# Nitrogen Losses from Dairy Manure Estimated Through Nitrogen Mass Balance and Chemical Markers

**Alexander N. Hristov*** Pennsylvania State University
**Shah Zaman and Mike Vander Pol** University of Idaho
**Pius Ndegwa** Washington State University
**Larry Campbell** University of Idaho
**Steven Silva** U.S. Geological Survey

Ammonia is an important air and water pollutant, but the spatial variation in its concentrations presents technical difficulties in accurate determination of ammonia emissions from animal feeding operations. The objectives of this study were to investigate the relationship between ammonia volatilization and $\delta^{15}N$ of dairy manure and the feasibility of estimating ammonia losses from a dairy facility using chemical markers. In Exp. 1, the N/P ratio in manure decreased by 30% in 14 d as cumulative ammonia losses increased exponentially. Delta $^{15}N$ of manure increased throughout the course of the experiment and $\delta^{15}N$ of emitted ammonia increased ($p < 0.001$) quadratically from –31‰ to –15 ‰. The relationship between cumulative ammonia losses and $\delta^{15}N$ of manure was highly significant ($p < 0.001$; $r^2 = 0.76$). In Exp. 2, using a mass balance approach, approximately half of the N excreted by dairy cows (*Bos taurus*) could not be accounted for in 24 h. Using N/P and N/K ratios in fresh and 24-h manure, an estimated 0.55 and 0.34 (respectively) of the N excreted with feces and urine could not be accounted for. This study demonstrated that chemical markers (P, K) can be successfully used to estimate ammonia losses from cattle manure. The relationship between manure $\delta^{15}N$ and cumulative ammonia loss may also be useful for estimating ammonia losses. Although promising, the latter approach needs to be further studied and verified in various experimental conditions and in the field.

Ammonia emitted from animal feeding operations (AFO) is a major air and water pollutant contributing to eutrophication, aerosol formation, acid rain, and impaired visibility (USEPA, 2004). The importance of mitigating ammonia emissions from livestock operations in the United States relates primarily to the contribution of ammonia to formation of $PM_{2.5}$, which are particles with diameter <2.5 μm and have significant detrimental effect on human health (from pulmonary to cardiovascular diseases; Oberdorster, 2000; Miller et al., 2007). Ammonia contribution to $PM_{2.5}$ is through formation of nitrate and sulfates and reduction of ammonia emissions has been suggested as an effective $PM_{2.5}$ mitigation practice (Pinder et al., 2007). Across different regions and weather conditions, $PM_{2.5}$ formed from ammonia emitted from livestock operations were estimated to contribute on average from 9 (if ammonium bisulfate is formed) to 11% (if ammonium sulfate is formed) of the total $PM_{2.5}$ concentrations in the United States (A.N. Hristov, unpublished data, 2009). Factors affecting ammonia emissions from animal manure are complex and have been thoroughly reviewed (Ndegwa et al., 2008).

In the United States, farm animals are considered the greatest contributor to gaseous ammonia emissions (51% of the total; R. Huntley, EPA, personal communication, 2009), with ruminants contributing about half of the total emissions. Ruminant animals are relatively inefficient utilizers of dietary N. The efficiency of transfer of feed N into milk protein N has been determined to be on average 0.25 (Hristov et al., 2005), as the remaining N is being excreted with urine and feces. Nitrogen excreted with urine represents more than half of all N losses, 0.63 (Tamminga, 1992). Urinary N is primarily in the form of urea (0.50–0.90 of the total N; Bristow et al., 1992) and following hydrolysis is the main contributor to ammonia emission from livestock facilities (Rom and Dahl, 1997; Bussink and Oenema, 1998; Thomsen, 2000). Most of the urea N in manure is lost as ammonia in the first few days following excretion (James et al., 1999; Meisinger et al., 2002).



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*Corresponding author (anh13@psu.edu).
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677 S. Segoe Rd., Madison, WI 53711 USA

A.N. Hristov, Dep. of Dairy and Animal Science, Pennsylvania State Univ., University Park, PA 16802. S. Zaman, M. Vander Pol, and L. Campbell, Dep. of Animal and Veterinary Science, Univ. of Idaho, Moscow, ID 83844. P. Ndegwa, Biological Systems Engineering Dep., Washington State Univ., Pullman, WA 99164. S. Silva, U.S. Geological Survey, Menlo Park, CA 94025.

**Abbreviations**: AFO, animal feeding operations; AIA, acid-insoluble ash; DM, dry matter; DTRC, Dairy Research and Teaching Center; TMR, total mixed ration.

Thus, in warm weather, up to 0.70 of the urinary urea N may be lost as ammonia (Muck and Richards, 1983; Bussink and Oenema, 1998). Volatilization losses of fecal N are considerably smaller (0.01–0.13; Bussink and Oenema, 1998).

A recent review of the literature on ammonia sampling and measurement at animal facilities identified various technical possibilities that would satisfy different research and monitoring requirements for AFO (Ni and Heber, 2008). The sampling and measurement technologies identified in this review indicated merits as well as limitations and suggested that selection of the methodology should be based on study objectives, budget limit, and available equipment and expertise. In general, this review pointed out that the three categories of sampling methods namely; closed, point, and open path, are adequate for assessing human and animal exposure, baseline emissions, building structure and mitigation technologies, and model pollutant dispersions. However, these sampling methods only cover limited sampling points or sampling paths, leaving significant uncertainties for the ammonia concentrations at uncovered spaces. The spatial variation of ammonia concentrations is thus still a major technical difficulty in accurate determination of ammonia losses in AFO. These uncertainties are reflected in the wide range of ammonia emission data found in the literature (Ndegwa et al., 2008; Ni and Heber, 2008). Estimates for annual ammonia N emissions from dairy operations vary from 5 to 6 kg (Powell et al., 2008), through 41 to 82 kg (Rotz and Oenema, 2006), to 140 kg per cow (Rumburg et al., 2004) with some estimates as low as 1.2 kg per cow (E. Wheeler, Pennsylvania State University, personal communication, 2008).

Alternative, indirect approaches to estimate ammonia emissions, which overcome spatial and temporal variations of ammonia loss in AFO have been suggested. Moreira and Satter (2006) proposed the use of N/P ratios in fresh and aged manure for estimating volatile N losses, which include ammonia, N, nitrous oxide, and small amounts of nitric oxide (Oenema et al., 2007). Estimates for the European Union countries suggest that ammonia represents about 74% of all gaseous N emissions from manure (with $N_2$ accounting for 21%; Oenema et al., 2007). As ammonia and other nitrogenous gases volatilize from manure, N/P ratio decreases and the decrease can be used to estimate total volatile N losses. This may be also true for other, nonvolatile macrominerals in manure (e.g., K). In an earlier publication (Hristov et al., 2006a) we reported a significant change in $\delta^{15}N$ of manure as nitrogenous compounds volatilize. Due to isotope fractionation, ammonia N volatilized from manure is highly depleted in $^{15}N$ and the resulting manure becomes increasingly enriched in $^{15}N$ as ammonia is being emitted. These relationships can potentially be used to model and predict ammonia losses from manure based on simple chemical analyses.

Thus, the objectives of this study were to: (i) Characterize the relationship between ammonia volatilization and $\delta^{15}N$ of dairy manure (Exp. 1); and (ii) Estimate ammonia losses from a dairy facility based on N mass balance or using markers, such as P and K (Exp. 2).

# Materials and Methods

## Experiment 1

The objective of this experiment was to monitor the changes in N/P ratio and $\delta^{15}N$ occurring in cattle manure as a result of ammonia N volatilization under controlled conditions.

Fecal and urine samples were collected from four commercial dairies in eastern Washington and from both the University of Idaho and Washington State University research dairy farms (a total of six dairy farms). Sampling procedures were approved by the University of Idaho Institutional Animal Care and Use Committee. Fresh spot fecal and urine samples were collected from randomly selected lactating dairy cows fed on the same diet (15 cows per diet). Two of the commercial dairies fed separate diets to high and low producing cows and from these dairies two separate fecal and urine samples were collected. Thus, a total of eight sets of fecal and urine samples were used in this experiment. Fecal samples were collected from the rectum or from the ground, when fresh. Urine samples were collected by massaging the vulva. Both fecal and urine samples were collected in the morning when cows were locked for managerial purposes. Details on sampling technique and sample processing are given in Hristov et al. (2006b). Fecal and urine samples were transported refrigerated to the laboratory, composited, and used to study ammonia losses in a closed system in vitro apparatus similar to the one described by Derikx and Aarnink (1993). Briefly, the system consisted of an 11.4 L simulated-manure-storage, acid bottles to trap the emitted ammonia, a flow-meter to regulate sweep-air, and a vacuum pump to pull air through the system. Feces were mixed with urine (a total of 2 kg on as is basis; 32% urine and 68% feces by weight) and incubated for 14 d. The urine/feces ratio was chosen based on in vivo experiments with lactating dairy cows conducted in our laboratory, in which total urine collections were performed. Each simulation was conducted in triplicate for each dairy or diet combination in a temperature-controlled environment maintained at 21°C, at a fixed air-flow rate (3.7 L $min^{-1}$). Ammonia was trapped in two consecutive graduated cylinders containing 2 L of 0.5 mol $L^{-}$ sulfuric acid solution each. Acid solution was exchanged daily during the experiment. Manure samples (10 g each) were collected daily by quickly opening and closing the storage containers. Air flow to the containers was stopped during sampling. Daily manure and sulfuric acid solution samples were analyzed for $\delta^{15}N$ of total N and ammonia N, respectively. Day 1 and 14 manure samples were also analyzed for N and P.

Ammonia N lost during the course of the incubation was estimated based on N mass balance (N in manure on Day 1–14-d cumulative ammonia N emission) and N/P ratio in fresh and 14-d manure samples: {1 – [(N/P ratio in aged manure)/(N/P ratio in fresh manure)] × 100} (Moreira and Satter, 2006; Hristov et al., 2006b).

## Experiment 2

This study was designed to estimate ammonia losses from a free-stall dairy barn based on N mass balance and using chemical markers.

The experiment was conducted in May-June 2006 at the University of Idaho Dairy Research and Teaching Center (DRTC). All procedures involving animals were approved by the University of Idaho Institutional Animal Care and Use Committee. The DRTC is a 120-cow capacity, free-stall facility with a scraping system for waste removal and a concrete pit for solid manure storage. Runoff water is directed through a drain pipe into a nearby pond. The facility has four separated, partially covered pens. The study was conducted in one of the pens, in which 18 lactating Holstein cows (various parity) were maintained for 30 d. Cows did not leave the pen except for milking, twice daily for approximately 15 min each time. Feed (total mixed ration; TMR) was delivered to the cows twice daily, weighed, and a representative sample was collected for analyses. A 30-d composite sample of the TMR was oven-dried at 60°C and analyzed for N, P, K, and acid-insoluble ash (AIA; see Chemical Analyses) and nutrient composition (Cumberland Valley Analytical Services, Inc., Maugansville, MD). Refusals were weighed daily and composited for chemical analyses. Intake of N, P, and K was corrected for composition of the refusals. Manure from the experimental pen floor was collected once daily. Manure from the alley leading to the milking parlor, the waiting area, and the milking parlor was not collected. The floor of the pen was scraped once daily around 0700 h using a front-end loader (which was the usual practice at the DRTC). The manure collected was transferred to a plastic sheet-lined trailer, weighed, and a composite sample (five 200-g subsamples from different locations were composited) was freeze-dried and analyzed for N, P, K, and $\delta^{15}N$. Then, the manure was transferred to the concrete pit for further storage. At the end of the experiment (30 d), all manure from the pit was removed, weighed, and a representative sample was freeze-dried and analyzed as for the trailer samples. Ambient temperature and precipitations at DRTC were monitored continuously throughout the experiment.

Individual cow milk yield was recorded daily and milk samples were collected from each cow during Weeks 2 and 4 of the experiment. Whole milk samples were freeze-dried and analyzed for N. Phosphorus and K content of milk was not analyzed but assumed based on NRC (2001).

Spot fecal (approximately 400 g per sample) and urine (approximately 300 mL per sample) samples were collected from each cow in 2 d (about 12-h apart) during Weeks 2, 3, and 4 of the experiment as described for Exp. 1. Urine aliquots were diluted 1:10 with 0.072 N sulfuric acid and frozen for later analyses. Another aliquot was diluted 1:5, stored frozen, and later analyzed for creatinine. Individual fecal and urine samples were composited per cow on a weight or volume basis. The composited fecal samples were freeze-dried, ground through a 1-mm sieve, and analyzed for AIA, N, $\delta^{15}N$, P, and K. Fecal dry matter (DM) output, respectively N excretion, was estimated using AIA as an intrinsic digestibility marker (Foley et al., 2006). Daily urinary N excretion was estimated using creatinine as a urine volume marker assuming creatinine excretion of 25.8 mg $kg^{-1}$ body weight (Vander Pol et al., 2008). Nitrogen, P, and K excreted with feces and urine was also estimated as: Nutrient in feces and urine = nutrient intake with feed– (nutrient in milk + nutrient in body weight gain).

**Table 1. Chemical composition (dry matter basis) of manure from commercial dairies (*n* = 8) on incubation Days 1 and 14 (Exp. 1).**

| | Average | SD | Minimum | Maximum |
|---|---|---|---|---|
| Day 1 | | | | |
| P, g $kg^{-1}$ | 6.0 | 1.0 | 4.8 | 7.6 |
| N, g $kg^{-1}$ | 30.6 | 4.2 | 23.8 | 34.7 |
| Total manure N, g | 9.5 | 0.35 | 9.1 | 10.2 |
| N/P ratio | 5.24 | 1.12 | 3.14 | 7.03 |
| Day 14 | | | | |
| P, g $kg^{-1}$ | 6.6† | 1.1 | 5.6 | 8.5 |
| N, g $kg^{-1}$ | 23.7‡ | 2.2 | 19.6 | 26.4 |
| N/P ratio | 3.65§ | 0.67 | 2.87 | 4.54 |

† Day 14 vs. Day 1, $p = 0.018$; SE = 0.46.

‡ Day 14 vs. Day 1, $p < 0.001$; SE = 1.39.

§ Day 14 vs. d 1, $p < 0.001$; SE = 0.371.

Body weight of the cows was recorded in 2 consecutive days at the beginning and at the end of the trial. Composition of body weight gain was assumed based on Hristov et al. (2006b) (N and P) and NRC (2001) (K).

### Chemical Analyses

Nitrogen concentration and $\delta^{15}N$ of samples were analyzed on a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technol., Inc., Valencia, CA) interfaced to a DELTA^plus isotope ratio mass-spectrometer (Thermo Finnigan MAT GmbH, Bremen, Germany). Samples were pulverized (Retsch MM200 micro mill; F. Kurt Retsch GmbH & Co. K. G., Haan, Germany) before the analysis. Ammonia was collected for $\delta^{15}N$ analysis through diffusion (Hristov et al., 2001). Phosphorus in samples was analyzed by the University of Wisconsin Soil and Forage Analysis Laboratory (Marshfield, WI) using a colorimetric assay (APHA, 1998). Potassium was analyzed on an Iris ICP atomic emission spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA). Samples were prepared according to Soon (1998). Diet and fecal samples were analyzed for AIA according to Van Keulen and Young (1977). Creatinine in urine samples was analyzed according to Broderick et al. (2007).

### Statistical Analyses

Individual feed, feces, urine, and manure sample analyses from commercial dairy farms were averaged per farm and the mean values were used in the statistical analysis in Exp. 1. Descriptive statistics (chemical composition, N/P and N/K ratios, and N mass balance data) were performed using PROC MEANS procedure of the SAS software system (SAS Inst. Inc., 2004). Difference in manure composition and the relationship between cumulative ammonia losses and $\delta^{15}N$ of manure (Exp. 1) were analyzed using PROC MIXED procedure of SAS with farm as a random effect.

## Results and Discussion

### Experiment 1

Phosphorus concentration in manure DM increased ($p = 0.018$) by 10% from Day 1 to Day 14, while N concentration decreased ($p < 0.001$) by 29% (Table 1). As a result, N/P ratio in manure decreased ($p < 0.001$) by 30% from d 1 to d 14 of the incubation.

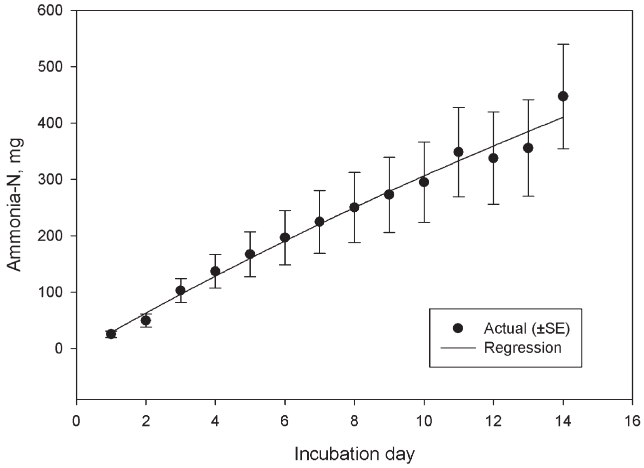


**Fig. 1. Average ($n$ = 8) cumulative ammonia-N losses during the course of Exp. 1.**

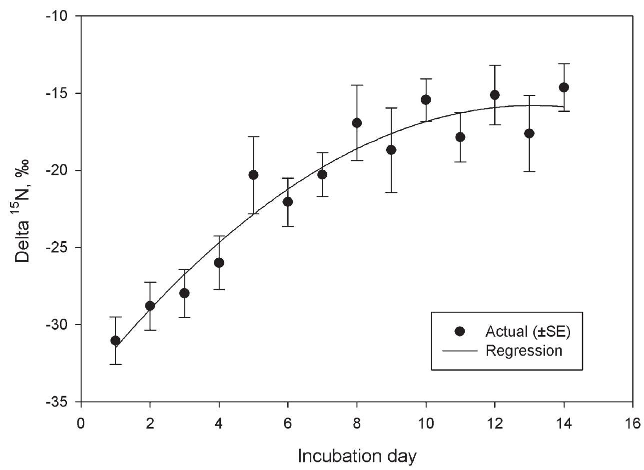


**Fig. 2. Average ($n$ = 8) $\delta^{15}N$ of ammonia N emitted during the course of Exp. 1**

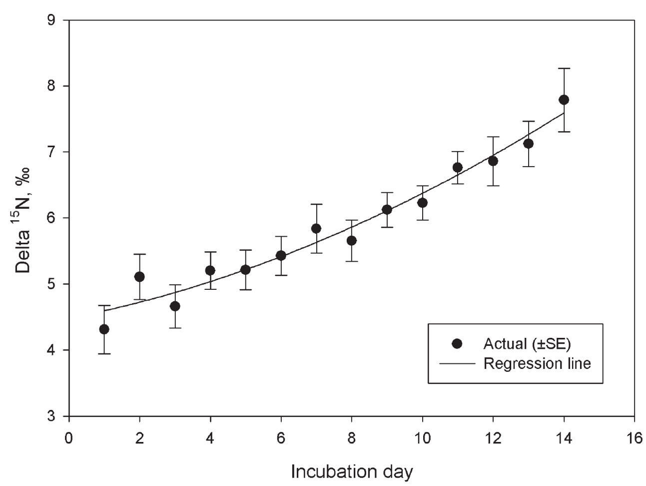


**Fig. 3. Average ($n$ = 8) $\delta^{15}N$ of manure N during the course of Exp. 1.**

Cumulative ammonia losses from manure increased ($p < 0.001$) exponentially ($r^2$ = 0.98) in 14 d to an average of 447 mg (Fig. 1). Delta $^{15}N$ of ammonia emitted during the incubation (average $\delta^{15}N$ of –21 ± 1.5 ‰) increased ($p < 0.001$) quadratically (Fig. 2; $r^2$ = 0.92) from –31‰ (Day 1) to –15 ‰ (Day 14). As a result, $\delta^{15}N$ of manure also increased ($p < 0.001$) quadratically (Fig. 3; $r^2$ = 0.96) from $\delta^{15}N$ of 5.64 (Day 1) to 7.22 ‰ (Day 14). The relationship between cumulative ammonia losses and $\delta^{15}N$ of manure (Fig. 4) was highly significant ($p < 0.001$; $r^2$ = 0.76): Cumulative ammonia N loss (mg) = –396.5 (SE = 65.43) + 106.3 (SE = 8.40) manure $\delta^{15}N$ (farm was a random effect).

Using the mass balance method, in 14 d, 0.36 of manure N was lost as ammonia (Fig. 5). Using N/P ratios (Moreira and Satter, 2006), ammonia N loss could be estimated at 0.28 of the original manure N. The estimated losses derived using these two approaches were not statistically different ($p$ = 0.50).

## Experiment 2

The amount and composition of dietary protein fed to dairy cows (and ruminants in general) is an important factor determining the efficiency of N utilization, urinary N losses, and ammonia emissions from manure (Colmenero and Broderick, 2006; Ndegwa et al., 2008; Agle et al., 2008; Van der Stelt et al., 2008). Compared to common feeding practices in commercial dairies in the United States (Hristov et al., 2006b), cows in Exp. 2 were fed a relatively low crude protein diet with typical distribution of ruminally-degradable and undegradable protein (Table 2). The diet included 140 g $kg^{-1}$ field peas (*Pisum sativum* L.), which have relative high ruminal protein degradability (Vander Pol et al., 2008). The diet also contained relatively high concentration of nonfiber carbohydrates, which would ensure efficient utilization of ruminal ammonia N for microbial protein synthesis, particularly with high-producing dairy cows (cows produced on average 38.4 ± 2.17 kg $d^{-1}$ milk during the duration of the trial). As demonstrated in a recent meta-analysis (Huhtanen and Hristov, 2009), milk yield is one of the factors (although considerably less powerful than dietary protein content) determining the efficiency of utilization of feed N for milk protein synthesis in dairy cows. Similar conclusion was drawn from a much smaller dataset by Yan et al. (2006). Average milk crude protein content (estimated as: milk N × 6.38) during the trial was 31.3 ± 0.89 g $kg^{-1}$.

Nitrogen mass balance data for Exp. 2 are shown in Table 3. During the study, on average 0.26 of the N consumed was secreted in milk. This figure corresponds well to published data and meta-analyses of large North American and North European datasets (Hristov et al., 2005; Huhtanen and Hristov, 2009). A relatively small proportion of the N consumed (0.03) was deposited as body weight gain, which is expected in dairy cows at various parity and stages of the lactation cycle (NRC, 2001). Thus, the estimated N excretion with urine and feces (N mass balance) represented 0.70 of the feed N consumed. As indicated in Materials and Methods, urinary and fecal N excretion was also estimated using digestibility and urine volume markers. Based on urinary creatinine concentration, average daily urine output was estimated at 28.7 ± 1.91 kg per cow, urine N concentration was 8.9 ± 0.22 g $kg^{-1}$, and thus average urinary N excretion per cow during the duration of the trial was 254 ± 16.9 g $d^{-1}$ (or 0.57 of the total N excretion). Using AIA as a marker, average fecal DM output was estimated at 8.1 ± 0.37 kg $d^{-1}$. With average N concentration in fecal DM of 23.6 ± 0.45 g kg$^{-1}$, fecal N excretion per cow was estimated to be 191 ± 8.6 g d$^{-1}$ (0.43 of the total N excretion). Thus, the total daily N excretion with feces and urine was estimated at 445 ± 21.4 g d$^{-1}$, which totaled 240.5 kg N for the 30 d of the experiment. This figure was 11% lower than the fecal and urinary N excretion estimated using the N mass balance approach (Table 3). Therefore, the two methods gave similar estimates for N excretion. Variability should be expected as neither the digestibility or urine volume markers are absolutely accurate, nor would the mass balance approach assure absolutely accurate estimation of N intake and N secretion with milk. In addition, N content of tissues was not determined in this trial and this may also contribute, even though minimally, to variability in the estimates. Manure N collected in a 24-h period represented 0.50 of the N excreted with feces and urine estimated using the N mass balance approach. If the marker approach was used, manure N recovered in 24 h represented 0.56 of the total N excreted. Thus, within 24 h, approximately half of the excreted urine and fecal N could not be recovered in the manure collected, if the mass balance approach was used. Using the marker approach, the unaccounted manure N in 24 h was 0.44 of the excreted {[N excreted with urine and feces (240.5 kg) – N recovered in 24 h manure (134.3 kg)]/ [N excreted with urine and feces (240.5 kg) × 100]}. In 30 d, the excreted unaccounted manure N was 0.61 and 0.56 of the N excreted for the two approaches, respectively. Thus, in the conditions of Exp. 2, most of the unaccounted N was lost (presumably volatilized as ammonia) during the first 24 h after excretion with an additional 22% lost in up to 30 d of manure storage. Similarly high initial (within 26 h of excretion) ammonia volatilization rates from manure were reported by James et al. (1999; 38–48% of the initial N) and more recently by Lee et al. (2009; approximately 40% of manure N volatilized in 48 h). It has to be pointed out, however, that, due to the design of the barn, manure excreted from the cow in Exp. 2 could not be completely recovered. As indicated earlier, manure in the alleys and milking parlor was not collected. We do not have an estimate of the proportion of this manure of the total excreted, but, based on the amount of time cows spent in these areas (approximately 0.5 h per milking), we assume that it was insignificant. Nevertheless, the N losses data reported here (using N mass balance or N/P ratios) are likely an overestimation of the actual losses. It is probably safe to assume that most of the early losses of volatile N originate from urinary urea (Rom and Dahl, 1997; Bussink and Oenema, 1998; Thomsen, 2000) with possible contribution of fecal N in the later stages of manure storage. Based on the estimated urinary and fecal N excretions (markers approach), it can be estimated that approximately 0.77 of the excreted urinary N was lost in the first 24 h [(manure N unaccounted in 24 h)/ (proportion of urinary N in total excreted N)], i.e., 0.44/0.57 = 0.77). In 30 d, this proportion would be 0.98 (0.56/0.57 = 0.98). According to this model, fecal N losses were minimal within the 30 d duration of the experiment. Fecal N is primarily microbial N and undigested feed N and its contribution to volatile N emissions is relatively small (from 0.02–0.36 of the total losses, depending on manure management; Thomsen, 2000).

As in Exp.1, the Moreira and Satter (2006) approach was used to estimate volatile N losses from manure in Exp. 2. Phosphorus mass balance data and estimated excretion are shown in Table 4. The P content of the diet fed was typical for western dairies (Hristov et al., 2006b). Approximately 0.29 of the P consumed was secreted in milk. The assumed P accumulation in body tissues was a relatively small proportion (0.05) of the P consumed. The estimated P excretion in feces and urine (mass balance method) was 0.65 of the P consumed. Excretion of P was also estimated based on fecal DM output (AIA marker method). Average P concentration in fecal DM was analyzed to be 8.3 ± 0.20 g kg$^{-1}$. Using the estimated fecal DM output (8.1 ± 0.37 kg d$^{-1}$), the total P excreted with feces was estimated to be 36.3 kg d$^{-1}$, which is about 12% lower that the estimation based on P mass balance (Table 4). Phosphorus losses with urine were assumed to be zero. This assumption was based on data from Wu et al. (2000), Knowlton and Herbein (2002), and Hristov et al. (2006b) who found very low urinary P concentrations and negligible contribution of urinary P to total P excretion in cattle. Nitrogen/P ratio in fresh manure was 6.57 in this experiment, which was within the range found in Exp. 1 (Table 1) and reported by Moreira and Satter (2006) (5.61–9.24) and Hristov et

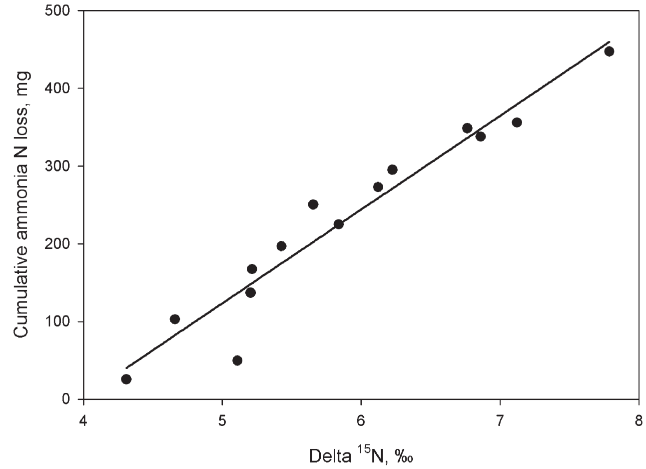


**Fig. 4. Relationship between cumulative losses of ammonia N from manure and $\delta^{15}$N of manure (Exp. 1, *n* = 8).**

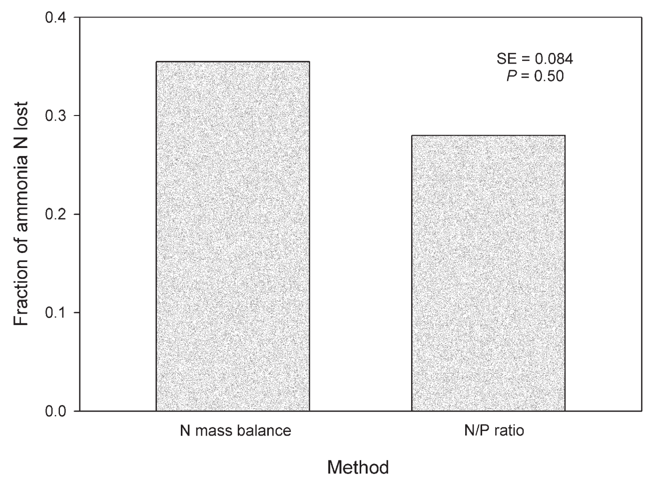


**Fig. 5. Ammonia N losses (*n* = 8) estimated using N/P ratio vs. mass N balance methods (Exp. 1).**

**Table 2. Composition of the diet fed to the cows during the mass N balance study (Exp. 2).**

| Ingredient | g kg$^{-1}$, Dry matter basis |
|---|---|
| Alfalfa hay† | 349 |
| Corn silage‡ | 218 |
| Field peas | 140 |
| Corn grain, steam-rolled | 87 |
| Cottonseed, whole with lint | 53 |
| Dry distiller grain with solubles | 54 |
| Barley grain, steam-rolled | 60 |
| Beef tallow | 16 |
| Mineral/vitamin mix§ | 23 |
| Chemical composition¶ | |
| Crude protein | 160 |
| Ruminally degradable protein | 105 |
| Ruminally undegradable protein | 67 |
| Net energy of lactation, Mcal kg$^{-1}$ DM | 1.56 |
| Neutral detergent fiber | 314 |
| Nonfiber carbohydrates | 438 |

† Alfalfa hay was 840 g kg$^{-1}$ DM and (DM basis): 185 g kg$^{-1}$ crude protein, and 434 g kg$^{-1}$ neutral detergent fiber.

‡ Corn silage was 300 g kg$^{-1}$ DM and (DM basis): 60% g kg$^{-1}$ crude protein, and 482 g kg$^{-1}$ neutral detergent fiber.

§ Mineral vitamin premix (Land O'Lakes, Saint Paul, MN) composition (as is basis): Ca, 158 g kg$^{-1}$; P, 47 g kg$^{-1}$; Mg, 52 g kg$^{-1}$; Cl, 28 g kg$^{-1}$; K, 3 g kg$^{-1}$; Na, 65 g kg$^{-1}$; S, 3 g kg$^{-1}$; Co, 4.36 mg kg$^{-1}$; Cu, 928 mg kg$^{-1}$; I, 42 mg kg$^{-1}$; Fe, 189 mg kg$^{-1}$; Mn, 4.7 mg kg$^{-1}$; Se, 15.7 mg kg$^{-1}$; Zn, 4649 mg kg$^{-1}$; vit. A, 258,000 IU kg$^{-1}$; vit. D, 45,900 IU kg$^{-1}$; and vit. E, 1839 IU kg$^{-1}$.

¶ Ruminally degradable and undegradable protein, net energy of lactation, neutral detergent fiber, and nonfiber carbohydrates were estimated by NRC (2001). Crude protein was estimated as N × 6.25. Forage composition was analyzed by Cumberland Valley Analytical Services, Inc. (Maugansville, MD).

al. (2006b) (8.09). If the marker approach was used to estimate fecal P excretion, the N/P ratio in fresh manure would be 6.62. In manure collected 24 h after excretion, N/P ratio decreased to 2.98, presumably as a result of volatile N losses. This ratio was within the range found in vitro in Exp. 1 and close to the one reported for manure samples from commercial dairy operations (2.62; Hristov et al., 2006b). The N/P ratios in aged manure published by Moreira and Satter (2006) were higher (4.14–6.39, depending on season and study year) than the ratios reported here. It has to be pointed out, however, that in the Moreira and Satter (2006) study, manure was collected (scraped) two to six times daily, which would result in a significantly lower volatile N losses compared to the current study (24 h collection). Hollmann et al. (2008) reported N/P ratio in manure leaving the dairy barn of 3.80 (calculated from data in Table 3; Hollmann et al., 2008). In this latter study, manure was removed from the barn in a 6-h interval. Using N/P ratios in fresh and 24-h manure, 24-h volatile N losses in Exp. 2 represented 0.55 (or 148.9 kg) of the N excreted with urine and feces (Table 5). This figure was 10% higher than the one derived using the N mass balance approach (Table 3). If the N/P ratio derived from the marker methods (6.62) was used, the proportion of N lost in 24 h would be identical (0.55). These figures are similarly high to the losses reported by Moreira and Satter (2006): 0.37 to 0.50 of the N excreted by the cows for the summer months (N losses were estimated at 0.16–0.19 for the winter trial). Based on excreta and manure N/P ratios calculated from the Hollmann et al. (2008) data, N losses in this latter study were 0.39 (in 6 h).

Nitrogen to P ratio further decreased in 30 d of manure storage in Exp. 2. Using the same approach, N losses in 30-d manure were estimated to be 0.65 of the N excreted with feces and urine. Thus, only an additional 18% of the N was lost after the first 24 h of manure storage, which is comparable to the additional 22% N unaccounted in 30 d found using the N mass balance approach. It has to be pointed out, however, that N losses estimated using N/P ratios may have been overestimated in Exp. 2. The reason for this statement is the unrealistically high recovery of P in manure. Based on total manure DM collected (in 24 h; Table 3) and manure P concentration (Table 4), P recovered in 24-h manure can be calculated at 45.7 kg, which is 11% greater than the estimated total excretion of P in feces and urine. This high recovery is difficult to explain. It could be due to variability in P analyses and manure weight data. A disproportionally high recovery of P (as a result of disproportional and unaccounted runoff losses of soluble manure nutrients other than P, for example) would result in lower N/P ration in manure and consequently high estimated volatile N losses.

In this experiment, we investigated the usefulness of another nonvolatile macromineral, K, as a marker for volatile N losses from manure. Unlike P, K is excreted in both feces and urine (Berry et al., 2001; Gustafson and Olsson, 2004; Hristov et al., 2006b) with excess dietary K being primarily excreted in urine (Underwood and Suttle, 2001). Another distinct difference between P and K metabolism is that body K reserves are low (unlike the large bone P reserves) in the dairy cow and K must be supplied daily in the diet (NRC, 2001). As a result of both P intake and bone P deposition/resorption (Valk et al., 2000; Valk, 2002) fecal P concentration can be highly variable (Wu et al., 2000). The Moreira and Satter (2006) approach for estimating volatile N losses from manure is based on the assumption that there are no volatile P losses and runoff. As ammonia (and to a considerably smaller extent other nitrogenous compounds; de Vries et al., 2003) volatilizes from manure, N/P ratio changes proportionally and can be used to estimate total volatile N losses. Similar assumptions can be made for K. If runoff occurs, the N/P ratios cannot be reliably used to estimate volatile N losses as P is primarily excreted in feces and is mostly insoluble. Also, it is likely that to a large extent runoff N would be of urinary origin and the N/P ratio of runoff would be disproportionally greater than the N/P ratio of manure. On the contrary, as K, similar to N, is found in both feces and urine and, again similar to N, is preferably excreted with urine when consumed in excess, its losses with runoff are expected to simulate N losses and would likely not change N/K ratio in manure.

Potassium balance data are shown in Table 4. Dairy diet K concentrations can vary significantly due mainly to variability in forage K concentration and K salts supplementation (Underwood and Suttle, 2001). Potassium concentration in the diet fed to the cows in Exp. 2, however, was within the range of dietary K reported for a large segment of commercial western dairies (Hristov et al., 2006b). Potassium secreted in milk represented 0.15 of the K consumed, which was close to the 0.11 reported by Hristov et al. (2006b). Potassium deposition in body tissues was negligible. The estimated excretion of K in feces and urine was 0.84 of the dietary K consumed by the cows. Potassium concentration in 24-h manure was within the range reported by Hristov et al. (2006b). Potassium recovered in 24-h manure (122 kg) was 0.76 of the estimated K excretion with feces and urine. Compared to the estimated N/K ratio in fresh manure (1.69), N/K ratio in 24-h manure decreased by 34%. Based on these ratios and using the Moreira and Satter (2006) approach, 24-h volatile N losses from manure could be estimated at 0.34 of the N excreted in feces and urine (Table 5). Similar to N/P, N/K ratio further decreased in 30-d manure. Based on this ratio, the unaccounted N in 30-d manure was estimated to be 0.46 of the N excreted.

The N losses derived using N/K ratios were lower than those derived based on N/P ratios and N mass balance data. As discussed earlier, the N/K approach may have an advantage over the N mass balance and N/P approaches as it would account for disproportionate nonvolatile or runoff N losses (urinary N, for example). Runoff losses would not be expected during the period that this research was conducted because the average precipitation was only 0.03 ± 0.07 mm. Nonvolatile losses of N not recovered in scraped manure, however, could not be discounted. Manure from the alley leading to the milking parlor, the waiting area, and the milking parlor was not collected in this experiment. In addition, losses (mainly urine) through the pen drain pipe were not accounted for, which may have overestimated volatile N losses derived based on the mass balance and N/P method. As the N/K approach would be less susceptible to unaccounted, nonvolatile N losses, the 0.34 N losses derived by this method should closely represent the true volatile (i.e., ammonia) N losses in the conditions of Exp. 2. The difference of 38% between N losses estimated using N/P (0.55) and N/K (0.34) ratios likely represent the nonvolatile losses of N in 24-h manure. Similar to the N/K estimates, Hollmann et al. (2008) reported 0.39 of the excreted N as unaccounted in manure leaving the barn (in a 6-h scraping interval). Based on the N/K approach, daily ammonia N losses in Exp. 2 could be estimated at 0.171 kg per cow [270.8 kg N excreted [(from Table 3)/30 d/18 cows) × 0.34]. Extrapolated over a 365-d period, this would represent 62 kg N annual ammonia N emission per cow. This figure is comparable to our earlier estimates (74 kg per cow annual ammonia N emission) for commercial dairies in Idaho (Hristov et al., 2006b; based on N/P ratios), but is higher than those reported by Demmers et al. (1998; using measurements of ammonia concentration and estimated ventilation rate for cattle buildings) and Koerkamp et al. (1998; cattle housing of various types in several Northern European countries) and published by the U.S. Environmental Protection Agency (USEPA, 2004). Emission data from studies varying in design, cow productivity, housing, and mainly diet composition (see Ndegwa et al., 2008) are difficult to compare. It is noteworthy that ammonia emissions measured off the barn floor may be significantly lower than emissions estimated by N mass balance or using the N/P ratio approach. For example, a chamber study by Powell et al. (2008), reported annual ammonia N emissions of 5 to 6 kg per cow in spring to as low as 1.8 kg in the winter months. A recent study conducted at the Pennsylvania State University's Dairy Center estimated annual ammonia N emissions off the barn floor as low as 1.2 kg per cow (E. Wheeler, personal communication, 2008). However, using component prediction models and a whole-farm simulation model, Rotz and Oenema (2006) estimated total (barn and manure handling) annual ammonia N losses from northeastern U.S. dairies at 41 to 82 kg per cow. A case study by Rumburg et al. (2004) estimated annual ammonia N emissions from a dairy (using the $SF_6$ tracer technique) as high as 140 kg per cow. Recent European data reported significantly lower ammonia emissions from dairy barns bedded with various amounts of straw (0.03–0.15 of the N excreted; Gilhespy et al., 2009). There is little doubt that volatile N losses from manure are large and this is particularly true for the initial stages of manure storage and certain manure management

**Table 3. Nitrogen imports and exports from the experimental pen in a 30-d period (Exp. 2).**

| Item | kg (or as indicated) |
|---|---|
| N imported with feed† | |
| Feed delivered | 27,149 |
| Feed DM (g kg$^{-1}$) | 598 |
| Feed N (g kg$^{-1}$, DM basis) | 25.6 ± 0.20 |
| Feed DM delivered | 16,235 |
| Feed N delivered | 416 |
| Feed refusals (DM basis) | 1252 |
| Feed refusals N (g kg$^{-1}$, DM basis) | 23.8 ± 0.55 |
| Feed refusals N | 30 |
| Feed N consumed | 385.8 |
| Exports | |
| Milk produced per cow during the experiment | 1151 ± 65.1 |
| Total milk produced | 20,714 |
| Milk DM (g kg$^{-1}$) | 125.5 ± 3.22 |
| Milk N‡ content (g kg$^{-1}$ milk DM) | 39.2 ± 0.83 |
| Milk N produced | 101.8 |
| Body weight gain (BWG) | 459 |
| Assumed N content of BWG§ (g kg$^{-1}$) | 28.8 |
| N in BWG | 13.2 |
| N excretion with feces and urine¶ | 270.8 |
| Manure collected within 24 h# | 40,669 |
| Manure DM (g kg$^{-1}$) | 216 ± 6.5 |
| Manure DM | 8785 |
| Manure N concentration (g kg$^{-1}$ DM) | 15.5 ± 0.54 |
| Manure N collected within 24 h | 134.3 |
| Manure collected in 30 d | 34,583 |
| Manure DM (g kg$^{-1}$) | 268 |
| Manure N (g kg$^{-1}$ DM) | 11.4 |
| Manure N collected in 30 d | 105.7 |
| Unaccounted N in 24-h manure†† | 136.5 |
| As fraction of N excreted in feces and urine (kg kg$^{-1}$) | 0.50 |
| Unaccounted N in 30-d manure ‡‡ | 165.1 |
| As fraction of N excreted in feces and urine (kg kg$^{-1}$) | 0.61 |

† As a total mixed ration.

‡ Total N.

§ Hristov et al. (2006b).

¶ Estimated using the N mass balance approach: Feed N consumed – (Milk N produced + N in body weight gain).

# Manure recovered from the experimental pen within 24 h of excretion.

†† Estimated N excretion with feces and urine (N mass balance approach) – N recovered in manure within 24 h of excretion.

‡‡ Estimated N excretion with feces and urine (N mass balance approach) – N recovered in manure in 30 d.

**Table 4. Phosphorus and potassium balance from the experimental pen in a 30-d period (Exp. 2).**

| Item | kg (or as indicated) |
|---|---|
| Phosphorus | |
| Feed P (g kg$^{-1}$, DM basis) | 4.2 ± 0.3 |
| Feed P delivered† | 67.7 |
| Feed refusals P (g kg$^{-1}$, DM basis) | 3.8 ± 0.2 |
| Feed refusals P | 4.7 |
| Feed P consumed | 63.1 |
| P secreted in milk‡ | 18.6 |
| P in body weight gain§ | 3.3 |
| P excreted in feces and urine¶ | 41.2 |
| N/P ratio in fresh manure# | 6.57 |
| P concentration in manure collected within 24 h†† (g kg$^{-1}$, DM basis) | 5.2 ± 0.2 |
| N/P ratio in 24-h manure‡‡ | 2.98 |
| Fraction of N unaccounted in 24-h manure (kg kg$^{-1}$) §§ | 0.55 |
| Unaccounted N in 24 h¶¶ | 148.9 |
| P concentration in 30-d manure (g kg$^{-1}$, DM basis) | 4.9 |
| N:P ratio in 30-d manure## | 2.33 |
| Fraction of N unaccounted in 30 d (kg kg$^{-1}$) ††† | 0.65 |
| Unaccounted N in 30 d‡‡‡ | 176.0 |
| Potassium | |
| Feed K (g kg$^{-1}$, DM basis) | 12.7 ± 0.63 |
| Feed K delivered | 206.2 |
| Feed refusals K (g kg$^{-1}$, DM basis) | 13.1 ± 0.78 |
| Feed refusals K | 16.4 |
| Feed K consumed | 189.8 |
| K secreted in milk§§§ | 29.0 |
| K in body weight gain¶¶¶ | 0.83 |
| K excreted in feces and urine### | 160.0 |
| N/K ratio in fresh manure†††† | 1.69 |
| K concentration in manure collected within 24 h (g kg$^{-1}$, DM basis) | 13.9 ± 0.39 |
| N/K ratio in 24-h manure‡‡‡‡ | 1.12 |
| Fraction of N unaccounted in 24-h manure (kg kg$^{-1}$)§§§§ | 0.34 |
| Unaccounted N in 24 h¶¶¶¶ | 92.1 |
| K concentration in 30-d manure (g kg$^{-1}$, DM basis) | 12.4 |
| N/K ratio in 30-d manure#### | 0.92 |
| Fraction of N unaccounted in 30-d manure (kg kg$^{-1}$) ††††† | 0.46 |
| Unaccounted N in 30 d‡‡‡‡‡ | 124.6 |

† As a total mixed ration.

‡ Assumed P concentration in milk, 0.9 g kg$^{-1}$ (NRC, 2001).

§ Assumed P concentration in body weight gain 7.2 g kg$^{-1}$ (Hristov et al., 2006b).

¶ Estimated as: Feed P consumed – (Milk P produced + P in body weight gain).

# Estimated as: N excreted in feces and urine (Table 3) ÷ P excreted in feces and urine

†† This is manure recovered from the experimental pen within 24 h of excretion.

‡‡ Estimated as: N concentration in 24-h manure (Table 3) ÷ P concentration in 24-h manure.

§§ Estimated as: 1 – (N/P ratio in 24-h manure ÷ N/P ratio in fresh manure).

¶¶ Estimated as: N excreted in feces and urine (Table 3) × fraction of N unaccounted in 24-h manure.

## Estimated as: N concentration in 30-d manure (Table 3) ÷ P concentration in 30-d manure.

††† Estimated as: 1 – (N/P ratio in 30-d manure ÷ N/P ratio in fresh manure).

‡‡‡ Estimated as: N excreted in feces and urine (Table 3) × fraction of N unaccounted in 30 d.

§§§ Assumed K concentration in milk, 1.4 g kg$^{-1}$ (NRC, 2001).

¶¶¶ Assumed K concentration in body weight gain 1.8 g kg$^{-1}$ (NRC, 2001).

### Estimated as: Feed K consumed – (Milk K produced + K in body weight gain).

†††† Estimated as: N excreted in feces and urine (Table 3) ÷ K excreted in feces and urine.

‡‡‡‡ Estimated as: N concentration in 24-h manure (Table 3) ÷ K concentration in 24-h manure.

§§§§ Estimated as: 1 – (N/K ratio in 24-h manure ÷ N/K ratio in fresh manure).

¶¶¶¶ Estimated as: N excreted in feces and urine (Table 3) × fraction of N unaccounted in 24 h.

#### Estimated as: N concentration in 30-d manure (Table 3) ÷ K concentration in 30-d manure.

††††† Estimated as: 1 – (N/K ratio in 30-d manure ÷ N/K ratio in fresh manure).

‡‡‡‡‡ Estimated as: N excreted in feces and urine (Table 3) × fraction of N unaccounted in 30 d.

practices. An elegant study with composted $^{15}$N-labeled feces and urine demonstrated that the majority (0.79) of the volatile N losses during the first 7 d of composting sheep (*Ovis aries*) manure originate from urine N and that 0.46 of the manure N was lost in 86 d (Thomsen, 2000). Remarkably, only 0.18 of the N was lost if manure was stored anaerobically. Our recent data (Lee et al., 2009) showed very low ammonium concentration in fresh manure, a rapid hydrolysis of urinary urea (within the first 24 h), and consequently a sharp increase in ammonium concentration in manure and ammonia volatilization rates.

Fecal samples from Exp. 2 were slightly enriched (average $\delta^{15}$N of 3.98 ± 0.11 ‰) and urine was slightly depleted in $^{15}$N (average $\delta^{15}$N of –0.87 ± 0.14 ‰; Fig. 6). Average $\delta^{15}$N of reconstituted fresh manure was 1.23 ± 0.15 ‰. These data confirm our previous observations that cattle urine is usually depleted ($\delta^{15}$N = –1.87 to 0.51‰) in $^{15}$N compared to feces ($\delta^{15}$N = 2.26 to 3.01‰) and the diet consumed ($\delta^{15}$N = 1.09 to 4.17‰). In a recent study we analyzed feces, urine, milk protein, and muscle tissue (*Longissimus dorsi*) from an individual cow and found similar trends: $\delta^{15}$N of 3.69, –0.06, 5.00, and 5.78 ‰, respectively (A.N. Hristov, unpublished data). Similar to Exp. 1, manure $\delta^{15}$N increased in Exp. 2 (Fig. 6) as manure aged, presumably due to the loss of $^{15}$N depleted ammonia; $\delta^{15}$N of 24-h manure was on average 4.89 ± 0.08 ‰ and further increased in 30-d manure to 5.48 ± 0.27 ‰ (the composite sample collected on Day 30 had $\delta^{15}$N of 6.25 ‰).

We first reported these effects of ammonia volatilization on cattle manure $\delta^{15}$N in Hristov et al. (2006a). Similar trends were observed for hog manure (C. Kendall, personal communication) and recently confirmed for cattle manure (Aguerre et al., 2008; Lee et al., 2009). It is known that animals, including ruminants, fractionate N isotopes, such that heavier isotope signatures correspond to animal waste. Steele and Daniel (1978) showed evidence of tissue and gut fractionation of N isotopes in cattle; urine was depleted ($\delta^{15}$N of –2.6 ‰) and feces and milk were enriched with $^{15}$N ($\delta^{15}$N of 2.6 and 4.3 ‰, respectively) as compared to the diet consumed. These processes have been used to distinguish manure-derived N from indigenous soil N (Selles and Karamanos, 1986; Kerley and Jarvis,1996; Kendall, 1998) and manure contribution to ground- and surface-water nitrate (Karr et al., 2001, 2003). Studies with compost have similarly demonstrated N isotope fractionation in animal manure. Choi et al. (2007), for example, observed $\delta^{15}N$ of composted manure as high as 45 ‰ (compared to $\delta^{15}N$ of 6.8 to 9.2 ‰ for cattle or pig manures). A more recent study by the same group (Choi et al., 2007), reported analogous trends; $\delta^{15}N$ of total N in manure increased from 7.6 to 9.9 ‰ and from 11.4 to 14.3 ‰ (depending on the bedding material), within 10 d of composting. The increase in the compost $\delta^{15}N$ was attributed primarily to ammonia volatilization and denitrification processes. Similarly, composting of corn (*Zea mays* L.) silage steadily increased $\delta^{15}N$ from 0.3 to 8.2 ‰ as ammonium and nitrate N decreased from 17.3 to 0.6 g $kg^{-1}$ ash (Lynch et al., 2006).

During manure storage, chemical kinetic isotope fractionation results in $^{15}N$ enrichment of the substrate and depletion of the product (ammonia) as lighter isotope molecules tend to react faster than molecules containing the heavier N isotope (Mariotti et al., 1981). The isotope fractionation factor associated with ammonia volatilization is one of the highest in the N cycle (~1.029, Högberg, 1997), which would results, when conditions are favorable, in a rapid increase in manure $^{15}N$ enrichment during storage. As stated by Högberg (1997), $\delta^{15}N$ of ammonia volatilized changes with time; $\delta^{15}N$ of ammonia volatilized from manure in Exp.1 tended to reach equilibrium around Day 14 of the incubation (Fig. 2). This phenomenon, however, deserves further investigation as in a more recent experiment, $\delta^{15}N$ of volatilized ammonia continued to increase (quadratically, $r^2$ = 88) through Day 20 of the manure ageing process (Lee et al., 2009): from –22.5 (Day 1), to –16.5 (Day 5) and –1.3 ‰ (Day 20).

The fractionation of N isotopes during ammonia volatilization in a closed system at standard temperature and pressure initially produces ammonia gas ($NH_3$) depleted in $^{15}N$ than the substrate dissolved ammonium ($NH_4$). The $\delta^{15}N$ of the residual ammonium therefore increases because of the loss of isotopically light N with the ammonia. As the process proceeds, $\delta^{15}N$ of both the increasing pool of ammonia gas and the decreasing pool of dissolved ammonium will continue to increase until the ammonium is exhausted and the ammonia gas obtains the $\delta^{15}N$ value of the original ammonium. The $\delta^{15}N$ values of both species at any point in time are a function of the reaction process (often characterized as the fraction of reactant remaining). In Exp. 1, this general trend can be seen in both $\delta^{15}N$ of ammonium and manure. The system in Exp. 1 is more complicated than a simple volatilizing pool of ammonium principally because of hydrolysis of urea forming new ammonium and the presence of other organic N species in manure that are measured along with the residual ammonium. In addition, and particularly in a field setting, reaction rates and fractionation factors are influenced by pH, temperature, wind, and humidity. Even considering these complicating factors, Exp. 1 shows a remarkable correlation between manure $\delta^{15}N$ and the cumulative ammonia loss suggesting that the trend of manure $\delta^{15}N$ may be useful for estimating ammonia losses. Although promising, this approach needs to be further and rigorously studied and verified in various experimental conditions and in the field under variable environment and manure management practices.

**Table 5. Nitrogen losses estimated using different approaches (Exp. 1 and 2).**

| Method | Proportion of N lost |
|---|---|
| Experiment 1† | |
| N mass balance | 0.36 |
| N/P ratios | 0.28 |
| Experiment 2‡ | |
| N mass balance, 24 h | 0.50 |
| N mass balance, 30 d | 0.61 |
| Marker/N mass balance method§, 24 h | 0.44 |
| Marker/N mass balance method§, 30 d | 0.56 |
| N/P ratios, 24 h | 0.55 |
| N/P ratios, 30 d | 0.65 |
| N/K ratios, 24 h | 0.34 |
| N/K ratios, 30 d | 0.46 |

† 14 d, a closed system in vitro apparatus (see Materials and Methods).

‡ 24 h or 30 d, dairy barn (see Materials and Methods).

§ Markers (creatinine and acid-insoluble ash) were used to estimate urine and fecal output (see Materials and Methods).

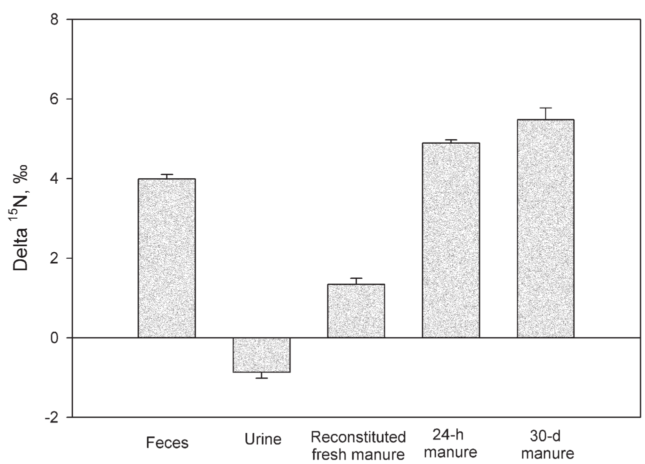


**Fig. 6. Average ( ± SE) $\delta^{15}N$ of manure samples in Exp. 2.**

## Summary and Conclusions

This study showed that under controlled conditions, changes in N/P ratio and $\delta^{15}N$ of cattle manure followed closely ammonia volatilization losses. There was a significant relationship between cumulative ammonia losses and $\delta^{15}N$ of aged manure, which was a result of volatilization of highly depleted in $^{15}N$ ammonia and perhaps other nitrogenous gases. In the specific conditions of Exp. 2, the process of ammonia volatilization was significantly accelerated and up to half of the N excreted by dairy cows could not be accounted for 24 h after excretion. Nitrogen mass balance and chemical marker approaches gave comparably high estimates for N losses (presumably as ammonia). Thus, N/P and N/K ratios can be successfully used to estimate ammonia losses from cattle manure. The observed fractionation of N isotopes in manure is remarkable and could potentially be useful to model ammonia losses. This approach, however, needs to be tested and verified in various experimental conditions and in the field.

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