NITROGEN VALUES OF LIQUID DAIRY MANURE AND DRY BROILER LITTER

AS AFFECTED BY PRESERVATION TREATMENT

by

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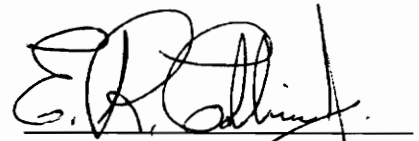in partial fulfillment of the requirements for the degree of
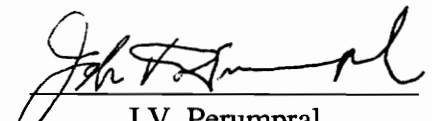
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(ABSTRACT)

Five liquid dairy manures and five dry broiler litters were tested in the laboratory to determine the effects of four preservation techniques on the forms and concentrations of nitrogen. 300 ml samples of fresh manure from five Virginia dairy and five poultry farms were analyzed for total Kjeldahl, ammonium, and nitrate/nitrite nitrogen within 24 hours of farm sampling, and at the end of seven days. Samples of the fresh manure were analyzed immediately as a control. The four preservation techniques were storage of the samples: at ambient temperature (26°C), by freezing (-22°C), by refrigeration (4°C), and by acidification with sulfuric acid (H_2SO_4) to pH < 2 plus refrigeration (4°C).

Concentrations of nitrogen fractions were tabulated on a dry-weight basis and statistically analyzed using a randomized block design, with subsampling of each treatment × farm combination. 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 (α=.05) the 7-day nitrogen concentrations of the ten manures. 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. Ambient storage was recommended as the most practical preservative technique because, of the three successful preservation methods, ambient storage provided the simplest procedure for 7-day preservation of nitrogen in liquid dairy manures and dry broiler litters.

Dedication

Bismillah   *In the name of God*

To my grandmother, who taught me how to be content, but not static...

to my father, who urged me to be good, to be fair, and to work hard...

and to my dear mother, who showed me kindness and beauty,

                              ...and taught me how to pray when I was little.

Acknowledgments

Read!
In the name of thy Lord and Cherisher,
Who created -
Created man, out of
A leech-like clot:
Read! And thy Lord
Is most Bountiful, -
He Who taught
(The use of) the Pen, -
Taught man that
Which he knew not.
Nay, but man doth
Transgress all bounds,
In that he looketh
Upon himself as self-sufficient.
Verily, to thy Lord
Is the return (of all).
                                                      Quran 96:1-8
                                                      (first verse revealed)

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M =   Molar (moles of solute/1000 ml solution)

N =   Normal (equivalent of solute/1000 ml solution)

M   =   Mega ($\times 10^6$)

μm   =   micron ($\times 10^{-6}$ m)

nm   =   nanometer ($\times 10^{-9}$ m)

ppm   =   parts per million (mg/l)

I. Introduction

A. Brief history of manure use

The use of animal manures for soil enrichment has been a traditional practice through much of the history of settled agriculture. Azevedo and Stout (1974) stated that, "returning nutrients and organic matter to the soil via animal manures completes the ancient and natural cycle on which all life depend." Since the beginnings of agriculture, it has been noted that manured crops grew better than crops without manure. Ancient civilizations such as China and Rome recognized the regenerative benefits of recycled manure nutrients and organic matter on cultivated soils. However, it is not known when or how manure fertilization actually began (Tisdale et al., 1993).

It was not until the late nineteenth and early twentieth centuries that the importance of plant nutrients, including nitrogen, was discovered. Economic incentives spurred developmental research in the area of mineral fertilization as food demands grew. The subsequent development of various mineral fertilizers to enrich depleted agricultural land added an enormous body of knowledge to the modern science of agronomy.

Lawes and Gilbert began the first long-term crop nutrition experiments at Rothamsted, England in 1843. The inorganic nutrients tested at Rothamsted were always compared to farmyard manure, because manure was the traditional source of soil fertility at the time. Lawes and Gilbert are credited with producing evidence of the paramount importance of nitrogen in plant nutrition, especially in the production of cereal crops (Jenkinson, 1991).

More costly fixed nitrate and ammonia fertilizers were developed as food demand continued to outstrip land and manure nutrient capabilities in the present century. Cheap natural gas became available for ammonia (NH_3) synthesis in the 1940's. Commercial

nitrogenous fertilizers eventually became less costly than the handling and spreading of equivalent amounts of animal manure nitrogen.

Modern chemical fertilizers offered several other advantages over animal manure. In addition to being less costly to transport and apply, commercial fertilizers have a standardized composition, are easier to store, and have a more predictable effect on crop growth than manure nutrients (Neeteson and Wadman, 1990). Subsequently, animal manure came to be perceived as a material with relatively little economic value as a fertilizer for production agriculture during the Green Revolution of the 1960's.

At the same time, pastured animals were more often being confined to meet increasing consumer demands for animal products. Today, animal nutrients in feedstuffs are increasingly imported from outside regions and then deposited locally as manure. The resulting concentration of nutrients associated with confined livestock operations is a potential source of surface and groundwater enrichment. Specifically, the land application of excessive amounts of nitrogenous organic solids from increasing domestic animal populations has been responsible for above normal levels of nitrates in regional and localized drinking water sources (Select Committee on Nonpoint Source Nutrient Management, 1990).

As modern growers must adhere to stringent water quality regulations to reduce off-farm environmental impacts, management of manure nutrients is increasingly sophisticated. Modern livestock production facilities, such as cattle feedlots and poultry operations, can concentrate thousands of animals in small areas. Elliott and Swanson (1976) identified four distinct manure handling problems associated with commercial animal feeding practices, including:

1) the need for adequate manure storage until application time corresponds with favorable crop, land, and weather conditions;

2) the social and economic problems associated with nutrient loss, especially in the form of ammonia;

3) the possible increased manure distribution costs due to long haul distances; and

4) the potential pathogen and pollution problems associated with gases (odors), dust, runoff, and deep percolation (leaching).

In spite of the above challenges that are faced by most modern livestock operations, Elliott and Swanson (1976) concluded that the benefits of using manure resources outweighed the disadvantages. The ultimate use of an organic amendment such as manure is to increase soil productivity through the natural cycle of decomposition. Soil productivity itself is dependent upon such factors as: soil structure, texture, fertility, pH, and moisture availability. The organic matter content of a soil is intimately related to its long-term soil productivity. Animal manures increase soil organic matter, which improves physical and chemical properties for root growth (Tisdale et al., 1993). Also, manure organic matter increases soil microbial activity and can improve the exchange capacity, tilth, aggregation, and water-holding capacity of a soil (Tisdale et al., 1993). Therefore, the enhancement of long-term soil productivity through the judicious application of animal manures makes these organic residues a valuable resource for crop production as well as land reclamation.

Admittedly, animal manures are often not as cost-effective or easy to apply as modern high-grade commercial fertilizers. The management and disposal of animal manures in industrialized agriculture requires scraping, processing, storing, hauling, and distribution. In addition, manures have a low nutrient content, are highly variable in nutrient composition, and often contain noxious weedseeds and odors. However, in spite of the negative attributes, farm manures have value as a soil amendment.

In addition, the current need to find practical and effective methods of long-term manure disposal has led to the increased use of land application as part of an environmentally and economically sound nutrient management strategy. Such an integrated approach to on-

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farm nutrient management has given hope to many that a more sustainable industrialized agriculture in the future is possible.

B. Role of manure nitrogen

Nitrogen is a significant constituent of the lithosphere, atmosphere, hydrosphere, and biosphere. After carbon, hydrogen, and oxygen, no other element is as intimately associated with the reactions carried out by living organisms, as is nitrogen (Stevenson, 1982). Nitrogen is required in relatively large amounts for adequate growth of most agricultural crops. Olsen and Kurtz (1982) identified the following six critical roles of N in plant nutrition as:

1) a component of chlorophyll;
2) a component of amino acids, the building blocks of proteins;
3) a component of enzymes, vitamins, and hormones;
4) a stimulative of root development and activity;
5) an essential element for carbohydrate utilization; and
6) a facilitator in the uptake of other nutrients (Olsen and Kurtz, 1982).

Nitrogen plays a unique and vital role among the elements essential for plant growth. The nitrogen available from farm manures provides an important link in the cycling of this valuable nutrient onto land.

Although the use of animal manures as the principal crop nitrogen source decreased in the years following World War II (Delwiche, 1970), dramatically higher energy costs in recent decades (with a corresponding rise in the price of industrially-fixed fertilizers) have made manure N reuse more attractive. Safley et al. (1983) reported that the economically recoverable nitrogen from manure in the United States had the potential of replacing approximately 15% of the nation's annual commercial fertilizer N requirement. The manufacture of fertilizers and pesticides consumes 39% of all the on-farm energy used in

U.S. production agriculture, and any increase in the utilization of manurial nutrients could reduce the energy use in agriculture (Safley et al., 1983).

One of the goals of sustainability in modern agriculture is to maximize the use of on-farm resources. Sweeten (1991) reported that from an estimated 26.5M t (dry weight) of dairy cow manure voided annually in the United States, 0.99M t of dry manure nitrogen is produced. According to Sweeten, as much as one-half to two-thirds of the total dairy manure produced may be voided directly onto pastures, leaving approximately 0.33-0.50M t of dry manure nitrogen available for managed reuse. Annual production of broiler nitrogen is estimated as 0.35M t from a total of 6.9M t of manure (Sweeten, 1991). Since essentially all poultry are raised in confinement, any estimated broiler manure nitrogen values represent nutrients available for managed reuse. The combined annual nitrogen production from these two manure sources (dairy and broiler manures) is approximately 1.4-1.5M t. Using a comparative farm cost of $0.44 / kg-N ($0.20 / lb-N) for commercial fertilizer N (Alley, 1994), the total value of the manure N managed by the nation's dairy and broiler producers is in excess of $700M. With efficient recycling of this manure N through land application, modern industrialized agriculture can maximize the use of this valuable on-farm resource in a way that both economic and environmental benefits are realized.

C. Manure N decomposition and nutrient management

Manure and urine begin to change in composition immediately upon being excreted, with physical, chemical, and biological processes influencing the various pathways by which animal manure decomposes (Smith and Peterson, 1982). Therefore, the manner in which a manure is handled or stored on the farm can affect its chemical composition. Several simplified pathways of manure N decomposition from stored animal manure and urine are illustrated below in Figure 1.1, and include the important nitrogen transformation

processes of ammonification, nitrification, immobilization, volatilization, and denitrification.

N_2 Denitrification   Organic Matter Immobilization   Surface Runoff

Microbial Decomposition   **Manure & Urine Farm Storage**   Removal to Soil

NH_4^+

Urease Ammonification

NO_3^- Nitrification

Anaerobic Metabolism

Groundwater   Surface Runoff NH_3 Volatilization   Odorous-reduced N compounds
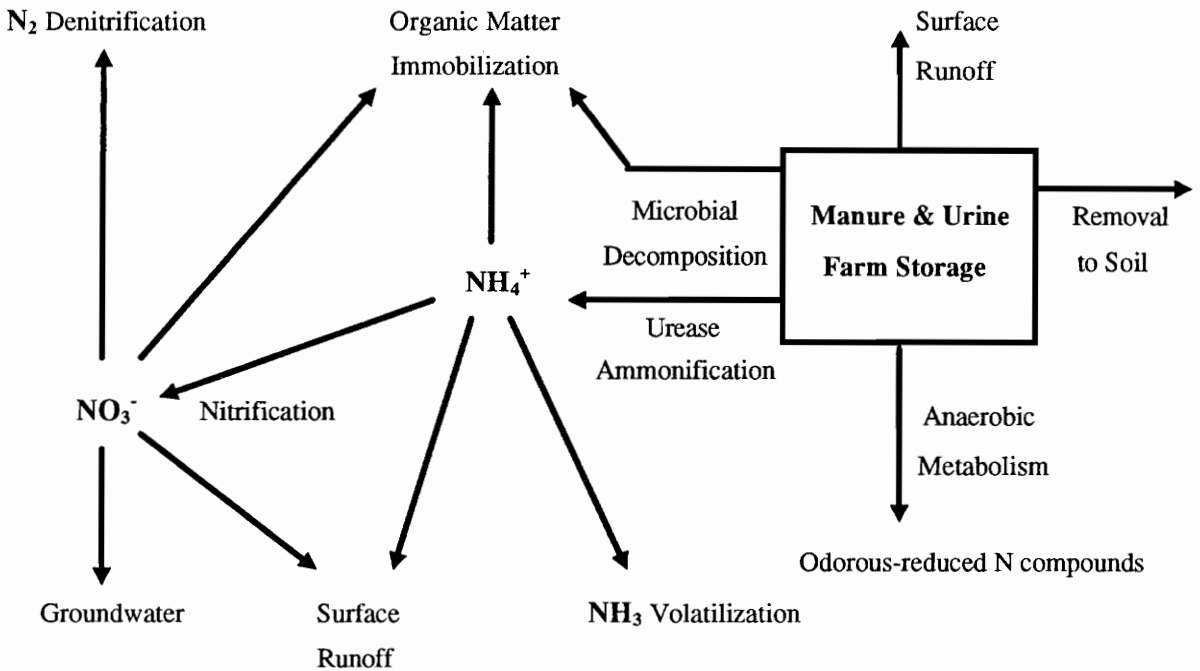
Figure 1.1.   Simplified manure N decomposition pathways in stored manure and urine (adapted from Elliott and Swanson, 1976).

Figure 1.1 illustrates how the mineral portion of a stored organic manure can increase as decomposition proceeds, with mineralized organic N (as NH_4^+) being converted to the highly mobile nitrate ion (NO_3^-). As mentioned previously, environmental awareness of the potential for surface and groundwater enrichment has led to increased concerns about the fate of mineral nitrogen. Subsequently, the mineralization of organic N in animal manures (through ammonification and nitrification) has become a basic premise in the continually improving discipline of manure nutrient management.

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Modern nutrient management planning utilizes an integrated scheme of management practices to achieve distinct goals. As an example, Moore and Gamroth (1994) listed seven manure management practices for each of two farming systems that had widely varying nitrogen balances. In the first system, which had minimal land area and a nutrient excess, farm management practices were recommended that promoted denitrification, high N crop uptake, and maximum ammonia volatilization. The second farming system, due to a limited nutrient supply, attempted to conserve nitrogen as a fertilizer by recommending manure management that achieved minimum N loss while matching the nutrient uptake of the crop. Manure timing, application method, and control of soil moisture were all managed to maximize the crop-efficiency of the mineralized N from the manure. The above example shows how important it is, in the design of manure management systems, that all possible nitrogen and other nutrient losses be recognized. Through sampling and laboratory analysis of farm manure, producers can have a better idea of the concentration of nutrients going onto their fields, making subsequent plant-availability and field-loss estimates more reliable.

D. Significance of project

1. Manure management and Virginia agriculture

The state of Virginia has a diversified agricultural industry, with cash receipts from poultry and dairy products accounting for almost 40% of the 1989 agricultural revenue in the state (Virginia Agricultural Statistics Service, 1989). Virginia ranked 10th in the United States in broiler production in 1990 with 182M birds, and ranked 19th in the United States in the number of milk cows on farm with a total of 143,000 milk cows (USDA, 1991). The combined cash receipts for broiler production and wholesale milk in Virginia totaled $577M in 1989, which exceeded the combined revenue from the top three cash crops

grown in the state (tobacco, soybeans, and peanuts) (Virginia Agricultural Statistics Service, 1989).

The efficient management of the liquid dairy manures and dry broiler litters produced by these Virginia livestock operations has become increasingly important as managers seek to recycle manure nutrients in the most environmental and economic manner. Laboratory nutrient analyses of animal manures in Virginia and neighboring mid-Atlantic states has allowed more accurate determination of manure nutrient levels for beneficial landuse, and has subsequently become an important tool in protecting regional water quality.

Manure nutrient analysis can provide application rate recommendations that permit efficient use of nutrients. Since 1987, many of the manure land application recommendations made in Virginia by Extension personnel have been based on the manure nutrient analyses performed by the Virginia Tech Water Quality Laboratory (Collins, 1994). Manure nutrient analysis in Virginia is offered for liquid dairy, dry broiler litter, dry turkey litter, layer or breeder litter, liquid poultry, semi-solid dairy, semi-solid beef, swine lagoon, and mixed swine manure. Estimates of the equivalent value of the manure as a nitrogen fertilizer have formed the basis for land application of animal manures, except when other nutrients are most limiting (Collins et al., 1989).

Data from the 1993 Nutrient Management Handbook (Virginia Department of Conservation and Recreation, Division of Soil and Water Conservation, 1993), shows that the majority of manure samples sent to the Virginia Tech Water Quality Laboratory from 1989-1992 were liquid dairy manure (40% of total samples) and dry broiler litter (23% of total samples). Liquid dairy manures generally consisted of a mixture of dairy cow feces and urine, feedlot scrapings, and milking parlor washwater, with varying amounts of site runoff. Liquid manures were classified as those having a moisture content greater than 87.5% (Virginia Tech Water Quality Laboratory, 1990). Virginia Tech utilizes Gale and

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Gilmour's (1986) definition of broiler litter as the mixture of bedding plus manure that has accumulated in a broiler house over at least one flock cycle. 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. More importantly, these two manure types were chosen because of the vital role played by poultry and dairy production in the economic well-being of Virginia agriculture.

2.   Statement of problem

Current manure sampling procedures in Virginia are aimed at obtaining a representative analysis of the manure by placing the manure sample in a Nalgene bottle or zip-lock plastic bag, and sending the sample to the 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 could 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 bottle during transit could conceivably reach 32°C (90°F) or higher. No preservation techniques are currently being used to stabilize the 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 (Environmental Protection Agency, 1986). Due to the dynamic nature of the nitrogen cycle, it is possible that the nitrogen concentrations of a manure sample could be affected by the combined in-transit environmental effects of time and temperature. Therefore, 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.

The project undertaken in this study 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 nutrient managers.

3.   Scope of project

The purpose of most manure nutrient analyses is to determine the nutrient content of a representative sample of a farm manure. In order to fully evaluate the accuracy of the manure nutrient analysis, however, it is important to determine how well the sample actually represents the manure being tested. Given the practical limitations of present-day manure sampling techniques, sampling error at the farm is difficult to avoid. The present study made no attempt to measure this normal sampling error; therefore the representativeness of the samples from manure storage was not critical in this experiment. Rather, this study evaluated the time-dependent environmental effects on the nitrogen values of individual liquid dairy manure and dry broiler litter samples.

The present study also determined whether preservation of manure samples could have significantly improved the accuracy of the nitrogen analysis procedures used in Virginia's free manure testing program from 1987-1994.

E. Objectives

The overall goal of the study reported herein was to examine the effects of sample preservation of liquid dairy manure and dry broiler litter on nitrogen concentrations. To accomplish this goal, the following objectives were undertaken:

1. To compare the forms and concentrations of nitrogen in liquid dairy manure and dry broiler litter over a 7-day period subjected to various manure preservation techniques.

2. To develop recommendations on the most applicable method of handling liquid dairy manure and dry broiler litter samples for making application rate recommendations from laboratory nitrogen analysis.

II. Review of Literature

A. Manure nitrogen composition

Numerous authors have identified the physical, chemical, and biological characteristics of animal manures. Azevedo (1974) described the composition and decomposition of farm manures by first characterizing the animal digestion processes unique to each class of livestock. He contrasted the ruminant fermentative predigestive system of cattle with the food-softening organs (crop and gizzard) of the avian digestive system, and made the fundamental statement that nitrogen in animal feces closely corresponds to dry-matter nitrogen intake. This important correlation between animal nitrogen intake and manure nitrogen composition, according to Azevedo, determines the nitrogenous components of animal manures, which he identified as undigested plant material (humus-like lignoproteins), synthesized microbial-cell protein, endogenous digested juices, and sloughed-off intestinal cells.

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. Azevedo (1974) cited numerous studies from the 1930's until 1974 that have characterized the recovery of various nutrients, including nitrogen, from animal manures. Although less than 40% of the total fresh weight of most manures in the studies cited by Azevedo were made up of urine, manure urine was found to be relatively more concentrated with respect to nitrogen than the feces (Azevedo, 1974). Adriano et al. (1974) reported that most of the N excreted by cattle is in the organic form, with approximately 50% in the urine as urea ($CO(NH_2)_2$), and the remainder in feces. Jarvis et al. (1987) stated that of the N ingested by ruminants, up to 80% is contained in the urine.

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Significantly, 90-95% 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, 1973; Jarvis et al., 1987), depending on the pH of the solution.

Azevedo (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. According to Krogdahl and Dalsgard, the range of uric acid found in the urine of eight White Leghorn layers was between 30% and 82% of total urinary nitrogen, while ammonia nitrogen varied from 6% to 23%. In this same experiment, urea nitrogen in the urine was found to range from 2% to 10% of total urinary nitrogen. Burnett and Dondero (1969) showed that most of the N in fresh poultry and animal manure is present mainly as urea or uric acid.

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. Henry and White (1990) reviewed six management variables that have been shown to affect the physical and chemical composition of broiler litters, including: type of bird raised, number of birds per unit area, type of feed and litter base, number of growing cycles the litter was used, and type of feeding and watering apparatus used. Collins et al. (1994a, 1994b) have shown that the subsequent storage and handling of liquid dairy manures and dry broiler litters can have a significant influence on their apparent nutrient composition.

A number of universities, including the University of Maryland, Virginia Polytechnic Institute and State University, The Pennsylvania State University, and North Carolina

State University, have compiled databases describing the various physical and chemical characteristics of animal manures. Among the chemical parameters most often reported to nutrient managers are the macronutrients N, P, and K, along with Ca, Mg, and S, and several micronutrients such as Mn, Zn, and Cu. Nutrient assays used for extension work are reported in elemental form except for P and K, which have traditionally been expressed as the oxides P_2O_5 and K_2O (Tisdale et al., 1993). Nutrient values are usually reported to farmers and nutrient managers on a wet basis, since that is the form handled at the farm in estimating application rates (Collins et al., 1994a, 1994b). Appendix 1 and 2 present examples of two manure nutrient reports from the Water Quality Laboratory at Virginia Tech in Blacksburg, Virginia. Average nutrient values of liquid dairy manure and dry broiler litter samples reported by the Virginia Tech Water Quality Lab (from 1989-1992) for the primary macronutrients and ammonium nitrogen are summarized in Table 2.1. (Note that nutrient values in Table 2.1 are given on a wet basis, as well as a dry weight basis, for purposes of comparison.)

Table 2.1.   Average nutrient values for liquid dairy manures and dry poultry litters in Virginia, 1989-1992.

	TKN	NH$_4$	P$_2$O$_5$	K$_2$O	MC †
Liquid dairy manure	22.6 * (47.5)	9.6 * (20.1)	12.1 * (25.4)	18.9 * (39.8)	94.3 %
Dry broiler litter	62.6 ** (43.7)	11.8 ** (8.2)	62.1 ** (43.4)	28.6 ** (20.0)	28.4 %

†   MC represents moisture content of wet material
*   values expressed as pounds/1000 gallons, wet basis
**   values expressed as pounds/ton, wet basis
( )   values in parentheses expressed as g/kg, dry weight (60° C) basis

Research investigations into the measurement of the nitrogen concentration of manures are numerous. Mahimairaja et al. (1990) identified four forms of nitrogen in their evaluation of analysis methods for fresh samples of poultry and animal manures. They reported average dairy manure N values of 33.6 g/kg, 5 g/kg, 0.03 g/kg, and 0.02 g/kg for Kjeldahl nitrogen (TKN), ammoniacal nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), and nitrite nitrogen (NO_2-N), respectively, with all results reported on a freeze dried weight basis. They calculated organic N as the difference between Kjeldahl N and extractable NH_4-N, and found that approximately 85% of the total N in a fresh sample of dairy manure was present in the organic form, while 15% of the total N was present in the inorganic form, mainly (99%) as NH_4-N (Mahimairaja et al., 1990).

Increased poultry production in recent decades has brought with it a large number of similar investigations into the nutrient value of broiler litter as a fertilizer source and soil amendment. Hileman (1967) reported the average N, P, K, pH, and moisture levels from 197 broiler houses in Arkansas, and was able to show that nearly all of the mineral elements essential for plant growth are present in broiler litter. He also stated that several factors influencing the chemical composition of broiler litter, such as moisture, temperature, and ventilation, all contribute to variation in the nutrient value of litter. Westerman et al. (1988) reported the physical and nutrient characteristics (including litter density and storage time) of three broiler litters and two turkey litters in a ten-month soil incubation study. Bitzer and Sims (1988) tested 16 broiler and four roaster litter samples as part of a study to evaluate the availability of nitrogen in poultry manure. They reported total N, NH_4-N, and NO_3-N, values for all twenty litters, with resulting average values of 53.2 g/kg, 20.6 g/kg, and 308 mg/kg, respectively, with wet weight concentrations expressed on a dry weight (104° C) basis (Bitzer and Sims, 1988). Bitzer and Sims, in the same study, also analyzed each of the litters for pH, C, and C/N (organic) ratio values, obtaining mean values of 8.7, 278 g/kg, and 9, respectively. They found the litters to be

alkaline, with the inorganic nitrogen fraction comprised primarily of NH_4-N, while corresponding NO_3-N levels were usually below 200 mg/kg (Bitzer and Sims, 1988).

Stephenson et al. (1990) analyzed litter samples from 106 Alabama broiler producers and calculated an average N:P:K ratio of 3:3:2. Nodar et al.(1990) studied the microbial populations of a poultry pine-sawdust litter and obtained nitrogen and pH values within the range of Bitzer and Sims (1988).

B. Transformations of manure nitrogen

1. Manure nitrogen mineralization

Azevedo (1974) reviewed the differences between nitrogen availability in manures and mineral nitrogen fertilizers. He explained that the relatively slow biological release of nitrogen from the complex organic compounds in manure is the main difference between the two types of fertilizers. Mehran and Tanji (1974) stressed the importance of evaluating rate coefficients to quantify N transformation processes in soils, but cautioned against making the assumption that these rate coefficients are constant when modeling these processes. They noted the important interactions between soil environmental factors, microorganism populations, and the estimated value of the N transformation rate coefficient. Nodar et al. (1990) cited a number of factors having a significant effect on the mineralization of organic substrates in a soil, including: pH, moisture content, C/N ratio, available nutrients, and particle size. Bonde and Lindberg (1988) studied nitrogen mineralization kinetics using models to evaluate mineralization rates in soil laboratory incubations using various types of organic solids. Serna and Pomares (1991) compared biological and chemical methods to predict nitrogen mineralization in animal manures. They identified two basic methods of quantifying N availability in soils, including:

incubation/plant uptake studies, and rapid chemical extraction processes (Serna and Pomares, 1991).

Hadas et al. (1983) and Bitzer and Sims (1988) previously found that manure N mineralization followed a two-phase process in poultry manure, and reported the presence of a rapidly mineralizeable organic N fraction. Gale and Gilmour (1986) reported a three-phase mineralization of poultry litter in their 28-day incubation study. Expressed as a percentage of the organic N added to the soil, Serna and Pomares (1991) reported a 16-week mineralized N range of 0% (cow manure) to 39% (poultry manure), and were able to predict N availability in the manures tested with several chemical extraction procedures. In general, chicken and pig manures were found to contain a higher percentage of mineralizeable N than sheep and cow manures, with results showing a marked variability in N mineralization with the type of manure and form of N present (Serna and Pomares, 1991).

Castellanos and Pratt (1981) had previously compared chemical indices to available manure N in a ten-month greenhouse trial with ten different manures. Significantly, they reported that the differences in N mineralization among the manures tested largely occurred during the first week of incubation. They also found potentially available nitrogen (PAN) to be related to the C/N ratio and the amount of C and N mineralized (Castellanos and Pratt, 1981). C/N ratios below 20 result in net mineralization, while C/N ratios above 20 result in net immobilization of nitrogen (Alley, 1994). King (1984) stated that incubation of soil-amendment mixtures is the most accurate method of estimating organic N availability under laboratory conditions. King conducted a 16-week study on various organic solids, and reported that available N was generally greater from aerobically digested municipal sewage sludges than from anaerobically digested sludges. He stated that the current method of estimating N availability in liquid organic samples is to assume that all the inorganic N in the liquid fraction is available (after making

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adjustments for NH_3 volatilization), then estimate the fraction of organic N in the solids that becomes available through the use of incubation studies.

In order to conduct their 26-week incubation study, Cheschier et al. (1986) divided liquid manure into four fractions, as follows: inorganic N (principally NH_3-N), rapidly mineralizeable organic N (urea or uric acid), near-term mineralizeable organic N (nitrogenous compounds mineralized by soil microorganisms within a few months), and very slowly mineralizeable organic N (more complex nitrogenous compounds resistant to microbial decomposition). They concluded that the use of chemical tests to predict actual available N must take into account the high NH_3-N losses that can occur with certain manures and soil types (Cheschier et al., 1986).

Bjarnason (1988) raised several cautions in the interpretation of data from N mineralization studies, including the important point that mineralization is a dynamic process. Bjarnason used a flexible simulation model to examine the validity of assumptions made in calculating nitrogen transformations. His results revealed that serious errors in the estimation of gross N transformations can result if remineralization of immobilized N is not taken into account. Bjarnason also indicated that decomposition of the immobilizing microorganisms begins about one week after immobilization. Figure 2.1 below, adapted from Bjarnason (1988), illustrates this dynamic internal recycling of nitrogen that occurs during organic decomposition.

Crawford and Chalk (1992) utilized the nomenclature proposed by Bjarnason (1988) to identify N pools and N transformation rates of soil fertilizer nitrogen as affected by nitrification inhibitors and solvents. Nitrogen pools used by Crawford and Chalk (1992) included: exchangeable NH_4-N, clay-fixed NH_4-N, (NO_3+NO_2)-N, total N, and organic N. They found nitrification inhibitors to have little or no effect on N transformations other

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than nitrification, while organic solvents were found to have only a temporary inhibition effect on nitrification due to the addition of an energy source (Crawford and Chalk, 1992).
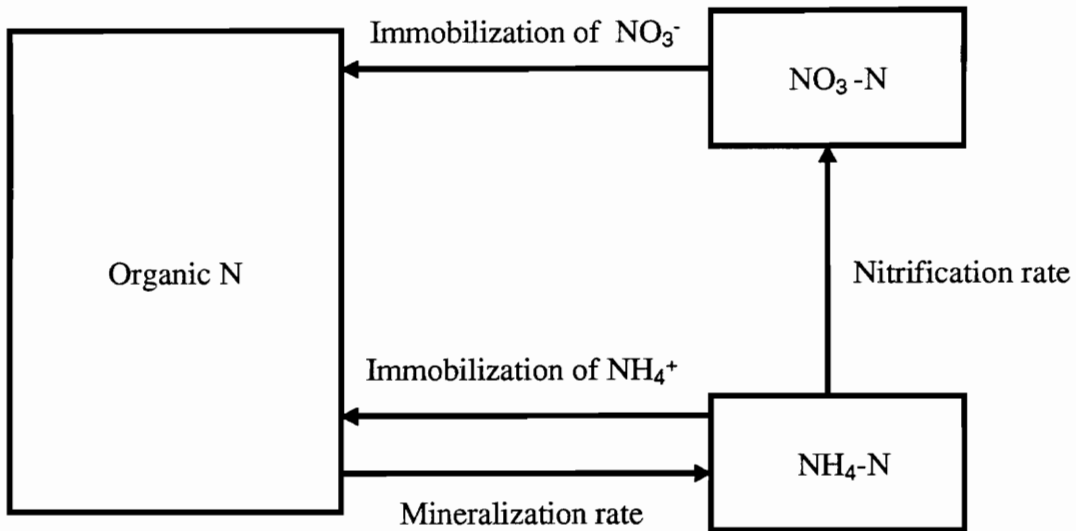
Figure 2.1 . Nitrogen pools and processes in the model used for calculating gross immobilization and gross mineralization (Bjarnason, 1988).

Burnett and Dondero (1969), in their study of the microbiological and chemical changes that occur during the decomposition of poultry manure, found that the matrix of microorganisms in poultry manure is complex in both composition and interactions. They reported that the decomposition of uric acid by both aerobic and anaerobic bacteria appeared to be related to the formation of significant quantities of ammonia. In another broiler litter study, Westerman et al. (1988) found that 40-50% of the organic nitrogen in broiler litter was available in the first few weeks of aerobic incubation. Bitzer and Sims (1988) calculated an average 66% net mineralization during the 140 days of their broiler litter incubation study.

2. Urea hydrolysis

Voss (1984) described the biochemical effects of the enzyme urease as a catalyst in the reaction of urea $(CO(NH_2)_2)$ with water to form ammonium carbonate $((NH_4)_2CO_3)$. Subsequent NH_4-N accumulation and rise in pH, according to Voss, occurs as the ammonium carbonate readily decomposes to CO_2 and OH^-, which can increase the loss of NH_3. Voss also stated that plants, bacteria, and soils are all sources of the urease enzyme, and that urease activity increases with increasing organic C, cation exchange capacity (CEC), or clay content of the soil, while temperatures above 80°C and below -20°C decrease urease activity.

Schefferle (1965) investigated the decomposition of uric acid in poultry litter and identified several strains of aerobic bacteria thought to be responsible. She found that uric acid, the main form of nitrogen excreted by poultry, was converted to ammonia by several organisms present in the litter. The majority of microorganisms, however, were found to convert uric acid only to urea, while subsequent hydrolysis of urea to ammonia was affected by other strains of bacteria which had no action on uric acid. Schefferle suggested that the high alkalinity of poultry litter results largely from this decomposition of uric acid to urea and ammonia. Burnett and Dondero (1969) reported a pH increase from 7.5 to 9.0 after 14 days of "dry" poultry manure incubation (moisture content 75%), and found that this increase in pH corresponded to a sharp decrease in uric acid content, and an increase in amine N, ammonia N content, and NH_3 evolution. They also stored a liquid manure slurry from the same laying hens to test the decomposition of a "liquid" manure at the same incubation temperature (22°C), and reported that the liquid manure showed a more rapid decrease in uric acid content after seven days, due to the presence of both aerobic and anaerobic uric acid decomposers. Burnett and Dondero (1969) reported that the ammonia N content of the liquid manure increased rapidly during the storage period. Unfortunately, they did not report the pH variation of the liquid mixture.

Delaune and Patrick (1970) investigated the conversion of a urea solution in waterlogged soils and reported that urea hydrolysis to ammonia proceeded at about the same rate in waterlogged soils as in soils kept at 33.3 kPa (1/3 bar) moisture tension. They found that maximum urea conversion occurred at about pH 8.0, with volatilization loss of ammonia from surface-applied urea slightly greater in soils at 33.3 kPa (1/3 bar) moisture tension. Malhi and Nyborg (1979) found that the rate of urea hydrolysis doubled from 1500 kPa (15 bar) to 700 kPa (7 bar) soil moisture tension in incubation studies at 20°C, but reported only a slight increase in urea hydrolysis after increasing soil moisture further to 33.3 kPa (1/3 bar) soil moisture tension.

Rapid mineralization of broiler litter N in a 24-hour incubation by Gale and Gilmour (1986) at 25°C was explained as decomposition of uric acid to urea, with subsequent hydrolysis to NH_3. Reynolds and Wolf (1987) determined that the mechanism responsible for reducing ammonia volatilization loss from surface-applied urea was the inhibition of urea hydrolysis under soil drying, but noted that the effects of initial soil water potential, changes in soil water potential, and air relative humidity on NH_3 loss were interrelated and difficult to isolate.

3. Pathways of N loss in liquid dairy manure

Pain et al. (1987), in their review of the use of additives to conserve nitrogen and reduce odors in livestock manure slurries, described four main pathways of N loss in a field, as follows; surface runoff, volatilization of ammonia (NH_3), leaching of nitrate (NO_3), and denitrification. Jarvis et al. (1987) identified three similar pathways of N loss from field-applied slurries and grazed pastures, including; gaseous emission (through NH_3 volatilization and denitrification), surface runoff, and leaching.

Jarvis et al. (1987) stated that from 60-80% of the urinary N applied to pastures can be lost to NH_3 volatilization, with highest losses occurring immediately after application. They stated that both volatilization and denitrification are dependent upon environmental conditions, and that pasture losses to volatilization in New Zealand were greatest during warm, dry conditions, and much reduced during cooler, wetter conditions. Denitrification rates were reported by Jarvis et al. to be dependent upon temperature, moisture, and nitrate status. They also reported that significant denitrification continued on a well-drained Hurley soil at temperatures below 5°C, and that the presence of a suitable carbon source in laboratory studies promoted significant rates of denitrification between 2° and 5° C (Jarvis et al., 1987).

Previous studies by Adriano et al. (1974) and Stewart (1970) investigated the losses of nitrogen from concentrated dairies and feedlot areas. Adriano et al. (1974) reported higher nitrogen losses from applied manure at higher moisture and temperature levels in a soil incubation experiment, and suggested that the losses occurred largely through volatilization of NH_3. They measured losses approaching 50% of applied N at a 90% water saturation percentage and 25°C temperature level, with most of the N losses occurring during the first four weeks of incubation. Stewart (1970), studying volatilization and denitrification losses from cattle urine in a laboratory feedlot simulation, reported several interesting soil/moisture interactions. He found that cattle urine added to a wet soil lost less than 25% of the added N as ammonia, while 65% was converted to nitrate. When urine was added to an initially dry soil, 90% of the added N was lost as ammonia (Stewart, 1970), suggesting that NH_3 volatilization occurs under dryer conditions.

4. Pathways of N loss in dry broiler litters

Research efforts into the losses of N from broiler litters have focused on mitigating the negative effects of high NH_3 levels in poultry houses. Moore et al. (1994) stated that researchers have known for over 30 years that high NH_3 levels in poultry facilities have an adverse effect on the health and production levels of chickens. Several studies in recent years, 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. They reported a trend towards decreased microbial density over their 14-week study, with a corresponding pH increase from 7.7 to 7.9. Nodar et al. (1990) also identified a large population of ammonifying microbes, which tended to decrease the C/N ratio of the litter piles over the course of their study (by consuming C for energy and releasing CO_2 in the process). They also reported that anaerobic free-nitrogen fixers were more abundant than aerobic ones (Nodar et al., 1990).

Kirchmann and Witter (1989) studied ammonia volatilization in a laboratory incubation experiment with fresh poultry manure to which increasing amounts of straw were added. They reported that increased additions of straw reduced ammonia volatilization during aerobic conditions, and also found that in aerobic manure, nitrogen was bound mainly in organic forms, while anaerobic manures were found to have about two-thirds of the N in ammonium form (Kirchmann and Witter, 1989). They stated that the amount of ammonia volatilized is influenced by several factors, the most important of which is the pH of the manure solution. Kirchmann and Witter stated that the pH value is the main factor regulating the equilibrium between NH_4^+ ions and NH_3 gas in the manure solution, but also noted the important effects of C/N ratio and adsorption amendments on NH_3 volatilization. Figure 2.2 below, taken from Kirchmann and Witter (1989), shows how the amount of litter, as well as the availability of its energy content, and solution pH,

determines the forms and concentrations of manure nitrogen that can be immobilized, mineralized, or volatilized in the absence of soil.
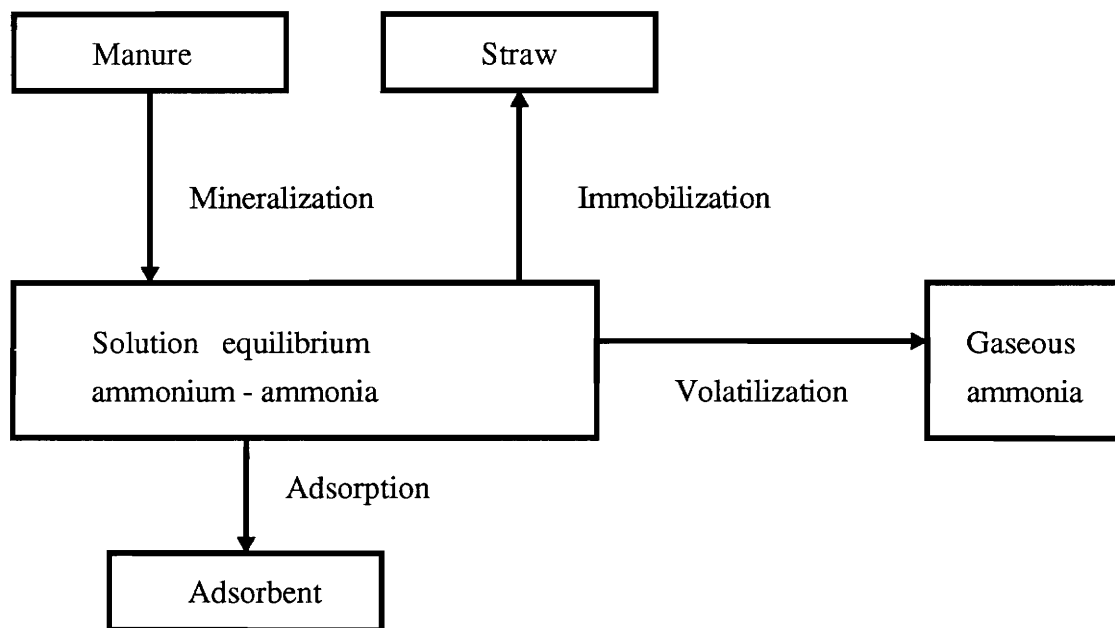
Figure 2.2.   Main processes affecting ammonia formation and volatilization during poultry manure decomposition (Kirchmann and Witter, 1989).

C. Manure N management in the mid-Atlantic states

Manures are normally evaluated for their ability to supply N to growing crops, either as an equivalent amount of fertilizer N, or as the amount of available N released from the manure (Hadas, 1983). Mathers and Goss (1979) developed a regression equation for various manure sources to estimate the amount of animal manure required to supply a desired amount of N into a soil. Their equations were based on the annual mineralization rates (decay constants) of previous researchers for poultry, dairy, swine, and beef manures (Pratt et al., 1973; Willrich et al., 1974). Values resulting from the equation of Mathers

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and Goss (1979) were found to be satisfactory for manures containing less than 4% N (wet basis).

Neeteson and Wadman (1990), in reviewing the detrimental environmental effects of N losses from excessive manure applications to agricultural crops, concluded that management of manure applications is the best solution to this pervasive problem. They offered several manure management guidelines which have become fundamental components of nutrient management philosophy in the mid-Atlantic states, including; manure application in spring and early summer only, incorporation of the manure immediately after application, and adjustment of manure application rates to the demand of the crop for nitrogen. Evanylo and Alley (1993) recommended a pre-sidedress nitrogen soil test for corn in Virginia to maximize the efficiency of crop nitrogen on soils that have received significant organic N contributions.

Prior to 1987 in Virginia, growers assigned nutrient credits to manure which were usually based on tabular values from MidWest Plan Service or similar references (Collins et al., 1994a, 1994b). In recent years, however, the prediction of plant available nitrogen from animal manures in the mid-Atlantic states of Delaware, Virginia, and Maryland has been estimated with a formula similar to that used by Bitzer and Sims (1988) for poultry manure, as follows;

$$PAN = 0.80\,N_i + 0.60\,N_o, \qquad\qquad (1)$$

where PAN = plant available nitrogen (kg/t),
   N_i = inorganic nitrogen (kg/t), and
   N_o = organic nitrogen (kg/t).

In equation (1) above, Bitzer and Sims (1988) predicted that 80% of the inorganic N and 60% of the organic N would be plant-available during the first year after application. Subsequent modifications to this formula by Sims and others (Sims et al., 1989; Collins, 1989; Bandel, 1989) have allowed for cumulative estimations of mineralization in the second or third years after organic applications, and have also accounted for the wide variations in ammonia volatilization as affected by the type and season of manure incorporation (Ritter, 1990).

Current recommendations from the Virginia Cooperative Extension Service for estimating manure N availability can be found in the 1993 Nutrient Management Handbook (Virginia Department of Conservation and Recreation, Division of Soil and Water Conservation, 1993). The following calculations for estimating the manure N availability of spring-applied liquid dairy manure and dry broiler litter represent current nutrient management formulas used in Virginia. Note that while the following nitrogen availability formulas include availability coefficients for both the inorganic and organic portions of manure nitrogen, the inorganic portion (NH_4-N) is the one most affected by application method.

Liquid dairy manure:

(Surface-applied)   Available N = (35% x organic N) + (25% x NH_4-N)

(Incorporated)   Available N = (35% x organic N) + (75% x NH_4-N)

Dry broiler litter:

(Surface-applied)   Available N = (60% x organic N) + (75% x NH_4-N)

(Incorporated)   Available N = (60% x organic N) + (95% x NH_4-N)

The 1993 Nutrient Management Handbook also includes tables of coefficients for adjustment of organic N availability by season, as well as manure residual factors that account for the previous manure history of a particular field. The above (or similar) nitrogen availability formulas and coefficients, when used together with specific laboratory nitrogen values from individual livestock producers, have provided nutrient managers in the mid-Atlantic states with an acceptable estimate of the nitrogen equivalent value of their manures (Ritter, 1990). *Most importantly, as a result of individual farm manure sampling and analysis, nitrogen availability formulas (such as those shown above) have been able to take into account the farm to farm variability inherent in a heterogeneous organic material such as manure.*

D. Manure sampling recommendations

Accurate laboratory analysis of the nitrogen value of a farm manure is dependent upon the sampling techniques used in the field, as well as the precision of the specific laboratory analyses. Manure sampling should ideally provide a representative sample of the farm manure being tested so that the laboratory can provide reliable nutrient values to the nutrient manager. Collins et al. (1994a, 1994b), among others, have addressed the inherent nonhomogeneity of farm manures, as well as the variability due to storage method and season. Sampling errors at the farm, and analytical errors in the laboratory, can affect the accuracy of all reported manure nutrient values.

Swanson and Gilbertson (1974) reviewed several fundamental requirements to be met when planning and conducting liquid and solid manure sampling for agricultural research. They strongly recommended preliminary laboratory analysis of sample results in order to quickly determine if the sampling procedures and equipment used in the experiment were adequate. Swanson and Gilbertson (1974) also made the important point that since conclusions for research study would ultimately depend on a sample that is very small

compared to the total from which it was drawn, it is critical that the samples be both random and representative. Converse (1974) stated that when sampling solid material, it is better to obtain two samples at the same time for separate analysis, rather than taking one sample for duplicate analysis. He stated that the former method will reflect both sampling variation and error in technique, while the latter will reflect only error in technique. By taking more samples, Converse concluded that one has a better chance of obtaining a representative sample, providing limitations of storage, equipment, and cost can be overcome. In any case, he stated that the goal of representative sampling is as or more important than good laboratory analysis (Converse, 1974).

Redell et al. (1974) discussed the design of several sampling devices to be used in obtaining samples of liquid manure. They reported that even after apparently effective agitation of a liquid manure pit, various concentrations of the liquid samples usually varied with sampling depth. They also discussed the sampling of solid animal manures and suggested that samples may be carefully composited into a single sample if no estimate of sampling error is required (Redell et al., 1974).

Current recommendations for environmental water and effluent sampling can be found in Standard Methods for the Examination of Water and Wastewater (Greenberg et al., 1992). The Virginia Cooperative Extension Service has prepared specific recommendations for liquid dairy manure and dry broiler litter sampling (Collins, 1989). Virginia's recommended procedures for sampling of liquid dairy slurries are as follows;

> 1) storage tanks or basins should be well agitated prior to sampling,
> 2) grab samples should be taken from at least six locations and thoroughly mixed in a bucket, and
> 3) the sample bottle should be filled about halfway with the composited sample to allow for air expansion during transit.

Current Virginia recommendations for sampling broiler litters include;

> 1) at least six representative samples should be taken from the broiler house or pile at a minimum 0.46 m (1.5 ft) depth,
> 2) the litter material should be thoroughly mixed in a bucket, and
> 3) a sample should be collected from the mixed composite for shipment to the laboratory.

Several authors have made specific suggestions on the collection and treatment of poultry samples. Krogdahl and Dalsgard (1981) recommended that collection of urine samples should be done under acid conditions for quantitative estimation of ammonia. Bitzer and Sims (1988) found that the addition of deionized water to broiler litter samples to achieve a moisture content of approximately 65% improved the reproducibility of their analyses (Gartley, 1994).

E. Manure nitrogen analysis

1. Review of methods available

Laboratory analysis of animal manures is not a standardized discipline at present, at least not in the same way as are the analyses of plants, soils, waters, wastewaters, feeds, and fertilizers. Solid and liquid manures do, however, share varying physical, chemical, and biological properties with many of the above materials. Analysis techniques have subsequently been "borrowed" and adapted over time to fit the particular needs of those wishing to obtain quantitative measurement of manurial nitrogen. Current nutrient management recommendations in Virginia for nitrogen-based land applications depend upon the quantitative analysis of total nitrogen and ammonium nitrogen, as well as other non-nitrogen nutrients. Organic nitrogen is estimated at present in Virginia by subtracting the ammonium (NH_4-N) content of the manure from its Kjeldahl nitrogen (TKN) content.

Although the more exact term for the calculated difference between TKN and NH_4-N levels in manures is non-ammonium nitrogen, the conventional term "organic nitrogen" will be used in the present study. 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., 1994a, 1994b). Nitrate nitrogen is often used, however, in water quality research dealing with nitrogen movements in the environment.

The most generally accepted methods for the analysis of total N are the Dumas and Kjeldahl procedures (Nelson and Sommers, 1980). The Dumas procedures are reported by the above authors to be slow and subject to interference by methane formed during combustion of the sample. Numerous sources can be found describing the Kjeldahl procedure, which involves two steps; a high-temperature digestion at 330°-350°C with concentrated sulfuric acid (H_2SO_4) and catalysts to convert organic and inorganic forms of N to NH_4^+, then NH_4^+ in the digest is determined by either acidimetric titration followed by alkaline distillation, colorimetric procedures, or ammonia-sensing electrodes (Nelson and Sommers, 1980; Bremner and Yeomans, 1988; Greenberg et al., 1992; Bremner and Mulvaney, 1982). Commonly used Kjeldahl procedures do not fully recover N from materials with high nitrate (NO_3-N) and nitrite (NO_2-N) levels unless modified by pretreatments such as potassium permanganate ($KMNO_4$) and reduced iron or salicylic acid and thiosulfate (Bremner and Mulvaney, 1982). Mahimairaja et al. (1990) evaluated seven pretreatments to the standard Kjeldahl procedure in the measurement of total N in poultry and animal manures. They reported that manure samples treated with $KMNO_4$ and acidified (H_2SO_4)-reduced iron (Fe) achieved nearly complete recovery of both NO_3-N and NO_2-N present in mineralized manures. In the case of fresh manures, however, they found that since over 95% of the total N was present as organic N and NH_4-N, modification of the standard Kjeldahl digestion with $KMNO_4$ and reduced Fe was unnecessary (Mahimairaja, 1990).

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Nelson and Sommers (1980) contrasted the advantages and disadvantages between the use of traditional macro-Kjeldahl flasks (350-500 ml) and the more recent Semi-micro-Kjeldahl procedures, which use 30-50 ml flasks. They noted that although semi-micro procedures utilize less space, the materials being analyzed must be finely ground to assure adequate reproducibility of the sample, especially in coarse, nonhomogeneous materials (Nelson and Sommers, 1980).

The most commonly used method for determining ammonium N in Kjeldahl digests for soil and plant samples is distillation under alkaline conditions, with collection of the distilled NH_3 in boric acid-indicator solution, followed by titration of the boric acid solution with a standard acid (Bremner and Mulvaney, 1982). Nelson and Sommers (1980) stated that colorimetric and electrode methods, although more rapid than distillation methods, usually require a more skilled technician to obtain high precision. They also reported that the heterogeneous nature of soil samples compared to plant materials decreases the relative precision of soil analysis (Nelson and Sommers, 1980).

Quantitative analysis of inorganic forms of nitrogen in soils was reviewed by Keeney and Nelson (1982). They concluded that the best method for most research to determine exchangeable NH_4^+, NO_3^-, and NO_2^- are the steam distillation methods proposed by Bremner and Keeney (1966). Steam distillation methods have been used for the past 20 years and have the advantage of being rapid, precise, simple, and not subject to interferences from colored extracts (Keeney and Nelson, 1982). However, one colorimetric method (the Griess-Ilosvay method of determining NO_2^-) has been used almost exclusively for determination of NO_2^- in soils and other biological materials (Keeney and Nelson, 1982). These authors recommended three analysis methods for the determination of NH_4^+, NO_2^-, and NO_3^- in soils which would minimize the disadvantages associated with colorimetric methods. In these three procedures (indophenol blue for NH_4^+, Griess-Ilosvay for NO_2^-, and cadmium reduction for NO_3^-), the inorganic nitrogen

forms are extracted by a $2M$ KCl solution for direct analysis, similar to procedures used in the analysis of natural waters (Keeney and Nelson, 1982).

In developing their extraction steam distillation methods for inorganic N in soils, Bremner and Keeney (1966) defined exchangeable ammonium as the ammonium extractable by $2M$ KCl at room temperature. Weatherburn (1967) demonstrated that the indophenol blue procedure (previously known as the Berthelot color reaction) could be intensified when 10 ml of the final solution contained about 60 mg of phenol, 0.25 mg of sodium nitroprusside, and at least 0.05 ml of sodium hypochlorite solution (5% NaOCl). Current descriptions of the phenol-hypochlorite reaction developed by Weatherburn (1967) for the colorimetric determination of ammonium can be found in Methods of Soil Analysis, Part 2 (Keeney and Nelson, 1982) as the Indophenol Blue Method, and in Standard Methods for the Examination of Water and Wastewater (Greenberg et al., 1992) as the Phenate and Automated Phenate Methods.

Keeney and Nelson (1982) reviewed the colorimetric procedures for NO_3^- and NO_2^- in soil extracts, and stated that the excellent sensitivity of the Griess-Ilosvay method for determination of NO_2^- has lead to the development of procedures whereby NO_3^- is also reduced to NO_2^- with various reagents for a combined quantitative analysis of NO_3^- and NO_2^- by the Griess-Ilosvay procedure. They also described the Copperized Cadmium Reduction Method to reduce NO_3^- to NO_2^- for quantification of NO_3^- by the Griess-Ilosvay method (Keeney and Nelson, 1982). Greenberg et al. (1992) described the Cadmium Reduction Method, Automated Cadmium Reduction Method, and a hydrazine and copper sulfate reagent version of the Griess-Ilosvay procedure, called the Automated Hydrazine Reduction Method (PROPOSED).

Gilbertson et al. (1974), in reviewing chemical analysis procedures for livestock and poultry manures, determined total N by micro-Kjeldahl procedures, with steam-distillation

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techniques used to determine NH_4^+, NO_3^-, and NO_2^-. They noted that colorimetric procedures, using a Technicon AutoAnalyzer, were satisfactory for determinations of inorganic N in their studies (Gilbertson et al., 1974). Overcash et al. (1974) discussed the procedures used to test for manure ammonia, organic, and total Kjeldahl nitrogen. They reported that total Kjeldahl nitrogen can be determined by digesting the sample with sulfuric acid (H_2SO_4), potassium sulfate (KSO_4), and a mercuric sulfate catalyst, with subsequent analysis for ammonia by distillation and titration. They recommended manure nitrite (NO_2^-) determination by the diazotization technique of the Griess-Ilosvay procedure, and recommended steam distillation using Devarda's alloy for nitrate (NO_3^-) analysis of highly colored or organic liquid animal manures (Overcash et al., 1974).

2. Precision and accuracy

Prakasam et al. (1974) evaluated the precision and accuracy of several analytical methods for determination of liquid manure nitrogen (as well as numerous other chemical and physical properties). They used the coefficient of variation (c.v.) as a measure of precision for replicate analyses, and used at least three replicate determinations in computing the precision of each analytical method. Accuracy was determined by the percent recovery of an added known amount of parameter being analyzed. Precision and accuracy of the micro-Kjeldahl method for the determination of TKN in liquid poultry manure were reported by Prakasam et al. (1974) as 2% and 98%, respectively. Prakasam et al. (1974) considered any coefficient of variation in replicate analysis under 5% to be satisfactory. The precision and accuracy of steam distillation for NH_4-N using magnesium oxide (MgO) were found to be 0.3% and 98.5%, respectively. Tests for NO_2-N using the diazotization method were reported by Prakasam et al. to correlate well with the steam distillation method of NO_2-N determination, using Devarda's alloy. Both methods (diazotization and steam distillation) were reported to be highly satisfactory for the determination of NO_2-N in liquid poultry manure (Prakasam et al., 1974).

Five methods of NO_3-N determination were also tested by Prakasam et al. (1974), including four colorimetric methods and one steam distillation method. Steam distillation was found to have the highest precision and accuracy of all the methods tested for NO_3-N analysis. Two phenol disulfonic acid (PDSA) methods and one salicylic acid method for colorimetric NO_3-N determination were reported to be satisfactory. The remaining colorimetric technique, using the brucine method, was found to have a poor accuracy (Prakasam et al., 1974). Keeney and Nelson (1982) reported that the brucine method, although generally simple and rapid, is highly susceptible to interference from organic matter, NO_2^-, and strong oxidizing and reducing agents. In the recent past, the PDSA procedure for NO_3-N has been the colorimetric technique that is most widely used for soil extracts, but it is time consuming and subject to interference by organic matter, NO_2^-, and chlorine (Cl^-) (Keeney and Nelson, 1982).

F. Manure N preservation

1. Laboratory preservation techniques

Smith (1993) stated that preservatives are used to maintain the chemical integrity of a sample, and may consist of chemical additives or simply a low storage temperature. Greenberg et al. (1992) admitted that, at best, preservation techniques only slow the chemical and biological changes that inevitably continue after a sample is collected. They noted the probable effect of microbiological activity within a sample on the nitrate, nitrite, and ammonia content of an environmental sample, and cited the nitrogen cycle as an example of how biological systems can influence sample composition. Current guidelines for preservation of nitrogen fractions, as listed in Standard Methods for the Examination of Water and Wastewater (Greenberg et al., 1992), are presented in Table 2.2 below.

Greenberg et al. (1992) stated that methods of sample preservation are relatively limited and are generally intended to slow biological processes, retard hydrolysis reactions, and reduce the volatility of sample constituents. They stated that preservation techniques for water and wastewater samples are limited to pH control, chemical addition, the use of amber and opaque bottles, refrigeration, filtration, and freezing (Greenberg et al., 1992).

Table 2.2.   Summary of water sample handling requirements when immediate laboratory analysis is impossible (Greenberg et al., 1992).

Nitrogen determination	Recommended preservation	Recommended maximum storage
Ammonia N	H_2SO_4 to pH<2, refrigerate	7 days
Nitrate N	refrigerate	48 hours
(Nitrate + Nitrite) N	H_2SO_4 to pH<2, refrigerate	none
Nitrite N	refrigerate	none
Organic N, Kjeldahl	H_2SO_4 to pH<2, refrigerate	7 days

Keeney and Nelson (1982) reported, in their review of preservation methods for inorganic N in soil samples, that the most common preservation methods are deep freezing or drying at laboratory temperatures. They stated that although soil samples should ideally be analyzed immediately for inorganic N fractions, some delays are nearly impossible to avoid. Keeney and Nelson (1982) also reported that various biocides have been recommended to minimize microbial transformations in soil inorganic N, but have generally been regarded as ineffective.

Klingaman and Nelson (1976) had previously evaluated several methods of preserving soluble inorganic phosphorus and nitrogen in unfiltered water samples. They stated that the soluble ammonium content of water samples can either increase (by biological

mineralization of organic N compounds) or decrease (by oxidation of ammonium to nitrite and nitrate due to nitrifying bacteria and by immobilization during growth of microbial bacteria). Klingaman and Nelson (1976) noted that the most common means of inhibiting these microbial activities were low temperature storage or addition of various bactericides such as mercuric chloride (HgCl), phenyl mercuric acetate (PMA), sulfuric acid (H_2SO_4), toluene, or chloroform. Their study compared the effectiveness of the above bactericides, as well as low temperature storage, on the preservation of soluble inorganic phosphorus and nitrogen, and concluded that storage at subzero temperatures was the most effective preservation treatment of those tested (Klingaman and Nelson, 1976).

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. Only one study (Moore et al., 1974) was found that attempted to establish the magnitude and rate of N transformations occurring in an animal manure sample as a function of standard laboratory preservative techniques. In the above study, six different types of animal manure samples were stored at room temperature, refrigerated (3°-10°C), frozen, and acidified (H_2SO_4 to pH<2). Nine different parameters were monitored in the 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. They reported that NH_3-N levels in some manures were marginally preserved by freezing and acidification. Moore et al. (1974) concluded that NH_3-N levels in cattle and fresh poultry samples cannot be preserved by refrigeration. They found that preservation of NO_3-N in poultry manure was most effective in the samples that were frozen. Moore et al. (1974) observed that poultry manure samples at room temperature experienced a significant decrease in NO_3-N, while the acidified samples showed a sharp initial increase in NO_3-N. None of the preservative treatments tested by Moore et al. (1974) was found to maintain initial NO_2-N levels in poultry oxidation ditch samples; in fact, all treatments that they tested showed a

drastic reduction in NO_2-N within three days. Based on the above observations, Moore et al. (1974) were unable to suggest any methods of preserving NO_2-N in animal manures; however, the preservation of samples by freezing was shown to conserve the combined oxidized nitrogen fraction (NO_3+NO_2)-N better than any other treatment. Moore et al. (1974) also reported that refrigerated fresh dairy manure increased in pH from 8.0 to 8.8 through the first week of storage, while an additional storage time of three weeks resulted in a general decline in pH values.

2. Reduction of ammonia volatilization

According to Carlile (1984), ammonia is a colorless, odorous gas produced from the nitrogenous fraction of animal wastes by microbial activity. She reported that ammonia odor is detectable by humans at concentrations greater than 25 ppm. Several authors have attributed the formation of ammonia in poultry houses to the microbial decomposition of uric acid in manure (Burnett and Dondero, 1969; Schefferle, 1965). Carlile (1984) stated that the decomposition of uric acid is dependent on factors such as litter moisture content, temperature, and pH. Schefferle (1965), however, did not observe a correlation between the above factors and the microbial population of uric acid decomposers in a built-up poultry litter. Moore et al. (1994) stated that the rate of NH_3 volatilization from poultry manure is dependent on pH, moisture content, wind speed, ammonium concentration, and temperature, with an increase in any of these variables resulting in increased volatilization. Carlile (1984) reviewed several techniques that have been developed to reduce ammonia losses in poultry manure and litter. She stated that effective methods of ammonia control act either by inhibiting microbial activity or by neutralizing ammonia that has been released.

Moore et al. (1994) cited several chemicals that have been tested for their effectiveness in reducing ammonia volatilization, including; acetic acid, antibiotics, ferrous sulfate,

gypsum, hydrated lime, limestone, paraformaldehyde, phosphoric acid, propionic acid, superphosphate, yucca plant extracts (saponin), and zeolites like clinoptilolite. In addition to the chemical additives mentioned by Moore et al. (1994) for broiler litter, Pain et al. (1987) reviewed the use of sulfuric acid to convert ammonium carbonate in manure slurries to more stable ammonium compounds.

Kirchmann and Witter (1989) identified three mechanisms to reduce ammonia losses, including; immobilization of ammonium with easily decomposable high carbon materials, adsorption of ammonium and ammonia on suitable amendments, and pH regulation of the manure solution itself. They concluded that the large quantities of high carbon straw required under aerobic decomposition to achieve significant reduction in ammonia loss was not practical on most farms. Kirchmann and Witter (1989) found that aerobic manure decomposition exhibited more ammonia losses than anaerobic decomposition during fresh poultry manure storage.

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; inhibition of urea hydrolysis, chemical stabilization, and adsorption. Relevant research in each of these three methods will be detailed separately in the following sections.

a) Inhibition of urea hydrolysis

Pain et al. (1987) stated that animal feces and urine contain little NH_4-N when voided, but because of the common enzyme urease, manure urea is quickly hydrolyzed to NH_4-N. They discounted the use of specific urease inhibitors for use with slurries as uneconomical, but reported that hydrated lime ($Ca(OH)_2$) was able to inhibit urea hydrolysis for several

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days by increasing the pH of a lime-manure mixture. Pain et al. (1987) also reported that various disinfectants have been used to inhibit ammonification, but with little success. Voss (1984) identified the major characteristics of a desirable urease inhibitor as cost-effective, nontoxic to other microorganisms, easily applied, and stable in storage. He stated that urease activity in soils can be inhibited by several compounds, including organic salts of Hg, Ag, and Cu, dihydric phenols, and certain quinones, but that no commercial urease inhibitors are currently available for use with urea fertilizers (Voss, 1984). Malhi and Nyborg (1979) tested the effects of thiourea and pellet size on urea hydrolysis in a number of field and incubation experiments. They reported that when thiourea (a metabolic inhibitor) was pelleted with urea at 33% (w/w), the rate of hydrolysis was halved. They estimated that hydrolysis of commercial urea could be inhibited for at least one week under field conditions (Malhi and Nyborg, 1979).

Nommik (1973) had previously studied the effects of large pellet size with the addition of urease inhibitors and nonspecific metabolic inhibitors. In his 28-day test on an experimental site of 90-year old Scots pines in Stockholm, he concluded that 5% (w/w) orthophosphoric acid inhibited the urease enzyme due to increased acidity, while 5% (w/w) boric acid, as a weakly dissociated acid, acted as a mild, nonspecific, metabolic inhibitor.

Carlile (1984) discussed the use of paraformaldehyde as an effective anti-microbial agent, but cited its instability and possible carcinogenic effects as major drawbacks. She also reported that the use of antibiotics such as thiopeptin and zinc bacitracin in poultry diets could be a useful method of controlling ammonia release through decreased microbial activity in the excreta. Carlile (1984) cited a number of other chemicals, such as sorbic acid, gentian violet, and calcium propionate which have been used in past studies to reduce microbial numbers in litter. Parkhurst et al. (1974) studied the use of volatile fatty acids (60% acetic acid and 40% propionic acid) to control microbial populations in pine

sawdust litter. They reported significant reduction of litter bacterial numbers and pH, but no effect on litter moisture content, temperature, or final nitrogen content (Parkhurst et al., 1974).

Goos (1985) and Goos et al. (1986) identified ammonium thiosulfate (ATS, 12-0-0-26S) as an effective inhibitor of the urease enzyme by adding one or two parts ATS per 100 parts of mixed urea ammonium nitrate (UAN) fertilizer. Goos et al. (1986) noted that ammonium polyphosphate (APP, 10-34-0) reduced ammonia loss from UAN due to its acidifying effect on the ammonia-ammonium equilibrium. Goos (1985), in soil incubation studies at 25°C, illustrated that the thiosulfate ion ($S_2O_3{}^{2-}$) is an inhibitor of nitrification as well as an inhibitor of urea hydrolysis, categorizing ATS as a general metabolic inhibitor on the order of boric acid. He contrasted ATS to the more effective but more expensive chemical phenyl phosphorodiamidate (PPD), and suggested that ATS may have application only with liquid fertilizers because of its hygroscopic nature (Goos, 1985).

b) Chemical stabilization of manure N

Chemical stabilization of manurial 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 manurial 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 and phosphoric acid 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. Moore et al. (1994) experimented with several chemical amendments to reduce ammonia volatilization from poultry litter. Compounds that were not effective in reducing volatilization in their laboratory study included calcium hydroxide (Ca (OH)$_2$), fly ash, acid

mine soil, and aluminum refinery waste products. Moore et al. (1994) also tested alum and ferrous sulfate chemical amendments, as well as four commercial products that claimed to reduce NH_3 volatilization in poultry litter. They concluded that acid-forming compounds such as alum and ferrous sulfate reduced volatilization of ammonia, while basic compounds such as "Multi-Purpose Litter Treatment" (MLT) actually increased volatilization. Poultry house atmospheric NH_3 levels in their study were highly correlated to litter pH, with the lowest NH_3 values observed under the most acidic conditions (litter + alum treatment). Moore et al. (1994) also noted that exchangeable ammonium levels were higher in the treatments with inhibited NH_3 volatilization. They concluded that the chemical amendments alum and ferrous sulfate had the added environmental advantage of immobilizing soluble P in the litter (Moore et al., 1994).

Pain et al. (1987) reported that in the early part of this century gypsum ($CaSO_4 \cdot 2H_2O$), superphosphate ($Ca(H_2PO_4)_2$), phosphoric acid (H_3PO_4), and kainite ($KMg (SO_4)Cl \cdot 3H_2O$) were tested for their ability to increase crop yields from applied manures. Superphosphate was found to be the most effective amendment by Salter and Schollenberger (1939), who discussed in detail the chemistry involved when adding various chemicals to manure. They presented the ammonium stabilizing equations for the chemical reactions of phosphoric acid and superphosphate, respectively, in equations (2) and (3), as follows;

$$NH_3 + H_3PO_4 \longrightarrow NH_4H_2PO_4 \qquad (2)$$

$$Ca(H_2PO_4)_2 \cdot H_2O + NH_3 \longrightarrow CaHPO_4 + HN_4H_2PO_4 + H_2O \quad (3)$$
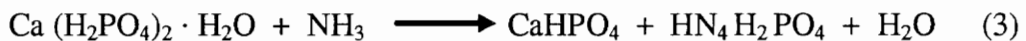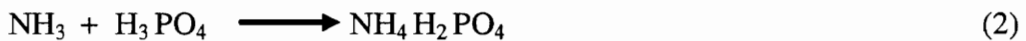
Safley et al. (1983) reproduced Salter and Schollenberger's 1939 experiments with gypsum, superphosphate, and phosphoric acid. They found that although both superphosphate and phosphoric acid were effective in producing stable ammonium

compounds, the two chemical amendments were not cost-effective. Safley et al. (1983) made the significant 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. Safley et al. (1983) noted that sulfuric acid can also be utilized with manure to stabilize the ammonia to ammonium sulfate. Pain et al. (1987) reported that sulfuric acid is relatively less expensive than either superphosphate or phosphoric acid. They reported that a batch of field-applied dairy slurry that had been reduced to a pH of 5.5 with sulfuric acid lost only 5% of its initial NH_4-N, compared to a 20% loss in a batch without acidification (Pain et al., 1987). Jurgens (1987), in pot trials of pig and cattle slurry, found that ground superphosphate (7.9% P) lowered the pH and reduced the ammonia content of the air space above the slurry, when compared with other slurry additives such as dicyandiamide (DCD), bentonite/organic mixtures, or a 50% cyanamide solution. Neeteson and Wadman (1990) reported that superphosphate or phosphoric acid is not added to slurries in the Netherlands because the rate of phosphorus would be too high for most soils. They noted that investigation was underway to determine whether the addition of nitric aid is effective in minimizing ammonia volatilization from animal manures (Neeteson and Wadman, 1990).

c) Adsorption

The third mechanism for controlling N losses through NH_3 volatilization, after inhibition of urea hydrolysis and chemical stabilization, is adsorption (Pain et al., 1987). Several materials such as kaolinite, bentonite, and zeolite are claimed to affect NH_3 adsorption through their relatively high cation exchange capacity (CEC); however, little research appears to have been reported on the use of these materials for preventing NH_3 loss from livestock slurries (Pain et al., 1987). Carlile (1984) has discussed the use of zeolites in particular by describing them as crystalline, hydrated aluminosilicates of alkaline earth

cations which are abundantly and easily obtained. She stated that these materials are the most effective ion exchangers known to man, and that they have been used extensively in Japan for controlling moisture and ammonia in manure. Carlile (1984) stated that the preference of one of the zeolites, clinoptilolite, for large cations such as ammonia makes it a practical adsorbent to control ammonia in poultry houses by either mixing directly with droppings or in suspended boxes containing the material.

3. Inhibition of nitrification in manure

Nitrification is a process in the decomposition of organic matter that has been defined as the biological oxidation of NH_4^+ released during mineralization to NO_3^- (Tisdale et al., 1993). The nitrification process is usually represented as a two-step process, as follows;

$$1) \qquad 2\,NH_4^+ + 3\,O_2 \xrightarrow{\quad Nitrosomonas \quad} 2\,NO_2^- + 2\,H_2O + 4\,H^+$$

$$2) \qquad 2\,NO_2^- + O_2 \xrightarrow{\quad Nitrobacter \quad} 2\,NO_3^-$$
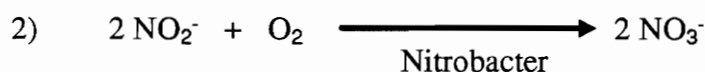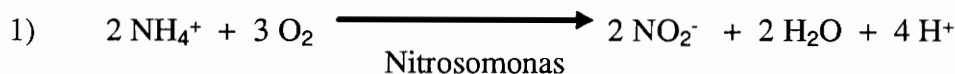
In the first reaction, according to Tisdale et al. (1993), the autotrophic bacteria Nitrosomonas oxidize NH_4^+ to NO_2^-. In the second reaction, NO_2^- is further oxidized to NO_3^- by the autotrophic bacteria Nitrobacter (Tisdale et al., 1993).

According to Goos et al. (1986), nitrification inhibitors slow the conversion of ammonium to nitrate, and may have agronomic value when conditions for nitrate leaching or denitrification occur after fertilizer application. Neeteson and Wadman (1990) stated that the inorganic nitrogen in animal manures consists almost entirely of ammonium, which must be nitrified before it can be leached as nitrate. Most of the work with nitrification inhibitors has sought to limit the environmental and economic consequences of excess

nitrates in surface and groundwater sources. According to Neeteson and Wadman (1970), of the many nitrification inhibitors tested on cattle slurries, dicyandiamide (DCD) appears to be the most effective in reduction of nitrate leaching. Pain et al. (1987) listed three characteristics for the ideal nitrification inhibitor, including that it be specific to NH_4^+ oxidation, resistant to degradation, and economical to use. Pain et al. (1987) stated that of seven commercially available nitrification inhibitors identified in 1983, only three have been frequently used in experiments with cattle slurries, including; nitrapyrin (N-serve), DCD, and Dwell (Terrazole). Pain et al. (1982) identified DCD as a bacteriostatic, which inhibits the multiplication of Nitrosomonas, while nitrapyrin is bactericidal, meaning it kills the target organisms. They reported that concerns over the possible toxic residues of nitrapyrin, and the high vapor pressures of nitrapyrin and Dwell compared with DCD, limit their use with surface-applied slurries (Pain et al., 1987). Geurink (1987) reported that the addition of DCD to pig slurry considerably improved the utilization of nitrogen.

Goos (1985) reported that a 5% (v/v) mixture of commercial grade ammonium thiosulfate (ATS) to a UAN solution inhibited nitrification by 51% in a 28-day incubation. Magalhaes and Chalk (1987) tested nitrapyrin and potassium chlorate in a 12-day incubation with prilled urea fertilizer. They determined that application of nitrapyrin decreased NO_2^- accumulation, but increased NH_3 volatilization (Magalhaes and Chalk, 1987). They also found that potassium chlorate increased NO_2^- accumulation, but did not affect emissions of NH_3 and N_2. Magalhaes and Chalk (1987), using soil pH as one measure of urea hydrolysis and nitrification, found that in all urea treatments, there was an initial increase in pH (due to urea hydrolysis), while pH decreased rapidly after three days (due to NH_4^+ oxidation by Nitrosomonas).

El-Shahawy and Ghazi (1983) reported the effects of three levels of four organic materials (starch, sawdust, wheat straw, and rice straw) and N-Serve on nitrification in soil. They reported that of the four organic materials tested, sawdust inhibited the nitrification

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process at a rate similar to N-serve, without a resultant accumulation of NO_2^- in the soil. Inhibition of nitrification by sawdust was explained by its high C/N ratio which caused the immobilization of soluble nitrogen (presumably ammonium), preventing further oxidation of ammonium ions to nitrate by Nitrosomonas (El-Shahawy and Ghazi, 1983).

Pain et al. (1987) pointed out the potential for high denitrification losses in injected cattle slurries as a function of soil NO_3^- content. They reported that the addition of nitrapyrin to soils treated with cattle slurry in December resulted in lower soil NO_3^- contents and lower rates of denitrification until the following April. Pain et al. (1987) therefore suggested that nitrification inhibitors could also be useful for their effects on denitrification, especially for slurries incorporated into well aerated soils late in the year (or even for spring applications of liquid manures on later maturing crops, such as maize).

4. Refrigeration / freezing / freeze drying of manure samples

Klingaman and Nelson (1976) reported that the best overall technique for preservation of unfiltered water samples for N and P analysis appeared to be storage at subzero temperatures. They found that storage of samples at either 4°C or 23°C resulted in large changes in soluble inorganic N and P fractions. Several interesting temperature-related transformations were reported by Klingaman and Nelson (1976) in their 84-day study. They observed, that after one week of storage at 23°C, the loss of NH_4-N in surface runoff samples was more than offset by a corresponding increase in NO_3-N, suggesting the biological nitrification of NH_4 into NO_3. They also reported that the NH_4-N content of river water samples stored at 4°C increased during the first six weeks of storage, and explained that the bacteria responsible for nitrification are more sensitive to low temperatures than the ammonifiers. This explanation was reinforced by their observation that NO_3-N levels in samples stored at 4°C showed no significant increase until after six weeks. Klingaman and Nelson (1976) also reported a large increase in total soluble

inorganic N in samples stored at 23°C, due to biological mineralization of organic nitrogen compounds present, with a smaller observed inorganic N increase in samples stored at 4° C. Storage at subzero temperature was found to be effective in minimizing changes in the total soluble inorganic N of river water, tile drainage, and surface runoff samples (Klingaman and Nelson, 1976).

Mahimairaja et al. (1990), in their evaluation of measurement methods of nitrogen in poultry and animal manures, suggested that fresh animal manures can be freeze dried for analysis of N. They tested the effects of drying on nitrogen content in fresh manures and found that freeze drying had no effect on total N content, while air drying caused the maximum reduction on total N, followed by drying at 105°C, and by microwave drying. Mahimairaja et al. (1990) indicated that air drying caused significant reduction in organic and ammoniacal N. Other authors have also shown that drying of soil and manure samples causes loss of N (Bremner and Mulvaney, 1982; Kirchmann and Witter, 1989). Mahimairaja et al. (1990) suggested that most of the N loss in drying occurs from the urinary compounds of urea and uric acid, while freezing followed by freeze drying inactivates microorganisms and results in less decomposition of uric acid.

Prakasam et al. (1974) noted that refrigeration of liquid poultry manure at 4°C was not satisfactory for preservation of inorganic nitrogen. Moore et al. (1974) evaluated four different temperature preservation techniques for manure samples, including; room temperature, refrigeration between 3° and 10°C, freezing (fast thaw), and freezing (slow thaw). They were unable to recommend consistent temperature preservation techniques for TKN or NH_3-N, but found that freezing preserved the oxidized $(NO_3 + NO_2)$-N fraction better than any other preservative treatment (Moore et al., 1974).

5. Acidification of manure samples

Acidification with H_2SO_4 to pH<2 is currently recommended for several inorganic and organic nitrogen fractions in water samples (Greenberg et al., 1992), as illustrated previously in Table 2.2. Klingaman and Nelson (1976) stated that most preservation techniques used have the goal of preventing biological transformations. They reviewed American Public Health Association recommendations for the seven-day preservation of soluble ammonium and nitrate in water samples using 0.8 ml of concentrated H_2SO_4 per liter coupled with storage at 4°C. They stated that H_2SO_4 promotes the decomposition of nitrite in water samples due to the formation of nitrous acid, which may yield nitrate and gaseous nitrogen oxides (Klingaman and Nelson, 1976).

Kirchmann and Witter (1989), in their laboratory incubation experiment with fresh poultry manure, showed that very low ammonia losses occurred during anaerobic decomposition of manure due to induced acid formation and subsequent reduced pH (5.0-6.2). They stated that the pH value is the main factor regulating the equilibrium between NH_4^+ ions and NH_3 gas in manure solutions, and that considerably higher ammonia concentrations under aerobic alkaline conditions produced a greater potential for ammonia volatilization. Parkhurst et al. (1974), treating pine sawdust litter with 60% acetic and 40% propionic acids, achieved a significant reduction in litter pH over a two- to three-week period. Carlile (1984) suggested that the reduction in litter by Parkhurst et al. (1974) also reduced ammonia release in the litter. Moore et al. (1994) found that acid-forming compounds such as alum and ferrous sulfate reduced volatilization in poultry litter, whereas basic compounds increased volatilization of NH_3.

Pain et al. (1987) reviewed the use of phosphoric and sulfuric acids to produce stable ammonium salts in manure slurries. They reported that acidification of a slurry to pH 5.5 with sulfuric acid reduced volatilization losses from 20% to 5%. They also reported that

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herbage yields were 10% greater on pastures treated with the acidified slurries. Muck and Richards (1983) used $3N$ HCl to reduce volatilization of NH_3 in dairy manure samples by lowering the pH to below 3. Prakasam et al. (1974) 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. Moore et al. (1974) were unable to recommend acidification of fresh manure samples to a pH<2 for preservation of any nitrogen forms. They reported that acidified samples of liquid poultry manure had a 300% increase in nitrate levels during the first week of storage, and a large reduction in nitrite within three days. Moore et al. (1974) noted that the above increases in NO_3-N were not stoichiometrically equivalent to the decreases in NO_2-N concentrations, and suggested that nitrogen losses were most likely occurring in the mixed liquor.

III. Materials and Methods

A. Experimental set-up

A total of 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 manures. By design, two different storage types for each manure were represented in this 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. Due to the strict hygiene implemented by Virginia poultry growers, only one of the five dry broiler litters in the study was sampled directly from a broiler house.

All liquid dairy manures and dry broiler litters were analyzed for initial organic and inorganic nitrogen fractions using triplicate replications. Manure samples were then subjected to four 7-day treatments, representing four manure N preservation techniques, as follows:

1) ambient storage at a temperature of 26°C (78°F),
2) freezing of the samples at -22°C (-7°F),
3) refrigeration of the samples at 4°C (39°F), and
4) acidification of the samples with sulfuric acid to pH<2, with refrigeration.

Duplicate 7-day manure nitrogen concentrations from the above preservative techniques were then 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 above comparison to determine whether the overall 7-day storage treatment effects (from each of the four nitrogen preservative treatments) were statistically significant for the manure types and nitrogen forms tested in this study.

The following sections will detail the materials and methods used in this experiment, including statistical procedures, manure sampling, and laboratory manure nitrogen preservation and analysis.

B. Statistical analysis procedures

The objective of the statistical experiment was to test whether 7-day preservation treatment effects were statistically significant across all ten farm manures for each of the four nitrogen fractions evaluated [TKN, organic N, NH_4-N, and (NO_3+NO_2)-N]. The experimental design was a randomized complete block, and the blocks (farms) were random effects. All preservative treatments were fixed effects. Resulting 7-day treatment levels (responses) of manure nitrogen were compared against corresponding control treatment levels using seven mixed-model ANOVAs (Ott, 1988). All laboratory nitrogen analyses were replicated through duplicate and triplicate subsampling in order to test whether interaction was present between the treatments and the variable manures from each farm. Interaction effects between farms and preservative treatments were anticipated due to the complex nature of the manures. The statistical model used in this study was;

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \qquad\qquad \varepsilon_{ijk} \cong \eta(0, \sigma),$$

where y_{ijk} predicts the response for a given treatment $_{(i)}$, farm $_{(j)}$, and replication $_{(k)}$,
        μ represents the grand mean of all nitrogen responses,
        α represents the treatment effect for a given preservation treatment,
        β represents the farm (block) effect for a given farm manure,
        $\alpha\beta$ represents the interaction effect for a given farm $\times$ treatment combination, and
        ε_{ijk} represents the experimental error (for a normal distribution with mean, μ).

For purposes of this experiment, the source of all sampling from each farm manure was a composite manure mixture collected at the farm in a single 19-liter (five-gallon) bucket. Due to the inherent heterogeneity of the manures, it was important to homogenize that portion of the manure being tested before individual treatment samples were taken. (Procedures for pre-mixing and sampling of the composited manure and individual treatments will be detailed in following sections). Substantial experimental error caused by random sampling of the heterogeneous manures was expected. Therefore, the laboratory procedures used in this study were designed to reduce both the between-treatment and within-treatment variability present in the farm manure samples tested. Reduced subsampling variability in the laboratory through systematic mixing procedures increased the precision of the comparative testing between the 7-day preservative treatments and the control treatments by lowering the within-treatment variance of each manure. In addition, conscientious mixing of the composited manure mixture at the farm decreased the initial between-treatment variance for each manure to assure that all five treatments more equally represented the single manure "source" from which they were taken.

Due to differences between the nitrogen ranges of the two different manure types, the five liquid dairy manures and five dry broiler litters tested in this study were analyzed in separate mixed-model ANOVAs. Separate statistical analysis was adopted in order to avoid the possibility of widely varying treatment effects between the two distinct types of manure (Myers, 1994). Each of the two generalized design blocks, shown below in Tables 3.1 and 3.2, depict the 25 individual farm × treatment combinations that were analyzed for all ten manures in this experiment. These generalized experimental design blocks also illustrate how the seven separate ANOVAs were prepared in this study. All control (day 0)treatment nitrogen analyses were replicated three times using laboratory subsamples, while each of the four preservative treatment analyses were replicated only twice. The replication procedures for triplicate and duplicate subsampling in the laboratory were

Table 3.1.   Experimental design block showing liquid dairy manures vs. manure control and preservative treatments.

Liquid dairy manure source	Control treatment	Ambient treatment	Freeze treatment	Refrigerated treatment	Acidification treatment
Farm #1	III	II	II	II	II
Farm #2	III	II	II	II	II
Farm #3	III	II	II	II	II
Farm #4	III	II	II	II	II
Farm #5	III	II	II	II	II

III   Depicts triplicate subsample N responses for each farm × treatment combination.

II   Depicts duplicate subsample N responses for each farm × treatment combination.

Note: Design blocks were repeated for each of three detectable nitrogen fractions.

Table 3.2.   Experimental design block showing dry broiler litters vs. manure control and preservative treatments.

Dry broiler litter source	Control treatment	Ambient treatment	Freeze treatment	Refrigerated treatment	Acidification treatment
Farm #6	III	II	II	II	II
Farm #7	III	II	II	II	II
Farm #8	III	II	II	II	II
Farm #9	III	II	II	II	II
Farm #10	III	II	II	II	II

III   Depicts triplicate subsample N responses for each farm × treatment combination.

II   Depicts duplicate subsample N responses for each farm × treatment combination.

Note: Design blocks were repeated for each of four detectable nitrogen fractions.

identical. Although triplicate subsampling for both control and 7-day preservative treatments would have been preferred, handling and analysis limitations in the laboratory necessitated a reduction of sample volume to manageable levels. A total of 770 separate farm × treatment combinations, representing ten farms, two manure types, five treatments, and four nitrogen fractions, were analyzed in this experiment. Quality control methods in the laboratory, including the routine use of concurrent blanks and elemental chemical standards, brought the total sample number to approximately 800.

Mixed-model ANOVA computations for this experiment were carried out using the SAS® System for data management and analysis (SAS Institute Inc., 1989). All duplicate preservative treatment means for each nitrogen fraction and manure type were compared to the corresponding triplicate control mean using Dunnett's test procedure. Dunnett's procedure, a specialized multiple-comparison test, evaluates a calculated critical difference ($C_{diff.}$) between the control group and the various experimental groups to determine significance. The computational formula for Dunnett's procedure used in the present study was:

$$C_{diff.} = d_r \sqrt{\frac{2 \, ms_{int.}}{n \text{ (per group)}}}$$

where d_r represents a table value from Appendix L in Bruning and Kintz (1977),
$ms_{int.}$ represents mean square interaction in the mixed-model, and
n equals the number of groups divided by the harmonic mean of the cell sizes.

To determine whether overall 7-day treatment levels of manure nitrogen were significantly different from the control means in this experiment, all preservative treatment means were compared to corresponding control means at an alpha level of 0.05.

As mentioned previously, results from the ANOVA analyses also revealed whether interaction was present between the preservation treatments and farms. Where interaction was present, individual one-way ANOVAs were analyzed for each farm manure and nitrogen form to better understand the interactions occurring. In manure types or nitrogen forms where no significant interaction occurred in the overall comparison of 7-day treatment means, more reliable conclusions were possible as to the consistent effects of the various preservation techniques on the nitrogen values of the liquid dairy manures and dry broiler litters tested in this study.

C. Manure sampling - field procedures

1. Description of participating farms

Dairy and broiler producers from Augusta, Franklin, and Page Counties were chosen because of their accessibility, and because they were representative of Virginia's dairy and broiler industry. Virginia Cooperative Extension and Division of Soil and Water Conservation personnel from six Virginia counties assisted in the scheduling of on-farm manure sampling with participating producers.

Table 3.3 below 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.

As Table 3.3 shows, all manure sampling was performed in the month of July in order to test the hypothesis that the manure samples were susceptible to nitrogen transformation under warm, moist conditions. Samples for each of the two manure types were numbered in the order they were taken, with the "D" and "B" prefix adopted to identify the manure sample as either "dairy" or "broiler". The milking herd size of the individual dairy

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producers participating in this experiment ranged from 50 to 100 Holstein milk cows. Participating broiler producers kept bird populations ranging from 28,000 to 140,000 birds.

Table 3.3.   Manure sampling summary and description of participating farms.

Farm	County (Virginia)	Manure type	Sample I.D.	Sample date	Livestock number	Manure storage
Farm #1	Augusta	Liquid dairy manure	D1	7/26/94	100 milk cows	Earthen lagoon
Farm #2	Augusta	Liquid dairy manure	D2	7/26/94	60 milk cows	Concrete tank
Farm #3	Franklin	Liquid dairy manure	D3	7/28/94	50 milk cows	Concrete tank
Farm #4	Franklin	Liquid dairy manure	D4	7/28/94	100 milk cows	Earthen lagoon
Farm #5	Franklin	Liquid dairy manure	D5	7/28/94	100 milk cows	Earthen lagoon
Farm #6	Augusta	Dry broiler litter	B6	7/26/94	28,000 birds	Broiler house
Farm #7	Augusta	Dry broiler litter	B7	7/26/94	60,000 birds	Roofed storage
Farm #8	Page	Dry broiler litter	B8	7/26/94	140,000 birds	Roofed storage
Farm #9	Page	Dry broiler litter	B9	7/26/94	128,000 birds	Roofed storage
Farm #10	Page	Dry broiler litter	B10	7/26/94	128,000 birds	Roofed storage

Broilers on all farms were housed in 154×13 m (504×42 ft) metal fabricated buildings, with each of these 2000 m^2 (21,000 ft^2) broiler houses capable of holding between 28,000 to 40,000 birds. As mentioned previously, on-farm sampling represented two types of storage for each of the two manure types, including earthen and concrete storage for liquid dairy manure, and fresh house litter and roofed storage for dry broiler litter.

Participating broiler growers raised a variety of breeds, depending on the commercial contractor supplying the stock. Four of the five growers in this study were raising commercial broiler mixes supplied by either "WLR Foods" or "Rocco", including such breeds as Arboracres, Avian, Ross, and Hubbard. One of the growers in the study, contracted to supply broilers to "Tyson Foods", was raising Tyson's proprietary breed, Cobb. Feed mixes for the broiler producers were also supplied by the contracting companies, and included a standard mix of available grains such as cracked corn/soybean or corn/wheat, but also included cookie and cracker meal mixes for birds contracted by "WLR Foods". Dairy producers, at the time of manure sampling, were feeding their milking herds a range of mixtures, including corn/wheat silage and corn distiller's grain, corn silage with rye and alfalfa hay, corn silage/grain with limited hay, or corn silage/alfalfa haylage with soybean meal.

All liquid dairy manures sampled in this study included twice-daily milking parlor washwater additions as well as various amounts of barnyard scrapings and flushings. 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. Broiler litters from all five poultry producers were removed from the houses after each seven-week growing cycle for disease prevention. Therefore, all dry broiler litters evaluated in this study had been utilized for no more than one growing cycle.

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2.   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 of 1994. Liquid dairy manures at the farm were stored in earthen or concrete impoundments ranging in capacity from an estimated 250 m^3 (67,000 gal) for the smallest concrete tank, to 3785 m^3 (1M gal) for the two largest earthen basins. Design depths in the above impoundments ranged from 1.8-2.4 m (6-8 ft). Liquid dairy manure samples were collected with the use of a fabricated 3.1 m×5 cm diam. (10 ft×2 in diam.) PVC sampler with plug-end attachment. The hollow sampler pipe was first inserted into the manure-filled basin or concrete tank in order to obtain a representative cross-section of the liquid manure. When the sampler reached the bottom of the impoundment, the plug-end was pulled shut and the full sampler, containing about 3.8 liters (1 gallon) of liquid dairy manure, was brought to the surface. Several such samples were taken in order to make a composite for experimental nitrogen analysis of the individual liquid dairy manure. The complete five-step sampling procedure used in this study for both manure types is listed at the end of this section.

Dry broiler litter sampled as part of the present experiment was taken from stored piles of varying size, with the exception of sample B1, 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. Basically, dry broiler litter sampling was completed by climbing on top of the 1.8-2.4 m (6-8 ft) high piles and, with a shovel, digging to an approximate depth of 46 cm (18 in). Generally, from five to six standard shovel-fulls of dry broiler litter were composited for manure nitrogen analysis at each of the five broiler farms.

The complete five-step sampling procedure that was followed in gathering the manure samples from all ten farms prior to laboratory analysis was as follows:

1. At each farm, a representative manure composite was placed in a 19-liter (five-gallon) bucket and mixed completely using a cordless electric power drill, paint stirrer, ladle, and fabricated portable blender.

2. While mixing or blending the manure in the bucket (and while still at the farm), the manure composite was carefully 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.

3. The five split samples were then labeled and transported to the laboratory within 24 hours under ambient temperature conditions. *One of the five representative bottles for each manure was used as a control (without storage) for immediate triplicate analysis.*

4. At the laboratory, the control sample from each farm was analyzed immediately for nitrogen concentrations, and each of the remaining four labeled manure bottles was treated with the appropriate preservative treatment.

5. Each treatment bottle was then stored (according to treatment) for one week before final nitrogen analyses.

D. Manure analysis - laboratory procedures

1.   Manure preservative treatments

The four preservative storage treatments described below were chosen in order to obtain a more accurate understanding of the potential nitrogen transformations in a fresh manure sample during shipment. A study period of seven days was chosen for this experiment because it was considered a reasonable sample shipment period in Virginia.

a)   Control treatment

All composited manure samples, after being split into five equally representative sample bottles at the farm, were transported to the laboratory under ambient conditions. Of the five sample bottles representing each farm manure, four were subjected to specific preservative storage treatments, as described below. The fifth sample bottle was used as a control for immediate triplicate analysis of organic and inorganic manure nitrogen forms, as well as for determination of average moisture content.

The control treatment bottles for each manure, along with the other treatment bottles, were subjected to approximately 24 hours of ambient storage during transfer from the farm to the laboratory. All samples, including the control treatment, were placed in a 5 cm (2 in)-thick sealed styrofoam container (without ice) during sampling and subsequent transport to the laboratory at Virginia Tech. Temperatures within the styrofoam container on July 26th and 28th, between sampling and transit to the laboratory, ranged from a low of 23°C (73°F) at 9:20 AM to a high of 31°C (87°F) at 6:24 PM, reflecting normal ambient temperature conditions during the summer in Virginia.

b)   Ambient storage treatment

Manure samples stored under 7-day ambient conditions were kept on a table in the 5th floor attic of Seitz Hall, approximately 1 m (3.3 ft) above the floor. The capped ambient treatment bottles were not disturbed during 7-day storage. Ambient temperatures in the 5th floor attic were monitored at least once daily during the storage period, from July 27 to August 4. During storage, week-long ambient temperatures on the 5th floor maintained a range of from 25-27°C (77-80°F), with little observed fluctuation.

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c) *Frozen storage treatment*

Seven-day treatment samples stored under subzero conditions were kept in a table-top freezer in the Water Quality Laboratory in Seitz Hall. The capped frozen treatment bottles were not disturbed during 7-day storage. Monitored temperatures inside the freezer during the storage period ranged from a low of -23°C (-9°F) to a high of -17°C (-1°F), with an average observed temperature of -22°C (-7°F).

d) *Refrigerated storage treatment*

Manure sample treatments stored under 7-day refrigeration were consigned space in the walk-in cooler located on the first floor of Seitz Hall. The capped refrigerated treatment bottles were not disturbed during 7-day storage. Temperatures in the cooler was a relatively constant 4°C (39°F) over the period of this study.

e) *Acidified storage treatment*

Seven-day acidified manure treatments consisted of the addition of concentrated sulfuric acid (H_2SO_4) to the labeled treatment bottles of each farm manure until a pH of below 2.0 was achieved. The techniques adopted for acidified storage of the manures were developed as part of the present study. The initial pH of each manure sample was determined by using a Corning desk-top pH meter (Model 360i) with hand-held electrode. Each liquid dairy manure was measured for initial pH by stirring the submerged electrode in the manure-filled Nalgene sample bottle at room temperature (25°C) until the instrument equilibrated. Concentrated H_2SO_4 was then added directly to the manure sample bottle using a calibrated burette with glass petcock (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 sample was then sealed in the same bottle and stored for seven days in the laboratory 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 (39°F). Total amounts of H_2SO_4 that were added to each manure as part of the acidification treatment were recorded, and are presented in Table 3.4.

Table 3.4. **Acidification treatment summary for liquid dairy manures and dry broiler litters.** †

Sample I.D.	Initial pH	H_2SO_4 added	Acidified pH
D1	6.7	1.1 ml	1.8
D2	7.3	2.9 ml	1.5
D3	7.5	1.7 ml	1.5
D4	7.4	4.6 ml	1.7
D5	7.8	1.3 ml	1.5
B6	7.9	2.1 ml	1.8
B7	7.9	3.9 ml	1.8
B8	5.7	3.3 ml	1.7
B9	6.6	2.8 ml	1.8
B10	4.2	2.3 ml	1.8

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

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 determination. Procedures identical to those used for soil pH testing at the

Virginia Tech Soil Testing and Plant Analysis Laboratory were used to determine the pH of the dry broiler litters (Donohue, 1994). Once the 1:1 (v/v) litter / DDI H_2O mixtures were made up, the mixtures were allowed to stand for at least 20 minutes before pH testing began. After an initial pH determination was made, concentrated H_2SO_4 was added as necessary (while stirring the mixtures) to lower the pH to below 2.0. The pH probe was rinsed with DDI H_2O throughout the analysis procedure. The pH-adjusted broiler litter mixtures were then sealed and refrigerated for one week, as described above for the liquid dairy manures, before final nitrogen analysis.

2. Manure analysis procedures

Laboratory analysis methods used in this study 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 $2M$ KCl solution, with subsequent analysis of the extract by the Automated Phenate and Automated Hydrazine Reduction Method [PROPOSED], respectively (Greenberg et al., 1992). Total organic N was obtained by subtracting inorganic N from the total Kjeldahl-N. Existing facilities at the Water Quality Laboratory in Seitz Hall on the campus of Virginia Tech were utilized for laboratory analysis during this study. In addition to manure nitrogen analyses, several other physical and chemical characteristics of the manures were determined, including; pH, moisture content (MC), and bulk density (BD). The following sections will detail the materials and methods employed in the determination of all laboratory results used in the present study.

a)   *TKN analysis*

All manure control and 7-day treatment samples were analyzed for total Kjeldahl nitrogen by first subsampling the appropriate treatment bottle. Three 1-2 g subsamples were removed from each 300 ml control treatment bottle immediately upon arrival at the laboratory, while only two subsamples were taken from the preservative treatment bottles (after seven days of storage). Because of the lower bulk density and higher wet-basis TKN content of the dry broiler litters, generally a 1 g subsample was used in dry broiler litter TKN determination. All treatment bottles being subsampled, whether liquid dairy manure or dry broiler litter, were shaken by hand twenty times (inverting the bottle completely each time) immediately prior to removing the 1-2 g subsample for TKN analysis. Where necessary, dry broiler litters were chopped (in the sample bottle) with an electric hand-mixer to achieve more representative subsampling. *Identical subsampling techniques were used on all subsequent nitrogen analyses carried out in this experiment*. After recording the weight of each subsample, the manures were placed in separate acid-washed 250 ml Kjeldahl digestion tubes. The following materials were added to each digestion tube, along with the individual manure subsample:

  1) one glass boiling bead,
  2) one catalyst tablet (99.9% potassium sulfate, 0.10% selenium), and
  3) 15 ml of concentrated sulfuric acid (using a fume hood and protective clothing).

Tubes were covered for refluxing and venting, then placed in an aluminum digestion block (Labconco® Digestor 25), where they were pre-heated at 200°C (392°F) for approximately 30 minutes. The temperature of the heating block was then increased to 380°C (716°F) for an additional three hours. Digestion of manure samples in groups of 25 (the capacity of the digestion block) required a minimum of 4-1/2 hours, including at least one hour of cooling after digestion while the tubes were still in the block. One National Institute of Standards and Technology (NIST, 1991) Standard Reference

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Material (#1547 Peach Leaves) and one concurrent blank (reagents with no manure sample) were digested with every group of samples that was analyzed.

After the digest was cooled, the tubes were removed from the block and slowly diluted with 50 ml of DDI H_2O in preparation for distillation. The 25 digest tubes were attached, one at a time, to the distillation head of a Labconco® RapidStill II (Model 65200), where 60 ml of 40% (w/w) sodium hydroxide solution was added, followed by eight minutes of vigorous steam distillation. The condensed distillate was collected in a 4% boric acid / indicator solution, which was subsequently titrated by hand with $0.1N$ HCl. Total Kjeldahl nitrogen concentration on a wet-basis (wb) for each sample was subsequently calculated as follows:

$$C_N \text{ (ppm)} = (14007) \, N_A \, \frac{V_S - V_B}{W_S}$$

where C_N represents the total nitrogen converted to NH_4^+ through digestion,
(14007) represents the equivalent weight of N, mg/eq.,
N_A represents the normality of the titrating acid, eq./liter,
V_S represents the volume of titrate used on the sample, ml,
V_B represents the volume of titrate required for the blank, ml, and
W_S represents the wet weight of the sample, g.

All resulting wet-basis TKN concentrations (ppm) were converted to a dry-basis (db) in order to eliminate any distortion of results caused by manures with different moisture contents. To determine dry matter TKN, all wet-basis nitrogen results (ppm) were divided by the average manure sample dry-matter content (determined from separate triplicate control subsamples that were oven-dried at 65°C to a constant weight). The only exception to this dry-basis conversion procedure was the acidified broiler litter treatments. Because the acidified dry broiler litters had previously been made up as a 1:1 (v/v) mixture

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as part of the pH adjustment for manure nitrogen preservation, it was necessary to assure that the TKN results for the acidified broiler litter treatments represented the same dry-matter concentrations as the other treatments. Therefore, for the purpose of accurately comparing preservative treatments, the following three-step computation was used to calculate dry-matter TKN (ppm) on all acidified dry broiler litter / DDI H_2O mixtures.

1. The wet-basis TKN concentration of the pH-adjusted litter mixture was determined using macro-Kjeldahl digestion, as described above.
2. The average moisture content (MC) of the pH-adjusted litter mixture was determined by oven-drying separate duplicate samples at 65°C (149°F) to a constant weight.
3. The wet-basis TKN concentration (from step 1) was divided by the average dry-matter content of the 1:1 (v/v) acidified litter mixture (from step 2) to determine the dry-basis TKN concentration (ppm) for each acidified broiler litter subsample.

The above procedures for calculating the dry-matter TKN concentrations of the acidified broiler litter mixtures in this study were also used to calculate corresponding dry-matter NH_4-N and (NO_3+NO_2)-N concentrations. Final dry-matter nitrogen concentrations for all farm manure treatments and nitrogen forms were subsequently converted from ppm to g/kg (using division by 1000) for final reporting and statistical analysis. The only exception was nitrate/nitrite nitrogen (reported as mg/kg) which needed no final conversion from ppm to mg/kg.

b) NH_4-N analysis

In the methods of analysis used in this experiment, NH_4-N was defined as ammonium nitrogen which was extractable by 2M 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 mineralized N (Hadas et al., 1983).

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Duplicate and triplicate subsamples of manure for NH_4-N analysis (weighing 1-2 g) were placed in individual acid-washed 250 ml Nalgene bottles. Exchangeable NH_4^+ was extracted from a manure sample by adding 100× the sample amount (by weight) of $2M$ KCl (i.e., for 1 g of sample, 100 ml of $2M$ KCl was added). The bottle was capped and shaken on a reciprocating mechanical shaker for one hour, then allowed to stand for at least 30 minutes. The samples were next filtered using unwashed Whatman 42 filter paper (2.5 μm) into individual 30 ml dilute-it vials suitable for sealed refrigerated storage (Virginia Tech Water Quality Laboratory, 1990). Separate $2M$ KCl extractions were performed for each of the triplicate and duplicate subsamples tested (110 total). Control testing of the unwashed Whatman 42 filters to determine ammonium contamination resulted in an average blank NH_4-N concentration of 0.059 ppm (per filter), which was approximately 1% of the lowest manure extract NH_4-N concentration (5.125 ppm). Therefore, washing of the filters was considered unnecessary for the purpose of this study.

Immediately preceding automated analysis, the manure filtrates were transferred to individual 5 ml auto-analyzer cups (one cup per sample). Liquid dairy manure filtrate analytes were diluted 10×, and dry broiler litter filtrate analytes diluted 100×, with DDI H_2O before NH_4^+ analysis using a Technicon TRAACS 800 auto analyzer (Industrial Method No. 780-86T). The additional dilutions with DDI H_2O were necessary because the expected NH_4-N concentrations (wb) of the two manure types (10-30 ppm for liquid dairy manure filtrates and 40-100 ppm for dry broiler litter filtrates) were well above the 2 ppm range of the analytical equipment used.

The analysis procedure utilized in this study for NH_4-N determination was referred to in Standard Methods for the Examination of Water and Wastewater as the Automated Phenate Method (Greenberg et al., 1992). In principle, the intensity of the blue color that developed after treatment of the filtrate with phenol (C_6H_5OH), nitroprusside, and other reagents under alkaline pH conditions was proportional to the NH_4^+ present (Keeney and

Nelson, 1982). Subsequent automated spectrometric measurements of the intensity of the colored complex (also called the Berthelot color reaction) were made at 660 nm. The resulting concentrations of NH_4-N for each filtrate analyte, reported in ppm, were corrected for the appropriate filtrate dilution as required (10× or 100×), and converted to a final dry-basis (g/kg) NH_4-N concentration, as described previously for total Kjeldahl-N.

All pH-adjusted subsamples (representing the acidified treatments), after seven days of storage and subsequent KCl extraction/filtration, were readjusted to an approximate pH of 6.0 with various concentrations of NaOH solution previous to analytical determination. Readjustment of pH in the acidified manure filtrates was considered necessary both to protect the analytical equipment from highly acidic solutions and to provide a final level of pH for analysis that was comparable to the filtrates representing the nonacidified treatments.

The pH readjustment of the 7-day acidified manure filtrates was completed approximately six days after filtration and about one month before automated colorimetric analysis could be scheduled. During this time, all manure subsample extracts were stored in the Water Quality Laboratory cooler at 4°C (39°F). *The refrigerated filtrates, sealed in 30 ml dilute-it vials, were thereafter utilized for all NH_4-N as well as (NO_3+NO_2)-N automated analyses, providing same-source analyte for both nitrogen determinations.*

Table 3.5 below indicates each of the pH adjustment operations conducted for all acidified extracts before final automated analysis. Manure subsample extracts from the pH-adjusted preservative treatments were labeled "P1" and "P2", to designate the two separate extractions from each farm manure. The six procedural steps carried out as part of the complete pH readjustment included; (1) initial manure pH determination, (2) initial adjustment of manure pH to < 2 with concentrated H_2SO_4, (3) manure pH determination at the completion of 7-day storage, (4) pH determination of the acidified filtrate six days

after extraction/filtration, (5) addition of NaOH for pH readjustment of the filtrate to approximately 6.0, and (6) final pH determination of the analyte before automated analysis.

Table 3.5.   Adjustment of pH for acidified manure treatment extracts. †

Sample I.D.	Initial pH 7/27/94	Adj. pH 7/27/94	7-day pH 8/3/94	Filtrate pH 8/9/94	NaOH added *	Final pH 8/9/94
D1-P1	6.7	1.8	2.6	3.5	2 drops °	5.4
D1-P2	"	"	"	3.6	2 drops °	5.6
D2-P1	7.3	1.5	1.7	2.8	4 drops δ	5.4
D2-P2	"	"	"	2.8	3 drops δ	6.3
D3-P1	7.5	1.5	1.5	2.8	4 drops ‡	7.1
D3-P2	"	"	"	2.8	4 drops ‡	6.2
D4-P1	7.4	1.7	2.4	3.3	3 drops ‡	5.8
D4-P2	"	"	"	3.2	3 drops ‡	5.7
D5-P1	7.8	1.5	1.8	3.0	2 drops ‡	7.0
D5-P2	"	"	"	3.0	2 drops ‡	6.4
B6-P1	7.9	1.8	2.7	3.3	3 drops ‡	6.1
B6-P2	"	"	"	3.4	3 drops ‡	6.1
B7-P1	7.9	1.8	2.5	3.1	5 drops δ	5.3
B7-P2	"	"	"	3.2	3 drops δ	5.9
B8-P1	5.7	1.7	2.5	3.1	3 drops ≡	5.3
B8-P2	"	"	"	3.1	3 drops ≡	5.6
B9-P1	6.6	1.8	2.8	3.3	2 drops ≡	5.7
B9-P2	"	"	"	3.3	2 drops ≡	5.6
B10-P1	4.2	1.8	3.1	3.4	3 drops ≡	5.7
B10-P2	"	"	"	3.4	3 drops ≡	5.4

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

* NaOH solutions (w/w) were added to approximately 25 ml of filtrate (20 drops ≈ 1 ml).

° 0.5% NaOH   ‡ 0.75% NaOH   δ 1.0% NaOH   ≡ 1.5% NaOH

c)   *(NO_3+NO_2)-N analysis*

As mentioned previously, the individual manure subsample extracts that were used for exchangeable NH_4-N determination were also utilized for analysis of exchangeable (NO_3+NO_2)-N. However, because the expected concentrations of nitrate/nitrite nitrogen in the manure extracts were at or below the 0.2 ppm detection limit of the Technicon TRAACS 800 auto analyzer (Industrial Method No. 782-86T) used in this analysis, no preliminary dilution of the extracts was considered necessary. *For the purpose of the present study, detection limit was defined as those concentrations at or below the lowest working standard used in the analysis procedure.* This practical detection level was considered by the instrument operator to be the lowest level achievable within the routine limits of the laboratory operations performed.

As an illustration of the difficulty encountered in detecting (NO_3+NO_2)-N concentrations (especially in the liquid dairy manures), Mahimairaja et al. (1990) reported combined (NO_3 +NO_2)-N levels of 50 ppm (db) in a fresh dairy slurry. Assuming a MC of 94.3% (Virginia manure testing program 3-year average), and including the fact that the manures in the above study were effectively diluted by a 10:1 $2M$ KCl extraction, the following calculations explain by example the weighty effect of dilution on laboratory detection.

50 ppm (db) $\times$ (1 - 0.943)   = 2.85 ppm (wb)   [(NO_3 +NO_2)-N in fresh sample]
2.85 ppm $\div$ (10:1 extraction) = 0.29 ppm (wb)   [(NO_3 +NO_2)-N in diluted analyte]

In the above example, the ten-fold difference in (NO_3 +NO_2)-N concentration would have placed both hypothetical samples (one diluted and one undiluted) near the extreme range of the analysis method used in the present study. Unfortunately, the 100:1 $2M$ KCl extraction which was adopted in the present experiment, chosen largely to eliminate the

need for separate NH_4-N and $(NO_3 + NO_2)$-N extractions, made manure $(NO_3 + NO_2)$-N analytical detection more difficult.

A second problem encountered in the analysis of the manure extracts for nitrate/nitrite nitrogen was the color remaining in the extracts after filtration, in spite of the 100:1 dilution resulting from the $2M$ KCl extraction procedure. Liquid dairy manure filtrates had colors ranging from mostly clear to one that was opaque (sample D4), while the dry broiler litter filtrates ranged in color from pale yellow to gold to dark amber, increasing the likelihood of colorimetric interference, as described below.

The analysis procedure used in this study for $(NO_3 + NO_2)$-N determination was referred to in Standard Methods for the Examination of Water and Wastewater (Greenberg et al., 1992) as the Automated Hydrazine Reduction Method [PROPOSED] (4500-NO_3^- H). In principle, the NO_2^- (originally present plus reduced NO_3^-) was determined by diazotization, whereby sulfanilamide under acidic conditions formed a highly colored soluble dye (also called the Griess-Ilosvay procedure). In this procedure, nitrate in the filtrates was first reduced to nitrite by an alkaline solution of hydrazine sulfate containing a copper catalyst. Subsequent colorimetric measurement of the soluble dye at 520 nm determined the concentration of $(NO_3 + NO_2)$-N present in the analyte. Because of the relatively low levels of nitrate/nitrite nitrogen anticipated in all the analytes, procedural interference in this analysis was a special concern.

In preliminary testing of similar manure filtrates, several manure extractions (generally from dry broiler litters) were found to absorb light in the photometric range used in the automated hydrazine procedure. Thereafter, separate absorbance tests on unused portions of the ten manures in this study were completed using a Milton Roy spectrophotometer (Spectronics-20). Experimental testing showed that all five broiler litter filtrates and one of the liquid dairy manure filtrates (sample D4) had in excess of 0.10 AU (Absorbance

Units), while the actual reagents used in the analysis procedure had an absorbance of only 0.03 AU (Industrial Method No. 782-86T). Observed absorbance for the other four liquid dairy manure filtrates were below 0.03 AU (all testing done at 520 nm).

Therefore, the subsampled filtrates from the five broiler litters, as well as dairy manure sample D4, were considered to be at risk of colorimetric interference. Consequently, an activated carbon technique was developed which reduced both the color and absorbance of these filtrates. Color removal procedures consisted of three inclusive steps, as follows:

1. Manure filtrates with absorbance greater than 0.10 AU (520 nm) were removed from refrigerated storage for color removal with activated carbon, including all eleven duplicate and triplicate filtrates from manure samples D4, B1, B2, B3, B4, and B5.
2. The colored filtrate in each of the 30 ml dilute-it vials from step 1 was mixed with 0.1g of Nuchar® SA-20 powdered activated carbon (Westvaco Chemicals) and shaken for 15 minutes on a mechanical shaker. The only exception was the filtrates representing litter sample B8, which required a total 0.2 g of activated carbon due to high color.
3. After shaking for 15 minutes, the carbon/filtrate mixtures were allowed to stand for an additional 15 minutes, after which they were filtered with a Gelman A/E glass fiber filter (1.5 μm) into individual 5 ml auto-analyzer cups for subsequent analysis.

Using the procedures outlined above, filtrate colors were cleared, and absorbance at 520 nm was reduced to 0.03 AU or less. Unfortunately, planned control testing of the colored filtrates (using steam distillation) to determine if activated carbon lowered extract N concentrations was not completed because of undelivered results from the collaborating laboratory.

[Note: The 66 colored filtrates were treated with activated carbon one week before automated $(NO_3 + NO_2)$-N analysis. Also, at the time the color removal procedures were carried out, analyte had already been removed from the dilute-it vials, for separate analysis of NH_4-N].

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d) *Bulk density analysis*

Bulk density analysis of the ten manures studied in this project was completed only to more fully describe the physical characteristics of the individual manures. Procedures for determination of all manure bulk densities, whether liquid dairy manure or dry broiler litter, where identical. Each of the fresh manure samples was placed, one at a time (without compaction), in a dry 100 ml graduated cylinder which had been previously weighed on a Mettler analytical balance (Type PC 2000-S2). A volume of 100 ml of manure sample was used in each bulk density determination. To obtain wet sample weight, the dry weight of the tared graduated cylinder was subtracted from the gross weight of the 100 ml manure sample and graduated cylinder. The resulting sample wet weight, in grams, was divided by the volume, in ml, (where 1 ml = 1 cm^3). Final bulk density (BD) results were reported as g/cm^3.

3. Precision and accuracy

Smith (1993) recommended calculating the accuracy of spikes into natural matrices by the following formula;

$$\%R = 100 \times \frac{\text{Observed value } - \text{ Background value}}{\text{Known value}}$$

where % R = percent recovery,
Observed value is the analytical result after spiking,
Background level is the analytical result of the matrix before spiking, and
Known value is the concentration of the spike.

Likewise, Smith (1993) suggested that precision be assessed through the following calculation;

$$RPD = 100 \times \frac{|A - B|}{(A + B)/2}$$

where RPD is the relative percent difference between duplicate determinations,

      A and B are the analytical results for the duplicate determinations,

      $| A - B |$ is the absolute difference between the determinations, and

      $(A + B)/2$ is the mean of the duplicate determinations.

The above formulas were utilized in this study to create control charts for precision assessment. The following calculations (using the average RPD and the standard deviation of the RPD) were used to construct a separate control chart for each nitrogen fraction and manure type analyzed, as follows;

$$RPDave = \frac{\sum_{i=1}^{n} RPDi}{n}$$

where RPDave is the average relative percent difference,

      RPDi is the relative percent difference of observation i, and

      n is the number of observations (Smith, 1993).

Greenberg et al. (1992) described the range (R) control chart as a necessary means for quality control of replicate analyses. They stated that the common practice is to use $\pm 2\,s$ and $\pm 3\,s$ limits for the warning limit (WL) and control limit (CL), respectively, where s represents standard deviation. The manual formula for calculating the standard deviation was given by Smith (1993) as;

$$S_{RPD} = \sqrt{\frac{\sum_{i=1}^{n}[RPDave - RPDi]^2}{n - 1}}$$

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The RPD ranges of each nitrogen fraction and manure type in this study were normalized (as in the above calculation for RPD) by dividing the absolute difference in replicate N concentrations by their average. The resulting normalized range was entered on an R control chart, and lines were drawn at RPD, RPD + 2 S_{RPD}, and RPD + 3 S_{RPD}. Perfect agreement between duplicate analyses resulted in a difference of zero when the values (A and B) were subtracted, so the base line of the R control chart was zero (Greenberg et al., 1992).

The following section outlines the methods used for assessing the precision and accuracy of all laboratory nitrogen determinations in this experiment.

Total Kjeldahl nitrogen (TKN):

1. One National Institute of Standard and Technology (NIST, 1991) certified standard (peach leaves) was run with each of the six digest batches. (Two of the six standards were lost due to incorrect laboratory procedures).

2. One method blank was run with each of the six digests. (Blank values were subtracted from measured values for reporting).

3. A separate Range (R) Control Chart (duplicate subsample analyses, using normalized range values), was prepared for each of the two types of manures in the study.

Ammonium nitrogen (NH4-N):

1. One EPA certified standard was run per every 20 samples.

2. One method blank was run per every 20 samples.

3. A separate R Control Chart (duplicate subsample analyses, using normalized range values) was prepared for each type of manure.

Nitrate/nitrite nitrogen ($(NO_3 + NO_2) - N$):

1. One EPA certified standard was run per every 20 samples.

2. One method blank was run per every 20 samples.

3. An R Control Chart (duplicate subsample analyses, using normalized range values) was prepared for broiler litter samples that were above the detection limit.

IV. Results and Discussion

A. Manure physical characteristics

Table 4.1 presents the results for selected physical characteristics of the ten farm manures tested in this study, including the type and duration of on-farm storage at the time of sampling.

Table 4.1.   **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	E	2-1/2 mo.
D2	94.8	7.3	1.01	C	2-1/2 mo.
D3	97.1	7.5	1.01	E	2-1/2 mo.
D4	87.3	7.4	0.88	C	2 mo.
D5	98.4	7.8	0.99	E	2 mo.
Average liquid dairy	**94.7**	**7.2 †**	**0.98**		
B6	29.5	7.9	0.38	H	0 wk.
B7	32.5	7.9	0.43	R	1 wk.
B8	23.3	5.7	0.42	R	1 mo.
B9	16.9	6.6	0.42	R	3 wk.
B10	13.8	4.2	0.47	R	4 mo.
Average dry broiler litter	**23.2**	**4.9 †**	**0.42**		

C   concrete liquid dairy manure storage

E   earthen liquid dairy manure storage

H   dry broiler litter directly from broiler house

R   dry broiler litter from roofed storage

†   average pH calculated from the [H$^+$] concentration of the manures

Average moisture contents (MC) for the five liquid dairy manure and dry broiler litter
samples were 94.7% and 23.2%, respectively. Average pH for the liquid dairy manures
tested was 7.2, while dry broiler litters had an average pH of 4.9. Bulk densities of the
liquid dairy manures were found to be similar to that of water, as would be expected, with
the exception of liquid dairy sample D4, which had physical characteristics closely related
to semi-solid manure, i.e., relatively lower moisture content and bulk density values.
Broiler litter samples tested in this study were found to have an average bulk density (BD)
of 0.42 g/cm^3, which corresponded to values reported by Westerman et al. (1988).

The overall farm-to-farm variability in moisture content and pH between the two types of
manures tested was found to be greater for the broiler litters than for the liquid dairy
manure samples (Table 4.2), reflecting greater physical differences between the broiler
litters tested than between the dairy manures. The coefficient of variation values reported
as percentages in Table 4.2 represent the standard deviation of the observed MC, pH, and
BD means for each of the five farms, divided by the overall mean for each manure type.

**Table 4.2.   Coefficient of variation values for selected manure physical
characteristics.**

	MC	pH †	BD
Liquid dairy manure	4.6 %	99.5 %	5.7 %
Dry broiler litter	34.4 %	191.4 %	7.6 %

†   Coefficient of variation values for pH represent the relative standard deviation
between the initial sample [H$^+$] concentrations for each manure type.

Individual bulk density values for the five liquid dairy and dry broiler litter samples
resulted in similar coefficient of variation (c.v.) values, due principally to the relatively low
bulk density value of sample D4, which could have been classified as a semi-solid manure.
Sample D4 was unique in that it was a "thicker" manure (more viscous); however, its bulk

density was found to be lower than the other four liquid manure samples due to its apparent "foaminess". This "foaminess" was the result of an increased solids content (hay, feed, feces, etc.) which displaced liquid in the sample. The increased solids content of sample D4 is partly attributable to the close proximity of the concrete manure storage pit to the feeding area at this farm, which were adjacent to each other. Therefore, excluding dairy sample D4 in bulk density comparisons, the overall farm-to-farm variability of the five liquid dairy manures tested would have been much less than the corresponding variability in dry broiler litter bulk densities (0.96% dairy vs. 7.64% broiler).

The relatively greater physical variation between the individual broiler litters can be explained not only by the higher probability of sampling error in a nonhomogeneous material such as broiler litter, but also by the fact that the broiler litters tested in this study varied in storage time from zero weeks to four months. Conversely, liquid dairy pits used in this study had all been last pumped in May of 1994, some two to three months prior to our July 1994 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.

Although this study was not designed to test for effects of storage time vs. manure physical characteristics, the initial random selection of farm producers, as well as the more flexible storage needs of poultry litter, provided the opportunity to compare litters of different age. Storage time effects on liquid dairy manure physical characteristics were not compared in this study because all five dairy manure samples had similar periods of on-farm storage prior to sampling. The physical characteristics of the five broiler litters, however, did suggest a relationship between litter storage time and initial sample pH. Although it was difficult to conclusively identify a positive relationship between litter storage time and pH with such a small number of samples, it was noted in this study that broiler litters stored for longer periods of time exhibited lower values of pH (Table 4.1).

Two possible reasons for a drop in stored litter pH over time have been alluded to by several researchers; and each of these explanations is intimately related to the microbiology of the litter pile. One possible reason for acidic conditions in stored broiler litter is the cessation of urea hydrolysis due to the eventual depletion of uric acid and urea in the pile, along with the subsequent mineralization and volatilization of the hydrolyzed ammonium, both of which are acidifying reactions. Schefferle (1965) observed that poultry manure/litter mixtures were alkaline and had high numbers of uric acid decomposers (uricase enzymes). Samples of unused litter (bedding without manure) were strongly acid (pH 4) and contained no bacteria that possessed uricase activity. These results suggest a direct relationship between rapid urea hydrolysis and fresh litter pH levels, one that results in temporarily increased litter pH levels only for as long as uric acid and urea are present.

Another possible explanation for the decrease in pH in used broiler litter over time relates to the bio-oxidative process called composting. De Bertoldi et al. (1985) defined composting as the microbial mineralization and partial humification of organic substances, dependent upon aerobic conditions (as well as optimum moisture, temperature, nutrient balance, and pH levels). They stated that when oxygen concentrations within composting pile interspaces fall below 15-20%, the concentration of anaerobic microorganisms begins to exceed aerobic ones (De Bertoldi et al., 1985). Kirchmann and Witter (1989) noted that aerobic poultry/straw mixtures in 120-day incubations developed alkaline conditions (pH 8.4-8.9), while anaerobic (50% water holding capacity) mixtures induced acid conditions (pH 5.8-6.2). The depletion of oxygen in a static pile of used poultry litter that has been stacked longer than one month most likely continues during even limited composting, at least until oxygen concentrations are reduced to critical levels within the pile interspaces. If anaerobic conditions subsequently develop within the pile, this could conceivably lead to acid conditions similar to those reported by Kirchmann and Witter (1989).

Two additional relationships were noted between the storage time and physical characteristics of the five broiler litters. Moisture content was found to generally decrease with increasing storage time. This result was not unexpected due to the fact that all litters were stored under roofed structures, during the heat of summer, when evaporation is normally highest. Collins et al. (1994a) reported lower overall moisture contents in Fall-sampled poultry litters compared to Spring litters. Lovell et al. (1970) reported an apparent relationship between moisture content and pH in 27-month old southern Ohio poultry litters, with lower pH values (pH<7.0) corresponding to litters with lower moisture contents (9.1%, 10.2%).

A closer look at the composting process reveals that the stored piles of broiler litter are most likely undergoing some form of accelerated drying over and above evaporation. Finstein and Miller (1985) described the "biological drying" that occurs during composting as a chain of events that generates heat, evaporates water, and subsequently causes drying through vaporization. Therefore, it seems likely that "in-pile" heating also contributed to the lower moisture contents of litter samples B8, B9, and B10, all of which were stored for longer than three weeks. Note that samples B9 and B10 differed ostensibly only in length of storage time, since both samples were taken from the same farm. The 19% drop in moisture content from three weeks to four months between samples B9 and B10 (both under roofed storage) provides some evidence that "biological drying" continues during extended storage of dry broiler litter. The lower initial pH value of sample B10 suggests that pH can continue to drop under induced anaerobic conditions.

The observed relationship between increased litter storage time and increased bulk density can be partly explained by the natural settling and compaction that occurs in most organic materials when stacked to depths of 1.8-2.4 m (6-8 ft). Also, this assumed composting would be expected to increase litter bulk densities by turning loose, bulky raw materials into crumbly, fine-textured material with a more soil-like texture (NRAES, 1992).

B. Manure nitrogen forms and concentrations

1. Manure nitrogen data quality

Total Kjeldahl nitrogen (TKN)
Accuracy: 92.2% recovery (n=4)
Precision: 8.74% c.v. (n=20)   Liquid dairy manure
             3.92% c.v. (n=20)   Dry broiler litter

Collaborative testing for TKN method evaluation was performed by the Forage Testing Lab at Virginia Tech. Accuracy achieved by the Forage Testing Lab, using the same NIST standard material as a control, was an average 99.7% recovery of certified total nitrogen (n=2). One possible reason for the higher average recoveries in the collaborative lab, excluding differences in laboratory equipment and technique, was the much shorter and hotter digestion used by the collaborative lab (approximately one hour @ 420°C vs. three hours @ 380°C used in this study). A third lab, at the Department of Plant and Soil Sciences at the University of Delaware, also reported using the shorter one-hour digestion (@430°C) for TKN analysis of dry broiler litters in macro-Kjeldahl digestion (Gartley, 1994). Page et al. (1982), in Methods of Soil Analysis, recommended five hours of Kjeldahl digestion after clearing when highly accurate results are desired. The TKN recoveries obtained in the present study, however, suggest that the three-hour digest that has been used in the Water Quality Laboratory at Virginia Tech for manure TKN determination should be re-evaluated in order to further test the accuracy of shorter digestion times.

Precision values obtained in this study compared very favorably to reported average c.v. values of 54.7% for duplicate synthetic macro-Kjeldahl determinations in 30 laboratories (Greenberg et al., 1992). The relatively higher average coefficient of variation in liquid dairy manures can be attributed to random subsampling error of the well-mixed, but

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unchopped organic solids present in the liquid dairy manures. Also, larger sample volumes were used for the less dense broiler litters, which would increase the repeatability of the litter subsampling by making each subsample more representative.

In the liquid dairy manure TKN analysis (n=20), one point was out of control. No points exceeded the statistical warning limit (Appendix 3). In the dry broiler litter TKN analysis (n=20), one point was out of control. No points exceeded the statistical warning limit (Appendix 4). All other subsampled points for TKN analysis fit the expected distribution for precision. A slight trend was noted in both manure types associated with sample sequence, possibly due to inconsistencies in batch digestions, and replacement reagents.

Ammonium nitrogen (NH_4-N)
Accuracy: NA
Precision: 4.02% c.v. (n=20)   Liquid dairy manure
              10.22% c.v. (n=20)   Dry broiler litter

Accuracy determination of the Automated Phenate Method used for NH_4-N analysis in this study was not possible because the EPA standard used for recovery calculations was determined to be out of date at the time of use. Fresh working standards used in the actual analysis, however, correlated well (R^2 = 0.999).

Precision values compared very favorably to the average 26.0% c.v. reported for duplicate synthetic samples in distilled water from 71 laboratories (Greenberg et al., 1992). The higher coefficient of variation value in the broiler litters most likely reflected the subsampling error encountered when extracting the unground and undissolved dry broiler litters with a 2M KCl solution. Conversely, liquid dairy manures would be expected to have more representative subsampling of exchangeable NH_4-N since much of the inorganic nitrogen had possibly been in soluble form for months.

In the liquid dairy manure NH4-N analysis, one point was out of control. No points exceeded the statistical warning limit (Appendix 5). In the dry broiler litter NH4-N analysis, no points were out of control. Two points exceeded the statistical warning limit (Appendix 6). All other subsampled points for NH4-N analysis fit the expected distribution for precision. No trends associated with sample sequence were evident.

Nitrate/nitrite nitrogen ($(NO_3 + NO_2)$ - N)

Accuracy:	NA	
Precision:	NA	Liquid dairy manure
	4.68% c.v. (n=12)	Dry broiler litter

Accuracy determination of the Automated Hydrazine Method (PROPOSED) used for $(NO_3 + NO_2)$-N analysis in this study was not possible because the EPA standard was made up of a different matrix than the $2M$ KCl solution analyzed. Working standards made up in a KCl matrix to match the undiluted manure extracts, however, correlated well $(R^2 = 1.000)$.

Precision results from independent laboratories using the Automated Hydrazine Method (PROPOSED) were unavailable for comparison at the time of this writing.

All of the initial liquid dairy samples and two of the dry broiler litters were found to have undetectable levels of $(NO_3 + NO_2)$-N. Of the three broiler litter samples that were detectable, no points were out of the control limit. One point exceeded the statistical warning limit (Appendix 7). All other detectable subsampled points for $(NO_3 + NO_2)$-N analysis fit the expected distribution for precision. No trends associated with sample sequence were evident.

2. Initial manure nitrogen levels

Mean nitrogen values and overall averages for the triplicate control samples of all ten farm manures are listed in Tables 4.3 and 4.4. Average relative standard deviation (coefficient of variation) values for each of the liquid dairy manure and dry broiler litter triplicate controls are presented in Appendix 8.

a) Total Kjeldahl, ammonium, and organic nitrogen

As Tables 4.3 and 4.4 show, concentrations of the major forms of nitrogen (TKN, NH_4-N, and organic N) found in the present study corresponded closely with recent average values from Virginia producers (Virginia Department of Conservation and Recreation, Division of Soil and Water Conservation, 1993), with the possible exception of broiler litter NH_4-N. One reason that NH_4-N values in the broiler litters tested in the present study were somewhat lower than NH_4-N averages in Virginia was their lower mean moisture content (due to storage time), as discussed previously. The random sampling that was done in this study resulted in broiler litters with an average moisture content of 23.2%, as compared to a three-year Virginia average of 28.4% (Table 2.1). Lower moisture litters could be expected to lose more nitrogen as volatilized NH_3 since air drying of poultry manure has been shown to cause significant reduction in both organic and ammoniacal N in manures (Mahimairaja, 1990; Gale et al., 1991).

Table 4.3. Mean nitrogen values for triplicate liquid dairy manure controls. †

Manure sample	TKN	NH$_4$-N	Organic N ‡	(NO$_3$+NO$_2$)-N
	g/kg	g/kg	g/kg	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 (1989-1992)	**47.5**	**20.1**	**27.4**	**not determined**

† All values expressed on a dry weight (65°C) basis.

‡ Organic N was calculated as the difference between total Kjeldahl nitrogen (TKN) and 2M KCl extractable NH$_4$-N.

Table 4.4. Mean nitrogen values for triplicate dry broiler litter controls. †

Manure sample	TKN	NH$_4$-N	Organic N ‡	(NO$_3$+NO$_2$)-N
	g/kg	g/kg	g/kg	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 (1989-1992)	**43.7**	**8.2**	**35.5**	**not determined**

† All values expressed on a dry weight (65°C) basis.

‡ Organic N was calculated as the difference between total Kjeldahl nitrogen (TKN) and 2M KCl extractable NH$_4$-N.

Differences in the organic and inorganic fractions of nitrogen between the two manure types also reflected 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). On a dry weight basis, the ammonium concentration of the average broiler litter in this study was 23% of the ammonium level in a comparable liquid dairy manure sample. 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. They found high ammonium levels in anaerobic manures, while organic nitrogen dominated aerobic manures, and concluded that the organic matter of anaerobic manure was poor in nitrogen and rich in carbon (Kirchmann and Witter, 1989). Total Kjeldahl nitrogen levels in the liquid dairy manures in the present study were about 15% higher than average broiler litter TKN levels, which followed the same trend as statewide averages in Virginia (Tables 4.3 and 4.4).

Figures 4.1 and 4.2 below present graphically the major forms and concentrations of nitrogen (organic and inorganic) in each of the ten farm manures tested, as well as initial manure pH. A trend was noted in the liquid dairy manures, relating pH to NH_4-N content. Higher pH values generally corresponded to higher NH_4-N levels within the dairy manures tested, possibly related to the production of hydroxyls (OH^-) during urea hydrolysis. Dairy manure studies by Muck and Richards (1983) suggested that at temperatures above 20°C, more organic N fractions than just urea are ammonified. Broiler litter NH_4-N levels followed the same general trend as dairy manures in the present study, with higher pH litters corresponding to relatively higher exchangeable ammonium levels. The exception, sample B6, was taken directly from a broiler house at five and one-half weeks into a seven-week cycle.
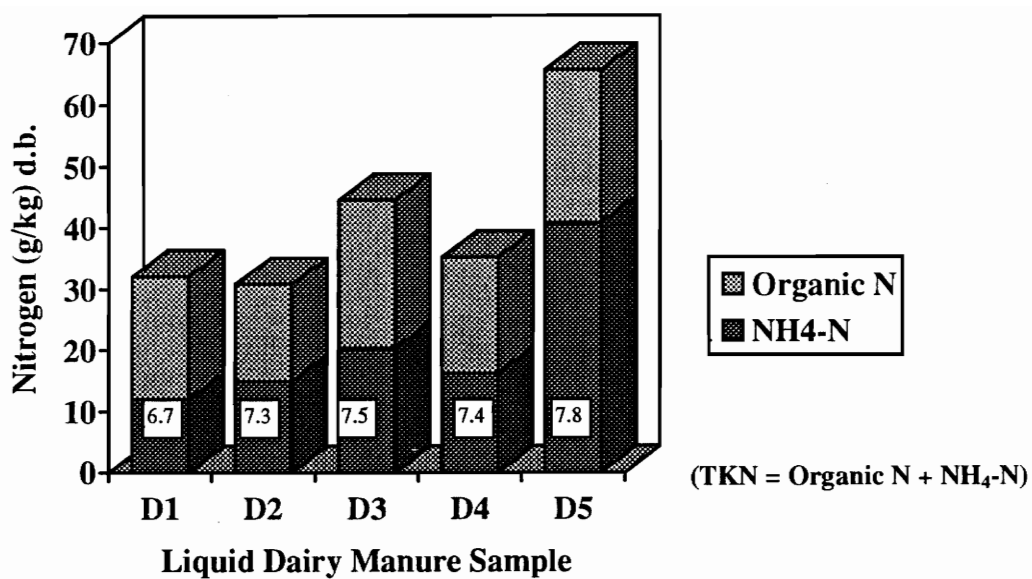
Figure 4.1. Mean initial nitrogen and pH values for liquid dairy manure samples.
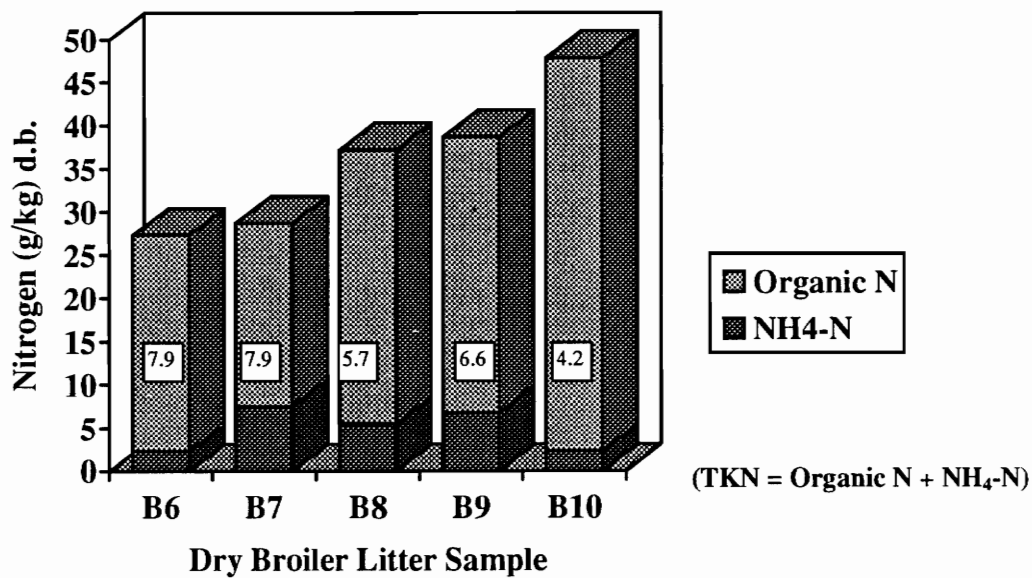
Figure 4.2. Mean initial nitrogen and pH values for dry broiler litter samples.

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A trend was also noted in the dry broiler litters relating total nitrogen to duration of storage. 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. Several researchers, including Kirchmann and Witter (1989), Nodar et al. (1990), and Moore et al. (1994), 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., 1994). 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 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).

Figures 4.1 and 4.2 also illustrate the nutrient variability between individual farm manures that makes individual manure nutrient analysis so important. A summary sheet of all manure results (on a wet basis) as reported to participating individual farm producers, can be found in Appendix 9. Corresponding nutrient management recommendations, based on the above manure nitrogen analyses, were provided to all nutrient management specialists and extension agents who participated in this study. These prescription application recommendations for each of the ten farm manures studied are presented in Appendix 10, and further emphasize the impact that accurate manure nitrogen analysis can have on efficient nutrient management.

b)   Nitrate / nitrite nitrogen

Initial nitrate/nitrite nitrogen results for three of the five broiler litter samples tested in this study (B8, B9, and B10) fell within the ranges reported by Bitzer and Sims (1988), Henry

and White (1990), and Nodar et al. (1990). However, two of the broiler litters and all five of the dairy manure samples were found to have combined $(NO_3 + NO_2)$-N levels below the detection limit of the procedures used in this study. Mahimairaja et al. (1990) determined that the combined nitrate/nitrite nitrogen component of a fresh dairy manure sample was 50 mg/kg (on a freeze dried basis), which was found to be below the detection limit of the automated hydrazine procedures used in this study. Adriano et al. (1974), using direct steam distillation procedures, reported practically no nitrate in freshly defecated dairy cow urine-feces mixtures. The liquid dairy manures tested in this study, at an average moisture content of 94.7%, included varying amounts of parlor wash water and runoff even before chemical analysis began. Unfortunately, further necessary dilution in the laboratory with a $2M$ KCl solution made accurate detection of $(NO_3 + NO_2)$-N even more difficult, especially in the liquid dairy manures. The two dry broiler litter samples (B6 and B7) that were found to have combined $(NO_3 + NO_2)$-N levels below the detection limit of this study also had the shortest on-farm storage periods (one week or less), suggesting that higher combined oxidized nitrogen levels in broiler litters are present following at least several weeks of storage.

Another possible reason for poor $(NO_3 + NO_2)$-N detectability in the broiler litter extracts was the use of activated carbon in the analysis procedure. Prakasam et el. (1974) reported that the use of activated carbon for removal of color in liquid poultry samples was unsatisfactory because significant amounts of nitrates were also removed. Because only partial results from the nitrate/nitrite nitrogen analyses for the broiler litters were obtained, and because this combined oxidized fraction of nitrogen makes up a relatively small portion of manure nitrogen, both the initial and seven-day broiler litter $(NO_3 + NO_2)$-N treatment results will be discussed together following the end of the next section.

3. 7-day manure nitrogen levels

Tables 4.5 and 4.6 below present the overall nitrogen means for the five dairy manures and broiler litters after treatment with four 7-day preservative techniques. (A complete listing of the resulting individual control and 7-day nitrogen concentrations in each of the ten subsampled farm manures can be found in Appendix 11). Treatment means for each nitrogen form and manure type were statistically tested in seven mixed-model ANOVAs against the corresponding control means to determine if there was a significant treatment effect on manure nitrogen across all five farms. Asterisks in the following two tables of results denote overall nitrogen preservative treatment means that were found to be significantly different (α=0.05) from the control mean after seven days of storage.

Table 4.5. Overall nitrogen means for liquid dairy manure control and corresponding 7-day preservative treatments. †

	Control	Ambient	Freeze	Refrig.	Acidific.	C.V.
TKN, g/kg	41.6	45.9 (0)	44.4 (1)	45.0 (1)	34.1 (1) *	10.0 %
NH$_4$-N, g/kg	20.8	21.7 (0)	20.7 (0)	20.9 (0)	16.4 (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

†   All values expressed on a dry weight (65°C) basis.
C.V. Represents the overall relative standard deviation between all triplicate and duplicate subsamples in the study.
( )   Represents the number of farms that exhibited significant treatment effects.
*   Represents overall mean significantly different (α=0.05) from the control mean.
ND   Represents nitrate / nitrite concentrations below the detection limit of this study.

Table 4.6 . **Overall nitrogen means for dry broiler litter control and corresponding 7-day preservation treatments. †**

	Control	Ambient	Freeze	Refrig.	Acidific.	C.V.
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 (4) *	15.7%
Organic N, g/kg	31.1	33.0 (1)	32.9 (1)	33.1 (0)	26.0 (2) *	4.7%
(NO_3+NO_2)-N, mg/kg ‡	704	652 (0)	688 (0)	655 (0)	538 (2)	8.2%

†   All values expressed on a dry weight (65°C) basis.

C.V.   Represents the overall relative standard deviation between all triplicate and duplicate subsamples in the study.

( )   Represents the number of farms that exhibited significant treatment effects.

*   Represents overall mean significantly different (α=0.05) from the control mean.

‡   Represents overall (NO_3+NO_2)-N means for samples B8, B9, and B10 only.

Results from Tables 4.5 and 4.6 show that among the ten farm manures and four preservative treatments tested in this study, acidification was the only treatment which had a significant effect (α= 0.05) on the 7-day mean concentrations of manurial nitrogen. None of the other 7-day treatment means, including ambient storage at an average 26°C (78°F), were found to be statistically different from the initial overall control concentrations of manure nitrogen for all ten manures.

Interaction between individual farms and preservative treatments was found to be generally strong in this study, as shown in Table 4.7. The major exception was the analysis of broiler litter NH_4 nitrogen. Strong farm $\times$ treatment interaction was anticipated in the present study, and was due principally to the farm-to-farm variability of the complex manures tested.

Table 4.7.   Level of significance for farm × treatment interaction. †

	Liquid dairy manure	Dry broiler litter
TKN	0.058	0.004
NH$_4$-N	0.011	0.540
Organic N	0.678	0.009
(NO$_3$+NO$_2$)-N	ND	0.004

†   Numerical values represent p-values, where level of significance < α =0.05
       indicates significant farm × treatment interaction.

ND   not detectable

Interaction effects observed in this study meant that comparisons of the overall means in
Tables 4.5 and 4.6 had to be made with caution, since interaction can mask main effects in
a statistical analysis (Ott, 1988). Subsequently, 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.

As an example, broiler litter NH$_4$-N preservative treatments did not exhibit significant
interaction, as mentioned above, meaning that generally consistent treatment effects for
NH$_4$-N concentrations were found across all five broiler litters. The importance of this
low farm × treatment interaction in the case of broiler litter NH$_4$-N is that the
corresponding treatment means from Table 4.6 could be used more directly in drawing
conclusions about the treatment effects on NH$_4$-N preservation in dry broiler litters. In
this study, acidification of the broiler litter samples to pH<2 at 4°C was the only treatment
that significantly affected overall broiler litter NH$_4$-N levels. In the five subsequent one-
way ANOVA analyses (one for each litter sample), it was found that 7-day acidification of
the five litters resulted in significant (α=0.05) increases in NH$_4$-N concentrations in four of
the five broiler litters. (Results from each of the four preservative treatment techniques
will be discussed separately in following sections).

A total of 33 similar one-way ANOVAs were analyzed in the second level of this study for each farm and nitrogen form, with complete results reported as individual farm × treatment means (Appendix 12). The number of significant individual farm × treatment effects for each nitrogen form and farm manure was tallied and included in Tables 4.5 and 4.6 as parenthetical subscripts to more fully explain 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 observed, results from this study reflected a generally high level of variability between individual manure subsamples when compared against the separate treatment effect means, which is why so few significant differences in 7-day treatment effects were found. Coefficient of variation values shown in Tables 4.5 and 4.6 represent two combined sources of variability measured in this study: laboratory subsampling error and analytical error. Relative standard deviations (c.v.) between all subsamples in the present study ranged from 3.2% in broiler litter TKN analysis to 21.7% in liquid dairy manure organic N analysis. Overall c.v. values of approximately 15% for the biological sciences are considered indicators of good technique and experimental design (Myers, 1994). Therefore, as a general conclusion, except for the overly aggressive acidification treatment, all preservative techniques tested in this study were shown to be successful in the 7-day preservation of liquid dairy manure and dry broiler litter nitrogen.

Graphical representation of the triplicate control and 7-day treatment means from Tables 4.5 and 4.6 are presented in Figures 4.3 and 4.4 below, and emphasize the significant impact of acidification (as tested in this study) on manurial nitrogen levels in liquid dairy manures and dry broiler litters. For graphical comparison, each of the five overall treatment means for the two manure types, including the control nitrogen means (at day 0) and the four preservative treatment nitrogen means (at day 7), are displayed side by side along the same (x) axis.

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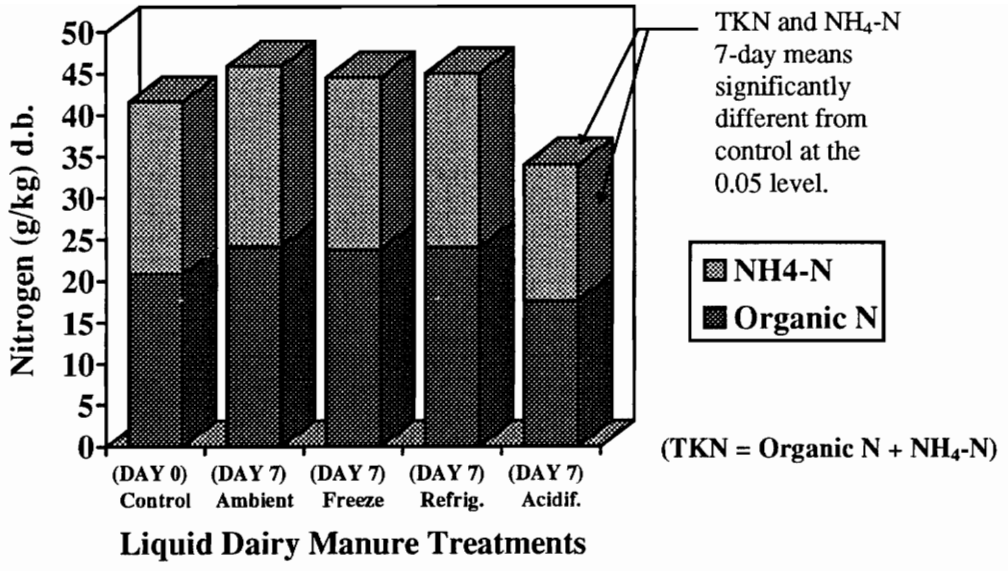
Figure 4.3. Overall nitrogen means for liquid dairy manure control and 7-day preservative treatments.
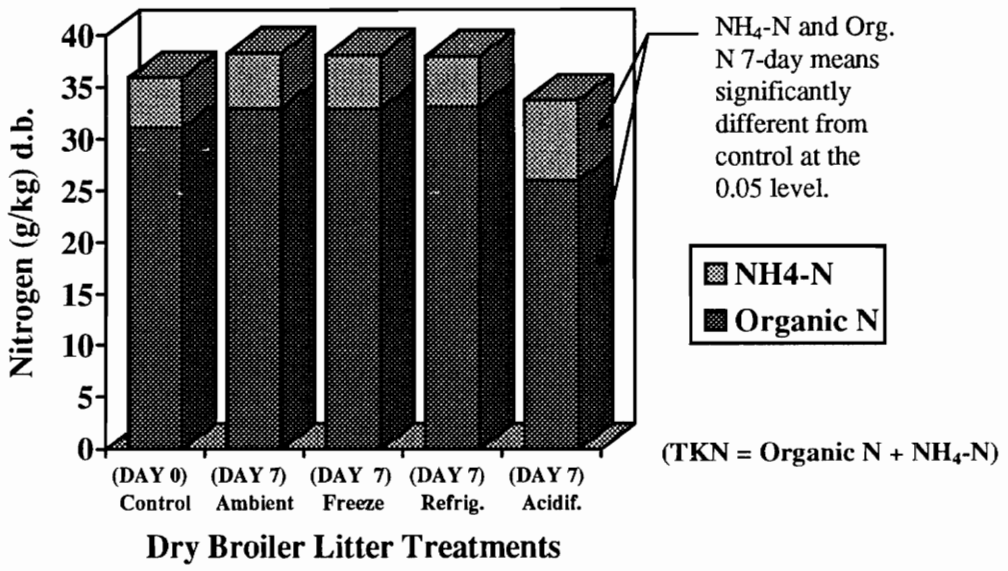
Figure 4.4. Overall nitrogen means for dry broiler litter control and 7-day preservative treatments.

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In the following sections, experimental 7-day nitrogen results from each of the four preservative treatments will be discussed separately, beginning with ambient storage. As mentioned previously, the combined nitrate/nitrite analysis results will be discussed at the end of this section.

a)   *Ambient storage*

Ambient storage at an average 7-day temperature of 26°C (78°F) was shown to be generally adequate for preservation of the major forms of nitrogen in the liquid dairy manures and dry broiler litters tested in this study. Of the five dairy manures tested at ambient storage, none were found to have 7-day TKN, NH_4-N, or organic N means significantly different (α=0.05) from corresponding fresh control means. Of the dry broiler litters tested under 7-day ambient storage conditions, only one litter sample of five had observed mean levels of nitrogen significantly different from the control. Sample B9, at 7-day ambient storage, had an observed TKN increase from 38.7 g/kg to 42.8 g/kg, with corresponding average organic N level increases from 31.9 g/kg to 36.5 g/kg. Nevertheless, the resulting overall nitrogen means for all samples of both manure types showed that 7-day ambient storage at 26°C had no significant effect on the major forms and concentrations of manure nitrogen determined in this study.

Manure pH was monitored throughout this study as one indicator of the microbiological hydrolysis of urea thought to be occurring within the manure samples. Table 4.7 below presents initial pH values for each of the manure samples, along with the corresponding 7-day pH values for ambient, freezing, and refrigeration treatments. Results from Table 4.7 suggest a relative chemical and microbiological stability in the anaerobic liquid dairy manures as compared to the more aerobic broiler litters. Average dairy manure pH levels were found to remain relatively stable over the 7-day study period, compared to a general rise in broiler litter pH for each of the preservative treatments tested.

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Table 4.8.   Initial and 7-day pH values of three preservative techniques in liquid dairy manure and dry broiler litters. †

Sample	Control	Ambient	Freeze	Refrigeration
D1	6.7	6.9	6.6	6.8
D2	7.3	7.3	7.1	7.3
D3	7.5	7.6	7.7	7.7
D4	7.4	7.4	7.3	7.5
D5	7.8	7.7	7.5	7.7
Average liquid dairy manure ‡	**7.2**	**7.3**	**7.1**	**7.3**
B6	7.9	7.5	8.4	8.4
B7	7.9	8.1	8.4	8.4
B8	5.7	6.1	6.1	6.1
B9	6.6	7.0	7.0	7.1
B10	4.2	4.6	4.5	4.5
Average dry broiler litter ‡	**4.9**	**5.3**	**5.2**	**5.2**

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

‡   Average pH was calculated from the $[H^+]$ concentration of the manure solutions.

b)   Frozen storage

Freezing of the liquid dairy manure and dry broiler litter samples at an average temperature of -22°C (-7°F) during the 7-day study period was shown to be generally effective as a nitrogen preservative technique. Of the five dairy manures tested, only sample D2 resulted in average nitrogen levels significantly different from its corresponding control mean after 7-day freezing, with increased TKN means from 30.7 g/kg to 34.1 g/kg. (A significant increase in 7-day TKN, which occurred only four times out of the 80 7-day TKN determinations in this study, was unexpected, and is largely unexplained in the

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analysis of a fixed laboratory sample). Frozen storage of broiler litter samples in this study was also found to be generally successful in maintaining initial nitrogen levels, with the exception of the four-month old litter sample, B10. Sample B10 was found to have significantly increased 7-day mean TKN and organic N levels, from initial values of 47.9 g/kg to 50.9 g/kg, and 45.5 g/kg to 48.6 g/kg, respectively. Nevertheless, as was the case with ambient storage, the resulting overall nitrogen means for all samples of both manure types showed that 7-day frozen storage at -22°C had no significant effect on the major forms and concentrations of manure nitrogen studied in this experiment.

c)   *Refrigerated storage*

Refrigerated storage of both liquid dairy manure and dry broiler litter at 4°C (39°F) was found to generally maintain the initial forms and concentrations of nitrogen over a 7-day period. Of the ten dairy manures and broiler litters tested, only liquid dairy sample D2 was shown to have significantly different 7-day TKN and organic N concentrations, with increased mean nitrogen levels of 30.7 g/kg to 34.5 g/kg and 15.9 g/kg to 19.4 g/kg, respectively. As Table 4.6 shows, no significant difference from control nitrogen means was found in 7-day refrigerated broiler litter samples.

Summarizing the first three preservative treatments, it is important to recall from Tables 4.5 and 4.6 that ambient storage at an average temperature of 26°C (78°F) for seven days, and frozen storage at an average temperature of -22°C (-7°F) for seven days, were also shown to have no significant effect on the overall forms of manurial nitrogen tested in this study. Therefore, the practical significance of the results of this study suggest that all three preservation techniques (ambient, frozen, and refrigerated storage) have overall 7-day treatment effects on manurial nitrogen that are less than the variability (error) incurred in the process of making the analytical nitrogen determinations. In other words, manure

storage under frozen (-22°C), refrigerated (4°C), or ambient (26°C) conditions were found to be equally effective in the 7-day preservation of the major forms of liquid dairy manure and dry broiler litter nitrogen. In this case, storage of the above manure samples at ambient temperatures of 26°C would be recommended as the simplest and therefore the most practical method of 7-day storage.

d) Acidification

A review of Figures 4.3 and 4.4 illustrates the overall 7-day treatment effects of acidification to pH<2 at 4°C (39°F) on the major manure nitrogen forms of the two manure types tested in this study. Overall mean concentrations of most major manure nitrogen forms decreased after seven days of refrigerated storage under acidified conditions, with the notable exception of broiler litter NH_4-N, which was found to increase significantly under acidified conditions. Significant differences in overall 7-day acidified nitrogen levels were observed in four of the seven manure/nitrogen randomized blocks analyzed in this study (Tables 4.5 and 4.6), 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 7-day nitrogen means that were significantly different from the initial nitrogen means of the corresponding control samples (Appendix 12). 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 the 7-day preservation of nitrogen in liquid dairy manures and dry broiler litters.

Another result found in this study was the opposite effect on mean and overall NH_4-N concentrations produced by acidification of the two manure types. While 7-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

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NH$_4$-N levels was observed. In fact, of the five individual one-way ANOVAs used to test 7-day acidified dry broiler litter NH$_4$-N means against initial control levels, four of the five litters were found to have significantly increased NH$_4$-N means (Table 4.6, Appendix 12). Reasons for the opposite effects of acidification on the two manure types are related to the initial nitrogen composition of the manures. 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%). Therefore, a relatively higher amount of inorganic N in the liquid dairy manures was available for possible NH$_3$ volatilization. Importantly, 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 7-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$_2$SO$_4$ as the factor responsible for this loss. During the acidification procedure, addition of concentrated H$_2$SO$_4$ was found to cause an aggressive foaming and heating of the liquid dairy manure samples, presumably with losses of nitrogen as NH$_3$ due at least partially to the physical effects of the exothermic reaction. Average moisture contents of the liquid dairy manures decreased from 94.7% to 93.2% after 7-day acidification, verifying H$_2$O evaporation.

Conclusions based upon the above results include the recommendation that acidification of liquid dairy manures with concentrated H$_2$SO$_4$ to pH<2 not be used as a nitrogen preservative treatment for 7-day storage, since losses of aqueous NH$_3$ appear to have been immediate in the present study.

Pain et al. (1987) reported that chemical stabilization of surface-applied liquid dairy slurries with H$_2$SO$_4$ to a pH of 5.5 reduced three-day NH$_3$ losses from 20% to 5%. Their results, as well as those of the present study, suggest that H$_2$SO$_4$ is more advantageous for liquid dairy manure nitrogen preservation as a chemical stabilizer (used to produce the stable ammonium sulfate compound) than as a general metabolic inhibitor.

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 7-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 (Table 4.5). 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 used in this study.

Overall 7-day dry broiler litter organic nitrogen concentrations appear to have 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 7-day increase in overall broiler litter NH_4-N under acidification treatment (Table 4.6) can be partially explained by the higher percentage of broiler litter nitrogen found in the organic form, as compared to liquid dairy manures (86% vs. 50%). Apparently, some 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. It was not possible from the results of this study to determine, or even estimate, that portion of organic nitrogen that was fixed as NH_4-N, since transformations in initial NH_4-N levels confound the calculated N balances. To illustrate (from Table 4.6), an observed mean decrease of 5.1 g/kg in organic N during 7-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 may have been occurring.

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It was observed that during the addition of H_2SO_4 to the 1:1 (v/v) DDI H_2O dry broiler litter mixtures, much less foaming and heat was generated than during liquid dairy manure acidification, probably due to the lower moisture content (MC) of the litter mixtures, which averaged 74.6% MC vs. 94.7% MC for the average liquid dairy manure sample. The reduced heating and foaming of the acidified broiler litter mixtures may partly explain the fact that overall 7-day TKN values for the acidified litters were not significantly reduced from control levels, as was the case with the liquid dairy manures (Table 4.5). As mentioned previously, aside from the significant loss of organic N in the broiler litter acidification treatment due to the mineralizing effect of H_2SO_4, it was noted that the resulting highly acidic litter mixture was at least partially effective as an inhibitor of NH_3 volatilization by equilibrium fixation (through chemical stabilization as ammonium salts).

Moore et al. (1994) tested several chemical amendments for their effect on NH_3 volatilization in poultry litter. They found that treated litters with lower pH values (6.8 and 6.6) had lower corresponding levels of atmospheric NH_3 when compared to control litters with pH values of 8.1 and 8.2. Results from Moore et al. (1994), as well as Pain et al. (1987) above, support the conclusions of this section, that the acidification of dry broiler litters and liquid dairy manures to pH<2, as tested in the present study, is unnecessarily aggressive and impractical for 7-day manure nitrogen preservation. However, the use of acidification as a chemical stabilizer for both liquid dairy manure and dry broiler litter at pH levels between 5.5 and 6.8 may have promise for longer term (>7 day) manure nitrogen preservation. Therefore, further research investigations into the long-term (>7 day) storage of manure nitrogen using less aggressive acidification techniques (minimum pH 5.5) may have merit.

4. Manure nitrate / nitrite testing

Figure 4.5 below represents the individual mean and overall (NO_3+NO_2)-N concentrations of the five broiler litter samples tested in this study. As discussed previously, all five liquid dairy manures, as well as two of the broiler litters, were found to have combined oxidized (NO_3+NO_2) nitrogen levels below the detection limit of the automated hydrazine procedures used in the laboratory analysis.
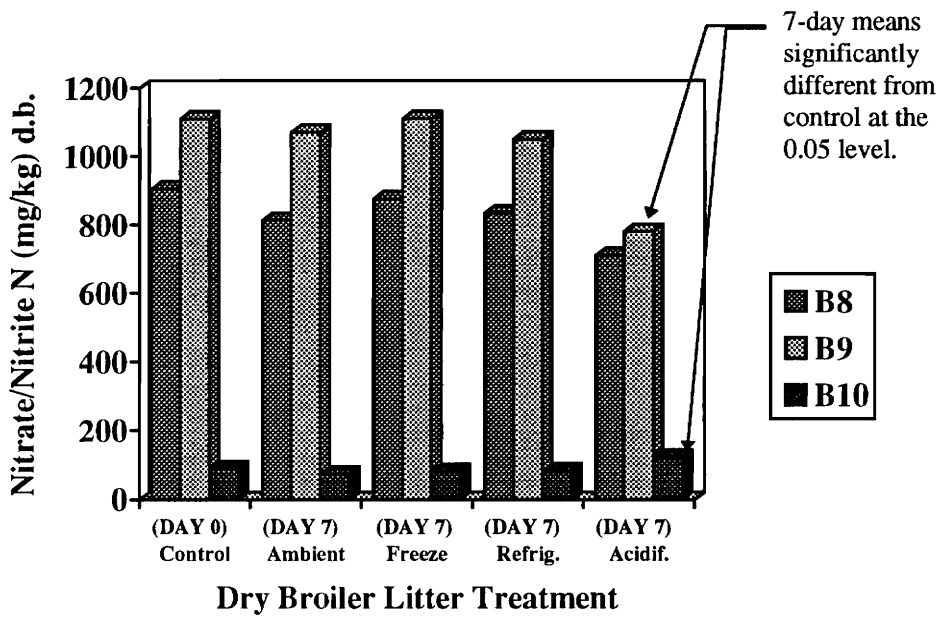
Figure 4.5. Mean nitrate / nitrite concentrations for individual broiler litter control and 7-day preservative treatments.

In analyzing Figure 4.5, it is interesting to note that broiler litter samples B6 and B7, which had nitrate/nitrite nitrogen concentrations below the detection limits of this study, also had the shortest storage times (0-1 week). Also, of the three broiler litters for which combined (NO_3+NO_2)-N concentrations were obtained, the litter sample with the longest storage (B10) had the lowest average (NO_3+NO_2)-N levels. Samples B9 and B8, at

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storage times of three weeks and one month, respectively, showed relatively higher levels of initial combined oxidized nitrogen. Therefore, it appears that the initial combined oxidized nitrogen concentrations of the broiler litters tested in this study were related to storage time, with increases in storage time leading to generally higher (NO_3+NO_2)-N levels. The exception was broiler litter sample B10 (storage period four months), which had lower overall inorganic nitrogen levels, including NH_4-N (Figure 4.2), possibly due to prolonged volatilization or leaching losses, as well as net immobilization during stabilization.

Figure 4.5 also reflects the relatively large differences in initial (control) (NO_3+NO_2)-N concentrations between the three litters, B8, B9, and B10. Although none of the overall 7-day treatment means for the three litters were found to be significantly different from the initial overall (NO_3+NO_2)-N control mean of 704 mg/kg from Table 4.9, subsequent analysis of individual farm $\times$ treatment combinations resulted in significant differences in the 7-day acidification treatments for two of the litters, B9 and B10. However, because the 7-day acidification treatments were found to have opposite effects on the combined oxidized (NO_3+NO_2)-N levels of samples B9 and B10, overall treatment effects were confounded. Sample B10, which had the longest on-farm storage time (four months), had significantly increased 7-day mean (NO_3+NO_2)-N levels under the acidification treatment. Conversely, sample B9, which was stored for less than one month, had significantly reduced 7-day acidified levels of (NO_3+NO_2)-N. Nelson and Sommers (1980), in their review of Kjeldahl digestion, reported that nitrite in soil samples (as HNO_2) decomposes to NO, NO_2, and H_2O on addition of H_2SO_4. A more complete analysis, possibly using steam distillation techniques, would be have been necessary in order to quantify the combined oxidized nitrogen transformations in the two types of manure tested in this study.

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Therefore, due to the incompleteness of the data for combined oxidized manure nitrogen fractions, and the confounding observed in the 7-day acidification treatments, overall results from the manure nitrate/nitrite nitrogen testing completed in the present study were inconclusive. In other words, it was difficult to provide evidence of a significant 7-day trend in manure (NO_3+NO_2)-N preservative storage levels using the limited results of the manure samples tested.

V. Summary and Conclusions

The nitrogen composition of five liquid dairy manure and five dry broiler litter samples was determined for four different preservation techniques, including: storage of the samples at ambient temperature (26°C), freezing of the samples (-22°C), refrigeration of the samples (4°C), and acidification with sulfuric acid (H_2SO_4) to pH<2 with refrigeration (4°C). All manure samples were treated with the above preservative techniques for seven days in order to determine the effects of the four treatments on the forms and concentrations of nitrogen present, including: total Kjeldahl, ammonium, and nitrate/nitrite nitrogen. Organic nitrogen was also tested in this study, and was determined as the difference between ammonium and total Kjeldahl nitrogen.

Control subsamples from each manure were analyzed prior to storage for determination of the fresh forms and concentrations of nitrogen. All initial and 7-day nitrogen fractions for the two manure types and five treatments (including the control) were tabulated on a dry weight basis and analyzed using a randomized block design, with separate mixed-model ANOVAs. Preservative treatment effects were compared against farms (blocks) in order to test whether the 7-day preservative treatment responses were statistically significant across the manures and nitrogen forms studied.

Three of the four preservative treatments (ambient storage, refrigeration, and freezing) were found to have no significant effect ($\alpha=0.05$) on the overall 7-day nitrogen values of the liquid dairy manures and dry broiler litters tested in this study. The fourth preservative treatment (acidification with H_2SO_4 to pH<2) resulted in significant overall decreases in most major nitrogen forms, with the notable exception of broiler litter NH_4-N, which increased significantly under acidified conditions due to fixation of mineralized organic N. Unfortunately, results from the combined oxidized manure nitrogen analyses in this study were inconclusive due to incomplete and confounded results in the manure samples tested.

Therefore, based on the main results of this study, the following conclusions were drawn:

1. Manure storage under ambient (26°C) conditions was recommended as sufficient for 7-day preservation of the major forms of liquid dairy manure and dry broiler litter nitrogen used for making application rate recommendations (i.e., 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).

2. Acidification with H_2SO_4 to pH<2 (with refrigeration) was found to be unacceptable as a preservative technique because of the aggressive physical action of the acid, which accelerated the volatilization of ammonia in liquid dairy manures, and the mineralization of organic N in dry broiler litters.

3. The TKN, organic N, and NH_4-N results reported to Virginia farmers and nutrient managers during the seven-year period from 1987-1994 (as part of the free manure testing program at Virginia Tech) would not have been significantly improved using any of the laboratory preservation techniques tested in this study.

Based on the information and experience gathered from this experiment, the following recommendations are made for further study:

1. The three-hour digest that has been used as part of Virginia's free manure testing program for TKN analysis should be re-evaluated in order to test the accuracy of shorter digestion times, since savings in both time and money could be realized in any future streamlining of the manure analysis procedures.

2. The use of steam distillation methods are recommended for research investigations of the combined oxidized nitrogen (NO_3+NO_2) forms in the two types of manure tested in this study. Steam distillation techniques, though time-consuming, are recommended because they are not subject to interference from colored extracts (Keeney and Nelson, 1982).

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APPENDIX

APPENDIX 1

```
********************************************************************
*                                                                  *
*           SOIL   AND   WATER   QUALITY   LABORATORY              *
*                                                                  *
*           DEPARTMENT   OF   AGRICULTURAL   ENGINEERING           *
*                                                                  *
*           BLACKSBURG   VIRGINIA   24061  (703-231-4334)          *
*                                                                  *
********************************************************************
```

                    Date Received : 04/15/94
                    Date of Report: 05/06/94

Submitted by: Landowner:

WINSTON PHILLIPS   KENNY BEARD
D.S.W.C.   RAPHINE, VA.
RT. 4, BOX 99-J
STAUNTON, VA. 24401


```
==================================================================

            SAMPLE IDENTIFICATION: LIQUID DAIRY / EARTHEN
                       LAB NUMBER: W3914
    --------------------------------------------------------------

        PERCENT MOISTURE:   97.65    NITROGEN (N)    :   11.40
        PHOSPHATE (P2O5):    6.79    POTASH (K2O)     :    9.34
        AMMONIA  (NH4-N):    5.11    MAGNESIUM (Mg)  :    2.45
        CALCIUM (Ca)    :    6.38    SULFUR (S)       :    1.16
        ZINC (Zn)       :    0.07    UNITS = POUNDS/1000 GALLONS


    --------------------------------------------------------------


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If surface applied, this manure will supply:

        PLANT-AVAILABLE NITROGEN (N)   :   3.48 POUNDS/1000 GALLONS
        PLANT-AVAILABLE PHOSPHATE (P2O5):   6.79 POUNDS/1000 GALLONS
        PLANT-AVAILABLE POTASH (K2O)   :   9.34 POUNDS/1000 GALLONS

If immediately incorporated or injected, this manure will supply:

        PLANT-AVAILABLE NITROGEN (N)   :   6.03 POUNDS/1000 GALLONS
        PLANT-AVAILABLE PHOSPHATE (P2O5):   6.79 POUNDS/1000 GALLONS
        PLANT-AVAILABLE POTASH (K2O)   :   9.34 POUNDS/1000 GALLONS

APPENDIX 2

```
****************************************************************************
*                                                                          *
*                SOIL   AND   WATER   QUALITY   LABORATORY                  *
*                                                                          *
*              DEPARTMENT   OF   AGRICULTURAL   ENGINEERING                 *
*                                                                          *
*              BLACKSBURG   VIRGINIA   24061   (703-231-4334)               *
*                                                                          *
****************************************************************************
```

Date Received : 09/14/93
Date of Report: 10/08/93

Submitted by: Landowner:

WINSTON PHILLIPS   NORTH POINT FARM
D.S.W.C.   RT. 1, BOX 121
RT. 4, BOX 99-J   WAYNESBORO, VA. 22980
STAUNTON, VA. 24401

```
===========================================================================
```

SAMPLE IDENTIFICATION: DRY BROILER LITTER / UNCOVERED STACK
LAB NUMBER: W3630

```
---------------------------------------------------------------------------
```

PERCENT MOISTURE:	25.21	NITROGEN (N)	:	57.48
PHOSPHATE (P2O5):	56.34	POTASH (K2O)	:	26.22
AMMONIA (NH4-N):	9.41	MAGNESIUM (Mg)	:	8.27
CALCIUM (Ca) :	32.48	SULFUR (S)	:	8.78
ZINC (Zn) :	0.67	UNITS = POUNDS/TON		

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---------------------------------------------------------------------------
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===========================================================================
```

If surface applied, this manure will supply:

PLANT-AVAILABLE NITROGEN (N)   : 35.90 POUNDS/TON
PLANT-AVAILABLE PHOSPHATE (P2O5): 56.34 POUNDS/TON
PLANT-AVAILABLE POTASH (K2O)   : 26.22 POUNDS/TON

If immediately incorporated or injected, this manure will supply:

PLANT-AVAILABLE NITROGEN (N)   : 37.78 POUNDS/TON
PLANT-AVAILABLE PHOSPHATE (P2O5): 56.34 POUNDS/TON
PLANT-AVAILABLE POTASH (K2O)   : 26.22 POUNDS/TON
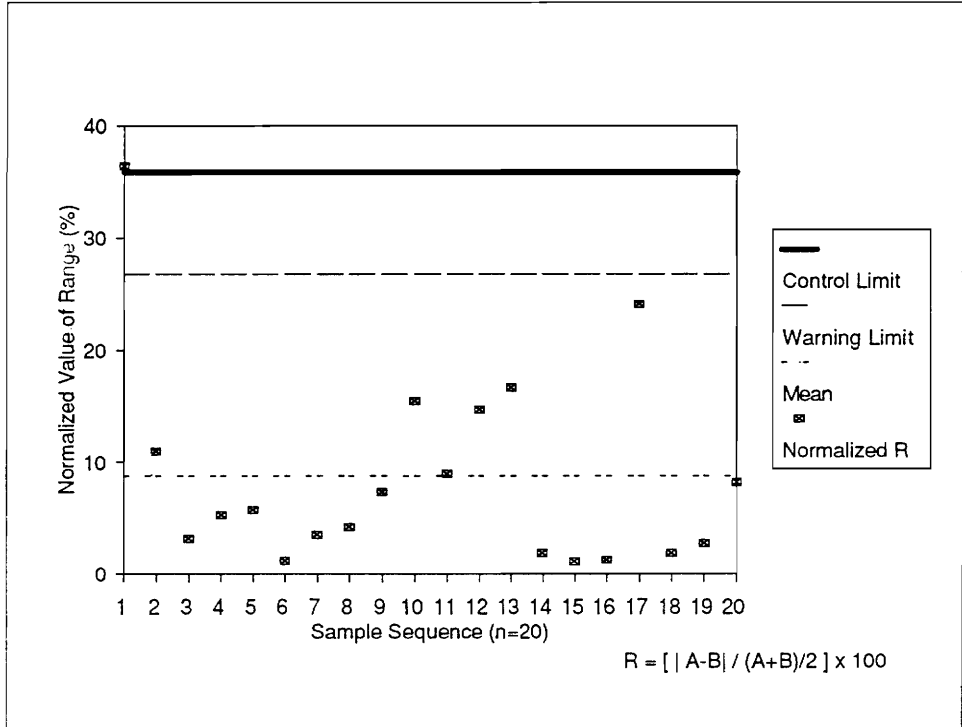
Liquid dairy manure TKN

R control chart (duplicate analysis)

The chart's y-axis is labeled "Normalized Value of Range (%)" ranging from 0 to 16. The x-axis is labeled "Sample Sequence (n=20)" ranging from 1 to 20.

Legend:
- Control Limit
- Warning Limit
- Mean
- Normalized R

$R = [|A-B| / (A+B)/2] \times 100$

Dry broiler litter TKN

R control chart (duplicate analysis)

Liquid dairy manure ammonium nitrogen

R control chart (duplicate analysis)

Dry broiler litter ammonium nitrogen

R control chart (duplicate analysis)

Dry broiler litter nitrate / nitrite nitrogen

R control chart (duplicate analysis)

APPENDIX 8

Coefficient of variation values for TKN triplicate controls. (d.b.)

triplicate samples	Control	TKN (g/kg)	mean	s.d.	c.v. (%)
D1		23.9			
D1		41.7	32.1	9.0	28.0
D1		30.6			
D2	D	30.3			
D2		31.8	30.7	0.9	3.0
D2	A	30.1			
D3		42.0			
D3	I	45.8	44.5	2.2	4.9
D3		45.8			
D4	R	36.1			
D4		35.3	35.2	1.0	2.9
D4	Y	34.1			
D5		64.7			
D5		68.6	65.6	2.7	4.1
D5		63.5			
	average	41.6	41.6	3.2	8.6
	std dev	13.9			
overall	c.v. (%)	33.3			
B6		28.4			
B6	B	26.5	27.3	1.0	3.6
B6		27.0			
B7	R	27.3			
B7		28.2	28.6	1.6	5.6
B7	O	30.4			
B8		36.8			
B8	I	38.2	37.3	0.8	2.2
B8		36.8			
B9	L	37.6			
B9		39.4	38.7	1.0	2.5
B9	E	39.1			
B10		48.6			
B10	R	47.5	47.9	0.6	1.3
B10		47.5			
	average	36.0	36.0	1.0	3.0
	std dev	7.8			
overall	c.v. (%)	21.7			

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Coefficient of variation values for NH4-N triplicate controls. (d.b.)

triplicate samples		Control NH4-N (g/kg)	mean	s.d.	c.v. (%)
D1		12.9			
D1		11.3	12.0	0.8	7.0
D1		11.7			
D2	D	15.1			
D2		14.8	14.9	0.2	1.4
D2	A	14.7			
D3		22.7			
D3	I	24.9	20.3	6.1	30.0
D3		13.4			
D4	R	16.2			
D4		16.1	16.2	0.1	0.4
D4	Y	16.2			
D5		38.5			
D5		42.4	40.8	2.1	5.0
D5		41.6			
	average	20.8	20.8	1.9	8.8
	std dev	11.0			
overall	c.v. (%)	52.8			
B6		2.3			
B6	B	2.4	2.3	0.1	3.5
B6		2.3			
B7	R	8.7			
B7		6.7	7.5	1.1	14.2
B7	O	7.0			
B8		4.8			
B8	I	6.2	5.5	0.7	13.2
B8		5.7			
B9	L	6.4			
B9		6.1	6.8	1.0	15.0
B9	E	8.0			
B10		1.9			
B10	R	2.8	2.3	0.4	19.2
B10		2.3			
	average	4.9	4.9	0.7	13.0
	std dev	2.4			
overall	c.v. (%)	48.2			

APPENDIX 8 (cont.)

Coefficient of variation values for Organic N triplicate controls. (d.b.)

triplicate samples		Control Organic N (g/kg)	mean	s.d.	c.v. (%)
D1		11.0			
D1		30.4	20.1	9.8	48.5
D1		18.9			
D2	D	15.2			
D2		17.0	15.9	1.0	6.2
D2	A	15.4			
D3		19.3			
D3	I	20.9	24.2	7.1	29.5
D3		32.4			
D4	R	19.9			
D4		19.2	19.0	1.0	5.3
D4	Y	17.9			
D5		26.2			
D5		26.2	24.8	2.5	10.0
D5		21.9			
	average	20.8	20.8	4.3	19.9
	std dev	5.8			
overall	c.v. (%)	28.0			
B6		26.1			
B6	B	24.1	25.0	1.1	4.2
B6		24.7			
B7	R	18.6			
B7		21.5	21.2	2.4	11.4
B7	O	23.4			
B8		32.1			
B8	I	32.0	31.7	0.5	1.7
B8		31.1			
B9	L	31.2			
B9		33.3	31.9	1.2	3.9
B9	E	31.1			
B10		46.7			
B10	R	44.7	45.6	1.0	2.3
B10		45.2			
	average	31.1	31.1	1.3	4.7
	std dev	8.7			
overall	c.v. (%)	28.0			

Coefficient of variation values for nitrate/nitrite triplicate controls. (d.b.)

triplicate samples	Control	(NO3+NO2)-N (mg/kg)	mean	s.d.	c.v. (%)
D1		not detectable			
D1		not detectable			
D1		not detectable			
D2	D	not detectable			
D2		not detectable			
D2	A	not detectable			
D3		not detectable			
D3	I	not detectable			
D3		not detectable			
D4	R	not detectable			
D4		not detectable			
D4	Y	not detectable			
D5		not detectable			
D5		not detectable			
D5		not detectable			
	average				
	std dev				
overall	c.v. (%)				
B6		not detectable			
B6	B	not detectable			
B6		not detectable			
B7	R	not detectable			
B7		not detectable			
B7	O	not detectable			
B8		933			
B8	I	914	906	32.2	3.6
B8		870			
B9	L	1132			
B9		1056	1114	51.8	4.6
B9	E	1155			
B10		105			
B10	R	82	92	12.1	13.2
B10		89			
	average	704	704	32.0	7.1
	std dev	469			
overall	c.v. (%)	66.6			

APPENDIX 9

Manure Project - Summary of Results for Farmers
Water Quality Lab, Seitz Hall, VPI, 8 September 1994

Landowner	Manure Type	Sample I.D.	Date Sampled	County	Moisture Content	Total Nitrogen	Ammonia Nitrogen	pH	Density
Danny Wampler	L. Dairy	D1	7/26/94	Augusta	95.85%	11.09 lb/1000gal	4.17 lb/1000gal	6.7	8.34 lb/gal
Marty Simmons	L.Dairy	D2	7/26/94	Augusta	94.78%	13.47 lb/1000gal	6.57 lb/1000gal	7.3	8.42 lb/gal
Allen Layman	L. Dairy	D3	7/28/94	Franklin	97.14%	10.69 lb/1000gal	4.88 lb/1000gal	7.5	8.42 lb/gal
Walter Kingery	L.Dairy	D4	7/28/94	Franklin	87.26%	32.88 lb/1000gal	15.12 lb/1000gal	7.4	7.34 lb/gal
Kenneth Bowman	L.Dairy	D5	7/28/94	Franklin	98.44%	8.43 lb/1000gal	5.29 lb/1000gal	7.8	8.26 lb/gal
AVERAGE LIQUID DAIRY SAMPLE					**94.69%**	**15.31 lb/1000gal**	**7.21 lb/1000gal**	**7.3**	**8.16 lb/gal**
Virginia L. Dairy Manure Averages (VPI&SU State Testing Program)					94.30%	22.61 lb/1000gal	9.57 lb/1000gal	N/A	N/A
Danny Wampler	B. Litter	B6	7/26/94	Augusta	29.50%	38.40 lb/ton	3.28 lb/ton	7.9	23.7 lb/cf
Dale Reeves	B. Litter	B7	7/26/94	Augusta	32.52%	38.60 lb/ton	10.06 lb/ton	7.9	26.8 lb/cf
Lynwood Moyer	B. Litter	B8	7/26/94	Page	23.29%	57.20 lb/ton	8.50 lb/ton	5.7	26.2 lb/cf
Richard Thomas	B. Litter	B9	7/26/94	Page	16.93%	64.20 lb/ton	11.84 lb/ton	6.6	26.2 lb/cf
Richard Thomas	B. Litter	B10	7/26/94	Page	13.75%	82.60 lb/ton	4.00 lb/ton	4.2	29.3 lb/cf
AVERAGE DRY BROILER LITTER SAMPLE					**23.20%**	**56.20 lb/ton**	**7.54 lb/ton**	**6.5**	**26.44 lb/cf**
Virginia D. Broiler Litter Averages (VPI&SU State Testing Program)					28.43%	62.58 lb/ton	11.75 lb/ton	N/A	N/A

APPENDIX 10

Manure Project - Summary of Results for Farmers
Water Quality Lab, Seitz Hall, VPI, 8 September 1994

NUTRIENT MANAGEMENT INFORMATION:
This manure will supply:

			if surface applied	if incorporated
Landowner	Manure type	Sample I.D.		
Danny Wampler	L. Dairy	D1	3.46 lb-N/1000gal	5.55 lb-N/1000gal
Marty Simmons	L.Dairy	D2	4.06 lb-N/1000gal	7.34 lb-N/1000gal
Allen Layman	L. Dairy	D3	3.25 lb-N/1000gal	5.69 lb-N/1000gal
Walter Kingery	L.Dairy	D4	10.00 lb-N/1000gal	17.56 lb-N/1000gal
Kenneth Bowman	L.Dairy	D5	2.42 lb-N/1000gal	5.07 lb-N/1000gal

Liquid Dairy Manure Calculations
Surface applied : Available N = (35% x org. N) + (25% x NH4-N)
Incorporated :   Available N = (35% x org. N) + (75% x NH4-N)

Danny Wampler	B. Litter	B6	23.53 lb-N/ton	24.19 lb-N/ton
Dale Reeves	B. Litter	B7	24.67 lb-N/ton	26.68 lb-N/ton
Lynwood Moyer	B. Litter	B8	35.60 lb-N/ton	37.30 lb-N/ton
Richard Thomas	B. Litter	B9	40.30 lb-N/ton	42.66 lb-N/ton
Richard Thomas	B. Litter	B10	50.16 lb-N/ton	50.96 lb-N/ton

Dry Broiler Litter Calculations
Surface applied : Available N = (60% x org. N) + (75% x NH4-N)
Incorporated :   Available N = (60% x org. N) + (95% x NH4-N)

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APPENDIX 11

Nitrogen Preservation Results (7-day treatment effects vs. control @ day 0)
25 October 1994

Sample	Control (triplicate)	Ambient	Freeze	Refrigeration	Acidification
	TKN Dairy (g/kg) d.b.				
DI	23.9	26.5	37.6	37.8	29.2
D1	41.7	38.3	33.7	39.0	27.7
D1	30.6				
D2	30.3	32.2	34.3	33.9	29.1
D2	31.8	34.1	33.9	35.1	27.9
D2	30.1				
D3	42.0	49.3	47.6	49.0	31.0
D3	45.8	45.8	55.6	44.8	35.9
D3	45.8				
D4	36.1	35.8	37.0	35.8	31.4
D4	35.3	42.3	36.3	35.4	31.0
D4	34.1				
D5	64.7	86.5	64.7	68.6	46.7
D5	68.6	67.9	63.5	70.5	50.7
D5	63.5				
AVERAGE	**41.6**	**45.9**	**44.4**	**45.0**	**34.1**
	TKN Broiler (g/kg) d.b.				
B6	28.4	28.2	29.4	26.7	24.4
B6	26.5	30.4	30.9	31.3	23.1
B6	27.0				
B7	27.3	30.2	29.3	29.8	26.5
B7	28.2	31.4	30.4	29.2	26.9
B7	30.4				
B8	36.8	39.5	38.7	39.4	38.7
B8	38.2	39.4	37.3	40.2	36.1
B8	36.8				
B9	37.6	43.0	42.0	43.8	39.3
B9	39.4	42.6	41.0	40.0	39.3
B9	39.1				
B10	48.6	48.5	51.1	50.2	40.3
B10	47.5	49.3	50.7	49.4	42.0
B10	47.5				
AVERAGE	**36.0**	**38.3**	**38.1**	**38.0**	**33.7**

Sample	Control (triplicate)	Ambient	Freeze	Refrigeration	Acidification
	NH4-N Dairy (g/kg) d.b.				
DI	12.9	13.1	12.6	12.5	9.6
D1	11.3	12.9	12.7	12.5	9.7
D1	11.7				
D2	15.1	16.1	15.6	14.9	14.1
D2	14.8	15.6	17.0	15.3	12.7
D2	14.7				
D3	22.7	19.9	18.0	19.1	13.9
D3	24.9	19.1	17.9	19.1	14.0
D3	13.4				
D4	16.2	15.9	17.0	20.4	15.4
D4	16.1	16.1	16.1	16.4	15.4
D4	16.2				
D5	38.5	44.0	41.9	40.7	29.5
D5	42.4	44.4	37.7	38.2	29.9
D5	41.6				
AVERAGE	20.8	21.7	20.7	20.9	16.4
	NH4-N Broiler (g/kg) d.b.				
B6	2.3	2.2	3.8	2.7	4.4
B6	2.4	3.6	3.2	3.1	4.5
B6	2.3				
B7	8.7	7.1	7.2	7.4	14.2
B7	6.7	9.9	7.6	7.2	9.5
B7	7.0				
B8	4.8	6.0	6.2	5.7	7.7
B8	6.2	6.2	6.1	6.0	7.6
B8	5.7				
B9	6.4	6.2	6.0	6.1	8.8
B9	6.1	6.4	6.8	6.4	8.9
B9	8.0				
B10	1.9	2.6	2.3	2.4	5.8
B10	2.8	2.5	2.4	2.4	5.5
B10	2.3				
AVERAGE	4.9	5.3	5.2	4.9	7.7

Sample	Control (triplicate)	Ambient	Freeze	Refrigeration	Acidification
	Organic N Dairy (g/kg) d.b.				
DI	11.0	13.4	25.0	25.3	19.6
D1	30.4	25.4	21.0	26.5	18.0
D1	18.9				
D2	15.2	16.1	18.7	19.0	15.0
D2	17.0	18.5	16.9	19.8	15.2
D2	15.4				
D3	19.3	29.4	29.6	29.9	17.1
D3	20.9	26.7	37.7	25.7	21.9
D3	32.4				
D4	19.9	19.9	20.0	15.4	16.0
D4	19.2	26.2	20.2	19.0	15.6
D4	17.9				
D5	26.2	42.5	22.8	27.9	17.2
D5	26.2	23.5	25.8	32.3	20.8
D5	21.9				
AVERAGE	**20.8**	**24.2**	**23.8**	**24.1**	**17.6**
	Organic N Broiler (g/kg) d.b.				
B6	26.1	26.0	25.6	24.0	20.0
B6	24.1	26.9	27.8	28.2	18.7
B6	24.7				
B7	18.6	23.1	22.1	22.4	12.3
B7	21.5	21.5	22.8	22.0	17.4
B7	23.4				
B8	32.1	33.5	32.5	33.7	31.0
B8	32.0	33.3	31.2	34.2	28.5
B8	31.1				
B9	31.2	36.8	36.0	37.7	30.5
B9	33.3	36.2	34.2	33.6	30.4
B9	31.1				
B10	46.7	45.9	48.8	47.8	34.5
B10	44.7	46.8	48.3	47.0	36.5
B10	45.2				
AVERAGE	**31.05**	**33.00**	**32.93**	**33.06**	**25.98**

Sample	Control (triplicate) (NO3+NO2)-N Broiler (mg/kg) d.b.	Ambient	Freeze	Refrigeration	Acidification
B8	933	783	888	804	848
B8	914	846	865	867	572
B8	870				
B9	1132	1060	1086	1058	791
B9	1056	1075	1127	1032	770
B9	1155				
B10	105	69	85	83	127
B10	82	78	80	85	119
B10	89				
AVERAGE	704	652	688	655	538

One Way ANOVA Results*
TKN (g/kg) d.b.

Sample	Control	Ambient	Freeze	Refrigeration	Acidification
D1	32.1	32.4	35.7	38.4	28.5
D2	30.7	33.2	**34.1**	**34.5**	28.5
D3	44.5	47.6	51.6	46.9	**33.5**
D4	35.2	39.1	36.7	35.6	31.2
D5	65.6	77.2	64.1	69.6	48.7
B6	27.3	29.3	30.2	29.0	23.8
B7	28.6	30.8	29.9	29.5	26.7
B8	37.3	39.5	38.0	39.8	37.4
B9	38.7	**42.8**	41.5	41.9	39.3
B10	47.9	48.9	**50.9**	49.8	**41.2**

NH4-N (g/kg) d.b.

Sample	Control	Ambient	Freeze	Refrigeration	Acidification
D1	12.0	13.0	12.7	12.5	**9.7**
D2	14.9	15.9	16.3	15.1	13.4
D3	20.3	19.5	18.0	19.1	14.0
D4	16.2	16.0	16.6	18.4	15.4
D5	40.8	44.2	39.8	39.5	**29.7**
B6	2.3	2.9	3.5	2.9	**4.4**
B7	7.5	8.5	7.4	7.3	11.8
B8	5.5	6.1	6.1	5.9	**7.7**
B9	6.8	6.3	6.4	6.3	**8.9**
B10	2.3	2.6	2.4	2.4	**5.7**

Organic N (g/kg) d.b.

Sample	Control	Ambient	Freeze	Refrigeration	Acidification
D1	20.1	19.4	23.0	25.9	18.8
D2	15.9	17.3	17.8	**19.4**	15.1
D3	24.2	28.1	33.7	27.8	19.5
D4	19.0	23.1	20.1	17.2	15.8
D5	24.8	33.0	24.3	30.1	19.0
B6	25.0	26.5	26.7	26.1	**19.4**
B7	21.2	22.3	22.5	22.2	14.9
B8	31.7	33.4	31.9	34.0	29.8
B9	31.9	**36.5**	35.1	35.7	30.5
B10	45.5	46.4	**48.6**	47.4	**35.5**

(NO3+NO2)-N (mg/kg) d.b.

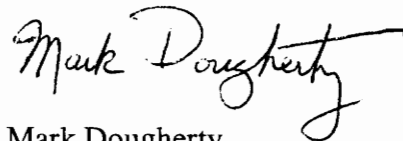| Sample	Control	Ambient	Freeze	Refrigeration	Acidification
B8	906	814	876	835	710
B9	1114	1067	1106	1045	**781**
B10	92	74	83	84	**123**

* bold values significantly different from control @ .05 level

Vita

If the average life span of a white male in the United States is a good indicator, then my time on this earth is likely about half over as of this writing, since I was born on January 4th, 1957. I have had the good fortune to be blessed with health and a loving family; two parents, three sisters, and two brothers. We kids were raised in the 60's and 70's with the values that our parents learned in the 30's and 40's. I was the second oldest in the family, was the first to move away from home, and have been fairly independent, since then.

After graduating from college in 1978 with a B.S. in Geography, I moved to Texas and became a surveyor's helper, a houseparent for deaf students, then an auto mechanic. I returned to college about 1986 with the idea of studying agriculture full-time, and ended up with a B.S. in Agricultural Engineering from Texas Tech University in 1990. From there, I took a job as an Assistant Design Engineer in Paris, Texas (the second largest Paris in the world), where I worked for three years. I returned to school at VPI in order to pick up my studies of agriculture (since I always knew that people couldn't eat without food).

Thanks be to God, now I will receive a Master's degree, which is something I have <u>often</u> dreamed about over the years.

Mark Dougherty

Blacksburg, Virginia

February 1995 C.E.

(Ramadan 1415 A.H.)