Nitrogen Values of Liquid Dairy Manure and Dry Broiler Litter as Affected by Preservation Treatment

M. Dougherty, D. H. Vaughan, G. K. Evanylo, E. R. Collins, Jr., A. H. AbdelGadir

Abstract. *Liquid dairy manure and dry broiler litter samples were laboratory tested to determine the effects of four preservation techniques on the form and concentration of nitrogen. 300-mL samples of fresh manure from Virginia dairy and poultry farms were analyzed for total Kjeldahl, ammonium, and nitrate/nitrite nitrogen within 24 h of farm sampling and at the end of seven days. The four preservation techniques considered were storage of the samples; (i) at ambient temperature (26°C), (ii) by refrigeration (4°C), (iii) by freezing (-22°C), and (iv) by acidification with concentrated sulfuric acid (H_2SO_4) to pH < 2 plus refrigeration (4°C). Organic and inorganic nitrogen concentrations from the preserved manures were compared to the corresponding fresh concentrations of nitrogen in each manure control. Ambient storage, refrigeration, and freezing did not significantly affect (α = 0.05) the seven-day nitrogen concentration of the 10 manures tested. Acidification reduced most N concentrations due to the aggressive physical action of the acid, which accelerated both mineralization of organic N and volatilization of ammonia. Conclusions of this study indicate ambient storage as the most practical nitrogen preservative technique for liquid dairy manure and dry poultry litter.*

Keywords. *Nitrogen value, Liquid manure, Broiler litter, Preservation.*

One of the goals of sustainability in modern agriculture is to maximize the use of on-farm resources. With current high farm input costs for energy and fertilizers, there is renewed interest and focus on sustainable reuse of on-farm nutrients such as manures. Efficient recycling of manure N through land application offers economic as well as environmental benefits since agricultural manures are major sources of greenhouse gas emissions (Brown et al., 2000; Kulling et al., 2001; Gay et al. 2003; Umetsu et al., 2005; Sagoo et al., 2007). In Virginia and neighboring mid-Atlantic states, laboratory nutrient analysis of animal manures has allowed more accurate determination of manure nutrient levels for beneficial landuse and has subsequently become an important tool in the reduction of surface and groundwater nutrient enrichment.

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Literature Review

Azevedo and Stout (1974) cited numerous studies from the 1930s until 1974 that characterized the recovery of various nutrients, including nitrogen, from animal manures. Pain et al. (1987) stated that about 80% of the N ingested by dairy cows is excreted in feces and urine. Krogdahl and Dalsgard (1981) related differences in the ammonia content of poultry feces to differences in feed composition, nutrient digestibility, and fiber content in the diet. Adriano et al. (1974) reported that most of the N excreted by cattle is in the organic form, with approximately 50% excreted in the urine as urea ($CO(NH_2)_2$), and the remainder in feces. Significantly, 90% to 95% of the N compounds of fresh dairy cow urine can be converted to the ammonium (NH_4^+) or ammonia (NH_3) form of N within one week (Luebs et al., 1973; Jarvis et al., 1987), depending on the pH of the solution.

Burnett and Dondero (1969) showed that most of the N in fresh poultry and animal manure is present mainly as urea or uric acid. Azevedo and Stout (1974) identified a relatively large proportion of fresh poultry manure nitrogen as uric acid, the water-insoluble organic substrate that forms a distinctive "white-cap" on poultry droppings. Using colostomized hens to separate urine from feces, Krogdahl and Dalsgard (1981) identified uric acid as the major nitrogen source in poultry urine, and protein as the major nitrogen source in feces.

Rapid mineralization of broiler litter N in a 24-h incubation by Gale and Gilmour (1986) at 25°C was explained as decomposition of uric acid to urea, with subsequent hydrolysis to NH_3. Smith and Kemper (1991) explained the dynamic changes undergone during manure chemical decomposition as attributable to the large number of microbes present in manure. They stated that the chemical composition of manures is not stabilized until either the material is above 88% dry matter content or the available nutrients are depleted. Collins et al. (1994, 1995) showed that the storage and handling of liquid dairy manures and dry broiler litters can have a significant influence on their apparent nutrient composition.

Several previous studies including Nodar et al. (1990) and Kirchmann and Witter (1989) have described the physical, chemical, and biological processes involved in the ammonia volatilization of broiler litter. Nodar et al. (1990) identified the microbial populations of a stored poultry pine-sawdust litter. Kirchmann and Witter (1989) found that the amount of ammonia volatilized from manures is influenced by several factors, the most important of which is the pH of the manure solution. They stated that pH is the main factor regulating the equilibrium between NH_4^+ ions and NH_3 gas in a manure solution.

Bitzer and Sims (1988) strongly recommended that laboratories involved in manure testing carefully assess currently used techniques to determine the effects of storage and handling on N loss. Mahimairaja et al. (1990), in their evaluation of measurement methods for nitrogen in poultry and animal manures, suggested that fresh animal manures can be freeze-dried for analysis of N. Muck and Richards (1983) used 3*N* HCl to reduce volatilization of NH_3 in dairy manure samples by lowering the pH to below 3.

At least two previous studies (Moore et al., 1974; Prakasam et al., 1974) attempted to establish the magnitude and rate of N transformations occurring in an animal manure sample as a function of laboratory preservative techniques. Moore et al. (1974) studied six different types of animal manure samples stored at room temperature, and under refrigerated, frozen, and acidified (H_2SO_4 to pH < 2) conditions. Several different parameters were monitored in their four-week study, including TKN, NH_3-N, NO_3-N, NO_2-N, and pH. Moore et al. (1974) reported that initial TKN concentrations in the poultry, swine, and cattle manures tested were not maintained by any of the preservation treatments tested, but that NH_3-N levels in some manures were marginally preserved by freezing and acidification. They concluded that NH_3-N levels in cattle and fresh poultry samples cannot be preserved by refrigeration. Prakasam et al. (1974) also noted that refrigeration of poultry manure at 4°C was not satisfactory for preservation of inorganic nitrogen, but concluded that for the determination of TKN and NH_4-N in manures, samples could be successfully stored after fixing them with 0.8 mL of concentrated H_2SO_4 per liter of sample.

Umetsu et al. (2005) examined the effect of ambient storage temperatures ranging from 5°C to 20°C on recovery of nutrients from dairy manure slurry after 150 days and found that more than 90% of TKN was recovered. In excess of 100% of NH_4-N was recovered due to mineralization of organic nitrogen. Pain et al. (1987) reviewed the use of various additives to livestock slurries to conserve nitrogen, improve flow properties, and reduce odors. They discussed the rapid hydrolysis of urea in urine to NH_4-N, with subsequent loss by NH_3 volatilization, and identified three main methods of controlling these losses, including chemical stabilization. Chemical stabilization of manure nitrogen can provide for the reaction of either free NH_3 or NH_4^+ ions in solution to form stable ammonium salts (Pain et al., 1987). Because of the nature of the reactions, much of the research in chemical stabilization of manure nitrogen has been done using liquid or semi-solid manures. However, Carlile (1984), in reviewing methods of ammonia preservation in poultry houses, noted the use of superphosphate ($Ca(H_2PO_4)_2$) and phosphoric acid (H_3PO_4) in at least one poultry study. She reported that phosphoric acid was more effective in controlling ammonia and reducing litter pH than superphosphate, but that all treatments tested were relatively ineffective in hardwood shavings litter after 17 days.

Safley et al. (1983) made the observation that maximum conservation of ammonia in manure using chemical amendments can only be realized if the chemicals are added in sufficient quantity to completely react with all of the ammonia present. They also noted that sulfuric acid can be utilized with manure to stabilize the ammonia to ammonium sulfate. Pain et al. (1987) reported that sulfuric acid is less expensive than either superphosphate or phosphoric acid.

Background

Many of the manure land application recommendations made in Virginia by Extension personnel have been based on the free manure nutrient analyses performed by the Virginia Tech Water Quality Laboratory (Collins et al., 1989). From 1989-1992, the majority of manure samples sent to the Virginia Tech Water Quality Laboratory were liquid dairy manure (40% of total 1,090 samples) and dry broiler litter (23% of total 1,090 samples) (Virginia Department of Conservation and Recreation, 1993). Between 2001 and 2004, the percentage of poultry litter samples increased to approximately 35% of a total 2,666 samples, with 19% of total samples coming from liquid dairy manure (Virginia Department of Conservation and Recreation, 2005).

In Virginia, liquid dairy manures generally consist of a mixture of dairy cow feces and urine, feedlot scrapings, and milking parlor washwater, with varying amounts of site runoff. Broiler litters consist of a mixture of bedding plus manure accumulated in a broiler house over one or more flock cycles of typically seven weeks. The scope of the present study was limited to the investigation of the above two major manure types, both of which were readily available, and both of which provided a substantial range of moisture content and nitrogen values for study.

Current nutrient management recommendations in Virginia for nitrogen-based land applications of manure depend upon the quantitative analysis of total nitrogen and ammonium nitrogen, as well as other non-nitrogen nutrients. Organic nitrogen is estimated by subtracting the ammonium (NH_4-N) content of the manure from its Kjeldahl nitrogen (TKN) content. Although the more precise term for the calculated difference between TKN and NH_4-N levels in manures is non-ammonium nitrogen, the conventional term "organic nitrogen" was used in the present study. The nitrate (NO_3-N) content of animal manures is not usually reported by commercial labs because it is generally a negligible part of the total N (Collins et al., 1994, 1995).

Typical manure sampling procedures in Virginia and elsewhere are aimed at obtaining a representative analysis of the manure by placing the manure sample in a Nalgene bottle or zip-lock plastic bag at the farm, and sending the sample to an analytical laboratory. Depending upon the circumstances (who took the sample, what day of the week the sample was taken, and in what part of the state the sample was taken), manure samples may be in transit for up to one week before they are received by the testing laboratory. During the warmer months of June through August, temperatures in the sample container during transit could conceivably reach 32°C or higher. No preservation techniques are currently used to stabilize manure samples while being shipped to the laboratory. In contrast, water and wastewater samples are routinely preserved for nutrient and microbiological analysis, including organic and inorganic nitrogen (Eaton et al., 2005; U.S. EPA, 2008).

Objectives

The hypothesis of the present study was that the concentrations of the various forms of nitrogen (both organic and inorganic) in a biological sample, such as manure, are particularly susceptible to microbiological transformation under warm, moist conditions during transit, especially during summer months. The project undertaken determined whether temperature and chemical preservation of manure samples during shipment from the farm to the laboratory would significantly improve the accuracy of the manure nitrogen assays provided to agricultural and other nutrient managers.

Given the practical limitations of manure sampling techniques, sampling error at the farm is difficult to avoid. Although no attempt was made to measure sampling error in this study; documented sampling procedures were used with the intent to obtain representative samples from manure storages. Consequently, the study evaluated only the time-dependent environmental effects of preservation technique on the nitrogen values of individual liquid dairy manure and dry broiler litter samples. The two main objectives of the study were:

- to compare the forms and concentrations of nitrogen in liquid dairy manure and dry broiler litter over a seven-day period subjected to various manure preservation techniques, and
- to develop recommendations on the most applicable method of handling liquid dairy manure and dry broiler litter samples for making application rate recommendations from standard laboratory nitrogen analyses.

Materials and Methods

Ten farms were chosen at random from Virginia dairy and broiler producers (five farms of each type) in order to obtain a representative variety of the two different manure types. Table 1 contains a complete list of participating farms and includes the farm location, manure type, sample I.D., sample date, number of livestock, and the type of on-farm storage facility from which the manure samples were collected. By design, two different storage types for each manure type were represented in the experiment. Three of the five liquid dairy manure samples were collected from earthen storage basins, while the remaining two were taken from on-farm concrete storage tanks. Of the five dry broiler litter samples tested in this experiment, four were collected from beneath roofed storage structures. Only one of the five dry broiler litter samples in the study was sampled directly from a broiler house.

After being split into separate treatment bottles, all liquid dairy manures and dry broiler litters were analyzed for initial organic and inorganic nitrogen fractions. Triplicate subsampling was used to replicate initial (control) N levels at day 0. Split manure samples were subjected to four seven-day treatments, representing four manure N preservation techniques, as follows:

- ambient storage at a temperature of 26°C,
- refrigeration of the samples at 4°C,
- freezing of the samples at -22°C, and
- acidification of the samples with sulfuric acid to pH < 2, with refrigeration.

Manure Types and Sources

The initial physical characteristics of all manures evaluated in this study are presented in table 2. All liquid dairy manures sampled included twice-daily milking parlor washwater additions as well as various amounts of barnyard scrapings and flushings. Liquid dairy manures at the farm were stored in earthen or concrete impoundments ranging in capacity from an estimated 250 m^3 for the smallest concrete tank, to 3785 m^3 for the two largest earthen basins. None of the liquid dairy manure storage structures were treatment lagoons. Design depths in the above impoundments ranged from 1.8 to 2.4 m.

Broiler litters included in the study were comprised of a mixture of various wood shavings (predominantly pine wood shavings) and the deposited manure from the flocks. All dry broiler litters evaluated had been utilized for no more than one growing cycle. Dry broiler litter samples were taken from stored piles of varying size, with the exception of sample B6, which was composited by the producer from six locations inside the broiler house. Broiler litter pile volumes varied widely depending upon the size of the operation and the utilization of the litter.

Manure Sampling Procedures

Farm sampling of the five liquid dairy manures and five dry broiler litters was completed over a period of three days in July. The source of all sampling from each farm manure was a composite manure mixture collected at the farm in a

Table 1. Manure sampling summary and description of participating farms.

Farm No.	County (Virginia)	Manure Type	Sample I.D.	Sample Date	Livestock Number	Manure Storage
1	Augusta	Liquid dairy manure	D1	26 July	100 milk cows	Earthen structure
2	Augusta	Liquid dairy manure	D2	26 July	60 milk cows	Concrete tank
3	Franklin	Liquid dairy manure	D3	28 July	50 milk cows	Concrete tank
4	Franklin	Liquid dairy manure	D4	28 July	100 milk cows	Earthen structure
5	Franklin	Liquid dairy manure	D5	28 July	100 milk cows	Earthen structure
6	Augusta	Dry broiler litter	B6	26 July	28,000 birds	Broiler house
7	Augusta	Dry broiler litter	B7	26 July	60,000 birds	Roofed storage
8	Page	Dry broiler litter	B8	26 July	140,000 birds	Roofed storage
9	Page	Dry broiler litter	B9	26 July	128,000 birds	Roofed storage
10	Page	Dry broiler litter	B10	26 July	128,000 birds	Roofed storage

Table 2. Initial physical characteristics of liquid dairy manure and dry broiler litter samples.

Manure Sample	Moisture Content (%)	pH	Bulk Density (g/cm^3)	Type of On-farm Storage	Time of On-farm Storage
D1	95.9	6.7	1.00	Earthen	2-1/2 months
D2	94.8	7.3	1.01	Concrete	2-1/2 months
D3	97.1	7.5	1.01	Earthen	2-1/2 months
D4	87.3	7.4	0.88	Concrete	2 months
D5	98.4	7.8	0.99	Earthen	2 months
Average ± SD	94.7 ± 4.3	7.3 ± 0.36[a]	0.98 ± 0.06		
CV (%)	4.6	4.9[a]	5.7		
B6	29.5	7.9	0.38	Broiler house	0 week
B7	32.5	7.9	0.43	Roofed	1 week
B8	23.3	5.7	0.42	Roofed	1 month
B9	16.9	6.6	0.42	Roofed	3 weeks
B10	13.8	4.2	0.47	Roofed	4 months
Average ± SD	23.2 ± 8.0	6.5 ± 1.40[a]	0.42 ± 0.03		
CV (%)	34.4	21.5[a]	7.6		

[a] Average and coefficient of variation of pH values were calculated from the [H+] concentration of the manures.

single 19-L bucket. Substantial experimental error caused by random sampling of the heterogeneous manures was expected. Therefore, the sampling procedures used in this study were designed to reduce both the between-treatment and within-treatment variability present in the farm manure samples tested. Liquid dairy manure samples were collected with the use of a fabricated 3.1- × 5-cm diameter PVC sampler with plug-end attachment. Dry broiler litter sampling was completed by climbing on top of the 1.8- to 2.4-m high piles and, with a shovel, digging to an approximate depth of 46 cm.

The two-step sampling procedure used to collect the manure samples from all 10 farms prior to laboratory analysis was as follows:

- At each farm, a representative manure composite was placed in a 19-L bucket and mixed completely using either a large stick (for poultry litter) or a cordless electric power drill, paint stirrer, ladle, and fabricated portable blender (for liquid dairy manure).
- While blending or mixing the manure in the bucket (and while still at the farm), the manure composite was split into five 500-mL Nalgene manure sampling bottles. The five plastic sampling bottles were filled with approximately 300 mL of the mixed manure in order to obtain five equally representative samples for separate treatment.

Manure Preservative Treatments

The five split samples taken at the farm were labeled and transported to the laboratory within 24 h under ambient temperature conditions. The control and each of the four preservative treatment bottles for each manure were subjected to approximately 24 h of ambient storage during transfer from the farm to the laboratory. All samples, including the control treatments, were placed in a 5-cm thick sealed styrofoam container (without ice) after sampling and during subsequent transport to the laboratory at Virginia Tech. Temperatures within the styrofoam container between sampling and transit to the laboratory ranged from a low of 23°C in the morning (approximately 9:00 A.M.) to a high of 31°C in the evening (approximately 6:30 P.M.), reflecting normal ambient summer temperatures in Virginia on the two sample dates (table 1).

At the laboratory, one of the five representative bottles of each manure was used as a control for immediate triplicate analysis. The control sample from each farm was analyzed immediately for nitrogen concentrations, while each of the remaining four labeled manure bottles was treated with the appropriate preservative treatment. Each treatment bottle was then stored (according to treatment) for seven days before final nitrogen analyses, using duplicate subsampling (1-2 g/subsample). The four preservation techniques evaluated were storage of the samples: (i) at ambient temperature (26°C), (ii) by refrigeration (4°C), (iii) by freezing (-22°C), and (iv) by acidification with sulfuric acid (H_2SO_4) to pH < 2 plus refrigeration (4°C).

Manure samples stored under seven-day ambient conditions were kept on a table in the fifth floor of Seitz Hall (not air-conditioned) on the Virginia Tech campus. Manure treatment samples stored under seven-day refrigeration were consigned space in a walk-in cooler on the first floor of Seitz Hall. Seven-day treatment samples stored under subzero conditions were kept in a table-top freezer in the Water Quality Laboratory in Seitz Hall. All samples were undisturbed during seven-day storage.

Seven-day acidified manure treatments consisted of the addition of concentrated sulfuric acid (18.4*M* H_2SO_4) to the appropriate treatment bottles for each farm manure (table 3). Initial manure pH was determined using a Corning desk-top pH meter (Model 360i) with hand-held electrode. Concentrated H_2SO_4 was added directly to the manure sample bottle using a calibrated burette (under a fume hood and with protective gloves and clothing). The liquid dairy manure was stirred while the acid was gradually added, until the pH registered below 2.0. The acidified manure mixture was then sealed in the sample bottle and stored for seven days in the refrigerated cooler in the Water Quality Laboratory. All acidified manure samples, including the dry broiler litters, were kept in the laboratory cooler at an average temperature of 4°C. The volume of H_2SO_4 added to each manure sample as part of the acidification treatment is presented in table 3.

The dry broiler litters were acidified in much the same way as the liquid dairy manures, except that, due to their lower moisture content, the litter samples used for acidification treatment had to first be made up in a 1:1 (v/v) mixture with distilled/deionized (DDI) water for pH

Table 3. Acidification treatment for liquid dairy manures and dry broiler litters.

Sample I.D.[a]	Initial pH	18.4M H_2SO_4 Added (mL)	Acidified pH
D1	6.7	1.1	1.8
D2	7.3	2.9	1.5
D3	7.5	1.7	1.5
D4	7.4	4.6	1.7
D5	7.8	1.3	1.5
B6	7.9	2.1	1.8
B7	7.9	3.9	1.8
B8	5.7	3.3	1.7
B9	6.6	2.8	1.8
B10	4.2	2.3	1.8

[a] All samples were brought to room temperature (25°C) before pH determination.

determination. Once the 1:1 (v/v) litter/DDI H_2O mixtures were made up, the mixtures were allowed to stand for at least 20 min before pH testing and adjustment began. The pH-adjusted broiler litter mixtures were then sealed and refrigerated for seven days, as described above for the liquid dairy manures, before final nitrogen analysis.

Manure Analysis Procedures

Laboratory analysis methods used for total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4-N), and nitrate/nitrite nitrogen ((NO_3+NO_2)-N) conformed to procedures used by the Virginia Tech Water Quality Laboratory (1990). Total Kjeldahl nitrogen was determined using a modified macro-Kjeldahl digestion procedure followed by alkaline distillation and acid titration with HCl (Bremner and Mulvaney, 1982). Laboratory analysis of the inorganic forms of nitrogen included ammonium and nitrate/nitrite extraction with a 2*M* KCl solution, with subsequent analysis of the extract by the Automated Phenate Method and Automated Hydrazine Reduction Method, respectively (Greenberg et al., 1992).

In this experiment, NH_4-N was defined as ammonium nitrogen which was extractable by 2*M* KCl at room temperature (Bremner and Keeney, 1966). The resulting concentrations of NH_4-N determined in this study were subsequently used as a measure of inorganic N. Total organic N was obtained by subtracting inorganic N from the total Kjeldahl-N.

All manure control and seven-day treatment samples were analyzed for total Kjeldahl nitrogen by subsampling the appropriate treatment bottle. Three 1- to 2-g subsamples were removed from each control treatment bottle immediately upon arrival at the laboratory. Only two subsamples were taken from the preservative treatment bottles after seven days of storage. All treatment bottles being subsampled, whether liquid dairy manure or dry broiler litter, were shaken by hand twenty times by inverting the bottle completely each time and immediately prior to removing the 1- to 2-g subsample for TKN analysis. Where necessary, dry broiler litters were chopped in the sample bottle using an electric hand-mixer to achieve more representative subsampling. Identical subsampling techniques were used on all nitrogen analyses carried out in this experiment.

Resulting wet-basis nitrogen concentrations (ppm) were converted to a dry-basis by dividing wet-basis nitrogen results (ppm) by the average manure sample dry-matter content determined by oven drying at 65°C to a constant weight. Final dry-matter nitrogen concentrations for all farm manure treatments and nitrogen forms were subsequently converted from ppm to g/kg for final reporting and statistical analysis, with the exception of nitrate/nitrite nitrogen (reported as mg/kg) which needed no final conversion from ppm to mg/kg.

Statistical Analysis

Duplicate manure N concentrations resulting from the seven-day preservative techniques were averaged on a dry weight (65°C) basis and compared against corresponding initial nitrogen means in a randomized block design. Mixed-model ANOVAs were used in the comparison to determine whether the overall seven-day storage treatment effects from each of the four nitrogen preservative treatments were statistically significant for the manure types and nitrogen forms tested.

Due to differences between the nitrogen ranges of the two different manure types, the five liquid dairy manures and five dry broiler were analyzed in separate mixed-model ANOVAs to avoid the possibility of widely varying treatment effects. All control (day 0) treatment nitrogen analyses were replicated three times using laboratory subsamples, while each of the four preservative treatment analyses (at day 7) were replicated twice,.

Mixed-model ANOVA and follow-up computations for this experiment were carried out using the SAS® System for data management and analysis (SAS Institute Inc., 1989) and Statistix 8 (Analytical Software, 2003). To determine whether overall seven-day treatment levels of manure nitrogen were significantly different from the control means, all preservative treatment means were compared to corresponding control means at an alpha level of 0.05. Results from the ANOVA analyses also revealed whether interaction was present between the preservation treatments and farms tested. Individual one-way ANOVAs were analyzed for each farm manure and nitrogen form to better evaluate the impact of the interactions occurring.

Results

Moisture content and pH of the 10 farm manures tested in this study (table 2) indicate greater variation between individual broiler litter samples than for liquid dairy manure samples. The wider variation in broiler litters can be explained not only by the higher probability of sampling error in the non-homogeneous broiler litters, but also because broiler litters tested in this study varied in storage time from zero weeks to four months. Conversely, liquid dairy basins sampled had all been last pumped two to three months prior to sampling. The liquid manures were also homogenized to a much greater degree before sampling than the solid broiler litters, further reducing the physical variability of the liquid dairy manure samples between farms.

Initial Manure Nitrogen Concentrations

Tables 4 and 5 indicate that initial concentrations of the major forms of nitrogen (TKN, NH_4-N, and organic N) found in the present study correspond with average values from Virginia producers (Virginia Department of Conservation

Table 4. Mean nitrogen values for triplicate liquid dairy manure controls.

Manure Sample[a]	TKN (g/kg)	NH_4-N (g/kg)	Organic N[b] (g/kg)	(NO_3+NO_2)-N (mg/kg)
D1	32.1	12.0	20.1	Not detectable
D2	30.7	14.9	15.9	Not detectable
D3	44.5	20.3	24.2	Not detectable
D4	35.2	16.2	19.0	Not detectable
D5	65.6	40.8	24.8	Not detectable
Overall average	41.6	20.8	20.8	---
VA average[c] (1989-1992)	47.5	20.1	27.4	Not determined
VA average[d] (2001-2004)	42.7	19.7	23.0	Not determined

[a] All values expressed on a dry weight (65°C) basis.
[b] Organic N was calculated as the difference between total Kjeldahl nitrogen (TKN) and 2M KCl extractable NH_4-N.
[c] Virginia Department of Conservation and Recreation (1993).
[d] Virginia Department of Conservation and Recreation (2005).

Table 5. Mean nitrogen values for triplicate dry broiler litter controls.

Manure Sample[a]	TKN (g/kg)	NH_4-N (g/kg)	Organic N[b] (g/kg)	(NO_3+NO_2)-N (mg/kg)
B6	27.3	2.3	25.0	Not detectable
B7	28.6	7.5	21.2	Not detectable
B8	37.3	5.5	31.7	906
B9	38.7	6.8	31.9	1110
B10	47.9	2.3	45.6	92
Overall average	36.0	4.9	31.1	704
VA average[c] (1989-1992)	43.7	8.2	35.5	Not determined
VA average[d] (2001-2004)	44.9	8.0	36.9	Not determined

[a] All values expressed on a dry weight (65°C) basis.
[b] Organic N was calculated as the difference between total Kjeldahl nitrogen (TKN) and 2M KCl extractable NH_4-N.
[c] Virginia Department of Conservation and Recreation (1993).
[d] Virginia Department of Conservation and Recreation (2005).

and Recreation, Division of Soil and Water Conservation, 1993, 2005). Differences in the organic and inorganic fractions of nitrogen between the two manure types reflect the physical characteristics of the manures. Each of the five liquid dairy manures were found to have approximately half of their total nitrogen (TKN) in the inorganic form, while broiler litters were found, on average, to have less than 20% of their TKN in the inorganic form. These results are in general agreement with previous work by Adriano et al. (1974), Azevedo and Stout (1974), and Bitzer and Sims (1988). Average organic N levels in the litters were 49% higher than corresponding liquid dairy manure samples. Kirchmann and Witter (1989) reported similar trends with incubated fresh poultry manure/straw mixtures. Furthermore, Lopez-Mosquera et al. (2008) reported that organic N in fresh broiler litter constituted 83% of total nitrogen on dry weight basis, which is comparable to the average organic N level reported in this study (86%).

A trend was noted in the dry broiler litters relating total nitrogen to duration of storage (table 2). Older litters in the present study were found to have generally higher dry-matter TKN values, due probably to the effects of in-pile composting (Dougherty, 1999). Several researchers have observed decreased C/N ratios with time in poultry litter incubation studies. The main reason cited for the drop in C/N ratios was the loss of C as CO_2 evolution from microbial decomposition (Moore et al., 1974). Kirchmann and Witter (1989) noted that after 201 days of decomposition, C/N ratios were halved in aerobic manures (from 18 to 9.5), while the corresponding C/N ratio decrease in anaerobic manures (from 18 to 13) was not as pronounced. Nodar et al. (1990) tracked C/N ratios in a pine-sawdust litter, and reported a gradual 14-week decrease in C/N ratio from 16 to 14 (from hatching, to bird harvest, to litter storage).

Detection of manure nitrate/nitrite nitrogen was variable and mostly unsuccessful. Two of the five broiler litters and all five of the dairy manure samples had combined (NO_3 + NO_2)-N levels below the detection limit of the analytical procedures used in the study. Consequently, it was difficult to evaluate a significant seven-day trend in manure (NO_3 + NO_2)-N preservative storage levels using the results of the manure samples tested.

Seven-Day Manure Nitrogen Levels

Tables 6 and 7 present overall nitrogen means for the five dairy manures and five broiler litters after treatment with four seven-day preservative techniques. Results indicate that among the 10 farm manures and four preservative treatments tested in this study, acidification was the only treatment which had a significant effect on the seven-day mean concentrations of manure nitrogen. None of the other seven-day treatment means, including ambient storage at an average 26°C, were found to be statistically different from the initial overall control (day 0) concentrations of manure nitrogen for the two manure types. Umetsu et al. (2005) examined the effect of similar storage temperatures (5°C -20°C) on recovery of nutrients from dairy manure slurry after 150-day period. They found that more than 90% of TKN was recovered after the storage period, and NH_4-N in excess of 100% was recovered due to mineralization of organic nitrogen. With the exception of acidification, results

Table 6. Overall nitrogen means for liquid dairy manure control and corresponding seven-day preservative treatments.

Nitrogen Form[a]	Control	Ambient[b]	Freezing	Refrigeration	Acidification	CV[c]
TKN (g/kg)	41.6	45.9 (0)	44.4 (1)	45.0 (1)	34.1[d] (1)	10.0%
NH_4-N (g/kg)	20.8	21.7 (0)	20.7 (1)	20.9 (0)	16.4[d] (2)	9.4%
Organic N (g/kg)	20.8	24.2 (0)	23.8 (0)	24.1 (1)	17.6 (0)	21.7%
(NO_3+NO_2)-N (mg/kg)	ND	ND	ND	ND	ND	ND

[a] All values expressed on a dry weight (65°C) basis.
[b] (0) Represents the number of farms that exhibited significant treatment effects.
ND Indicates nitrate/nitrite concentrations below the detection limit of this study.
[c] CV represents the coefficient of variation between all triplicate and duplicate subsamples in the study.
[d] Represents overall mean significantly different (α = 0.05) from the control mean.

Table 7. Overall nitrogen means for dry broiler litter control and corresponding seven-day preservation treatments.

Nitrogen form[a]	Control	Ambient[b]	Freezing	Refrigeration	Acidification	CV[c]
TKN (g/kg)	36.0	38.3 (1)	38.1 (1)	38.0 (0)	33.7 (1)	3.2%
NH_4-N (g/kg)	4.9	5.3 (0)	5.2 (0)	4.9 (0)	7.7[d] (4)	15.7%
Organic N (g/kg)	31.1	33.0 (1)	32.9 (1)	33.1 (0)	26.0[d] (2)	4.7%
(NO_3+NO_2)-N[e] (mg/kg)	704	652 (0)	688 (0)	655 (0)	538 (2)	8.2%

[a] All values expressed on a dry weight (65°C) basis.
[b] (1) Represents the number of farms that exhibited significant treatment effects.
[c] CV represents the coefficient of variation between all triplicate and duplicate subsamples in the study.
[d] Represents overall mean significantly different (α = 0.05) from the control mean.
[e] Represents overall (NO_3+NO_2)-N means for samples B8, B9, and B10 only.

of the present study suggest that the greater source of increase with preservation was in TKN due to increased organic N rather than ammonium N; however the differences were not significant.

Interaction Between Farms and Preservative Treatments

Strong farm × treatment interaction was anticipated in the present study due to farm-to-farm variability and was found to be generally strong in this experiment, as indicated in table 8. The major exceptions were the analysis of dairy manure organic N and broiler litter NH_4-N, both of which had relatively consistent treatment effects across all five farms. Interaction effects observed in the study mean that comparisons of the overall means in tables 6 and 7 have to be made with caution, keeping in mind that dairy manure NH_4-N and TKN are highly associated, as are poultry litter organic N and TKN.

In order to better explain the overall treatment effects as influenced by interaction, individual statistical analysis of the preservative treatments across individual farms was completed. Consequently, a total of 33 one-way ANOVAs were analyzed for each farm and nitrogen form. The number of individual farms exhibiting significant treatment effects was tallied and included within tables 6 and 7 as parenthetical subscripts to more fully indicate how the nitrogen forms and concentrations of the manures in this study were affected by sample preservation.

In spite of the strong farm × treatment interaction, results indicate relatively high variability between individual manure subsamples compared against treatment effect means. The general result is that, with the exception of the overly aggressive acidification treatment, all preservative techniques tested in this study were equally successful in the seven-day preservation of nitrogen in liquid dairy manure and dry broiler litter samples.

Table 8. Level of significance for farm × treatment interaction.

Nitrogen Form	Liquid Dairy Manure[a]	Dry Broiler Litter[a]
TKN	0.058	0.004
NH_4-N	0.011	0.540
Organic N	0.678	0.009
(NO_3+NO_2)-N	ND[b]	0.004

[a] Numerical values represent p-values, where level of significance < α = 0.05 indicates significant farm × treatment interaction.
[b] ND Indicates nitrate/nitrite concentrations below the detection limit of this study.

Discussion

Because observed seven-day treatment effects on manure nitrogen were found to be less than the variability (error) in the analytical nitrogen determinations themselves, manure storage under ambient (26°C), refrigerated (4°C), or frozen (-22°C) conditions were equally effective in the seven-day preservation of the major forms of liquid dairy manure and dry broiler litter nitrogen. As a result, if all other conditions were equal, storage of the above manure samples at ambient temperatures of 26°C would be recommended as the simplest and most practical method of seven-day storage for subsequent nitrogen analysis.

Acidification of Manures as a Preservative Treatment

Although the acidification treatment was unsuccessful as a preservative treatment, it proved instructive. Overall mean concentrations of most major manure nitrogen forms decreased after seven days of acidified conditions under refrigeration, with the notable exception of broiler litter NH_4-N, which increased significantly under acidified conditions. Significant differences in overall seven-day acidified nitrogen levels were observed in four of the seven manure/nitrogen randomized blocks analyzed, including: liquid dairy manure TKN and NH_4-N, and dry broiler litter NH_4-N and organic N. Of the 33 individual farm/nitrogen ANOVAs tested, a total of 12 resulted in acidified seven-day nitrogen means significantly different from initial nitrogen means. Therefore, acidification treatment with H_2SO_4 to pH < 2 at 4°C as tested in this study was determined to be an unacceptable technique for preserving the nitrogen forms of seven-day liquid dairy manure and dry broiler litter samples.

Further, while seven-day refrigeration of acidified liquid dairy manure samples resulted in significantly decreased overall NH_4-N concentrations, a significant increase in corresponding dry broiler litter NH_4-N levels was observed. In fact, of the five individual one-way ANOVAs used to test seven-day acidified dry broiler litter NH_4-N means against initial control levels, four of five litters had significantly increased NH_4-N means. Because liquid dairy manures tested in this study had a higher percentage of total nitrogen as NH_4-N when compared to the dry broiler litters (50% vs. 14%), a relatively higher amount of inorganic N in the liquid dairy manures was available for potential NH_3 volatilization. However, it was only in the acidification treatment that a significant decrease in overall NH_4-N levels was observed in liquid dairy manures. Since similar significant seven-day losses of NH_4-N were not observed in any of the refrigerated dairy manure treatments, it is reasonable to isolate the addition of H_2SO_4 as the factor responsible for this loss.

During the acidification procedure, addition of concentrated H_2SO_4 caused an aggressive foaming and heating of the liquid dairy manure samples. Average moisture contents of the liquid dairy manures decreased from 94.7% to 93.2% after seven-day acidification, verifying H_2O evaporation. Immediate losses of aqueous NH_3 was likely due to the physical effects of the exothermic reaction. During the addition of H_2SO_4 to the broiler litter mixtures, much less foaming and heat was generated than during liquid dairy manure acidification, due to the lower moisture content of the litter mixtures.

Another disadvantage of manure acidification to pH < 2 that was observed in both the liquid dairy manure and broiler litter samples was the apparent mineralization of manure organic nitrogen. Overall seven-day TKN means for the acidified liquid dairy manure samples in this study were found to be significantly lowered from corresponding initial TKN means, with 42% of the overall TKN losses apparently attributable to losses of organic nitrogen, since 58% of the overall TKN losses were directly attributed to the significant loss of liquid dairy manure NH_4-N under acidification. Therefore, not only was NH_3 volatilized by the physical reactions (foaming and heating) of the concentrated H_2SO_4 with liquid manure, but some portion of organic nitrogen was apparently mineralized by the H_2SO_4 treatment, which would result in a distorted nutrient determination.

Overall seven-day broiler litter organic nitrogen concentrations responded similarly to the decreases observed in liquid dairy manure organic nitrogen, except that in the case of broiler litters, the loss of organic N through mineralization was statistically significant. Also, the loss of organic N in the acidified broiler litters was accompanied by a significant increase in NH_4-N levels. This statistically significant seven-day increase in overall broiler litter NH_4-N under acidification treatment can be partly explained by the higher percentage of broiler litter nitrogen found in the organic form, as compared to liquid dairy manures (86% vs. 50%, respectively). Apparently, a portion of the broiler litter organic nitrogen that was mineralized by H_2SO_4 was effectively fixed as NH_4-N by equilibrium in the highly acidic treatment matrix. The observed mean decrease of 5.1 g/kg in organic N during seven-day broiler litter acidification was accompanied by only a 2.8 g/kg overall increase in NH_4-N, suggesting that other nitrogen losses or transformations occurred. Conclusions based upon the above results show that the acidification of dry broiler litters and liquid dairy manures to pH < 2, as tested in the present study, is an unnecessarily aggressive method for seven-day manure nitrogen preservation.

SUMMARY

The effects of four preservation techniques on the form and concentration of nitrogen was evaluated on liquid dairy manure and dry broiler litter samples from Virginia. Manure concentrations of total Kjeldahl, ammonium, and nitrate/nitrite nitrogen were analyzed within 24 h of farm sampling and at the end of seven days. The four preservation techniques evaluated were storage of the samples: (i) at ambient temperature (26°C), (ii) by refrigeration (4°C), (iii) by freezing (-22°C), and (iv) by acidification with sulfuric acid (H_2SO_4) to pH < 2 plus refrigeration (4°C).

Organic and inorganic nitrogen concentrations from the preserved manures were compared to the corresponding fresh concentrations of nitrogen in each manure control. Ambient storage, freezing, and refrigeration did not significantly affect ($\alpha = 0.05$) the seven-day nitrogen concentration of the ten manures tested. Acidification reduced most N concentrations due to the aggressive physical action of the acid, which accelerated both mineralization of organic N and volatilization of ammonia.

Based on the results of this study, two major conclusions can be drawn. First, manure storage under ambient (26°C) conditions can be recommended as sufficient for seven-day preservation of the major forms of nitrogen in liquid dairy manure and dry broiler litter. Since there was no practical difference, on average, between the nitrogen levels of the ambient preservative treatment and the nitrogen levels of corresponding fresh manure samples, nitrogen analysis of above manures stored under ambient conditions can be used for making nitrogen application rate recommendations. The second conclusion drawn from this study is that acidification with concentrated H_2SO_4 to pH < 2 (with refrigeration) was found to be an unacceptable preservative technique because of the aggressive physical action of the acid, which accelerated both the volatilization of ammonia in liquid dairy manures and the mineralization of organic N in dry broiler litters. In addition, use of a hazardous material such as concentrated acid as tested in this study is not a practical alternative for most producers. Further study quantifying phosphorus preservation techniques is recommended.

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