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# Dietary Manipulation in Dairy Cattle: Laboratory Experiments to Assess the Influence on Ammonia Emissions

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## ABSTRACT

Improvements to the efficiency of dietary nitrogen use by lactating dairy cattle can be made by altering the concentration and form of protein in the diet. This study collected urine and feces from dairy cows from selected crude protein (CP) treatments of 2 lactation studies. In the first trial, collections were made from cattle fed a diet with high (19.4%) or low (13.6%) CP content (HCP and LCP, respectively). In the second trial, collections were made from cattle fed diets in which the forage legume component was alfalfa (ALF) or birdsfoot trefoil with a low (BFTL) or high (BFTH) concentration of condensed tannins (CT). A system of small laboratory chambers was used to measure $NH_3$ emissions over 48 h from applications of equal quantities of urine and feces to cement (simulating a barn floor) and from applications of slurries, made by combining feces and urine in the proportions in which they were excreted for each treatment, to soil. Reducing dietary CP content resulted in less total N excretion and a smaller proportion of the excreted N being present in urine; urine N concentration was 90% greater for HCP than LCP. Surprisingly, $NH_3$ emissions from the barn floor were similar in absolute terms despite the great differences in urine urea-N concentrations, presumably because urease activity was limiting. Cumulative emissions from fresh slurries applied to soil represented 18% of applied N for both HCP and LCP. Following storage at 20°C for 2 wk, cumulative emissions from LCP were much lower than for HCP, representing 9 and 25% of applied N, respectively. Emissions were also lower when expressed as a proportion of slurry total ammoniacal N (TAN) content (24 and 31%, respectively) because of treatment differences in slurry pH. Increasing CT content of the dietary forage legume component resulted in a shift in N excretion from urine to feces. Cumulative $NH_3$ emissions from the barn floor were greater for ALF than for BFTL or BFTH. Emissions from fresh and stored slurries were in proportion to slurry TAN contents, with approximately 35% of applied TAN being lost for all treatments. Emissions expressed as a proportion of total N applied were consistently lower for BFTH than for ALF.
(**Key words:** dietary manipulation, crude protein, tannin, ammonia emission)

**Abbreviation key: ALF** = alfalfa, **BFTH** = birdsfoot trefoil with high tannin concentration, **BFTL** = birdsfoot trefoil with low tannin concentration, **CT** = condensed tannins, **HCP** = high CP diet, **LCP** = low CP diet, **TAN** = total ammoniacal N.

## INTRODUCTION

Dairy cows use feed N much more efficiently than many other livestock, but they excrete 3 times more N in manure than in milk. An average cow producing 8200 kg of milk annually excretes 21,000 kg of manure containing about 110 kg of N (van Horn et al., 1996), with approximately equal proportions excreted in feces and urine. The majority of urinary N (depending on diet and animal condition) is in the form of urea, which is hydrolyzed by fecal urease to $NH_3$. About 25% of dairy manure N is lost as $NH_3$ under current US practices (Pinder et al., 2004), contributing to the total annual $NH_3$ redeposition rates in the Upper Midwest of 23 to 40 kg of N/ha (Burkart and James, 1999). Environmental and potential human health impacts occur both from the relatively local redeposition of $NH_3$ and from aerosols that travel greater distances (Dockery et al., 1993; Davidson and Mosier, 2004).

Ammonia losses begin directly after urine deposition in the dairy barn and continue throughout manure handling, storage, and land application. Most efforts to reduce nutrient loss from dairy operations have focused on improved methods for land application of manure, where a large impact can be made at relatively low cost (Misselbrook et al., 1996; Smith et al., 2000; Huijsmans et al., 2001; Misselbrook et al., 2002; Thompson and Meisinger, 2002). However, reducing N excretion through dietary manipulation represents another opportunity where large impacts could be made, as subse-

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quent losses would be reduced throughout the manure management continuum, particularly if combined with other abatement strategies (e.g., at manure application).

A number of dietary studies have shown that reducing the CP content of the diet, above that needed to meet requirements, leads to better efficiency of N use i.e., a higher proportion of N intake is secreted in milk N and a lesser proportion excreted in urine and feces (Krober et al., 2000; Kulling et al., 2001; Broderick, 2003). Reducing urinary N excretion should lead to reductions in subsequent $NH_3$ emissions. Kebreab et al. (2002) presented a model of N metabolism for a lactating dairy cow that predicted significant reductions in $NH_3$ emissions (based on modeled urea-N outputs) from cattle associated with reducing CP content or increasing energy content of the diet. A number of studies using laboratory chamber systems measuring $NH_3$ emissions from slurries (mixtures of urine and feces) have shown reductions in $NH_3$ emission associated with lower CP content of the diet (Paul et al., 1998; James et al., 1999; Kulling et al., 2001; Frank and Swensson, 2002), as might be expected. However, Paul et al. (1998), working with dairy cattle, and Misselbrook et al. (1998), working with pigs, showed that diet might influence other manure characteristics, such as pH, thereby influencing the proportion of N that is lost as $NH_3$.

Brito and Broderick (2003) found that an equal mix of forage from alfalfa silage with corn silage in lactating dairy cows' diet gave the greatest improvement in N efficiency, without loss of yield of milk, fat, and protein, compared with diets dominated by either one of these forages. Beyond the improvements seen with proper mixes of alfalfa and corn silage, the feeding value of perennial forages is enhanced by condensed tannins (**CT**) and polyphenols, which are lacking in most feeds used in the United States. Modest amounts of CT (2 to 4% of DM), as is found in birdsfoot trefoil (*Lotus corniculatus*), reduce protein breakdown during ensiling and rumen fermentation by up to 50% (Albrecht and Muck, 1991; Broderick and Albrecht, 1997). Studies with sheep indicate that modest concentrations of tannin permit extensive protein digestion in the abomasum and small intestine, and greater subsequent absorption of amino acids, without adversely affecting feed consumption or digestion (Min et al., 2003). In a New Zealand study, tannins in birdsfoot trefoil increased milk production of nonsupplemented Holstein cows by 2.7 kg/d (Woodward et al., 1999). In addition to enhancing protein use by ruminants, experiments with forage and browse in Africa suggest that tannins and polyphenols shift N excretion from urine to feces and from soluble to insoluble N forms in feces (Powell et al., 1994).

Two recent trials were conducted to assess the influence of dietary protein concentration (manipulating the CP content of the diet) or protein form (different concentrations of CT in the forage legume component of the diet) on the performance of lactating dairy cows. Details of these studies are reported elsewhere (Olmos Colmenero and Broderick, 2003, 2004; Hymes-Fecht et al., 2004). Briefly, Olmos Colmenero and Broderick (2003) showed that poorer N use was associated with diets higher in CP, with no significant increase in milk yield for an increase in dietary CP content from 15 to 19%. Hymes-Fecht et al. (2004) suggested that improved use of CP in the forage legume component of the diet was associated with an increased concentration of CT in the silage. The objectives of the present study were to assess (using urine and feces from the above trials and a system of laboratory chambers) the influence of manipulating dairy cattle dietary protein concentration and form on $NH_3$ emissions from urine and fecal deposits to a concrete floor and from fresh and stored slurries applied to soil.

## MATERIALS AND METHODS

### Dietary Treatments

Urine and feces were collected from lactating Holstein cows housed in a tie-stall system, from 2 dietary trials varying either in dietary protein concentration or protein form. Urine and feces collections were made from a randomly selected subgroup of the cows on each diet after completion of the lactation trials, with each subgroup continuing to be fed the treatment diet until the completion of urine and feces collection.

In the first trial, cattle were fed diets with high (19%) or low (14%) CP content (treatments **HCP** and **LCP**, respectively) with 2 cows per dietary treatment. In the second trial, cows were fed diets of similar composition, with the exception of the forage legume component, which was alfalfa (**ALF**; *Medicago sativa*), or birdsfoot trefoil with low tannin (~2% of the forage, 1% of the total diet on a DM basis, **BFTL**), or high tannin (~7% of the forage, 3.5% of the total diet on a DM basis, **BFTH**) content, with 3 cows per diet. Details of the diets for both trials are given in Table 1. Total feces and urine were collected separately from the cows while in the tie stalls (i.e., excluding periods when the cows were being milked) over a period of 60 to 100 h. Feces were scraped by hand from metal catchment containers fitted into the tie-stall gutters; urine was collected via indwelling catheter tubes draining into plastic containers embedded in ice. Volume of urine and mass of feces were recorded on an individual cow basis and subsamples of material were retained for total N analyses. Composite fecal and urine samples for each dietary

**Table 1.** Composition of the diets used in the dietary protein concentration and protein form manipulation trials for lactating dairy cattle from which urine and feces samples were collected.

| | Dietary protein concentration trial | | Dietary protein form trial | | |
|---|---|---|---|---|---|
| Ingredient[1] | Low CP | High CP | Alfalfa | BFT,[2] low tannin | BFT, high tannin |
| Alfalfa silage | 25 | 25 | 50 | | |
| BFT, low tannin | | | | 50 | |
| BFT, high tannin | | | | | 50 |
| Corn silage | 25 | 25 | 10 | 10 | 10 |
| Rolled high-moisture shelled corn | 44.0 | 30.4 | 34.6 | 33.5 | 33.5 |
| Roasted soybeans | 2.5 | 2.5 | | | |
| Solvent-extracted soybean meal | 2.4 | 16.0 | 4.7 | 5.8 | 5.8 |
| Sodium bicarbonate | 0.6 | 0.6 | 0.3 | 0.3 | 0.3 |
| Salt | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Dicalcium phosphate | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 |
| Vitamin and minerals | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Chemical composition of TMR | | | | | |
| DM, % | 53.8 | 55.0 | 45.0 | 45.1 | 43.2 |
| N, % | 2.2 | 3.1 | 2.7 | 2.5 | 2.6 |
| CP, % | 13.6 | 19.4 | 17.1 | 15.8 | 16.4 |
| NDF, % | 26.2 | 26.2 | 26.1 | 26.0 | 26.3 |

[1]Percentage on a DM basis.
[2]BFT = Birdsfoot trefoil.

treatment were frozen after collection until required for the laboratory trials.

## Laboratory Chambers for Ammonia Emission Measurement

The laboratory set-up consisted of 6 chambers in which the manure was exposed to a constant airflow (Figure 1), similar to the system described by Chadwick et al. (2001). Air was drawn through the system by means of a vacuum pump, with the airflow rate through each chamber being controlled at 4 L/min. An acid trap (containing 0.075 L of 0.02 *M* orthophosphoric acid) before each chamber removed $NH_3$ from inlet air and a second acid trap on the outlet side of each chamber collected any $NH_3$ emitted during the measurement period. Glass or fluorinated ethylene propylene (FEP) tubing was used between the chamber and outlet acid trap to minimize adsorption of $NH_3$ to tubing walls. The set-up was housed in a large incubator such that all experiments were conducted at a constant temperature (15°C).

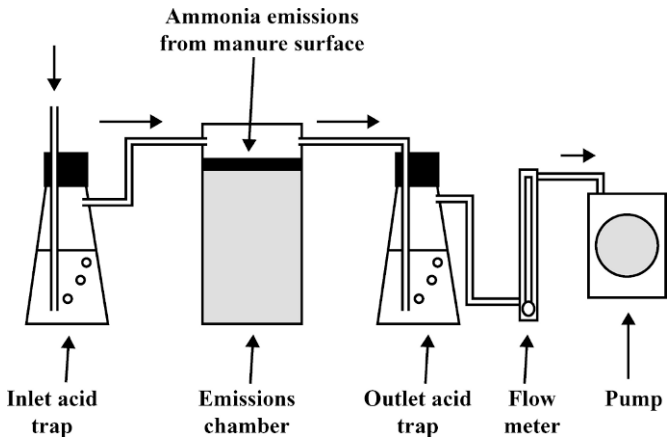


**Figure 1.** Schematic diagram of laboratory set-up of chambers for ammonia emission measurements.

The chambers were constructed from plastic drainage pipe of 10 cm diameter and 19 cm height. An end-cap was glued to the base of the chamber and a lid fitted to the top, with silicone grease used to ensure an air-tight seal. The internal surfaces of the lid were sprayed with Teflon coating to minimize adsorption of $NH_3$. Each chamber lid had 4 horizontally positioned inlet and outlet ports to ensure good mixing of air within the chamber. The main body of the chamber was filled with cement (to simulate a barn floor) or soil, leaving a headspace of approximately 0.35 L. The flow rate through the chamber (4 L/min) ensured that the number of headspace changes per minute was such that the emission rate would not be greatly influenced by small differences in flow rates between chambers (Kissel et al., 1977).

Tests were conducted to assess the quantitative recovery of $NH_3$ emitted from a solution within the chamber by the acid traps. Two acid traps were connected in series on the chamber outlets to determine whether a single acid trap was sufficient to trap all $NH_3$ in outflow air. Recovery tests were performed by placing

a shallow Petri dish containing 0.02 L of ammonium sulfate solution (2 g/L of N) in each chamber. The solution pH was raised by adding 1 mL of sodium carbonate/sodium bicarbonate mixture (1 *M*) through a port in the chamber lid, to promote $NH_3$ volatilization. The system was run with airflow of 4 L/min for a 4-h period. To stop volatilization, 1 mL of 2 *M* sulfuric acid was added to the solution in the chamber via the port in the chamber lid. Samples of the initial and final solutions in the Petri dishes within each chamber and the solutions in the outlet acid traps were analyzed for ammonium-N by automated colorimetry (Searle, 1984).

### Emissions from Simulated Deposits to Barn Floor

Deposits of urine and feces to a barn floor would normally be scraped, leaving a thin layer from which emission occurs. In the simulation experiments, therefore, a constant mass of feces (8 g) and volume of urine (8 mL) were applied to the chambers to achieve a thin emitting layer of approximately 1 mm above the cement surface [similar to the methodology used by Elzing and Monteny (1997)]. Immediately after adding the urine, the chamber lid was closed and sealed with silicon grease, and the airflow through the system started. Acid traps were changed after 1, 3, 6, 12, 24, and 36 h, and measurement was stopped at 48 h. At the end of each sampling period, acid from the outlet acid traps was made up to 0.1 L with deionized water and then analyzed for ammonium-N using automated colorimetry (Searle, 1984). Three replicate chambers were used for each of the selected dietary treatments. Samples of feces and urine were retained for chemical analyses.

Ammonia emission rates (*F*, mg of N/$m^2$ per h) for each sampling period were calculated as:

$$F = \frac{XV}{At} \quad [1]$$

where *X* is ammoniacal-N concentration of the acid trap solution (mg/L), *V* is the volume of acid trap solution (L), *A* is the exposed surface area of the chamber ($m^2$), and *t* is the duration of the sampling period (h). The total emission for the period (mg of N) is calculated as *XV*, and total emission for the duration of the experiment (48 h) is derived by summing emissions for each sampling period. Total emission was expressed as a proportion of the total N, urine N, or urea N applied to each chamber.

### Emissions from Slurry Applied to Soil

For simulated emissions from land applications, the urine and feces from each selected treatment were mixed in the proportions in which they were excreted to produce slurries, which were then standardized at 7% DM content by the addition of water. Two experiments were conducted in which $NH_3$ emission measurements were made from fresh (stored for 24 h at 4°C) or stored (2 wk at ambient temperature, mean 20°C) slurries applied to soil in the laboratory chambers. The chambers were packed with a sieved (to 2 mm) silt loam soil of the Plano series (Munoz et al., 2003) at a bulk density of 1.2 g/$cm^3$, leaving 0.35 L of headspace. Water was added to the soil to achieve 60% water-filled pore space. Following addition of water, the chambers were left for 24 h at 15°C to equilibrate before slurry application.

Slurry was applied to the soil at a standard rate of 40 mL to each chamber, equivalent to a field application rate of 50 $m^3$/ha. Lids were replaced and measurements commenced immediately after slurry application to each chamber. Measurement continued for 48 h, with acid traps being replaced after 1, 3, 6, 12, 24, and 36 h. Emission rates for each period and cumulative emissions were calculated as described above. Three replicate chambers were used for each of the slurry treatments. Samples of slurry were retained for chemical analyses.

### Chemical Analyses

Samples of feces used in the barn-floor simulation studies were analyzed in triplicate for DM content, pH, total N, total ammoniacal N (**TAN**), and undigested feed N content. Dry matter content was determined by drying in an oven to constant weight at 100°C. The pH of a water/feces mixture (2:1 ratio) was measured using a calibrated portable pH meter (Accumet AP61, Fisher Scientific, Pittsburgh, PA). Acidified samples of feces were freeze-dried and ground for total N determination by combustion assay (Leco FP-2000 nitrogen analyzer, Leco, St. Joseph, MI). Total ammoniacal N content was determined by automated colorimetry (Searle, 1984) following KCl extraction (5 g of feces in 50 mL of 2 *M* KCl, shaken for 2 h and filtered through Whatman no. 42 filter; Fisher Scientific). Cell wall components of feces were determined using the detergent system (Goering and van Soest, 1970) as NDF, and the N content of the NDF was determined by combustion assay (Leco FP-2000 nitrogen analyzer).

Samples of urine used in the barn-floor simulation studies were analyzed in triplicate for pH, total N, TAN, and urea N content. Following pH determination, samples were acidified (60 mL of 0.07 *N* $H_2SO_4$ and 15 mL of urine) before subsequent analyses. Total N was measured by combustion assay (Elementar Vario MAX CN analyzer, Elementar, Hanan, Germany), with 200 mg of sucrose being added to the 2.5-mL urine sample

**Table 2.** Proportion of N excretion in urine and feces as influenced by dietary protein concentration or form in lactating dairy cattle. Values are the mean for the 2 (protein concentration trial) or 3 (protein form trial) cows on each diet.

| | Dietary protein concentration trial | | Dietary protein form trial | | |
|---|---|---|---|---|---|
| | Low CP | High CP | Alfalfa | BFT,[1] low tannin | BFT, high tannin |
| Urine [total N], g/L | 4.5[b] | 8.5[a] | 7.5[b] | 8.8[a] | 6.7[b] |
| Feces [total N], g/kg of DM | 20.6 | 24.4 | 26.7 | 29.5 | 33.1 |
| Urine volume,[2] L/h | 1.09 | 1.05 | 0.93 | 1.09 | 1.07 |
| Feces volume,[2] kg of DM/h | 0.21 | 0.17 | 0.20 | 0.21 | 0.30 |
| Relative proportion of total N in | | | | | |
| Urine, % | 52[b] | 68[a] | 55[a] | 60[a] | 40[b] |
| Feces, % | 48[a] | 32[b] | 45[b] | 40[b] | 60[a] |

[a,b]Within each trial, values with different superscripts are significantly different ($P < 0.05$).

[1]BFT = Birdsfoot trefoil.

[2]Based on total amount collected per cow over the 60- to 100-h collection period, which excluded times when cows were being milked.

to aid combustion. Total ammoniacal N was measured following KCl extraction, as for the fecal samples. Urea N was determined using an automated colorimetric assay (Broderick and Clayton, 1997) adapted to a flow-injection analyzer.

Slurry samples were analyzed in triplicate for DM content, pH, total N, and TAN content, using the same procedures as for the fecal samples.

### Statistical Analyses

For each of the individual chamber measurements, a Michaelis-Menten type curve was fitted to the cumulative $NH_3$ loss with time, as used by Sommer and Ersboll (1994):

$$N(t) = N_{\max} \frac{t}{t + K_m} \quad [2]$$

where $N(t)$ (kg of N per ha) is the cumulative loss at time $t$ (h), and $N_{max}$ (kg of N per ha) and $K_m$ (h) are model parameters representing total loss as time approaches infinity and time at which loss reaches one-half of maximum, respectively. For each manure application, the parameters $N_{max}$ and $K_m$ were derived using the model-fitting procedure in GENSTAT (Lawes Agricultural Trust, 1993). Mean cumulative losses after 6, 12, 24, and 48 h and $N_{max}$ for the simulated barn floor trials and 6, 24, and 48 h and $N_{max}$ for the slurry to soil trials were compared between treatments (within the protein concentration or protein form trial) using the 1-way ANOVA procedure in GENSTAT (Lawes Agricultural Trust, 1993)

## RESULTS

### Laboratory Chamber Recovery Tests

Mean recovery of $NH_4^+$–N over 7 recovery tests was 97% (standard error = 1.0%), with a range from 92.1 to 100.2%. Mean capture of $NH_4^+$–N in the first of 2 acid traps on the outlet side of each chamber was 99.5% of the total captured in both acid traps, indicating that a single acid trap on each outlet was sufficient for measurements.

### Nitrogen Excretion

During the dietary protein concentration trial, urine N concentration in HCP was almost twice that in LCP (Table 2). There were no significant differences between the protein concentration treatments in fecal N concentrations or in the volumes of urine and mass of feces collected over the collection period ($P > 0.05$). The greater N concentrations in urine for HCP resulted in a shift in the relative proportion of N excreted in urine or feces from approximately equal amounts in LCP to a much greater proportion in the urine for HCP. Based on the concentration and volume outputs, mean hourly total N excretion per cow over the collection period was 30% lower for LCP than HCP ($P < 0.05$), with respective values of 9.2 and 13.1 g/cow per h. Urine N excretion was 45% higher ($P < 0.05$) for HCP than for LCP (8.9 vs. 4.9 g/cow per h, respectively).

From the protein form trial, urine N concentration was highest in BFTL, with no significant differences between that of ALF and BFTH (Table 2). There were no significant dietary effects ($P > 0.05$) on total N concentration in the feces or in the volumes of urine and mass of feces collected. Thus a greater proportion of the

**Table 3.** Analyses of composite urine and feces samples used in the ammonia emission studies.[1]

| | Dietary protein concentration trial | | Dietary protein form trial | | |
|---|---|---|---|---|---|
| | Low CP | High CP | Alfalfa | BFT,[2] low tannin | BFT, high tannin |
| Urine | | | | | |
| pH | 9.0 (0.01) | 8.8 (0.03) | 7.8 (0.02) | 7.8 (0.02) | 7.9 (0.02) |
| Total N, g/L | 4.50 (0.007) | 9.35 (0.041) | 6.38 (0.260) | 5.41 (0.016) | 5.57 (0.022) |
| Urea N, g/L | 1.91 (0.019) | 5.83 (0.563) | 4.23 (0.357) | 3.70 (0.075) | 3.54 (0.009) |
| TAN,[3] g/L | 0.43 (0.161) | 0.23 (0.090) | 0.43 (0.016) | 0.33 (0.006) | 0.26 (0.005) |
| Feces | | | | | |
| pH | 6.5 (0.03) | 6.8 (0.04) | 6.6 (0.02) | 6.6 (0.04) | 6.6 (0.08) |
| DM, % | 17.9 (0.35) | 18.0 (0.08) | 14.6 (0.10) | 14.8 (0.14) | 14.6 (0.41) |
| Total N, g/kg of DM | 26.9 (0.47) | 28.4 (0.53) | 23.8 (0.09) | 22.8 (0.36) | 24.7 (0.10) |
| TAN, g/kg of DM | 1.80 (0.730) | 0.90 (0.860) | 3.42 (0.090) | 2.95 (0.050) | 3.52 (0.070) |
| NDF N, g/kg of DM | 2.35 (0.244) | 2.16 (0.100) | 2.37 (0.090) | 2.95 (0.069) | 3.60 (0.088) |

[1]Values in parentheses are standard errors of the mean (n = 3).

[2]BFT = Birdsfoot trefoil.

[3]TAN = Total ammoniacal N.

N was excreted in the urine for ALF and BFTL and in the feces for BFTH (Table 2). Total N excretion per cow over the collection period did not differ significantly between treatments ($P > 0.05$), averaging 12.3, 15.8, and 17.1 g/cow per h for ALF, BFTL, and BFTH, respectively. Urine N excretion was significantly greater ($P < 0.05$) from BFTL than from ALF or BFTH (9.6 vs. 7.0 and 7.2 g/cow per h, respectively).

## Ammonia Emissions from Simulated Deposits to Barn Floor

***Protein concentration.*** Analyses of the urine used in the simulated barn floor emissions trials showed that HCP had a significantly higher total N and urea N concentration (Table 3). It should be noted that the urine and fecal N concentrations given in Table 3 are for composite samples of material and differ from the averages of individual animal values as given in Table 2. The proportion of urine N as urea N was also higher in HCP (62% compared with 42% for LCP). There were no significant differences between LCP and HCP in terms of fecal analyses, with the exception of pH. For both urine and feces, differences in pH were statistically significant, but small in absolute terms and likely to have been of little consequence in influencing $NH_3$ emissions.

Cumulative $NH_3$ emissions from the urine and feces in the chambers over the 48-h measurement period were not significantly different ($P > 0.05$) between LCP and HCP (Figure 2a). Thus when expressed as a proportion of either the total N applied (Figure 2b) or urea N applied (Figure 2c), losses were significantly greater from LCP as the urine from the LCP treatment had a lower total N and urea N concentration. Projected $N_{max}$ values could not be derived using equation [2] because there was insufficient curvature within the cumulative emission against time relationship to 48 h.

***Protein form.*** For the manure spread in the chambers, urine total N concentration was greater for ALF than for BFTL or BFTH, which were not significantly different (Table 3). There were no differences in urea N concentrations, but urine TAN concentration decreased with the increasing concentration of CT in the forage legume. There were small differences in fecal total N concentration, with that from BFTL being lower, and an increase in NDF-N content with increasing CT content of the forage legume, suggesting a greater amount of undigested feed N in those diets. Urine and fecal pH values were similar, as were fecal DM and TAN contents.

Cumulative $NH_3$ emission over the 48-h measurement period was significantly greater ($P < 0.05$) from ALF than from BFTL and BFTH, which were not significantly different in absolute terms or when expressed as a proportion of the total N or urea N applied (Figure 3). As the cumulative emission curves were of similar shapes, the predicted $N_{max}$ values were also higher for ALF than the other 2 treatments (Table 4).

## Ammonia Emissions from Slurry Applications to Soil

***Protein concentration.*** The DM content of the prepared fresh slurries was greater than the target value of 7% (Table 5). Differences in DM content and pH between the treatments were small in absolute terms. Total N and TAN concentrations were greater for HCP, although TAN represented a greater proportion of total N for LCP than HCP, with respective values of 33 and

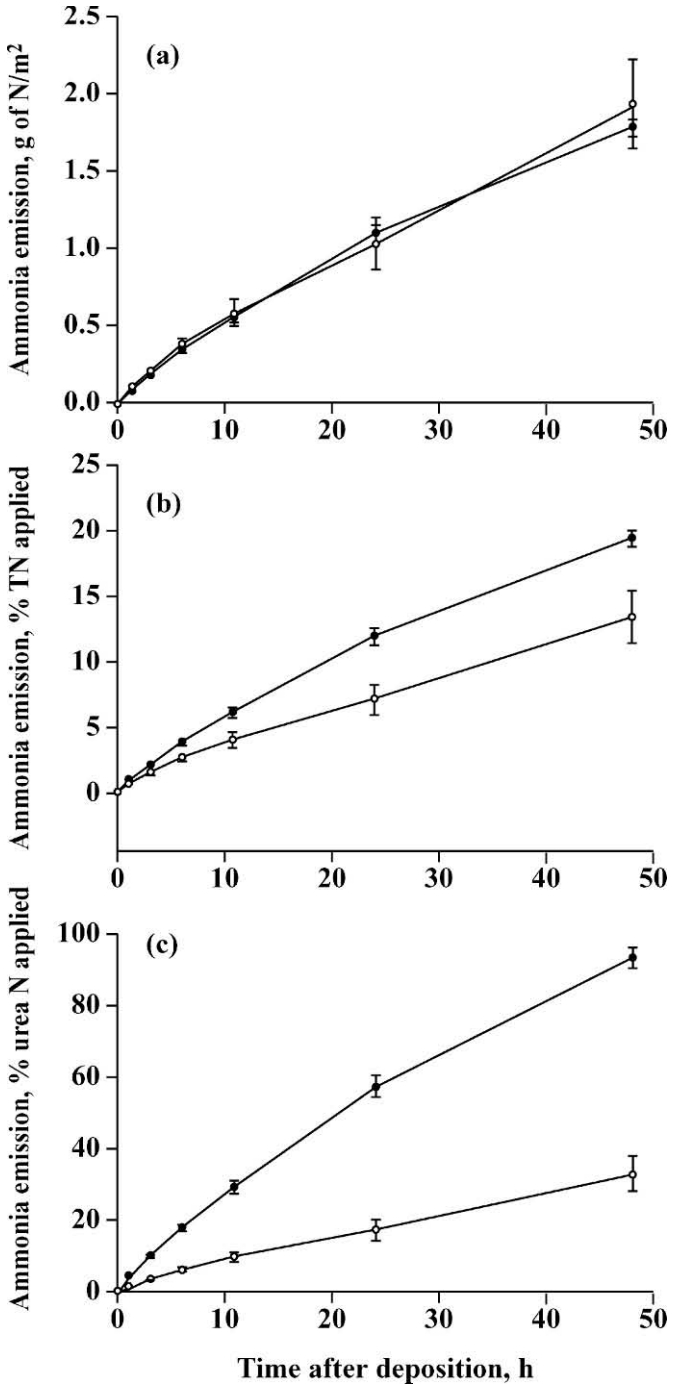


**Figure 2.** Influence of dietary CP content on ammonia emissions from dairy cattle urine (8 mL) and feces (8 g) deposited on a simulated barn floor: a) expressed as g of $N/m^2$; b) as percentage of total N applied; c) as percentage of the urea N applied. Dietary CP contents: 13.6% (●) and 19.4% (○). Error bars show ± 1 SE (n = 3).

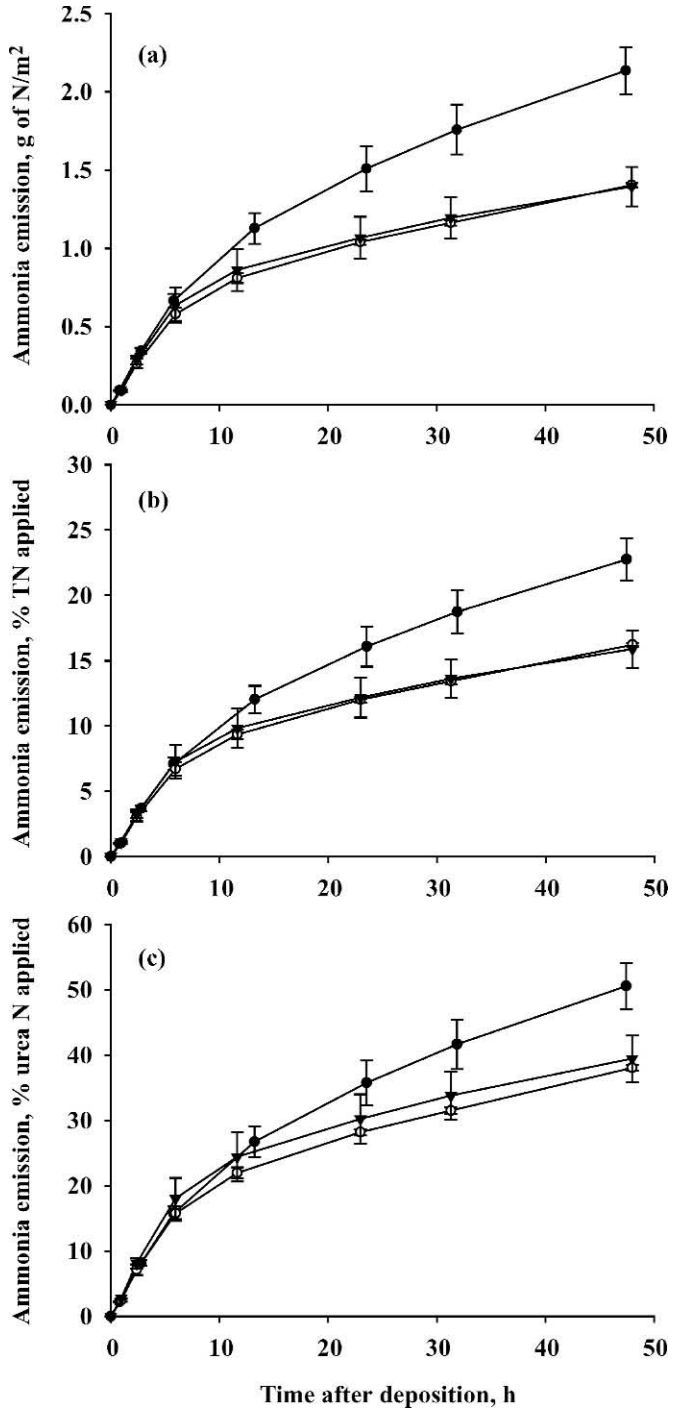


**Figure 3.** Influence of dietary protein form on ammonia emissions from dairy cattle urine (8 mL) and feces (8 g) deposited on a simulated barn floor: a) expressed as g of $N/m^2$; b) as percentage of total N applied; c) as percentage of the urea N applied. Dietary forage legume component: alfalfa (●); birdsfoot trefoil with low tannin content (○); birdsfoot trefoil with high tannin content (▼). Error bars show ± 1 SE (n = 3).

**Table 4.** Predicted maximum cumulative ammonia emissions ($N_{max}$), estimated from a fitted Michaelis-Menten function to the cumulative emissions curve, for urine and feces applied to a simulated barn floor and for fresh and stored slurries applied to soil.

| | Dietary protein concentration trial | | Dietary protein form trial | | |
|---|---|---|---|---|---|
| | Low CP | High CP | Alfalfa | BFT, low tannin | BFT, high tannin |
| Barn floor simulation | | | | | |
| g/m$^2$ | ND[3] | ND | 3.15[a] | 1.74[b] | 1.66[b] |
| % of total N applied | ND | ND | 34[a] | 20[b] | 19[b] |
| % of urea N applied | ND | ND | 75[a] | 47[b] | 47[b] |
| Fresh slurry to soil | | | | | |
| g/m$^2$ | ND | ND | 3.69[a] | 3.58[a] | 2.72[b] |
| % of total N applied | ND | ND | 31[a] | 33[a] | 25[b] |
| % of TAN[2] applied | ND | ND | 45 | 44 | 48 |
| Stored slurry to soil | | | | | |
| g/m$^2$ | 1.42[b] | 4.80[a] | 3.94[a] | 3.09[b] | 2.55[b] |
| % of total N applied | 12[b] | 29[a] | 30[a] | 23[b] | 19[b] |
| % of TAN applied | 32 | 36 | 45 | 41 | 47 |

[a,b]Within each trial, values with different superscripts are significantly different ($P < 0.05$).

[1]BFT = Birdsfoot trefoil.

[2]TAN = Total ammoniacal N.

[3]ND = Not determined.

24%. After 2 wk of storage, pH had increased in HCP (Table 5). Dry matter contents had decreased for both treatments. Total N and TAN concentrations were greater for HCP and there was a substantial change in the proportion of the total N represented by TAN, with values of 38 and 82% for LCP and HCP, respectively. Pre- and poststorage volume measurements were not made, so it was not possible to determine N loss during storage.

Cumulative $NH_3$ emissions over 48 h from the application of fresh slurries to soil were significantly greater ($P < 0.05$) for HCP than LCP, both in absolute terms and when expressed as a percentage of the initial TAN concentration, but not when expressed as a proportion of the total slurry N content (Figure 4). There were differences in the shapes of the cumulative emission curves, with that for HCP still rising steeply after 48 h. Projected $N_{max}$ values could not be derived using equation [2] because there was insufficient curvature within the cumulative emission against time relationship to 48 h. Cumulative emissions over 48 h from the application of stored slurries to soil were also significantly greater ($P < 0.05$) for HCP, in absolute terms and as a proportion of the initial total N or TAN (Figure 5). Predicted maximum emission from HCP as a proportion of the total N applied was more than twice that

**Table 5.** Analyses of slurries derived from urine and feces samples collected from lactating dairy cows fed diets varying in protein concentration and protein form.[1]

| | Dietary protein concentration trial | | Dietary protein form trial | | |
|---|---|---|---|---|---|
| | Low CP | High CP | Alfalfa | BFT,[2] low tannin | BFT, high tannin |
| Fresh slurry | | | | | |
| pH | 7.7 (0.02) | 8.1 (0.01) | 8.5 (0.02) | 8.3 (0.02) | 8.0 (0.02) |
| DM (%) | 7.7 (0.21) | 8.4 (0.20) | 7.9 (0.25) | 7.6 (0.01) | 7.4 (0.16) |
| Total N, g/L | 3.02 (0.031) | 4.97 (0.044) | 2.42 (0.051) | 2.18 (0.014) | 2.19 (0.018) |
| TAN,[3] g/L | 1.01 (0.028) | 1.20 (0.036) | 1.63 (0.020) | 1.61 (0.012) | 1.15 (0.034) |
| Stored slurry | | | | | |
| pH | 7.6 | 8.7 | 8.0 | 7.6 | 7.5 |
| DM (%) | 4.7 (0.73) | 6.3 (0.44) | 6.5 (1.11) | 5.6 (1.47) | 6.5 (0.35) |
| Total N, g/L | 2.37 (0.064) | 3.27 (0.071) | 2.61 (0.008) | 2.70 (0.044) | 2.68 (0.013) |
| TAN, g/L | 0.90 (0.021) | 2.69 (0.154) | 1.74 (0.030) | 1.50 (0.049) | 1.10 (0.001) |

[1]Values in parentheses are standard errors of the mean (n = 3).

[2]BFT = Birdsfoot trefoil.

[3]TAN = total ammoniacal N.

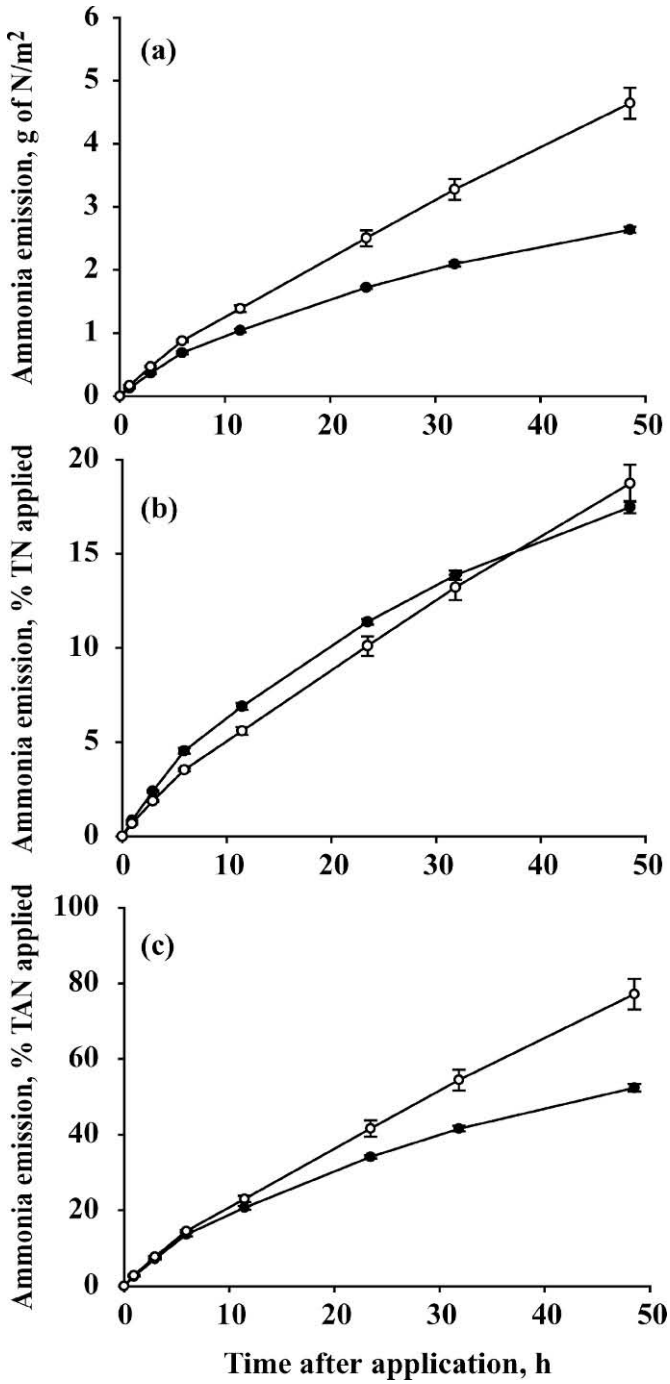


**Figure 4.** Influence of dietary CP content on ammonia emissions from fresh slurry applied to soil: a) expressed as g of $N/m^2$; b) as percentage of total N applied; c) as percentage of the total ammoniacal N (TAN) applied. Dietary CP contents: 13.6% (●) and 19.4% (○). Error bars show ± 1 SE (n = 3).

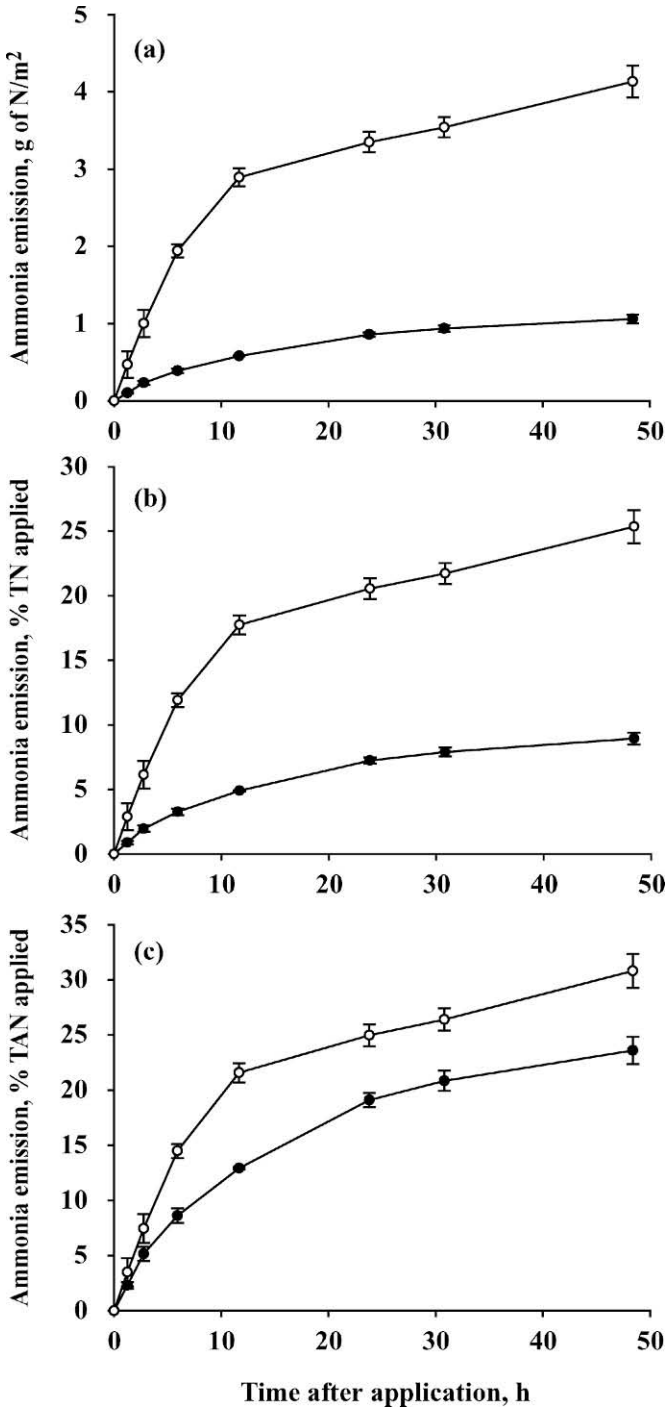


**Figure 5.** Influence of dietary CP content on ammonia emissions from stored slurry applied to soil: a) expressed as g of $N/m^2$; b) as percentage of total N applied; c) as percentage of the total ammoniacal N (TAN) applied. Dietary CP contents: 13.6% (●) and 19.4% (○). Error bars show ± 1 SE (n = 3).

for LCP but as a proportion of the TAN applied, there was no significant difference ($P > 0.05$) between treatments, with a mean loss across both treatments of approximately 34% of applied TAN.

***Protein form.*** There were no differences in the DM content of the fresh slurries prepared from the urine and feces from the protein form trial (Table 5) although

target DM content of 7% was marginally exceeded. There were small differences in fresh slurry pH, with the pH declining with increasing CT content of the dietary forage legume. Total N content was greater for ALF than for either BFTL or BFTH, whereas TAN content was similar in ALF and BFTL, both being greater than for BFTH. Total ammoniacal N expressed as a proportion of total N content was therefore greatest in BFTL (74%) and least in BFTH (52%). After 2 wk of storage, slurry DM contents were lower than for the fresh slurries with no differences between treatments, with a mean value of 6.2%. Slurry pH was lower for the BFTL and BFTH treatments than for ALF. Total N concentrations were similar, but TAN content declined with increasing CT content, so TAN expressed as a proportion of total N also declined with increasing condensed tannin content with values of 67, 56, and 41% for ALF, BFTL, and BFTH, respectively.

Cumulative $NH_3$ emissions over 48 h following application of the fresh slurries to soil were significantly greater ($P < 0.05$) for ALF and BFTL than for BFTH in absolute terms and as a proportion of the total N applied, but there were no treatment differences as a proportion of TAN applied (Figure 6). The cumulative emission curve shapes were similar between treatments and the predicted $N_{max}$ values followed the same pattern (Table 4). Following application of the stored slurries, cumulative emissions over 48 h were significantly greater ($P < 0.05$) from ALF than either BFTL or BFTH in absolute terms and as a proportion of total N applied, but again, there were no significant differences ($P > 0.05$) when expressed as a proportion of the TAN applied (Figure 7). Again, similarities in the emission curve shapes meant that treatment effects on predicted $N_{max}$ values (Table 4) were the same as those on cumulative emissions at 48 h.

## DISCUSSION

Nitrogen excretion was reduced by 30% and urinary N excretion by 45% when dietary CP content was lowered from 19.4 to 13.6%. These values are not based on a full daily collection of urine and feces and the possibility that there were differences in excretal volumes while the cows were away from the stalls cannot be excluded. In addition, the mean hourly rate of excretal output may have been different while the cows were being moved and milked, so mean daily output values were not predicted from our data. However, these results confirm the work of others that N excretion can be reduced by lowering dietary CP content and that the reduction is predominantly in the urea N content of the urine (Krober et al., 2000; Kulling et al., 2001; Broderick, 2003). The magnitude of the reduction in urinary N excretion was not as large as that reported by Castillo et al. (2000), who concluded from a number of published studies that reducing CP content from 20 to 15% would

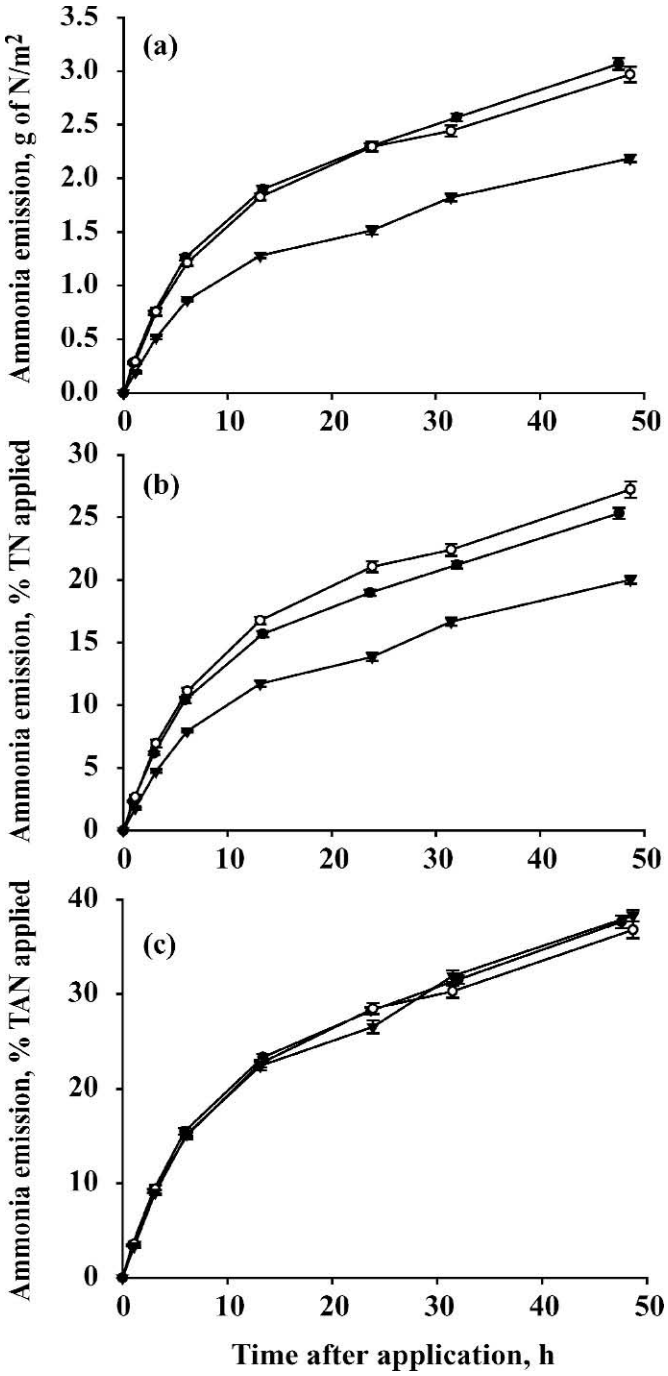


**Figure 6.** Influence of dietary protein form on ammonia emissions from fresh slurry applied to soil: a) expressed as g of $N/m^2$; b) as percentage of total N applied; c) as percentage of the total ammoniacal N (TAN) applied. Dietary forage legume component: alfalfa (●); birdsfoot trefoil with low tannin content (○); birdsfoot trefoil with high tannin content (▼). Error bars show ± 1 SE (n = 3).

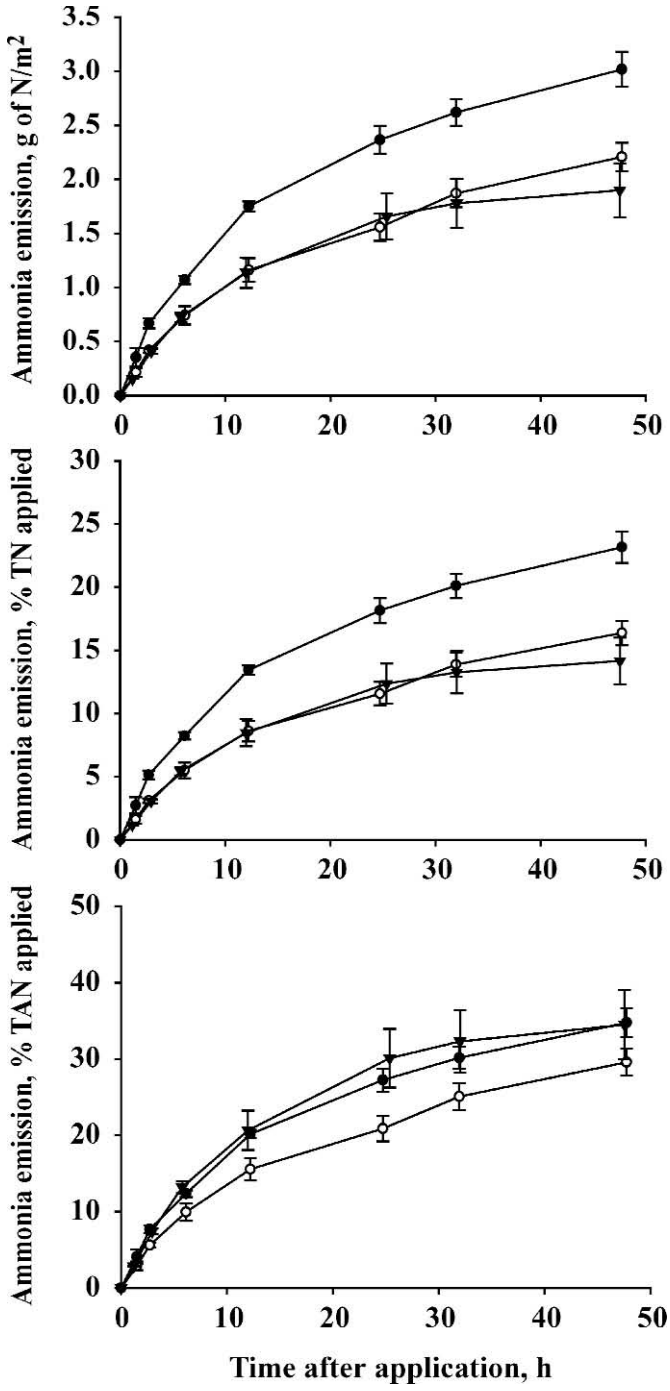


**Figure 7.** Influence of dietary protein form on ammonia emissions from stored slurry applied to soil: a) expressed as g of $N/m^2$; b) as percentage of total N applied; c) as percentage of the total ammoniacal N (TAN) applied. Dietary forage legume component: alfalfa (●); birdsfoot trefoil with low tannin content (○); birdsfoot trefoil with high tannin content (▼). Error bars show ± 1 SE (n = 3).

result in a 66% reduction in urinary excretion. Castillo et al. (2000) also reported reductions in fecal N excretion of up to 21%, but no significant reductions were found in the present study. Although not assessed in this study, increasing the energy content of the diet may improve efficiency of N use (e.g., Broderick, 2003), and replacing grass forage with maize or concentrates has been shown to improve N use (Valk, 1994; Kulling et al., 2003).

Increasing the CT content of the dietary forage legume component did not reduce total N excretion; indeed, it appeared to have the opposite effect, but the shift from urinary to fecal excretion between the BFTL and BFTH treatments was obvious. There were some differences in the CP content of the diets, with that for ALF being greater than that for the birdsfoot trefoil treatments, which may have led us to expect lower N excretion from the BFTL and BFTH treatments. Results from the lactation trial suggested no differences in N intake between diets but an improved milk N output for the birdsfoot trefoil diets (Hymes-Fecht et al., 2004), so, again, we might have expected less N excretion from the birdsfoot trefoil diets compared with ALF. Fewer cows were used for the manure collection for this study and intake measurements were not made, so differences in intakes cannot be excluded as a possible reason for differences in excretal N output. In addition, as discussed above, fecal and urine outputs as collected may not be representative of daily outputs. The amount of undigested feed N in feces increased with increasing concentration of CT in the diet (Table 3) and a balance is required between protecting sufficient protein from rumen degradation to improve postrumen absorption of essential amino acids and protecting too much protein such that it passes through the animal undigested. Previous research has shown that in sheep, feeding birdsfoot trefoil with medium concentrations of CT (3 to 5%) improved N use efficiency without reducing intake, whereas high concentrations (7.5 to 10%) depressed voluntary feed intake and rumen carbohydrate digestion (Barry and McNabb, 1999).

Measurements from the simulated barn floor trials indicated that cumulative $NH_3$ emissions would continue to increase beyond the 48-h measurement period (Figures 2 and 3). This is consistent with the time required for complete hydrolysis of the urea content of the urine, which has to occur before $NH_3$ volatilization can take place. Rate of hydrolysis is temperature-dependent but from the data given by Whitehead and Raistrick (1993), complete hydrolysis at 15°C (as used in the present study) would occur within 10 to 15 d. Muck (1981) reported much faster hydrolysis of urea on dairy barn floors, with >95% urea decomposition in urine within 6 h at 30°C and within 24 h at 10°C. Elzing and Monteny (1997) showed that peak emission rate (occurring within 1 to 5 h of urine application to a concrete floor) increased with increasing urea N concen-

tration of the urine. The results from the protein form trial are consistent with this, where cumulative emission after 48 h was greater from ALF, which had a higher urea N concentration than either BFTL or BFTH. However, in the protein concentration trial, the emission rates were similar over the first 48 h despite large differences in urea N concentration of urine for HCP and LCP. It is possible that urease activity was limiting in this case and that emissions would have continued for longer from HCP. The higher pH of the urine from the protein concentration trial (Table 3) may have influenced urease activity; Muck (1981) showed that maximum urease activity occurred between pH 6.8 and 7.6 and that activity decreased linearly with pH outside this range. Cumulative emission from LCP after 48 h accounted for almost 100% of the applied urea N and some of this emission probably derived from other urine and fecal N components, as was noted by Whitehead and Raistrick (1993) and Muck and Richards (1983). Actual losses from a dairy barn floor will depend on a number of variables including temperature, airflow, cleaning frequency, urease activity, and urine puddle replenishment rate (Monteny et al., 1998), but the results of the present study suggest that dietary manipulation may not always result in a reduction in emissions proportional to the reduction in excreted urea N.

Urea hydrolysis appeared to be a limiting factor controlling emission rates from the fresh slurries applied to soil in the protein concentration trial. Slurry TAN content was only 20% higher for HCP compared with LCP, whereas a much greater difference would be expected based on differences in urine urea N concentrations. Continued hydrolysis over the 48-h measurement period, replenishing the slurry TAN content, resulted in the cumulative emissions curve for HCP rising more steeply than that for LCP (Figure 4). Two weeks of storage at 20°C was sufficient for complete hydrolysis to have occurred and consequently there was a much greater difference in TAN contents between the 2 treatments in the stored slurries. The stored HCP slurry had a higher pH, resulting in a greater proportional loss of $NH_3$ (Figure 5c). A higher slurry pH associated with higher dietary CP content was noted in cattle by Paul et al. (1998) and in pigs by Misselbrook et al. (1998). Slurry pH is largely determined by the relative concentrations of VFA and TAN and increases as the VFA:TAN ratio decreases (Paul and Beauchamp, 1989). Reducing the CP content of the diet, resulting in a lower slurry TAN content, would not necessarily reduce slurry VFA content. For the protein form trial, there were no additional effects of other slurry compositional changes on $NH_3$ emission and differences in losses were related to the differences in slurry TAN contents.

## CONCLUSIONS

Manipulating the concentration and form of protein in the diet of lactating cows influenced the amount and form of N excretion and subsequent $NH_3$ emissions from the barn floor and manure management. Reducing dietary CP content from 19 to 14% reduced total N excretion and resulted in a greater proportion of the N excretion in urine, with an increase in urine N concentration of 90%. Surprisingly, losses from a simulated barn floor were similar from both treatments in the short term (48 h), presumably because urease activity was limiting, but losses from slurries applied to soil were lower for the LCP treatment both in absolute terms and as a proportion of the TAN applied. Increasing the concentration of CT in the forage legume component of the diet shifted N excretion from urine to feces and led to reduced losses from the barn floor (in absolute terms and as a proportion of urine urea N applied) and slurries applied to soil (in proportion to the reduction in the TAN content of the slurries).

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