by lactating cows also affects urinary N excretions and fecal N chemistry (Powell et al., 2009), which affects NH₃ loss from barn floors and from soil after manure application (Broderick, 2009).

Tannins are considered to have adverse and beneficial effects, depending on their concentration and nature, the animal species, the physiological state of the animal, and ration composition. The 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., 2010a) but increased feed N use efficiency and decreased N excretion in urine (Aguerre et al., 2010b). The excreta and tannin extracts from those studies were used in the present study (i) to determine effects of feeding tannin at two levels of dietary CP on NH₃ emissions from excreta deposited on a simulated concrete barn floor, (ii) to determine effects of feeding tannin extracts on the urease activity in feces, and (iii) to determine if the application of tannin extract directly to the barn floor (rather than feeding it to the dairy cows) affects NH₃ emission from dairy manure.

Materials and Methods

The experimental diets fed to lactating dairy cows were total mixed rations containing (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). Additional information on the diets can be found in Aguerre et al. (2010a). The tannin extract (ByProQ, 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, as determined by the polyvinylpyrrolidone powder method (Tempel, 1982): tannin concentration of 792 g kg⁻¹ and pH of 3.72 (Silvateam, Indunor S.A., Argentina). Feces and urine were applied in the mass ratio they were excreted (Aguerre et al., 2010b) to concrete-filled, lab-scale ventilated chambers, and NH₃ emissions were measured periodically over a 48-h period.

Collection of Feces and Urine

Feces and urine were collected from eight multiparous lactating Holstein cows, which had the following characteristics at the onset of the feeding trial: body weights of 708 ± 41 kg cow⁻¹, days in milk of 125 ± 41 d, and daily milk production of 44.6 ± 6.9 kg cow⁻¹. Cows were fed eight diets: four tannin extract levels of 0, 4.5, 9.0, and 18.0 g kg⁻¹ (corresponding to 0T, LT, MT, and HT, respectively), each fed at dietary CP levels of 155 and 168 g kg⁻¹ (corresponding to LCP and HCP, respectively). The feeding trial (Aguerre et al., 2010a) was a split-plot within a 4×4 Latin Square design, with a replication being four cows assigned to each of the four tannin levels within the LCP diet and four other cows assigned to the four tannin levels within the HCP diet. Feces and urine for the present trial were collected (Aguerre et al., 2010b) during the last day of a 20-d replication period, just before reassigning diets to the eight cows for the next replication (total of four replications). All feces produced during the 24-h collection period were combined within a diet, and the same procedure was followed for urine. Samples of 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 by Misselbrook et al. (2005). Briefly, the chambers were constructed from a plastic drainage pipe of 10 cm diameter and 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. The main body of the chamber was filled with concrete (to simulate a barn floor), leaving a headspace of approximately 0.35 L. Air was drawn through the system by means of a vacuum pump (Thomas Industries, Monroe, LA), with the airflow rate through each chamber being controlled at 4 L min⁻¹. An acid trap containing 0.075 L of 0.02 mol L⁻¹ orthophosphoric acid before each chamber removed any NH₃ from inlet air, and a second acid trap on the outlet side of each chamber collected the NH₃ emitted during the measurement period. Glass or Teflon tubing was used between the chamber and outlet acid trap to minimize adsorption of NH₃ to the tubing walls. The set-up was housed in a large incubator, and all trials were conducted at the same temperature (15°C). Preliminary tests were conducted to determine whether a single acid trap was sufficient to trap all applied N in outflow air (Misselbrook et al., 2005).
Application of Feces and Urine to Emission Chambers

Three NH₃ emission trials were conducted. Trial 1 was designed to determine possible interactions between dietary tannin and CP levels on NH₃ emissions from excreta deposited on a concrete barn floor. For this trial, feces and urine were applied (total weight of 16 g chamber⁻¹) in the mass ratio (g g⁻¹) that they were excreted (Table 1). Trial 2 was designed to determine the possible effects of tannin feeding on urease activity in feces. For this trial, a procedure outlined by Muck (1981) was followed. A urea solution (8 mL containing 36 mg urea) was added to feces (8 g wet feces chamber⁻¹) from cows fed 0T, MT, and HT within the HCP diet. The HCP diet was selected because this CP concentration (168 g kg⁻¹) is close to the recommended level for diets fed to high-producing dairy cows (Broderick, 2003). The urea N application rate was the average amount of urea N applied to 0T, MT, and HT treatments at HCP level in Trial 1. Trial 3 was designed to determine if the application of tannin extract directly to barn floor (rather than feeding it) would affect NH₃ emissions. For this trial, excreta from the 0T, HCP diet was used. Feces (8 g wet feces chamber⁻¹) were applied first, followed by application of one of three tannin extract levels: 0 mg (0TEx), 45 mg (MTEx), or 90 mg (HTEx). Urine (8 g chamber⁻¹) was then added to the feces–tannin mixtures, and after a brief, slight mixing, chambers were closed for determination of NH₃ emissions. The three tannin extract levels of 0TEx, MTEx, and HTEx used in Trial 3 were calculated from a Trial 1 relationship between tannin fed and total N (feces + urine) excreted during the lactation trial (Aguerre et al., 2010b). Cows on the HCP-MT diet consumed approximately 206 g of tannin, and cows on the HCP-MT and the HCP-HT diets excreted similar amounts of total N (average of 435 g d⁻¹). The resulting consumed tannin/excreta N ratio of 0.474 (i.e., 206/435) was multiplied by the 95 g of excreta N applied to the HCP-0TEx (Table 1) to provide the MTEx application of 45 mg, and this was doubled to arrive at the HTEx application of 90 mg.

Ammonia Emissions from Excreta Deposits on Barn Floor

For Trial 1, 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 four tannin extract treatments within the LCP level. At the end of replication 1 determinations, chambers were scraped and cleaned with distilled water and dried, and feces–urine from the same diets was applied again and emissions were recorded for 48 h (replication 2). The same procedure was followed for replications 3 and 4. Trial 1 therefore had a total of eight blocks: four replicates of feces–urine from 0T, LT, MT, and HT at LCP and four replicates of feces–urine from 0T, LT, MT, and HT at HCP. For Trial 2, a block of six chambers was used so that two replicates of 0T, MT, and HT urea treatments could be run at the same time. The block was repeated to achieve four replicates per treatment. Likewise, for Trial 3, a block of six chambers was used, corresponding to two replicates of the 0TEx, MTEX, and HTEx treatments. The block was repeated to achieve a total of four replicates per treatment.

Deposits of urine and feces to a free-stall barn floor would normally be scraped, leaving a thin layer from which emission occurs. In the present trials, therefore, a constant mass of slurry (16 g) was applied to the chambers to achieve a thin emitting layer of approximately 1 mm above the concrete surface, similar to the methodology used by Misselbrook et al. (2005). For all three trials, feces were spread evenly on the concrete surface, followed by application of urine (Trial 1), urea (Trial 2), or tannin extract plus urine (Trial 3). Immediately after applications were complete, 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 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 ammonium (NH₄⁺) by flow injection analysis (QuickChem Methods 12–107–06–2–A; Lachat Instruments, 1996). The total emission (mg N) for 1, 3, 6, 12, 24, 36 and 48 h was calculated as the product of ammoniacal

Table 1. Dietary crude protein and tannin extract impacts on relative mass of feces and urine applied, total nitrogen applied, fecal pH, fecal total nitrogen, fecal ammonical nitrogen, urine pH, urine total nitrogen, 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>Feces§</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g kg⁻¹</td>
<td>g g⁻¹</td>
<td>Mg</td>
<td>g kg⁻¹</td>
<td>pH</td>
</tr>
<tr>
<td>155</td>
<td>0</td>
<td>2.01</td>
<td>75</td>
<td>5.60</td>
<td>4.12</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
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<td>69</td>
<td>6.09</td>
<td>3.83</td>
</tr>
<tr>
<td></td>
<td>18.0</td>
<td>2.41</td>
<td>71</td>
<td>5.77</td>
<td>4.42</td>
</tr>
<tr>
<td>168</td>
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<td>1.64</td>
<td>95</td>
<td>6.75</td>
<td>4.24</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
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<td>6.50</td>
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<td>6.63</td>
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<tr>
<td></td>
<td>18.0</td>
<td>2.13</td>
<td>86</td>
<td>6.70</td>
<td>4.54</td>
</tr>
</tbody>
</table>

| † Crude protein.       |
| ‡ As excreted (wet basis). 16 g of total feces+urine added to each chamber. |
| § Wet basis. Average fecal dry matter content (153.5 g kg⁻¹) was similar among all dietary tannin extract and CP levels. |
| ¶ Amount of total N (feces + urine) applied to each chamber. |
| # Fecal total N.       |
| †† Fecal ammonical N.   |
| ‡‡ Urine total N.       |

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N (NH₃ plus NH₄⁺) concentration of the acid trap solution (mg L⁻¹) and the volume of acid trap solution (L). Cumulative emission for each 48-h period was derived by summing emissions determined at each sampling interval.

**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 an Accumet AP61 portable pH meter [Fisher Scientific, Pittsburgh, PA]), fecal total N (FN) by combustion assay (Elementar VarioMax CN analyzer; Elementar Vario, Hanan, Germany), and fecal ammoniacal N (5 g fresh feces shaken in 50 mL 2 mol L⁻¹ KCl for 2 h and filtered through Whatman no. 42 and analyzed on a Lachat automated N analyzer) (QuickChem Methods 12-107-06-2-A; Lachat Instruments, 1996). Samples of urine were analyzed in triplicate for pH, 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 analysis. Total urinary N (UN) was measured by combustion assay (Elementar VarioMax CN analyzer; Elementar Vario, Hanan, Germany), with 200 mg sucrose 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 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 fecal TN 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 (Table 1) was the sum of FN and UN.

**Statistical Analyses**

Statistical analyses were performed using the SAS statistical package (SAS Institute, 2007). A repeated measures model within PROC MIXED was used to account for auto-correlated errors. A spatial power error structure was included to account for the uneven time intervals between NH₃ emission measurements. Because of possible non-normal variances associated with percentages, percentage NH₃ emissions (of excreta N applied) were transformed into “percent” before data analyses. For Trial 1, the model included all interactions between levels of tannin extract, CP, and time of NH₃ emission measurement. For Trials 2 and 3, the models included interactions between levels of tannin extract and time of NH₃ emission measurement. The protected LSD (P < 0.05) test was used to separate treatment means among tannin extract and CP levels.

**Results and Discussion**

Preliminary analyses of dietary tannin extract and CP level impacts on N intake, milk production, and N excretion were summarized recently by Aguerre et al. (2010a,b). There were no significant (P < 0.05) interactions between dietary tannin and CP on N intake, milk production, or total N excretion, and neither tannin extract nor CP level significantly affected N intake or milk production. Compared with the 0T diet, feeding tannin (average of LT, MT, and HT) increased fecal N excretion by 18%. Compared with the other tannin levels, HT decreased urine N excretion by 16%. Compared with the LCP diet, the HCP diet decreased N excretion in feces by 20% but increased urea N excretion by 40%.

The composite feces and urine applied to the present study emission chambers had variable 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. Average concentrations (g kg⁻¹) of FN (4.1), UN (5.2), and urea N (1.8) in the excreta of cows fed LCP were lower than average FN (4.4), UN (7.5), and urea N (4.5) in the excreta of cows fed HCP. Urea N comprised only 23 to 44% of UN excreted by cows fed LCP and 53 to 70% of UN excreted by cows fed HCP diets (Table 1). The range of urea N concentrations within the LCP diet was much lower than what would be expected from cows fed similar diets without tannin extract (Broderick, 2003, 2009). For the HCP diet, urea N concentrations decreased as the level of tannin extract increased (Table 1). These results indicate that feeding tannin extract decreases UN and urea N concentrations.

The patterns of NH₃ emissions during the 48-h monitoring period were similar for all three trials (Fig. 1, 3, and 5). Emissions increased rapidly to a maximum at 24 h and then declined precipitously. These NH₃ emission patterns and cumulative NH₃ emissions (Fig. 2, 4, and 6) corroborate findings from similar laboratory chamber studies that measured losses of NH₃ from dairy barn floors (Missetbrook et al., 2005; Missetbrook and Powell, 2005).

**Impacts of Dietary Tannin and Crude Protein Levels on Ammonia Emission**

Results from Trial 1 provided evidence that feeding tannin extracts produced excreta that altered the pattern of NH₃ emissions (Fig. 1) and cumulative NH₃ loss (Fig. 2) from simulated barn floors. Relative reductions in NH₃ emission due to tannin feeding were greatest when the extract was fed at LCP. Compared with 0T treatments, the average (LT, MT, and HT) cumulative NH₃ emission was 16% less from LCP excreta and 8% less from HCP excreta. This 2-fold difference in NH₃ emission reduction may be attributed to two factors: (i) the lower amounts of excreta N applied at LCP than HCP and (ii) the lower pH of applied feces and urine from cows fed LCP (Table 1). Somewhat similar to the present study, Paul et al. (1998) and Missetbrook et al. (2005) compared NH₃ emissions from manure of cows fed a low-CP diet with emissions from manure of cows fed a high-CP diet. In those studies, the lower NH₃ emissions from the lower-CP diets were attributed to lower manure pH, which was hypothesized to reduce urease activity. Muck (1981) showed that maximum urease activity occurs between pH 6.8 and 7.6 and that activity decreased linearly with pH outside this range. In the present study, the average pH of feces (5.73) and urine (8.84) from cows fed the LCP diets was lower than the pH of feces (6.64) and urine (9.04) from the HCP diet (Table 1). The lower pH of feces and urine from LCP likely contributed to less NH₃ being emitted from...
the LCP treatments compared with the HCP treatments (Fig. 1 and 2).

For the LCP diet at 24 h, NH$_3$ emissions from LT, MT, and HT were similar and on average were 33.3% less than ($P < 0.001$) emissions from 0T (Fig. 1). For the HCP diet at 24 h, NH$_3$ emissions from LT, MT, and HT were 24.6, 18.4, and 37.0% less than emissions from 0T ($P < 0.001$). For the HCP diet at 36 h, NH$_3$ emissions from LT, MT, and HT were similar and were on average 43.7% less than ($P < 0.001$) emissions from 0T. For the LCP at 24, 36, and 48 h, cumulative NH$_3$ emissions from LT and HT were similar and were on average 30.5% less than ($P < 0.001$) emissions from 0T and MT, which had similar cumulative NH$_3$ emissions (Fig. 2). For the HCP diets at 36 and 48 h, cumulative NH$_3$ emissions from LT, MT, and HT were generally similar and on average 13.0% less than ($P < 0.001$) emissions from 0T (Fig. 2).

Of the total excreta N applied (TN) (Table 1), relative cumulative NH$_3$ emissions from the LCP chambers (16.7%) was significantly ($P < 0.001$) less than relative cumulative NH$_3$ emissions from the HCP chambers (29.0%). Calculated as a percentage of urine N applied, relative cumulative NH$_3$ emissions from LCP chambers (45.5%) were significantly ($P < 0.001$) less than relative cumulative NH$_3$ emissions from the HCP chambers (60.7%). These differences in relative NH$_3$ emissions can be explained by the impacts of dietary CP on urine N excretion and therefore differences in urine N applications to the chambers. Urine N additions (21–26 mg) to the LCP chambers were on average 45% less than urine N additions (36–45 mg) to the HCP chambers (Table 1). The impact of high-CP diets on urine N excretions and NH$_3$ emissions from dairy barn floors has been determined under similar laboratory conditions (Misselbrook et al., 2005) and in-barn conditions with dairy cows (Van Duinkerken et al., 2005; Powell et al., 2008; Aguerre et al., 2010c).

Tannin level had differential impacts on NH$_3$ emissions. Of the total excreta N applied to the LCP chambers, the LT and HT treatments lost a similar relative amount of N (14.5%), which was significantly ($P < 0.05$) less than relative N loss (19.0%) from 0T and MT treatments. The impacts of tannins on urine N loss depended on the level of CP fed. Tannin level did not significantly affect relative urine N loss (45.0%) from LCP chambers but did affect emissions from HCP chambers. For the HCP chambers, relative urine N loss from the 0T and LT treatments were similar (53.5%) and significantly ($P < 0.001$) lower than the HT treatment (64.0%). Lower relative urine N loss from 0T and LT compared with HT can be
explained by tannin level impacts on urine N excretion and therefore urine N applications to chambers. Urine N additions to the 0T (18 mg) and LT (26 mg) chambers were on average 39% less than urine N additions to the HT (36 mg) chambers (calculated from Table 1).

**Impacts of Dietary Tannin on Urease Activity in Feces**

The antecedent lactation trial (Aguerre et al., 2010a) and N balance study (Aguerre et al., 2010b) revealed that feeding tannin extract reduced urine N excretion, which partially explained the reductions in NH₃ emissions from simulated dairy barn floors in the present trial. The objective of Trial 2 was to determine if feeding tannin extract also reduced urease activity in dairy feces, which would have contributed to the reductions in NH₃ emissions observed in Trial 1 (Fig. 1 and 2). Ammonia emissions from feces of the MT and HT diets at 24 h were similar and on average 12.5% less than (P < 0.05) NH₃ emissions from feces of the 0T diet (Fig. 3). Likewise, at 36 h, NH₃ emissions from feces of the MT and HT diets were similar and were on average 37.7% less than (P < 0.05) NH₃ emissions from feces of the 0T diet. The significant impacts of feeding tannin extract on reduced urease activity in feces also became evident in cumulative NH₃ loss (Fig. 4). At the 36-h and 48-h sampling times, feces from the MT and HT diets had on average 11.5% less NH₃ loss compared with feces from the 0T diet. Results of Trial 2 indicate that a portion of tannin extract effects on reducing NH₃ loss from simulated barns floors (Fig. 1 and 2) could be attributed to reductions in urease activity in dairy feces.

**Impacts of Direct Application of Tannin Extract on Ammonia Emission**

The objective of Trial 3 was to determine the impact of direct tannin application (rather than feeding it) on NH₃ emissions. Ammonia emissions from HTEx at 24 h were 12.4% less than emissions from 0TEx (P = 0.09) (Fig. 5). The impact of tannin extract application became more significant when cumulative NH₃ losses were considered (Fig. 6). Compared with feces from 0TEx and MTEx, the application of HTEx reduced cumulative NH₃ loss by 16.2% (P < 0.05) at the 36-h sampling period and by 19.0% (P < 0.001) at the 48-h sampling period. Of the total excreta N (95 mg) applied, 20.0% was emitted as NH₃ from HTEx chambers, which was significantly (P < 0.05) less than emissions from 0TEx and MTEx chambers (25.1%). Calculated as a percentage of urine N applied, relative NH₃ emissions (52.7%) from HTEx chambers were significantly (P < 0.05) less than NH₃ emissions (66.2%) from the 0TEx and MTEx chambers.

The precise nature of tannin’s inhibitory effects on urease activity and subsequent reductions in NH₃ emissions is unknown. Tannins have been found, however, to inhibit an array of enzymatic activities in plants, livestock, and soils. For example, during fruit ripening, tannins’ inhibitory effects on enzymatic activity have been linked to two main factors: (i) the formation of substrate–tannin complexes, which prevents attack by enzymes, and (ii) the strong inhibitory effects of tannins alone (Goldstein and Swain, 1965). Tannins have been found to reduce urease activity in Jack bean (*Canavalia ensiformis*) (Hamid, 2010) and lichen (*Evernia prunastri (L.*) (Legaz and Brown, 1983), attributable to the same combinations of factors listed above. Somewhat similar tannin impacts on enzymatic activities have been determined in ruminant livestock. Tannins bind to rumen bacterial surfaces or to forage proteins to reduce enzyme access and activity (Waghorn, 2008). As noted for plants and ruminants, similar factors influence tannin’s inhibitory effects on enzymatic activity in soils (Joanisse et al., 2007).

In the present study, tannin impacts were assessed on two sources of urease: (i) urease contained in fresh feces and (ii) urease present on the barn floor (derived from fresh and previously deposited feces). What is uncertain in our interpretation of the results from Trial 2 is the relative amount of consumed tannin that formed complexes with urease or inhibited urease production pre-excretion versus the amount of tannin that may have remained active postexcretion. Results from Trial 2 (Fig. 3 and 4) and Trial 3 (Fig. 5 and 6) indicate that fed and unfed tannin extract appeared to reduce urease activity in feces.

![Fig. 3. Patterns of NH₃–N emissions from feces of cows fed different levels of tannin extract. 0T, MT, and HT refer to dietary tannin extract levels of 0, 9.0, and 18.0 g kg⁻¹ of diet dry matter intake, respectively. *Significantly different at P < 0.05.](image)

![Fig. 4. Cumulative NH₃–N emissions from feces of cows fed different levels of tannin extract. 0T, MT, and HT refer to dietary tannin extract levels of 0, 9.0, and 18.0 g kg⁻¹ of diet dry matter intake, respectively. *Significantly different at P < 0.05. Cumulative NH₃–N at 48 h with different letters differ significantly (P < 0.05). Numbers in parentheses are SE.](image)
and on barn floors, which apparently contributed to reductions in NH₃ emissions.

Conclusions

The effectiveness of tannin extract in reducing NH₃ emissions from excreta deposited on simulated barn floors can be attributed to two factors: (i) reductions in urinary N excretion by dairy cows (Aguerre et al., 2010b) (Table 1), and therefore the pool of N available for transformation to NH₃, and (ii) reductions in urease activity in feces (Fig. 3 and 4). The direct application of tannin extract (rather than feeding it) to barn floors also appeared to reduce urease activity and NH₃ emissions. The relative effectiveness of feeding tannin in reducing NH₃ emissions depended on the concentration of CP in the diet. Relative reductions in NH₃ emission were greater at LCP than HCP. This difference may be attributed to (i) lower amounts of urine N excreted, and therefore applied to barn floors when cows are fed lower CP diets, and (ii) lower pH of excreta derived from LCP diets. Reductions in NH₃ emission due to decreased urease activity were likely associated with substrate–tannin complexes, which prevented urea attack by urease enzymes, or to an inhibitory effect of the tannin extract. The next step in this research would be to ascertain the impact of feeding tannin or its application directly onto barn floors in larger-scale trials that simulate more closely commercial dairy barn floors.

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References


