NITROGEN RELEASED FROM POULTRY LITTER: ANALYSIS AND PREDICTION

by

JASON E. MOWRER

(Under the Direction of Miguel Cabrera)

ABSTRACT

Poultry waste in the form of broiler litter (BL) has intrinsic value to crop producers for its phosphorus (P), potassium (K), and nitrogen (N) content. This dissertation research is focused on the N content. It improves a method for analyzing uric acid N from poultry waste. Use of 0.1 M sodium acetate to extract the waste, separation by HPLC, and detection by UV/VIS at 290 nm resulted in substantially improved recoveries ranging from 88.7 to 109.1% (n = 22; mean = 100.1%, median = 98.8, and $\sigma$ = 4.8). Stability of the analyte in extraction solution was improved to > 2 days. Near infrared reflectance (NIR) spectroscopy was used to develop rapid analysis methods for the prediction of important forms of N in BL samples. Calibrations were developed for total-N ($r^2$ = 0.896), $NH_4$-N ($r^2$ = 0.795), $NO_3$-N ($r^2$ = 0.926), uric acid-N (UAN) ($r^2$ = 0.909), organic-N (ON) ($r^2$ = 0.821), water soluble organic-N (WSON) ($r^2$ = 0.897), potentially mineralizable-N (PMN) ($r^2$ = 0.842), and initial plant available (PAN + PMN) ($r^2$ = 0.888). Incubation experiments for PMN used to calibrate the NIR instrument suggested that stored poultry wastes release less PAN (mean = 33%) than those collected fresh from poultry houses (mean = 50-60%). Changes in UAN and xanthine-N (XN) in BL showed early increases in concentration followed by declines over the course of 38 days. A strong relationship was found between the first-order decay-rate constants for these compounds and water potential ($\psi$) in the BL samples: $k_{UAN} = 0.0054(\psi) + 0.101$ ($r^2$ = 0.9987); and $k_{XN} = 0.0066(\psi) + 0.1101$ ($r^2$ = 0.9285).

INDEX WORDS: Broiler litter, Near infrared reflectance, Nitrogen, Plant available nitrogen, Potentially mineralizable nitrogen, Uric acid, Xanthine

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# DEDICATION

Dedicated to some people I admire greatly:  Olen Mowrer, Ana Mowrer, Charles Mowrer, Doris Allen, Gitta Pap, Herman Kimmich, Diane Marchik, Pat Metheny, Bill Evans, Keith Jarrett, Steely Dan, Niels-Henning Ørsted Pedersen, Isaac Newton, Robert Hooke, Albert Einstein, and Gottfried Leibniz.

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# CHAPTER 1

# INTRODUCTION

Improvement of soil fertility through additions of organic wastes is a widespread practice in agricultural production. This practice enhances soil organic matter content, adds macro- and micro-nutrients necessary for crop growth, and generally promotes sustainability in the industry that produces the waste as well as in the one that accepts it. Long-term cultivation of arable soils has historically been recognized as a contributor to the deterioration of soil fertility. Management practices that promote sustainability are given increasing priority. Benefits of additions of organic wastes to soils managed for crop production include increased capacity to hold water and nutrients, improved structure and microbial ecology, resilience against drought, erosion reduction, and a long-term increase in nitrogen (N) supply (Diacono and Montemurro, 2010). One example of organic waste that holds the potential to provide a substantial amount of nitrogen to soils is broiler litter (BL) (Nahm, 2005). BL is a waste material produced in large quantities by the poultry production industry in the United States. BL is composed of avian feces, bedding material (e.g. wood shavings, peanut hulls, and/or rice hulls), spilled feed, feathers, and soil mixed in from the floor of the growing facility. Perkins et al. (1964) estimated 1.46 kg of BL is produced per bird. At current rates of broiler chicken production (USDA, 2013), the U.S. generated approximately 11.3 Tg of BL each year. Turkey production in the U.S. generates approximately 2.8 Tg of litter a year (Flora and Riahi-Nezhad, 2006). Though more difficult to provide estimates for, egg production and breeding facilities also produce prodigious amounts of waste. Therefore, land application of BL and other poultry wastes represents an important avenue of disposal for the poultry industry.

In addition to enhancement of organic matter and long-term nitrogen supply, BL provides many plant-essential nutrients to cropped soils. Total phosphorus in BL has been reported in the range of 10 g

$kg^{-1}$ (Codling et al., 2008) and 14 g $kg^{-1}$ (Kleinman et al., 2002). Total potassium in BL has been reported in the range of 10 g $kg^{-1}$ (Bolan et al., 2010) and 17 g $kg^{-1}$ (Hirzel and Walter, 2008). Other macro-nutrients present in BL and their approximate concentrations include calcium (18 g $kg^{-1}$), magnesium (4 g $kg^{-1}$), and sulfur (6 g $kg^{-1}$) (Bolan et al., 2010). Micronutrients such as copper (0.33 g $kg^{-1}$), manganese (0.33 g $kg^{-1}$), zinc (0.35 g $kg^{-1}$) (Codling et al., 2008), selenium (0.38 mg $kg^{-1}$), boron (35 mg $kg^{-1}$), and nickel (10 mg $kg^{-1}$) (Bolan et al., 2010) can also be supplied.

In practice, it is the nitrogen contained in BL that is the locus of economic value for the crop producer accepting this waste material for the improvement of soil fertility. More specifically, the producer is interested in the amount of nitrogen from BL that will be available to a crop over the growing season. A substantial portion of the nitrogen found in BL will be inaccessible to plants for use in the growth process. To put this into perspective, most or all of the phosphorus and potassium found in BL is expected to be available to a crop to be taken up and used for plant growth and maintenance with substantial build up of residual supply available in subsequent years (Mitchell and Tu, 2006; Bolan et al., 2010). The percentage of nitrogen from BL that will be taken up by a crop can vary from 0 to100% of the total amount of N present in the first year with little or no residual supply expected in subsequent years (Bitzer and Sims, 1988; Gordillo and Cabrera, 1997a; Gordillo and Cabrera, 1997b; Qafoku et al., 2001; Ruiz-Diaz et al., 2008; Ruiz-Diaz et al., 2012). These authors summarily attribute the lack of predictability in available N supply from BL to the chemical nature of the nitrogen compounds present, the biology of the material, and its interaction with the soil environment.

To start, only the mineral forms of N are available for plant uptake. These mineral forms include ammonia ($NH_3$/$NH_4^+$) and nitrate ($NO_3^-$), which combined may comprise a negligible portion to approximately half of the total N present in the BL (Gordillo and Cabrera, 1997a; Qafoku et al., 2001; Sistani et al., 2008). Ammonia may volatilize and be lost to the atmosphere prior to plant uptake and nitrate may be lost through leaching below the root zone or through denitrification (Moore et al., 1996). Organic N makes up the remainder of the total supply. While organic N is not immediately available for plant uptake, a portion of this N pool may become available as it is mineralized. Mineralization of

organic N is a biologically mediated process. The subsequent breakdown of complex organic compounds via enzymatically-catalyzed reactions into simpler compounds terminates in the mineral form ammonia (Carlile, 1984), which may be further oxidized to nitrate via the nitrification pathway (Pote et al., 2002) (Figure 2.1). Ammonia and nitrate loss can be mitigated by proper management practices (Bolan et al., 2010). However, current inaccuracies in N release prediction are rooted in our poor understanding of the details of organic N mineralization.

Noting that initial nitrogen in avian feces at the time of deposition is 70% uric acid and 30% undigested proteins, Carlile (1984) and Groot Koerkamp (1994) have identified the specific organic compounds of interest in the mineralization pathway that arise from the breakdown of these two important pools of organic N (Figure 2.1). These include guanine, adenine, hypoxanthine, xanthine, uric acid, allantoin, allantoic acid, glyoxylic acid, and urea. Considerable research has gone into understanding the mineralization of N from BL in the context of total organic N converted to mineral N (Bitzer and Sims, 1988; Gordillo and Cabrera, 1997a; Gordillo and Cabrera, 1997b; Qafoku et al., 2001; Ruiz-Diaz et al., 2008; Ruiz-Diaz et al., 2012). These authors have measured initial and final organic N, total N, and ammonia N. Some have tracked changes in nitrate and urea as well. Gordillo and Cabrera (1997a, 1997b), Qafoku et al. (2001), and Ruiz-Diaz (2008) have measured initial uric acid N in their samples and successfully related their results to final PAN. However, little has been reported concerning the patterns of change with time.

Rothrock et al. (2010) measured uric acid from a single BL sample treated with three common chemical amendments at 0, 2, 4, and 6 weeks and compared the rate of change at each time point. Initial uric acid nitrogen was found to be between 1.3 and 1.7 g $kg^{-1}$ BL. Their results suggested that small changes in all treatments (increases or decreases) occur in the first two weeks. The most rapid changes (net decreases in concentration in all samples) occurred between two and four weeks. The final period of time between four and six weeks was characterized by net decreases in all samples that were smaller in magnitude. Final concentrations ranged from 0.03 to 4.7 g uric acid nitrogen $kg^{-1}$ BL. Bao et al. (2008) measured allantoin in poultry manure at 0, 1, 2, 4, 6, 8, and 10 weeks. Initial concentrations were

reported to be 8.77 and 11.1 g $kg^{-1}$ manure. These authors observed small net increases in the manures over the first two periods followed by significant net decreases for the remaining times sampled. Final concentrations of 5.5 and 6.6 g $kg^{-1}$ BL were reported. They suggested that a first-order reaction equation was appropriate to fit to these patterns of allantoin N decay which provided rate constants (k) in the range of -0.0012 to -0.0015 $day^{-1}$ and half-lives of 46 to 58 days.

These two studies provide the summation of work to date on changes in organic N in poultry waste with time specific to the compounds in Figure 2.1 that fall above urea in the N mineralization pathway. Such a relative dearth of knowledge is likely due to a lack of standardized methods of analysis and the amount of time required to measure these compounds. The original research that follows is dedicated to improving our understanding of the changes these compounds undergo over time. This work is also dedicated to improving the methods of measurement necessary not only to understand the changes better, but to communicate these patterns of change to the crop producers who manage their soils with inputs of BL.

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# CHAPTER 2

# LITERATURE REVIEW

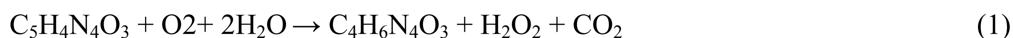
## The Biochemistry of Uric Acid Decomposition in PL

Any accounting of the nitrogen (N) found in poultry litter (PL), or broiler litter (BL) specifically, must begin with the N composition of the fecal matter deposited by the birds. All birds (class aves) are incapable of producing the uricase enzyme that hydrolyzes uric acid. They must excrete uric acid as a final form of nitrogenous waste (Schefferle, 1965). This waste N is shed along with the feces as birds do not produce urine, nor do they urinate as many other animals do. The N contained in uric acid represents the largest portion of N present in avian feces. Groot Koerkamp (1994) estimated that the fresh fecal matter from poultry feces is 70% uric acid and 30% undigested proteins. Physiological production of uric acid in poultry results from the degradation of the purine nucleotide guanine through the intermediate compound xanthine (Yuan et al., 1999). Its formation from the deamination of the nucleoside adenosine through the intermediate compounds inosine, hypoxanthine and xanthine has also been described (Xi et al., 2000). Deamination of the nucleotide adenine to hypoxanthine, xanthine, and uric acid was outlined by Berg and Jørgensen (2006), who found that the formation of urea from the guanine pathway was substantially more rapid than from adenine.

From the moment that feces are deposited, the initial forms of N present (uric acid and proteins) begin a process of chemical transformation. Although urea and ammonia can be released from the breakdown of pyrimidine nucleic bases and proteins, the catabolysis of purine nucleic bases and subsequent breakdown of uric acid appears to be the most important source of mineralizable nitrogen arising from PL or BL. When water is present in sufficient amounts and temperature is adequate, uric acid in BL will be mineralized relatively rapidly through the compounds allantoin, allantoic acid, glyoxylic acid, and urea to ammonia (Carlile, 1984) (Figure 2.1). Ammonia from BL may be further oxidized to

nitrite and nitrate via nitrification (Pote et al., 2002); or it may be released from BL and enter the atmosphere through volatilization (Moore et al., 1996). From Figure 2.1, it can be seen that all reactions involving the decomposition of organic N compounds are mediated by biologically produced enzymes. Therefore, all of the reactions require the presence of water; and many require the participation of water as reactant as well. It can also be seen that many of these reactions are aerobic, necessitating the presence of oxygen as reactant. This section of the review presents basic available information on the biochemistry of the breakdown of uric acid and its products to ammonia. Descriptions of molecular structure, enzymatic structure, and reaction details are provided where available.

**Uric acid**, as the principal component of initial N in PL, provides a logical starting point for an examination of the chemical composition of nitrogen in PL. Uric acid is a purine similar in structure to guanine and xanthine (Figure 2.2). It contains one pyrimidine (six-member ring with N atoms at the 1 and 3 positions) fused to an imidazole (five-member ring with N atoms at the 1 and 3 positions) (Stryer, 1988). Uric acid is hydrolyzed (Eq. 1) in the presence of the enzyme uricase (urate oxidase), water, and oxygen to allantoin, carbon dioxide and hydrogen peroxide (Fraisse et al., 2002).



$$C_5H_4N_4O_3 + O2 + 2H_2O \rightarrow C_4H_6N_4O_3 + H_2O_2 + CO_2 \quad (1)$$

Atalrsha et al. (2009) estimated the free energy value of the reaction in Eq. 1 at -269 kJ $mol^{-1}$. Kahn et al. (1997) described the presence of a short-lived radical intermediate that rapidly degrades spontaneously to allantoin. The authors identified the reaction intermediate as hydroxyisouric acid which has since been confirmed by others (Fraisse et al., 2002: Retailleau et al., 2005) as 5-hydroxyisourate. Wang and Königsberger (1998) describe the solubility of uric acid as low [$K_{sp} = 10^{-5.4}$ mol $L^{-1}$ ($\Delta H_r = 14.5$ kJ $mol^{-1}$) with Na as the cation], increasing with increasing pH. In the pH range 7 to 8.5 $K_{sp}$ was found to be relatively insensitive to changes in temperature. Uric acid will therefore remain largely in the form of precipitated solid when in PL.

The presence and importance of uric acid specific to PL was investigated by Shefferle (1965) and found to be degraded as a result of the action of various bacterial organisms naturally present in the litter. The strains of bacteria found to participate in the decomposition of uric acid were described as primarily gram negative and aerobic. This degradation, catalyzed by the uricase enzyme, proceeded at a wide range of temperatures that included temperatures near 0°C. The uricase enzyme is a protein that has been isolated from a multitude of ecological niches. Some bacteria (e.g. *Bacillus fastidiosus*) can utilize urate as a sole carbon and nitrogen source following hydrolysis while leguminous plants such as soybean utilize the enzyme to convert 'fixed' nitrogen to ureides for translocation within the plant (Busi et al., 2007). Uricase is an unusual protein in that its biological function and specificity for substrate are reportedly consistent regardless of source organism: however, its structure and molecular size have been shown to differ from source to source. For instance, early findings place importance on the presence of Cu(II) in the enzyme extracted from pig liver (Baum et al., 1956). More recent work has shown that copper is absent from other source forms of urate oxidase. Bongaerts et al. (1978) described an uricase isolated from the bacterium *Bacillus fastidiosus* (M = 145-150 kDa) as a tetramer composed of two kinds of subunits (Mr = 36,000 and 39,000) as determined by decomposition followed by gel filtration. These authors found no evidence of metal ions. Fraisse et al. (2002) worked with rasburicase common in mammals, and reported a tetramer with identical subunits of 34 kDa not linked by disulfide bridges. X-ray analysis revealed that the tetramer is composed of two dimmers superimposed face-to-face to form a tunnel-shaped protein with four active sites (1 per monomer). The form of uricase isolated from the fungal organism *Aspergillis flavus* is also described as a homotetramer (M = 135 kDa) with four active sites at dimeric interfaces (Gabison et al., 2008; Retailleau et al., 2005). The study by Retailleau and co-authors further revealed details on the structure of the subunits. These were reported to contain 301 amino acids each. Use of crystal-packing contacts showed the active site of this enzyme (dimeric interface) to be between the amino acids glutamine (Gln228) and arginine (Arg176) that form a structure analogous to 'tweezers' that can physically hold the urate molecule for catalysis.

Uricase can be considered a ubiquitous enzyme in natural systems, including PL. It has high substrate specificity, yet can be inhibited by a number of compounds also naturally present in PL (Baum et al., 1956; Fridovich, 1965). Michaelis-Menten constants ($K_m$) for the degradation of urate in solution by uricase have been reported by several authors. Baum et al. (1956) determined a $K_m$ value of $2 \cdot 10^{-5}$ M. This is in relatively good agreement with the range of values of $3.9\text{-}4.8 \cdot 10^{-5}$ M found by Fraisse et al. (2002), though Fridovich (1965) placed this at the lower value of $0.5 \cdot 10^{-5}$ M. The $R_{max}$ value in the study by Baum et al. (1956) was found to be pH dependent with an optimum pH of 8.5. These authors also noted the inhibition of uricase by cyanide and several structural analogs of uric acid such as methyl substituted urates and other purines. Inhibition constants ($K_i$) were determined by Bongaerts et al. (1978) for cyanide ($5 \times 10^{-6}$ M), xanthine ($2 \times 10^{-5}$ M), and oxonate ($1.85 \times 10^{-6}$ M). Baum et al. (1956) reported 100% inhibition of uricase activity by oxonate. Fridovich (1965) investigated this inhibition as well and reported an interesting phenomenon in his study. The rapid degradation of uric acid in alkaline solutions was characterized as an oxidation of urate to oxonate. Fridovich presented an inhibition constant for oxonate as $K_i = 1 \cdot 10^{-7}$. Uricase activity has been reported to be 13.1 units $mg^{-1}$ (Huang and Wu, 2004).

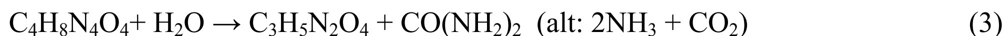
**Allantoin** is the product of the uricase-catalyzed oxidation of uric acid. The primary molecular structure change arising from the reaction involves the disruption of the pyrimidine ring at Position 1 (Figure 2.2). The solubility of the new compound is increased over that of the substrate uric acid. Allantoin is hydrolyzed to allantoic acid by cleavage of the five member ring through the enzyme allantoinase (Eq. 2).

$$C_4H_6N_4O_3 + H_2O \rightarrow C_4H_8N_4O_4 \qquad (2)$$

Like uricase, allantoinase is ubiquitous and can be found in bacteria, fungi, plants, and animals (Hayashi et al., 2000; De Windt et al., 2002; Raso et al., 2007). Hayashi et al. (2000) described allantoinase isolated from fish liver as a single peptide with a molecular weight of 54 kDa. These authors found that allantoinase in amphibians was structured as a dimer of two 54-kDa units that were complexed

with the allantoicase enzyme. The activity of allantoinase from frog liver was estimated at 0.09 units $mg^{-1}$. De Windt et al. (2002) reported the activity of allantoinase from mushrooms at 33 pkat $mg^{-1}$ (~ 0.5 units $mg^{-1}$). Raso et al., (2007) reported an activity in allantoinase extracted from French bean (*Phaseolus vulgaris*) at 2 units $mg^{-1}$. Mulroony and Hausinger (2002) found that allantoinase is a metalloenzyme most likely containing zinc, but possibly cobalt. pH optima for the activity of allantoinase was reported to be 5.0 by Tajima and Yamamoto (1974) and 7.5 by Lee and Roush (1964). Both of these values are lower than the alkaline conditions that favor uricase activity.

**Allantoic acid** (allantoate) produced from the hydrolysis of allantoin possesses no ring structures. Theoretically, its solubility should be further increased in aqueous solution due to the increased polarity and potential for interaction with water molecules. The enzyme allantoicase (allantoate amidohydrolase) catalyzes the hydrolytic conversion of allantoic acid into uriedoglycolate and either urea or two ammonia molecules and one carbon dioxide (Eq. 3).



$$C_4H_8N_4O_4 + H_2O \rightarrow C_3H_5N_2O_4 + CO(NH_2)_2 \ \ \text{(alt: } 2NH_3 + CO_2\text{)} \qquad (3)$$

Little is known about this particular reaction and its enzyme as compared to other segments of the purine degradation pathway. Certainly, little has been published concerning the details of reaction mechanism or allantoicase structures. However, Leulliot et al. (2004) proposed a bimodal organization of a cylindrically shaped monomer with a jelly-roll shape. Hayashi et al. (2000) measured the molecular size at 48 kDa (fish liver allantoicase) and estimated an enzymatic activity of 0.013 units $mg^{-1}$.

**Ureidoglycolate** is formed from the release of two amide groups resulting from the cleavage of a C-N bond. Agreement upon the formation of two ammonia molecules and carbon dioxide as opposed to a single urea molecule is not consistent in the literature. Ureidoglycolate is considered more soluble in aqueous solution and is degraded to glyoxylate and urea by the enzyme ureidoglycolase (Muñoz et al., 2006) according to the reaction presented in Eq. 4.

$$C_3H_5N_2O_4 \rightarrow C_2H_2O_3 + CO(NH_2)_2 \qquad (4)$$

Activity of ureidoglycolate purified from chickpea (*Cicer arietinum*) has been reported at 8.6 units $mg^{-1}$ (Muñoz et al., 2001). These authors found a Km value of 6 µM in the presence of $Mn^{2+}$, a molecular mass of 180 kDa, and reported the optimum pH between 7 and 8 for this source of the enzyme. Subsequent purifications from French bean (*Phaseolus vulgaris*) have yielded activity estimates of 4.4 units $mg^{-1}$, a Km value of 3.9 mM, and a molecular mass of 240 kDa (Muñoz et al., 2006). This is different from that purified from the chickpea source, indicating that ureidoglycolase may vary by source as uricase does.

**Urea** is a soluble compound in aqueous solution (~1.3 kg $L^{-1}$ $H_2O$) (Lee, and Lahti, 1972). Urea may be formed from the deamination of a number of nitrogen containing compounds. Sirko and Brodzik (2000) described its derivation from arginine catalyzed by argenase. Although this is outside the purine catabolysis pathway, it may represent an important source of urea formation originating from the amino acid glutamine, which the authors describe as important in general nitrogen metabolism. Formation from the deamination of ureidoglycolate is outlined in Eq. 4 and its potential formation from the hydrolysis of allantoic acid is outlined in Eq. 3. Urea is the final and simplest of the organic nitrogen compounds in the purine catabolysis / N mineralization pathway. It is composed of a single carbonyl group attached to two amines and is easily hydrolyzed by the enzyme urease (urea amidohydrolase) to form 2 molecules of ammonia and one molecule of carbon dioxide according to Eq. 5.

$$CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2 \qquad (5)$$

Mobley et al. (1995) proposed that this is the summary result of two reactions. The first reaction produces one ammonia and one carbamate ($COOHNH_2$). The second reaction decomposes carbomate to produce one ammonia and one carbonic acid ($H_2CO_3$). The deprotonation of carbonic acid and the protonation of both ammonia molecules results in a net increase in pH at the rate of one mole of hydroxide ions for every mole of urea hydrolyzed. The free energy of this reaction is estimated to be -940

kJ $M^{-1}$ (Estriu et al., 2004). Urease is a nickel containing metalloenzyme ubiquitous in nature (Sirko and Brodzik, 2000). Found in bacteria, fungi, and plants, urease was first isolated from the seeds of the jack bean plant (*Canavalia ensiformis*) in 1926 (Jabri et al., 1995). This now-common source of urease for many applications catalyzes the hydrolysis of urea at a rate $10^{14}$ times faster than the uncatalyzed reaction. The early isolation and discovery of useful applications of the urea-urease reaction system has led to its being more thoroughly studied than any of the other reactions in the purine catabolism - N mineralization pathway described herein. Jabir et al. (1995) characterized the bacterial urease isolated from *Klebsiella aerogenes* as having three subunits of 60.3 kDa, 11.7 kDa, and 11.1 kDa each, and the jack-bean-derived urease as either a trimer or hexamer of identical 91 kDa subunits. Plant and fungal ureases generally consist of identical subunits, while bacterial ureases are multimers formed from a mixture of two or three different types of subunits. However, certain structural similarities suggest that all such ureases are variants of the same enzyme with similar catalytic mechanisms (Sirko and Brodzik, 2000).

Urease kinetics follow a Michaelis-Menten-type behavior with no evidence of substrate inhibition or allosteric behavior (Mobley et al., 1995). These authors give Km values in the range of 2.2 to 130 mM and activities in the range of 1000 to 5,500 units $mg^{-1}$ for microbial ureases. Inhibition of the enzyme is affected by several classes of compounds, including hydroxamic acids, phosphoramides, and thiols (Mobley et al., 1995; Sirko and Brodzik, 2000). In agriculture, urease inhibitors are exploited to control loss of N from the formation and volatilization of ammonia under alkaline conditions. Specifically, N-(n-butyl) thiopohsphoric triamide (NBPT) is used to control ammonia loss from fertilizer urea applications (Sanz-Cobena et al., 2011), and the use of phenyl phosphorodiamidate (PPDA) and Cyclohexylphosphoric triamide (CHPT) are described by Varel (1997) as effective in preventing urea hydrolysis in animal wastes.

The overall biochemistry of the N mineralization pathway from uric acid to ammonia is characterized by incremental hydrolyses of organic bonds, which serve to simplify the molecular structures at each step (Figure 2.2). In general, each reaction produces a compound with greater aqueous solubility than uric acid. Urea and ammonia, the simplest of these molecules, are utilizable by bacterial

and fungal organisms as sole sources of nitrogen. The rates of reactant decomposition and product formation within the context of poultry litter for each of the reactions described in this section would benefit from addtional study. To date, the vast majority of studies have focused on the total formation of urea-N or ammonia-N from the sum of organic N (Bitzer and Sims, 1988; Tyson and Cabrera, 1993; Gordillo and Cabrera, 1997; Qafoku et al., 2001; Hartz et al., 2000; Preusch et al., 2002; Fujiwara and Murakami, 2007; Ruiz-Diaz, 2008). The results of these studies suggest that the release of N from PL for the purpose of crop production is highly variable with an average of 50% to 60% of the total N.

Detailed accounting of the individual compounds present in the organic N fraction may improve the accuracy of prediction of N released from PL. However, only two studies could be identified that specifically characterize the decay of these compounds. Rothrock et al. (2010) measured uric acid in a single incubated BL sample treated with poultry litter chemical amendments at 0, 2, 4, and 6 weeks. Initial uric acid concentrations in the samples used in their study ranged from 1.3 to 1.6 g N $kg^{-1}$ BL (dry weight basis). These authors reported a decline in UAN in all treatments incubated at 25°C over the six-week period with final concentrations ranging from 0.03 to 0.47 g UAN $kg^{-1}$ BL. Bao et al. (2008) measured allantoin-N concentrations in six composted poultry, swine, and dairy manures at 0, 1, 2, 4, 6, 8, and 10 weeks. Initial allantoin-N in two poultry manures was 8.77 and 11.14 g $kg^{-1}$ and declined to 5.5 and 6.6 g $kg^{-1}$ respectively. The study performed by Bao et al., (2008) suggests that allantoin-N in fresh poultry manure may average close to 30% of TN. Aside from these two studies, no further information is available on the time dependent concentrations of guanine, adenine, hypoxanthine, xanthine, uric acid, allantoin, allantoic acid, or ureidoglycolate from PL.

**Analysis and Prediction of Nitrogen from Poultry Litter:**

The analysis of nitrogen from PL and prediction of the portion that may be released as mineral N for crop uptake has historically focused on total N and its fractions of mineral and organic N. However, methodologies for quantifying all of the compounds listed in the previous section do exist. In this section, these methodologies and their application to the prediction of PAN will be reviewed and discussed.

**Total N** in PL is most frequently analyzed by either by Kjeldahl digestion or by the method of combustion. Kjeldahl digestion uses heat in excess of 360°C and concentrated sulfuric acid in the presence of catalysts such as potassium, copper, and titanium oxides to decompose organic N compounds to ammonia (Bremner, 1965). Salicylic acid may be added to account for nitrate N (Bitzer and Sims, 1988). The pre-existing ammonia and the ammonia produced by the digestion procedure are determined by titration of the steam distillate (Bremner, 1965), colorimetry (Willis et al., 1996), or gas-diffusion conductance (Carlson, 1978). The method of combustion (Nelson and Sommers, 1982) is a more rapid, single-step measurement of all forms of N present in a PL sample. Briefly, a small portion of the sample (< 1g) is placed in an oven at 1350°C. The sample is combusted and all gases formed are swept via a helium gas stream through a copper catalyst that converts all N to gaseous $N_2$. Detection of $N_2$ is achieved by thermal conductivity. Total N values in fresh collected PL reported in the literature include the following: 18 to 81 g $kg^{-1}$ (Bitzer and Sims, 1988), 27 to 60 g $kg^{-1}$ (Gordillo and Cabrera, 1997a), 24 to 50 g $kg^{-1}$ (Qafoku et al., 2001), 2 to 64.8 g $kg^{-1}$ (Reeves, 2001) and 23 to 66 g $kg^{-1}$ (Fujiwara and Murakami, 2007). Composting and storage of PL causes a substantial decrease in total N over time when compared to fresh PL. Cooperband et al. (2002) reported a decline in total N concentrations of 45 and 57 g $kg^{-1}$ in fresh PL to a level of 6 to 15 g $kg^{-1}$ in PL aged from 1 to 15 months. Tyson and Cabrera (1993) reported levels of 13 and 14 g N $kg^{-1}$ in composted BL.

**Mineral N** forms in poultry litter include nitrate ($NO_3^-$) and ammonia. Nitrite ($NO_2^-$) and other transitory forms of mineral N exist, but are of lesser importance to the subject of PL as fertilizer (Cameron et al., 2013). The latter exists in equilibrium in solution phase as $NH_4^+$ and $NH_3^0$. Additionally, the non-polar form ammonia will maintain equilibrium between the gaseous and dissolved forms. For the purposes of this review, $NH_3$-N will be used to denote all forms of ammonia or ammonium unless otherwise specified.

Ammonia volatilization from poultry litter is undesirable. In the production facility, volatilization and accumulation of gaseous ammonia can be detrimental to bird health (Carlile 1984; Moore et al., 1996; Ritz et al., 2004). Large plumes of gaseous ammonia can cause significant and undesirable odors that

affect a large area surrounding the facility (Wheeler et al., 2006). Ultimately, though, the reduction in total N through loss of ammonia reduces the economic value of litter as a source of fertilizer N (Marshall et al., 1998).

$NO_3$-N and $NH_3$-N represent a very important fraction of the total N in PL. These two compounds are often considered the only forms available for plant uptake (Cameron et al., 2013), however, Nashölm et al. (2009) recently provided evidence that amino acids are also available for uptake. As this area of research continues develops, it may shed some light on our understanding of the release of N from PL when used to fertilize crops.

Plant available nitrogen (PAN) is a term best applied to the mineral N that is immediately available for plant uptake (Gordillo and Cabrera 1997a; Qafoku et al., 2001). Some authors have included into this term the amount of N that may mineralize from the organic fraction, transforming over time to PAN (Ruiz-Diaz et al., 2008; Shah et al., 2013). It is important to distinguish between that pool of N that is immediately available for plant use and that pool of N that will release slowly over the crop growth cycle. The term potentially mineralizable nitrogen (PMN) best describes the latter pool (Gordillo and Cabrera, 1997a; Gordillo and Cabrera, 1997b; Qafoku et al., 2001). However, it is suggested here that the term PAN may still be appropriately applied to the sum of these pools if the distinction is made between the initial available N ($PAN_i$) and the N that will be available at some point in time ($PAN_t$).

The analysis of mineral N from PL is most frequently achieved by first extracting the compounds from solid matrix with the use of an aqueous solution. Both compounds are soluble in water, and sorption interactions with other compounds or active surfaces in the organic or mineral fractions within the PL can be negated with the addition of a chemical salt. Extracting solutions of 1M KCl, 2M KCl, and 0.01M $CaCl_2$ at ratios ranging from 1:10 to 1:200 (mass:vol) have been described in the literature (Gordillo and Cabrera 1997a; Qafoku et al., 2001; Ruiz-Diaz et al., 2008). Following filtration to remove solids, $NH_3$-N was measured in these studies by the colorimetric indophenol method. $NO_3$-N was measured from the extracting solution by the Greiss-Ilosvay colorimetric procedure. Although this method is the preferred approach, it requires the use of cadmium reduction to first convert $NO_3^-$ to $NO_2^-$ prior to color

development. This reduction process is known to be sensitive to interference from the presence of high concentrations of organic compounds (Crutchfield and Grove, 2011). As this is an unavoidable characteristic of PL extracts, additional steps have been suggested to improve this procedure. The suggestions include individual reduction for each sample by addition of cadmium reducing agent in granular or wire form, and cleanup of high organic matter-containing samples with Somogyi's reagent. The relative effectiveness of these approaches has been described by Crutchfield and Grove (2011). $NH_3$-N in PL extracts may alternatively be measured by gas diffusion-conductance or gas-diffusion colorimetry (Carlson, 1978). Mineral N as $NH_3$-N or as $NH_3$-N + $NO_3$-N may also be measured by steam distillation without extraction where $NO_3$-N is accounted for by addition of salicylic acid as previously described for the total N procedure (Bitzer and Sims, 1988; Reeves, 2001; Chen et al., 2009).

$NH_3$-N concentrations in PL have been reported in a number of publications. Reeves (2001) found a range of 4.2 to 13.1 g kg-1 in his survey of 207 samples. Other ranges reported in the literature from studies of multiple PL and/or BL samples include 2.1 to 33.4 g $kg^{-1}$ (Bitzer and Sims, 1988) 2.5 to 5.7 g $kg^{-1}$ (Gordillo and Cabrera, 1997a), 0.8 to 6 g $kg^{-1}$ (Qafoku et al., 2001). $NO_3$-N concentrations in PL are lower than $NH_3$-N concentrations and are occasionally negligible or below analytic detection limits for the methods described above. $NO_3$-N in PL values reported in the literature include 0.013 to 3.7 g $kg^{-1}$ (Bitzer and Sims, 1988), <0.01 to 1.82 g $kg^{-1}$ (Gordillo and Cabrera, 1997a), and <0.01 to 7.4 g $kg^{-1}$ (Qafoku et al., 2001).

**Organic N,** *in toto* as a fraction of total N, is calculated as the difference between mineral N and total N by combustion (Reeves, 2001), or as the difference between $NH_3$-N and total Kjeldahl N (Bitzer and Sims, 1988). In the case of individual constituents of the organic N pool (e.g. urea, ureidoglycolate, allantoic acid, allantoin, uric acid, xanthine, hypoxanthine, guanine, adenine, etc.) methodologies for measurement from PL have not been described in the literature or are rare. Despite prominent placement on the importance of urea in the organic fraction of N in PL contained in recent reviews (Nahm, 2003; Nahm, 2005; Bolan et al., 2010), no specific reference is given on studies that provide direct evidence of its relative concentration and concomitant contribution to this pool. A recent study by Cook et al. (2008)

on the abundance of ureolytic microbial organisms in PL and their importance in the production of ammonia fails to provide measurements of urea. Only $NH_3$-N concentrations are presented in this work. Singh et al., (2009) studied the inhibition of urease as an approach to reducing ammonia volatilization in poultry houses by measuring rates of ammonia production. No data were provided on urea buildup to balance the argument.

Varel et al. (1997) measured urea in cattle slurries by using a colorimetric procedure employing a carbamido-diacetyl reaction with urea in the presence of acidic ferric ions and thiosemicarbazide. The similarities in materials with respect to organic matter content indicate that the procedure may be useful for measuring urea from PL. No method has been reported for the measurement of ureidoglycolate in PL nor have any concentrations in PL been previously published. Eitemann et al., (1994) described a method for the simultaneous measurement of oxonic acid, uric acid, creatine, allantoin, xanthine, and hypoxanthine in PL. Alkaline extraction with 0.025 M $Li_2CO_3$ was first used to solubilize the compounds of interest. This was followed with separation by HPLC and measurement with UV/VIS detection. The authors reported concentrations in fresh poultry feces for allantoin (2.7 mg $kg^{-1}$), oxonic acid (2.4 mg $kg^{-1}$), creatine (3.9 mg $kg^{-1}$), uric acid (270 mg $kg^{-1}$), and xanthine (0.8 mg $kg^{-1}$). Hypoxanthine was not detected in the fresh fecal material but was reported to increase to 10.8 mg $kg^{-1}$ following storage at room temperature for eight weeks. Oxonic acid and allantoin increased as well during this time period while creatine, uric acid and xanthine decreased. Allantoic acid has not been reported in published studies on PL. Bao et al. (2008) used 2,4-nitrobenzene hydrazine colorimetry to determine allantoin in poultry manures. Whether the manures contained bedding material or not was not specified. The conditions of collection were not reported either. The results of this study with respect to allantoin concentrations are described in a preceding section. Uric acid from PL has been measured by the method of Eitemann et al. (1994) in studies by Gordillo and Cabrera (1997a) and Rothrock et al. (2010) who reported concentrations of 3.5 to 20.5 g and 4.1 to 4.7 g UAN $kg^{-1}$ PL respectively. Fujiwara and Murakami (2007) and Ruiz-Diaz et al. (2008) used a colorimetric method described in Alumot and Bielorai (1979). In this method, uric acid is reacted with the enzyme uricase, and the stoichiometric

reaction product hydrogen peroxide is oxidatively coupled with 3-methyl-2-benzothiazolinone hydrozone and N,N-dimethylaniline to produce a stable indamine dye. Guanine and adenine from PL have not been reported in the existing literature on the subject. As clear, sequential components in what has been identified as an important pathway for the mineralization of organic N from PL, these compounds have received little detailed attention in scientific investigations to this point. In the section of the review that follows, it will be made clear that the majority of studies to date on mineralization of organic N from PL suffer from a wide margin of error that must surely arise from a conceptual approach that has struggled to relate final mineral N to initial organic N, rather than to any of the individual components of the heretofore identified purine catabolysis - N mineralization pathway.

**Measurement of mineralizable N from PL** can be made directly by application of the material to crop land. A nitrogen balance can be calculated from plant uptake and residual soil N (Bitzer and Sims, 1988; Sistani et al., 2008; Ruiz-Diaz, 2012). The costs and time involved in this procedure preclude its usefulness in predicting PMN for practical application. Laboratory incubation studies where PL is placed in soil and the release of mineral N is measured provide a realistic and intuitively relatable alternative to field studies (Bitzer and Sims, 1988, Tyson and Cabrera, 1993; Gordillo and Cabrera, 1997b; Qafoku et al., 2001, Ruiz-Diaz et al., 2008). Laboratory incubations remain costly in terms of time, requiring months for complete aerobic decomposition. Their usefulness can be measured in the number of samples that can be evaluated simultaneously. In this way it is theoretically possible to make more frequent measurements of mineralized N or individual components of organic N. In practice, however, most agricultural laboratories use average data from Bitzer and Sims (1988) based on total N to provide an estimate to end users of PL. Reported values for PMN from fresh PL reported in the literature from studies using multiple samples include ranges from 213 to 1096 g N $kg^{-1}$ organic N (Bitzer and Sims, 1988), 254 to 398 g N $kg^{-1}$ organic N (Tyson and Cabrera, 1993), 465 to 868 g N $kg^{-1}$ organic N (Gordillo and Cabrera, 1997a), and 239 to 736 g N $kg^{-1}$ organic N (Qafoku et al., 2001). In composted PL, values for PMN have been reported in the range of 28 to 58 g N $kg^{-1}$ organic N (Tyson and Cabrera, 1993), and < 0.0 to 270 g N $kg^{-1}$ organic N (Hartz et al., 2000). The process of composting, therefore,

results in a much lower release of organic and total N as compared to fresh PL. Litters that have been stacked or stored, as is the normal practice in the field (Delaune et al., 2004; Mitchell and Tu, 2005), without having undergone formal composting have received no scientific attention to date. The changes in total N, organic N, PMN, and their respective sub-components with time during storage remain un-investigated. This represents an area of study that is of immediate and practical importance to providers and end-users of PL as an economic commodity.

Some research has been devoted to developing more rapidly measurable indices of PMN. Ruiz-Diaz et al. (2008) found that the average PAN in chicken (66% of TN) and turkey manure (58%) was 'basically the same' as the water soluble nitrogen (WSN) fraction of total N. Gordillo and Cabrera (1997) reported that PMN in BL (112 day soil incubation) was positively correlated with total N (TN) and uric acid N (UAN) and negatively correlated with the carbon:nitrogen (C:N) ratio (PMN = 26.68 + 1.04 UAN(g N $kg^{-1}$ PL) – 1.22 C/N; $r^2 = 0.95$). Qafoku et. al. (2001) found a good relationship (PMN = -0.018 + 1.293 WSON (g N $kg^{-1}$ PL); $r^2 = 0.87$) between water soluble organic nitrogen (WSON) and PMN from BL (also 112 day soil incubation). WSN and WSON are measured by shaking the PL sample with water and collecting the filtrate for digestion using persulfate to oxidize the soluble organic N, $NH_3$-N and $NO_3$-N (Cabrera and Beare, 1993). WSON is calculated by subtracting the concentration of mineral N constituents from WSN.

**The use of Near-Infrared Reflectance (NIR)** spectroscopy to measure N from PL has shown potential in the last 15 years. Because NIR is a 'secondary' analytic technique and because it has possibility for such broad application to all of the N forms in PL discussed so far, a description of its use in the measurement of N from PL provides an appropriate *coda* to a review of analysis and prediction. NIR is considered a secondary analytic technique because it requires the pairing of spectral data gathered by the NIR instrument with reference chemical composition data generated by a 'primary' laboratory analytical technique. A software program is typically used to identify differences in sample spectra over a range of wavelengths (e.g. 400 to 2500 nm). These are statistically regressed against reference chemical data for each sample using partial least squares (PLS), principal components analysis (PCR), artificial

neural network (ANN), or other data analysis approach to generate a prediction equation for samples of unknown composition.

Reeves (2001) reported good results on the calibration of NIR for $NH_4$-N ($r^2$ = 0.960; RMSD = 0.024%), TN ($r^2$ = 0.943; RMSD = 0.204 %), organic N ($r^2$ = 0.944; RMSD = 0.200 %), and dry matter ($r^2$ = 0.986; RMSD = 0.012 %) in BL gathered from the University of Maryland Soil Testing Laboratory (College Park, MD). The authors noted that the samples had to be reanalyzed to improve results as $NH_4$-N concentrations had changed since measurement at the Soil Laboratory. Tasistro et al. (2003) published similar results for $\psi$ and TN in BL samples received by the University of Georgia Soil Plant and Water Testing Laboratory (Athens, GA). As previously mentioned Qafoku et al. (2001) measured PMN directly and reported a good relationship between NIR-predicted and lab values ($r^2$ = 0.82; SECV = 2.01 g N $kg^{-1}$) on fresh BL gathered from poultry houses. Fujiwara et al. (2007) developed a calibration for UAN in composted poultry manure using NIR. These authors described an close relationship between UAN and mineralized N from soil incubations ($r^2$ = 0.99999, SE = 0.61 mg $g^{-1}$, n = 91). Xing et al. (2008) obtained successful calibration for water content ($\theta$) ($r^2$ = 0.88, RMSEC = 20.10 g $kg^{-1}$), organic matter (OM) ($r^2$ = 0.88, RMSEC = 6.32 g $kg^{-1}$), total N ($r^2$= 0.92, RMSEC = 1.08 g $kg^{-1}$), and $NH_4$-N ($r^2$= 0.92, RMSEC = 0.77 g $kg^{-1}$) in layer manure. Chen et al. (2009) demonstrated that artificial neural networks (ANN) produced an NIR calibration equation for $NH_4$-N ($r^2$ = 0.93; RMSEC = 0.71 g $kg^{-1}$) slightly superior to that produced by the partial least squares (PLS) approach ($r^2$ = 0.92; RMSEC = 0.77 g $kg^{-1}$).

**Areas of research of importance** have been identified in this review. One area of research that will improve our understanding of PL as organic amendment is the measurement of individual compounds of the purine catabolysis - N mineralization pathway (Figure 2.1; Figure 2.2) with time during storage of litter. A second area involves developing a better understanding of the relationship between these compounds and the ultimate release of mineral N following land application to soils under crop and pasture production. Finally, new and rapid methodologies for laboratories to asses these qualities in PL are paramount to relating the potential value of this material to both the provider and to the end-user.

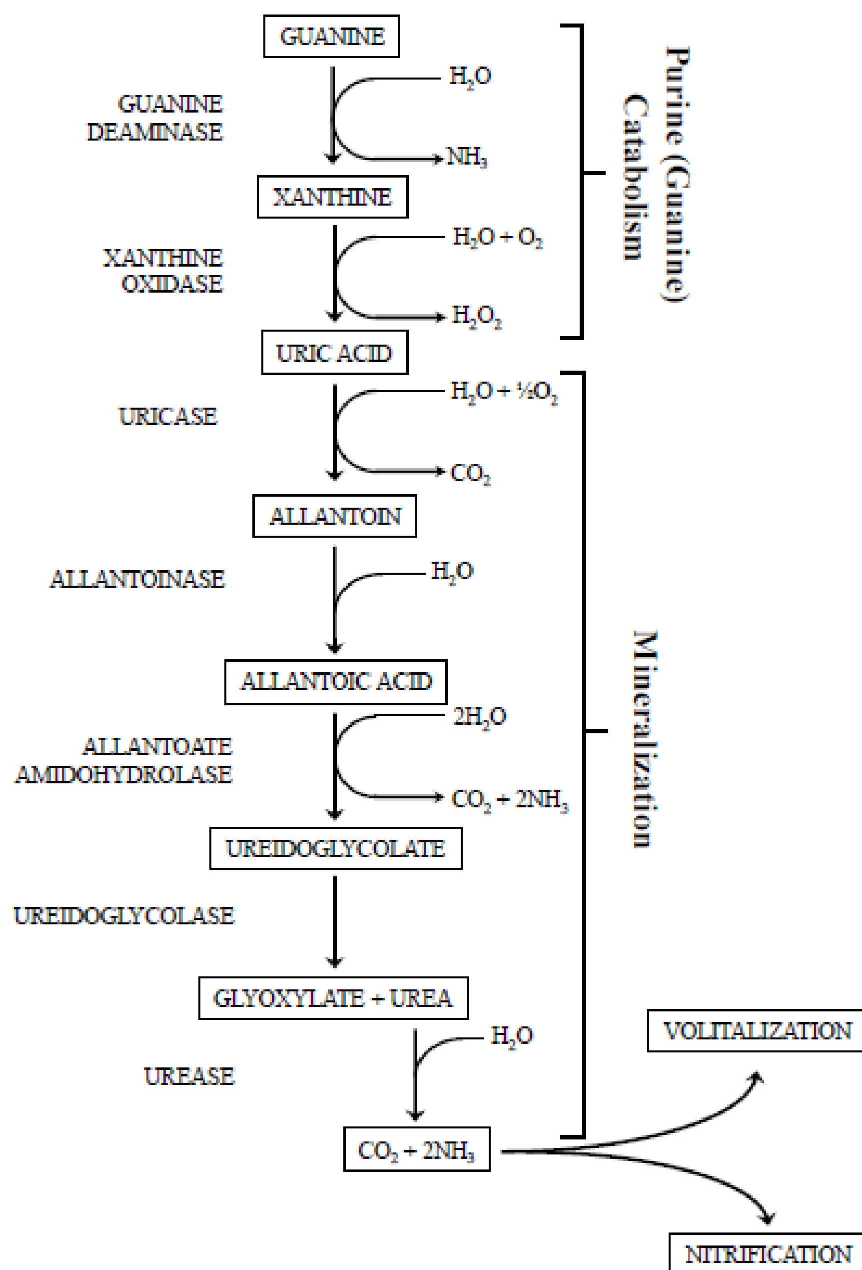



Figure 2.1: Simplified purine (guanine) catabolysis – nitrogen mineralization pathway. Modified from Carlile (1984) and Yuan et al. (1999).

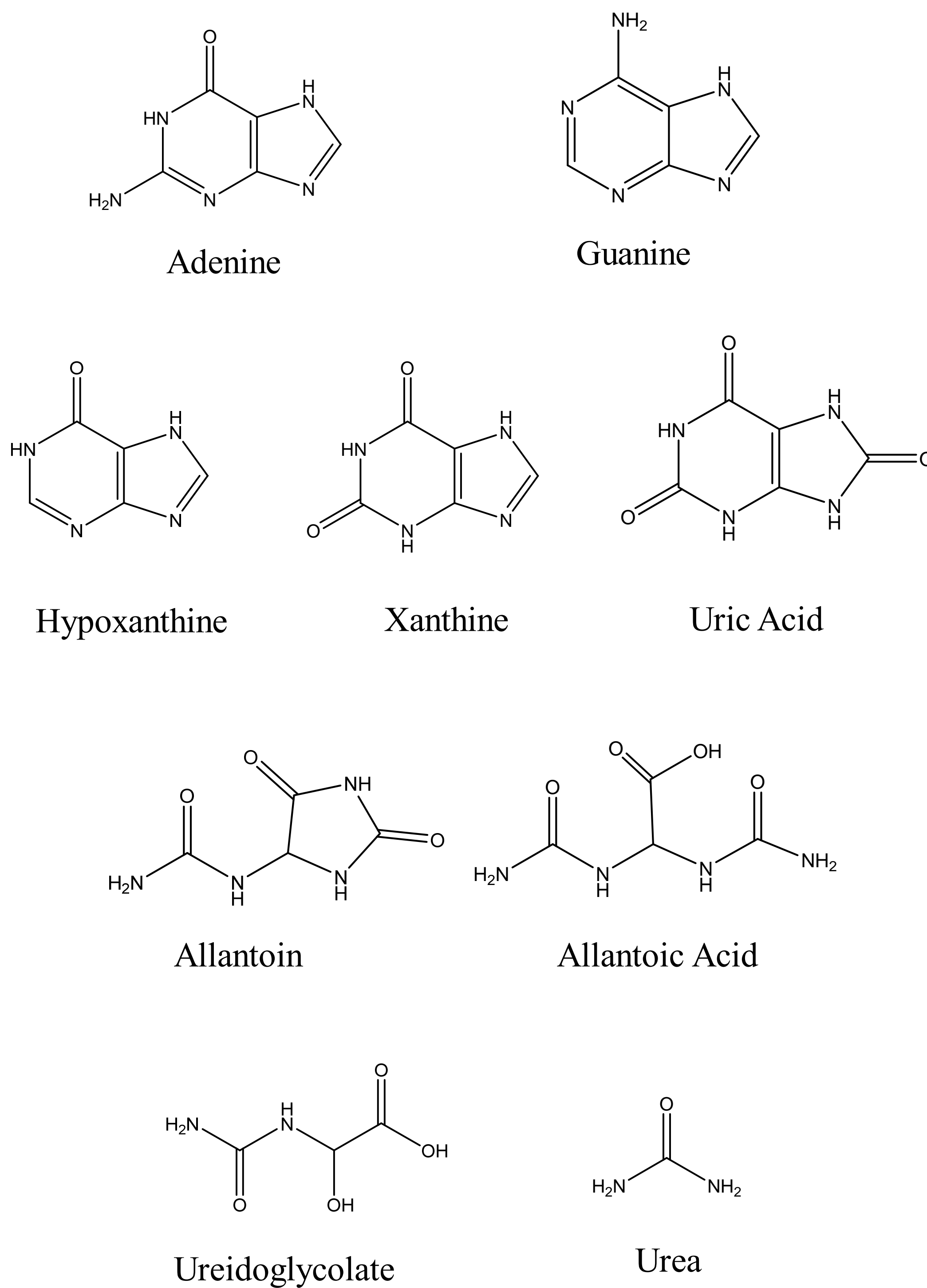



Figure 2.2: Molecular structures of nitrogen compounds in the purine catabolysis - nitrogen mineralization pathway as suggested by Munoz et al. (2001) and Berg and Jorgensen (2006).

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# CHAPTER 3

# NON-DEGRADATIVE EXTRACTION AND MEASUREMENT OF URIC ACID FROM POULTRY LITTER[1]

[1]Mowrer, J., D. Kissel, M. Cabrera, and S. Hassan. 2013. Soil Sci. Soc. Am. J. 77:1413-1417.
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Abstract

Due to limited solubility of uric acid from poultry litter (PL), alkaline solutions are frequently used to extract uric acid from PL for measurement, but the high pH can cause oxidation of the analyte. Our objective was to compare 0.1 M sodium acetate (pH 7.4), 0.025 M $Li_2CO_3$ (pH 11.2) and a NaOH/$Na_2PO_4$ buffer (pH 12.4) as a means of extracting uric acid from PL for analysis by high pressure liquid chromatography (HPLC). In laboratory experiments, extractant-induced degradation of reagent uric acid proceeded at a rate of approximately 80 g $kg^{-1}$ $day^{-1}$ in 0.025 M $Li_2CO_3$ (pH 11.2) and a NaOH/$Na_2PO_4$ buffer (pH 12.4) compared with 0 g $kg^{-1}$ $day^{-1}$ in sodium acetate (pH 7.4) over a two-day period. In extracted PL samples, the alkaline extractants were found to enhance the enzymatic oxidation of uric acid to completion in $Li_2CO_3$ and by 800 g $kg^{-1}$ in NaOH/$Na_2PO_4$ buffer over the same two-day period. Sodium acetate extraction resulted in minimal loss of analyte (30 g $kg^{-1}$), possibly due to thermal inactivation of the responsible enzyme uricase. Stabilization of the dissolved uric acid extracted from PL samples allowed for the development of a very accurate measurement procedure by HPLC with UV/VIS detection at 290 nm. The method detection and reporting limits described in this work are 2.83 mg and 8.5 mg uric acid-nitrogen $kg^{-1}$ fresh litter respectively. Spike recoveries (n=22) ranged from 88.7 to 109.1%, with mean = 100.1%, median = 98.8, and $\sigma$ = 4.8). Chromatographic separation and measurement took 15 min per sample. Of the 118 PL samples that were analyzed in this study, uric acid-, ammonium-, and nitrate-nitrogen concentrations accounted for an average of 9.4, 17.4, and 1.2 percent respectively of the total nitrogen present in PL.


Abbreviations:

HPLC – High pressure liquid chromatography; MDL – Method detection limit; N – Nitrogen; NIR – Near infrared reflectance; PL – Poultry litter, RPD – Relative percent difference

## Introduction

Uric acid is the final product in the breakdown of nucleotides in avian digestive systems, representing 70% of the total nitrogen (N) in fresh poultry fecal matter (Groot Koerkamp, 1994). Poultry litter (PL, a mixture of poultry manure and bedding material) has been reported to contain concentrations from 1.9 to 27.8 g uric acid-N $kg^{-1}$ (Baker, 1946; Gale et al., 1991; Eiteman and et al., 1994; Gordillo and Cabrera, 1997). Decomposition of uric acid in poultry houses releases volatile ammonia (Rothrock et al.,2010) , which can be harmful to the health of flocks in production. Furthermore, when PL is applied to agricultural or grazing lands, uric acid is an important source of plant-available N and a useful chemical index for estimating mineralizable N that can be used as a nutrient by crops and forages (Gordillo and Cabrera, 1997). These authors found that the mineralizable N in PL could be closely estimated (r2 = 0.95) from uric acid and the carbon: nitrogen ratio. Therefore, the accurate measurement of uric acid in PL is important to researchers in the fields of poultry science and nutrient management.

Several methods for the determination of uric acid have been published. Clinical applications for measurement of biological fluids have used colorimetric detection of uric acid oxidation byproducts (Kageyama, 1971), or have utilized electrochemical-oxidation-electrode methods (Manea et al., 2006; Safavi et al. 2006). The clinical methods are reported to be sensitive to the presence of organic materials that may interfere with uric acid oxidation. Thus, the high concentration of organic-oxidizing compounds in PL raised concerns about these approaches. Consequently, a logical approach appeared to be chromatographic separation coupled with UV/VIS detection as done by Eiteman et al. (1994). In preliminary studies with the method of Eiteman et al. (1994), however, we found spike recoveries in the range of 73 to 91%. Laboratories that analyze PL and provide recommendations for fertilization with this material are interested in developing a calibration for the measurement of uric acid in PL by near infrared reflectance (NIR) spectroscopy. NIR offers rapid, accurate analysis of a wide range of compounds in a diverse set of materials; however, the calibration process requires a laboratory reference method with a high degree of accuracy (Workman, Jr., 2001). The desired level accuracy was not achieved with the method of Eiteman et al. (1994) as indicated by the low spike recoveries. The goal of Eiteman et al.

(1994) was to measure multiple compounds in poultry litter including uric acid, oxonic acid, allantoin, and others. The authors reported that some compromises had been made in the measurement of some of the analytes so that simultaneous analysis of all could be achieved. In our preliminary study using the method of Eiteman et al. (1994), we suspected that the observed low recoveries of uric acid in spiked samples were due to the degradation of analyte in the presence of alkaline extractant solution. Fridovich (1965) described the oxidation of uric acid to oxonic acid when stored in alkaline solutions. The extractant suggested by the method of Eiteman et al. (1994) is 0.025 M $Li_2CO_3$ with a pH of 11.2. This presented a problem because uric acid is only sparingly soluble in neutral and acidic solutions (Finlayson and Smith, 1974). Extractants of neutral pH have been used to avoid alkaline degradation, including lead- and sodium acetate solutions (Terpstra and De Hart, 1974). We chose sodium acetate because it is the less hazardous of the two. To eliminate the possibility that final solution pH between samples might be causing variable solubility of the uric acid, a buffered alkaline extractant was additionally considered. Consequently, the objective of our study was to improve the uric acid extraction procedure by comparing 0.1 M sodium acetate (pH 7.4), 0.025 M $Li_2CO_3$ (pH 11.2) and a NaOH/$Na_2PO_4$ buffer (pH 12.4) as a means of extracting uric acid from PL for analysis by HPLC.

**Materials and Methods**

To obtain poultry litter samples that were representative of a wide range of properties (Table 2.1), we randomly selected 118 samples that were submitted by poultry producers for analysis of nutrient content to the University of Georgia Soil, Plant, and Water Analysis Laboratory (2400 College Station Rd., Athens, Ga.) over a period of 4 months. An amount of litter equaling approximately 2/3 the volume of a sealable 1L plastic bag was prepared by placing it in a commercially available food grinder (Asia Kitchen Machine, Sumeet Centre Inc, Toronto, ON) at low power for ~ 30 s. Although no attempt was made to truly homogenize the ground material, the resulting fineness was such that the largest particles could pass through a number 5 U.S.A. Standard Testing Sieve (4.00 mm). Samples were stored in a refrigerator (< 4°C) in the bags until extraction.

**Extraction:** Three extraction treatments were tested in this study. In each, two grams of poultry litter (fresh weight) were weighed into 500-mL conical flasks. The neutral extracting solution (500 mL 0.1 M sodium acetate) was added and stirred vigorously with glass stir rods for 10 seconds to fully suspend the ground PL. The top of the flask was covered with aluminum foil and placed in a water bath at 50°C for 2 h. This was followed by sonication for 15 min. All standards solutions were made by the same procedure. When preparing calibration standards in this manner with reagent grade uric acid (Acros Organics; > 99%), we observed that portions of larger granules of uric acid reagent could occasionally persist after 2 h. Addition of the sonication step was found to complete the dissolution when it was performed following the water bath procedure. Extraction with the two alkaline extractants (0.025 M $Li_2CO_3$ and $NaOH/Na_2PO_4$ buffer) was performed as per Eiteman et al. (1994). This method does not employ heating, but we did include the final sonication step. Calibration standards for each extraction treatment were made up in the same solution as the treatment. Observations reported in Figure 3.1 were the result of repeated analyses of A) pure reagent grade uric acid (100 mg $L^{-1}$) dissolved in each of the three extractants and B) uric acid extracted from PL in each of the three extractants over a 44 hour period of the same samples placed on the HPLC autosampler.

**Dilution:** Following extraction, a 1-mL aliquot was taken from the extraction solution and diluted to 10 mL final volume with 0.1 M sodium acetate. This dilution placed most samples in the appropriate concentration range for analysis, though lesser or no dilution was occasionally required when the analyte concentrations were very low. A portion of this solution was filtered directly into a 2- mL target vial for HPLC analysis using a Luer-type syringe fitted with a 0.45-µm polypropylene filter.

**Analysis** of uric acid in diluted extracts was performed on a Hewlett-Packard series 1100 HPLC instrument with degasser and autosampler (Hewlett-Packard, Palo Alto, CA). The injection volume was 20 µL with a flow rate of 1 mL $min^{-1}$. Separation was achieved using an ODS-2 Hypersil LC column (250 x 4.6 mm; 5 µm particle size). Mobile phase was 0.05 M $KH_2PO_4$ prepared from HPLC grade crystalline reagent. A UV/VIS detector (290 nm) was used to quantify the extracted uric acid as it was eluted. The wavelength of detection (215 nm) used by Eiteman et al. (1994) was described by the authors

as a compromise to measure all of the analytes of interest. However, our goal was to measure uric acid alone as accurately as possible. For this purpose we chose a wavelength (290 nm) with a high specificity for uric acid, as demonstrated by Finlayson and Smith (1974), with the result that we were able to eliminate potential interfering peaks resulting from compounds unrelated to the present study. This modification also allowed us to reduce the analysis time from 30 to 15 min with no carry-over from previous samples. Column pressure and elution time varied from 8.3 to 9.4 bar MPa and 8.5 to 10 min respectively during the course of the study.

Ammonium and nitrate were extracted from litter samples (prepared as previously described) by shaking 1 g with 100 mL 2 M KCl for 30 min. Shaking was immediately followed by gravimetric filtration through a Whatman number 1 filter (GE Healthcare Life Sciences, 800 Centennial Ave. Piscataway, NJ 08854). Ammonium was analyzed by gas-diffusion conductance (Carlson, 1978) on the Timberline TL2800 ammonia analyzer (Timberline Instruments, LLC, 1880 S. Flatiron Ct. Unit 1, Boulder, Co. 80301). Nitrate was determined using the Griess-Ilosvay technique (Keeney and Nelson, 1982) on an ALPKEM RFA 300 autoanalyzer. ALPKEM has since been purchased by OI Analytical (P.O. Box 9010, College Station, Tx 77842-9010). Total N was analyzed by the Dumas combustion method (Nelson and Sommers, 1982) on the LECO CNS 2000 (LECO Corporation, 3000 Lakeview Ave. St. Joseph, Mi 49085). Total N was measured on an as is basis.

**Calibration**: The method of external calibration was used to calibrate the HPLC instrument response as peak area. Calibration curves were calculated using a linear equation and constructed from the following concentrations of uric acid: 0.1, 0.5, 1.0, 5.0, and 10.0 mg $L^{-1}$ in 0.1 M sodium acetate. Calibration concentrations were prepared by appropriate dilution from a stock solution of 100 mg $L^{-1}$ taken through the same procedure as sample extraction described above. Calibrations were performed daily and immediately prior to analysis of samples. Baselines were set manually with a consistent procedure for all calibrants, quality controls, and samples. Calibrants, standards and sample fortifications were prepared from reagent grade uric acid (Acros Organics, reagent grade, >99% purity).

**Detection Limit**: An effective method detection limit (MDL) for the procedure was calculated using the last 8 values of the 0.5 mg $L^{-1}$ standard and multiplying the standard deviation of these values by the single tailed t-statistic. This is a modification of the method described in the EPA document SW 846 (USEPA, 1984).

**Quality Control**: Measures taken to ensure the integrity of the data were as follows. Demonstration of accuracy in measurement for the procedure was accomplished through fortifying and mixing at least one litter sample per analytic batch with 50 mg uric acid reagent in the flasks prior to addition of extractant solution. Spike recoveries were calculated by subtracting the original sample concentration from the spiked sample and dividing this result by the concentration of added analyte. Additionally, a modified method of standard additions (Bader, 1980) was employed to test the validity of external calibration against the presence of potential matrix interferences. Incremental amounts of uric acid reagent (0, 20, 40, 60, 80 & 100 mg) were added and mixed with litter samples as in the sample fortification procedure. All samples not receiving fortification were analyzed in duplicate to test the precision of the procedure.

**Results and Discussion**

**Preliminary work**: initial attempts to measure uric acid in poultry litter samples using the method of Eiteman et al. (1994) with detection at 290 nm resulted in unsatisfactory recoveries of fortified samples (n = 10; range = 73.1 - 91.4%; mean = 80.8%; median = 81.2%). The method employed an extracting solution of 0.025 M $Li_2CO_3$ at pH 11.2, but variation in litter composition resulted in variable pH values for the final extractant solution. To reduce the influence of variable pH, we used a buffered solution of 0.01 M $Na_2HPO_4$ and 1 mL 50/50 NaOH $L^{-1}$ at pH 12.35, but saw no improvement in results.

We analyzed 118 litter samples (Table 3.1) using 0.1 M sodium acetate, which was found to be very effective compared to alkaline solutions in preventing analyte degradation (Fig. 1). Degradation of reagent grade uric acid in both alkaline extractants was very similar. Disappearance of uric acid proceeded at a rate of approximately 80 g $kg^{-1}$ $day^{-1}$ in 0.025 M $Li_2CO_3$ and the NaOH/$Na_2PO_4$ buffer over a 44 hour time period. During the same period, we observed no analyte loss in the sodium acetate

solution. In solutions containing extracted PL samples and reagent grade uric acid, the loss of analyte in the two alkaline extractants was even more pronounced. Use of the NaOH/$Na_2PO_4$ buffer (pH 12.4) to extract PL resulted in a loss of 800 g analyte $kg^{-1}$ PL, while use of the $Li_2CO_3$ solution (pH 11.2) resulted in complete loss of the analyte in 44 hours. The difference may possibly be attributed to a pH in the $Li_2CO_3$ solution closer to the reported optimum pH of 9 for uricase activity (Baum et. al., 1956). Furthermore, it was observed that the analyte in sodium acetate sample extractions was protected from enzymatic degradation for a period of at least two days. This is assumed to be due to thermal inactivation of uric acid oxidase (Finlayson and Smith, 1974). Uric acid concentrations in fresh litter averaged 7789 mg $kg^{-1}$. Uric acid-N averaged 3769 mg $kg^{-1}$ in dry litter. This is in the range reported by previous authors (Gordillo and Cabrera, 1997: Baker, 1946; Eiteman et al., 1994; and Gale et al., 1991). The average uric acid-N accounted for 9.4% of the total N available in the samples used in this study, as compared to 17.4% ammonium-N and 1.2% nitrate-N.

**Calibration:** external calibration was performed immediately prior to each of the 22 analytic batches. The standard linear dynamic response range extended from 0.1 mg $L^{-1}$ to 100 mg $L^{-1}$. Parameters for the linear calibration models are presented in Table 3.2 in chronological order. The slopes (m) show good stability over the course of the study; however the general trend from 0.0102 to 0.0092 might be explained by equilibration of the column or increased facility with the procedure over time. Y-Intercepts (b) are generally positive but always below the proposed reporting limit in solution of 0.1 ppm uric acid. The model fit parameters (r2) are all exceptional over the calibration range (0.1 – 10 ppm).

**Detection Limit:** Analysis of the calibration models indicates that 0.5 mg uric acid $L^{-1}$ is a conservative reporting limit for this procedure as all standards at this level were measured to be within 20% of the expected value. Inclusion of a standard at 0.1 mg $L^{-1}$ occasionally returned a residual exceeding 30% of the expected value. In every case, forcing the model through the origin improved this residual to within 20% of the expected value. However, the data gathered in this study were calculated without forcing the intercept through the origin. An effective method detection limit (MDL) for the procedure was calculated using the last 8 values of the 0.5 mg $L^{-1}$ standard. Multiplying the standard

deviation of these values by the single tailed t-statistic (n-1 = 7; p = 0.99) returned an MDL of 0.034 mg $L^{-1}$ uric acid. The 'in sample' MDL for the procedure is therefore 8.5 mg uric acid or 2.8 mg uric acid-nitrogen $kg^{-1}$ PL. Estimation of a practical quantitation or reporting limit was made by application of a 3x factor to the MDL resulting in minimum reportable value of 25.5 mg $kg^{-1}$ uric acid (8.5 mg $kg^{-1}$ uric acid-nitrogen) in the litter sample.

**Quality Control:** Accuracy of the procedure as measured by recovery of added analyte indicates that it is suitable for routine analysis of uric acid in PL. Spike recoveries (n=22) ranged from 88.7 to 109.1%, with mean = 100.1%, median = 98.8, and $\sigma$ = 4.8).

**Matrix interference** on the accuracy of the procedure was found to be minimal as investigated by a modified method of standard additions. Results are presented in Figure 3.2. Slopes and intercepts of four samples are within the range observed by the method of external calibration. The slope of a fifth sample identified as 1121 is outside this range, though the calculated concentration of uric acid for this sample differs only by 16% RPD (Table 3.3).

**Precision** was found to be very good despite the minimal homogenization of the litter samples. Duplication was also satisfactory. Whenever the relative percent difference between replicate analyses exceeded 15%, a third replicate of the sample was analyzed. This occurred with seven samples and in all cases the third replicate was sufficient to reduce the relative standard deviation to less than 15%.

**Conclusions**

Improvements to the procedure described in Eiteman et al. (1994) were effected by applying the following modifications:

1) Alkaline degradation of uric acid by the extractant is eliminated by the change to 0.1 M sodium acetate.
2) Change in wavelength of measurement from 215 nm to 290 nm takes advantage of high absorbance specificity for uric acid and minimizes detection of interfering compounds. This modification allowed for a reduction in analysis time from 30 to 15 minutes.

3) Substitution of a separation column of similar chemical and physical properties at significantly lowered cost produces high quality and reproducible results over hundreds of analyses.

Modification of the extractant and measurement conditions described by Eiteman et al. (1994) has resulted in a non-degradative procedure for the analysis of uric acid in poultry litter. This new method leaves the analyte abiotically stable in neutral solution for several days and protected from biotic degradation through thermal inactivation of uric acid oxidase. This effect of temperature on the activity of the enzyme was explored by Pitts et al., (1974). A brief examination of the effect of temperature on extraction indicated that thermal inactivation could be avoided by extraction at 30°C for 2 hours, though comparative recoveries were approximately 85 to 90% (data not presented). The relatively short time required for the separation step will allow for a large number of analyses in a given day and the sensitivity and dynamic range allows for the generation of quality data on uric acid content of poultry litter. The MDL for this procedure is estimated to be 2.83 mg uric acid-nitrogen $kg^{-1}$ 'as is' litter with a lower limit for reporting at 8.5 mg uric acid-nitrogen $kg^{-1}$ 'as is' litter.

118 samples of PL ready for field application were analyzed by the method outlined in this study. The inorganic plant available forms of nitrogen, ammonium and nitrate, together accounted for less than 20% of the total N in the samples. Uric acid, an important reservoir in PL of potentially mineralizable organic N, accounted for 10% of the total N, or up to 30% of the N that may be plant available within a growing season.

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# TABLES

Table 3.1: Selected characteristics of poultry litter. Given are the minimum, maximum, mean, median, and standard deviation values for uric acid in fresh litter and uric acid-nitrogen dry weight litter. Water content of the litters in the study is provided, as well as percentage of the total nitrogen in the litters studied supplied as uric acid-, ammonium-, and nitrate-N.

| | **Uric Acid** | **Uric Acid-N** | **Water Content** |
|---|---|---|---|
| | mg $kg^{-1}$ Fresh Litter | mg $kg^{-1}$ Dry Litter | g $H_2O$ $kg^{-1}$ Dry Litter |
| **Min** | 26 | 15 | 153 |
| **Max** | 43528 | 17910 | 1276 |
| **Median** | 7474 | 3625 | 418 |
| **Mean** | 7789 | 3769 | 458 |
| **SD** | 5764 | 2603 | 177 |

| | **Uric Acid-N** | **Ammonium-N** | **Nitrate-N** |
|---|---|---|---|
| | % of Total Nitrogen | % of Total Nitrogen | % of Total Nitrogen |
| **Min** | 0.07 | 3.46 | 0.00 |
| **Max** | 31.81 | 55.58 | 21.74 |
| **Median** | 9.36 | 13.82 | 1.01 |
| **Mean** | 9.43 | 17.37 | 1.15 |
| **SD** | 5.46 | 7.65 | 4.03 |

Table 3.2: Parameters for 22 5-point linear calibration models of the type y = mx + b, where y is the concentration of uric acid in extraction solution (mg $L^{-1}$), m is the slope, x is the instrument response as peak area, and b is the line intercept with the y-axis. Order = chronological.

| **analytic batch** | **Slope (m)** | **y-intercept (b)** | **$r^2$** |
|---|---|---|---|
| 1 | 0.0102 | -0.0065 | 1.0000 |
| 2 | 0.0103 | 0.0311 | 1.0000 |
| 3 | 0.0107 | -0.0089 | 1.0000 |
| 4 | 0.0103 | 0.0348 | 0.9998 |
| 5 | 0.0101 | 0.0220 | 0.9997 |
| 6 | 0.0101 | 0.0373 | 0.9996 |
| 7 | 0.0100 | -0.0048 | 1.0000 |
| 8 | 0.0095 | 0.0053 | 1.0000 |
| 9 | 0.0095 | 0.0044 | 1.0000 |
| 10 | 0.0097 | 0.0286 | 0.9998 |
| 11 | 0.0097 | 0.0261 | 0.9999 |
| 12 | 0.0098 | 0.0316 | 1.0000 |
| 13 | 0.0091 | 0.0080 | 1.0000 |
| 14 | 0.0101 | 0.0079 | 1.0000 |
| 15 | 0.0091 | 0.0095 | 1.0000 |
| 16 | 0.0094 | -0.0154 | 0.9998 |
| 17 | 0.0094 | 0.0340 | 0.9999 |
| 18 | 0.0093 | -0.0079 | 0.9999 |
| 19 | 0.0094 | 0.0336 | 1.0000 |
| 20 | 0.0093 | 0.0116 | 1.0000 |
| 21 | 0.0093 | -0.0150 | 0.9998 |
| 22 | 0.0092 | 0.0345 | 1.0000 |
| **Mean** | 0.00970 | 0.01372 | 0.99992 |
| **Median** | 0.00960 | 0.01055 | 0.99999 |
| **SD** | 0.00046 | 0.01820 | 0.00012 |

Table 3.3: Comparison of sample uric acid concentrations (mg $kg^{-1}$ fresh litter ) calculated by the method of external calibration and standard additions. Relative percent difference (RPD) = $|x-y|/(|x+y|/2)$.

| Sample Number | Standard Additions | External Calibration | Relative Percent Difference |
|---|---|---|---|
| **1097** | 7010 | 7978 | 12.92 |
| **1102** | 3721 | 3742 | 0.58 |
| **1103** | 6278 | 7018 | 11.13 |
| **1111** | 6825 | 6234 | 9.06 |
| **1121** | 3625 | 4278 | 16.53 |

FIGURES

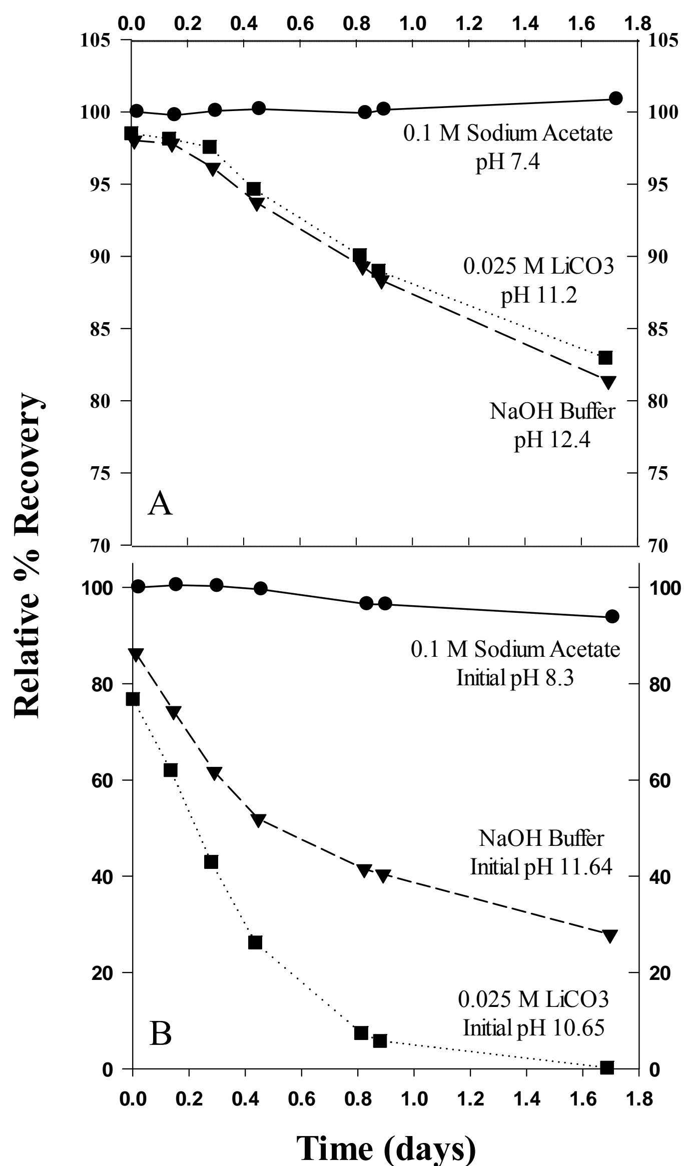


Figure 3.1A: Reagent uric acid degradation in three extractants. The stability of the analyte is compared in the three extractants 0.01 M sodium acetate, 0.025 M lithium carbonate, and a buffered sodium hydroxide solution.

Figure 3.1B: Sample uric acid degradation in three extractants. The stability of the analyte is compared in the presence of the same three extractants and enzymes native to the extracted sample.

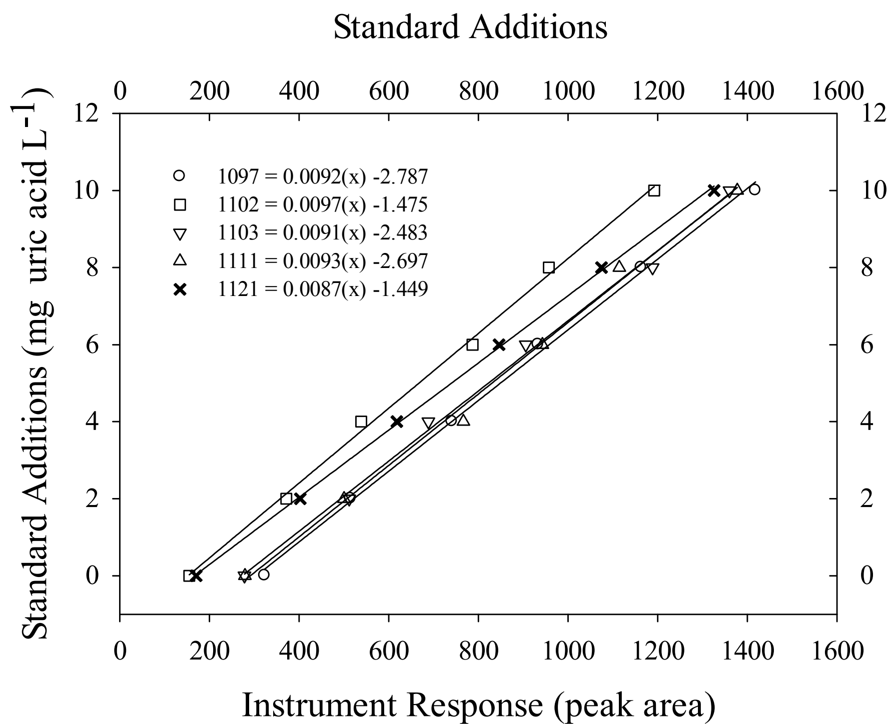


Figure 3.2: Graph of standard additions results for extraction with 0.01 M Sodium Acetate. Plots for samples 1097, 1102, 1103, 1111, and 1121 fortified with 0, 20, 40, 60, 80, and 100 mg $L^{-1}$ extractant fit with linear model of the type y = mx + b.

# CHAPTER 4

# NIR CALIBRATIONS FOR ORGANIC, INORGANIC, AND MINERALIZED NITROGEN FROM POULTRY LITTER[2]

[2]Mowrer, J., D. Kissel, M. Cabrera, and S. Hassan. 2014. Submitted to Soil Sci. Soc. Am. J. 12/14/13

Abstract

Near infrared reflectance (NIR) spectroscopy was evaluated for use in measuring nitrogen (N) forms in poultry litter (PL) important to predicting the mineral plant available nitrogen (PAN), and organic potentially mineralizable nitrogen (PMN). NIR analysis was performed on PL samples of 'as received' variable water content without drying. The samples were not fresh but of varied ages and closely representative of the PL applied to crop and pasture soils in Georgia (U.S.). NIR scans of 118 PL samples were paired with direct measurements and calibration models were developed using modified partial least squares (PLS) regression analysis. The results show that NIR calibration models can be developed on PL with variable $\theta$ for total-N ($r^2$ = 0.896), $NH_4$-N ($r^2$ = 0.795), $NO_3$-N ($r^2$ = 0.926), uric acid-N ($r^2$ = 0.909), organic-N ($r^2$ = 0.821), water soluble organic-N (WSON) ($r^2$ = 0.897), PMN ($r^2$ = 0.842), initial PAN + PMN ($r^2$ = 0.888), total carbon ($r^2$ = 0.926), and water content ($r^2$ = 0.996). PMN was determined in 100-day soil incubations with a Bonifay and an Orangeburg soil. The results of the mineralization study suggest that PMN from PL that has been stored for some period of time prior to land application may be lower than PMN from fresh PL samples. We found that an average of 19% of the organic-N mineralized and an average of 33% of the total N would be available for plant uptake.

Introduction

The poultry industry in the United States generates large quantities of poultry litter (PL), which is a mixture of poultry feces, bedding material (e.g. pine straw, wood shavings, peanut hulls, rice hulls, etc.), wasted feed, feathers, and soil minerals from the floor of the rearing facilities. Though it is difficult to accurately measure the total amount of litter produced by the industry, commonly referenced estimates are 1.46 kg of litter per broiler chicken (Perkins et al., 1964) and 11.2 kg of litter per turkey (Flora and Riahi-Nezhad, 2006). Using these estimates and the Poultry - Production and Value Summary (USDA, 2013), U.S. producers generated approximately 11.3 Tg of broiler litter and 2.8 Tg of turkey litter in 2012.

PL is an increasingly valuable resource for improving soil fertility for crop and forage production as it contains many essential plant macro- and micro-nutrients. In practice, agronomic producers frequently choose to use this material as a less expensive alternative to chemical sources of nitrogen (N) fertilizer. The development of reliable estimates of the N fertilizer value of PL relative to chemical sources is therefore important in preventing wasteful application and in optimizing the economic investment of the producer. However, predicting the N-fertilizer value of PL is made difficult by the nature and variability of the N forms present in the litter at the time of land application. In fresh avian feces, uric acid comprises an estimated 70% of the initial organic N, while undigested proteins make up the remaining 30% (Groot Koerkamp, 1994). With time, these organic N compounds decompose, mineralize, stabilize and/or become immobilized into active biomass. Only the mineral forms ammonium/ammonia ($NH_4^+$/$NH_3$) and nitrate ($NO_3^-$) are immediately available for plant uptake. $NH_4$-N concentrations in the literature range from 0.0045 to 14 g $kg^{-1}$ on a dry matter basis, whereas $NO_3$-N concentrations range from <0.001 to 2 g $kg^{-1}$ (Gordillo and Cabrera, 1997; Qafoku et al., 2001; Ruiz-Diaz et al., 2008; Sistani et al., 2008, and Kokoasse and Genus, 2012). The amounts of $NH_4$-N and $NO_3$-N in PL vary but may average approximately 17% and 1% respectively of the total mass of N present in the dry matter (Mowrer et al., 2013). Aside from these plant available nitrogen (PAN) forms, there is a pool of organic nitrogen which may potentially mineralize to become PAN. This potentially mineralizable

nitrogen (PMN) is expected to be comprised mainly of the more easily hydrolysable organic nitrogen-containing compounds including nucleic material, proteins, amino acids, uric acid, allantoin, glyoxilic acid, and urea (Vogels and Van der Drift, 1976; Nahm, 2005; Rothrock et al., 2010).

The degree to which the organic N pool may mineralize depends upon properties such as pH, water content, and temperature; as well as the chemical, physical, and microbiological properties of the soil to which the PL is applied. Nitrogen mineralization in fresh broiler litter (BL) has been reported to average 50 to 60% of the organic N (Bitzer and Sims, 1988; Gordillo and Cabrera, 1997; Qafoku et al., 2001) with a range of approximately 20 to 100%. Composted litters and manures may only mineralize around 5 to 15% of the organic N present (Hartz et al., 2000; Preusch et al., 2002). In stored or otherwise aged PL, the amount of PMN is expected to be somewhere between that of fresh and composted, depending on the length and conditions of storage.

Currently, most agricultural analysis laboratories recommend that agronomic producers applying BL consider approximately 50 to 60% of the total N (usually measured as total combustion N or total Kjeldahl N) to be available to any particular crop during the first growing year. This includes the sum of initial PAN (PANi) and PMN. Residual available N for subsequent years is assumed to be very low or negligible (Ruiz-Diaz et. al., 2012). The predictive power of this approach has high rate of error as evidenced by the ranges of PMN reported in the above studies. However, direct measurement of PMN takes a considerable amount of time. For instance, Tyson and Cabrera (1993) reported that it takes 56 days for *most* of the PMN in BL to be released in laboratory soil incubations. Subsequent research has suggested that certain components of the PL organic N fraction that are more easily measured may be useful in predicting the PMN available to crops. Agricultural analysis laboratories interested in improving N-fertilizer based recommendations for PL application would benefit from a more rapid measurement of PMN or a suitably related index. Ruiz-Diaz et al. (2008) found that the average PAN in chicken (66% of TN) and turkey manure (58%) was ‘basically the same’ as the water soluble nitrogen (WSN) fraction of total N. Gordillo and Cabrera (1997) reported that PMN in BL (112 day soil incubation) was positively correlated with total N (TN) and uric acid N (UAN) and negatively correlated

with the carbon:nitrogen (C:N) ratio (PMN = 26.68 + 1.04 UAN(g N $kg^{-1}$ PL) – 1.22 C/N; $r^2$ = 0.95). Qafoku et. al. (2001) found a good relationship (PMN = -0.018 + 1.293 WSON (g N $kg^{-1}$ PL); $r^2$ = 0.87) between water soluble organic nitrogen (WSON) and PMN from BL (also 112 day soil incubation). More importantly, this study also resulted in a successful calibration for directly measured PMN in BL using a near infrared reflectance (NIR) spectroscopy instrument.

NIR is an analytical approach that offers the potential for rapid and accurate analysis of many compounds that may take considerably more time when measured by conventional, or 'wet chemistry', methods. Initial investment in the equipment can be high for a routine agricultural laboratory, but cost per analysis is very low. It is more safely operated by technicians than many conventional laboratory procedures and generates no hazardous waste. NIR is a 'secondary' analytical technique that requires the pairing of spectral data gathered by the NIR instrument with reference chemical composition data generated by a 'primary' laboratory analytical technique. Briefly, differences in sample spectra over a range of wavelengths (e.g. 400 to 2500 nm) are statistically regressed against reference chemical data for each sample using partial least squares (PLS), principal components analysis (PCR), artificial neural network (ANN), or other data analysis approach to generate a prediction equation for samples of unknown composition. Because the conventional measurement of the organic N compounds in PL that contribute to PMN can be time consuming and often costly for agricultural laboratories, the procedures are rarely requested by paying clients. Instead, inaccurate estimates of available N from measurements of total N are currently standard practice. Calibration of NIR for initial PAN and PMN in PL, either by direct measurement or some reliable index or portion of the organic N pool, to develop a prediction equation for use in routine analysis would improve the ability of such laboratories to provide accurate estimates of the N-fertilizer value of PL applied to crops.

Reeves (2001) reported good results on the calibration of NIR for $NH_4$-N ($r^2$ = 0.960; RMSD = 0.024%), TN ($r^2$ = 0.943; RMSD = 0.204 %), organic N ($r^2$ = 0.944; RMSD = 0.200 %), and dry matter ($r^2$ = 0.986; RMSD = 0.012 %) in BL gathered from the University of Maryland Soil Testing Laboratory (College Park, MD). The authors noted that the samples had to be reanalyzed to improve results as $NH_4$-N

concentrations had changed since measurement at the Soil Laboratory. Tasistro et al., (2003) published similar results for θ and TN in BL samples received by the University of Georgia Soil Plant and Water Testing Laboratory (Athens, GA). As previously mentioned Qafoku et al., (2001) measured PMN directly and reported a good relationship between NIR-predicted and lab values ($r^2$ = 0.82; SECV = 2.01 g N $kg^{-1}$) on fresh BL gathered from poultry houses. Fujiwara et al. (2007) developed a calibration for UAN in composted poultry manure using NIR. These authors described an extremely close relationship between UAN and mineralized N from soil incubations ($r^2$ = 0.99999, SE = 0.61 mg $g^{-1}$). Xing et al. (2008) obtained successful calibration for water content (θ) ($r^2$ = 0.88, RMSEC = 20.10 g $kg^{-1}$), organic matter (OM) ($r^2$ = 0.88, RMSEC = 6.32 g $kg^{-1}$), total N ($r^2$= 0.92, RMSEC = 1.08 g $kg^{-1}$), and $NH_4$-N ($r^2$= 0.92, RMSEC = 0.77 g $kg^{-1}$) in layer manure. Chen et al. (2009) demonstrated that artificial neural networks (ANN) produced an NIR calibration equation for $NH_4$-N ($r^2$ = 0.93; RMSEC = 0.71 g $kg^{-1}$) slightly superior to that produced by the partial least squares (PLS) approach ($r^2$ = 0.92; RMSEC = 0.77 g $kg^{-1}$).

Aside from the studies by Reeves (2001) and Tasistro et al. (2003), the above NIR calibration experiments were performed using litters that were dried (most often lyophilized) and finely ground. The purpose of drying samples for NIR analysis is to remove spectral interference arising from differences in water content between samples in a calibration or prediction set (Workman, Jr., 2001). The freeze drying process requires expensive equipment and takes 36 to 48 hours for complete lyophilization of small batches of samples. The use of low temperature ovens and air drying processes can improve the speed of this step for routine analysis laboratories; however, any form of drying during the sample preparation process has been demonstrated to cause losses of $NH_4$-N and changes in the organic N and carbon (C) content of the sample (Mahimairaja et al., 1990; Wood and Hall, 1991). Homogenization of samples by finely grinding improves repeatability of analyses and has been recommended to reduce NIR spectral noise arising from differences in particle size between samples (Workman, Jr., 2001), yet grinding PL to such fine particle size increases the surface area to volume ratio of the material in such a way as to potentially increase the availability of the organic N fraction to microorganisms responsible for

mineralization. Van Kessel et al. (1999) found drying and grinding of manure slurries to be "unsuitable" for the purpose of investigating N mineralization. Laboratory and field studies performed on samples prepared in this way may therefore not reflect the behavior of materials being used under actual crop and forage production conditions. Moreover, sufficient evidence has been presented to call into question the value of routine laboratory analyses conducted on samples prepared by drying and finely grinding.

In this work, we sought to determine the potential for developing NIR calibrations for N fractions and/or indices related to the prediction of PMN from PL on samples receiving only minimal homogenization and no drying. Because agricultural and State Extension laboratories are often called upon to make N fertilizer equivalence recommendations on all forms of PL, we sought to study various sources of chicken PL, identified as broiler, hen (layer), breeder, and pullet. Total N, organic N, $NH_4$-N, $NO_3$-N, UAN, PMN, WSON, θ, and total carbon (TC) were directly measured on over 100 poultry litter samples prepared in this way. NIR calibrations developed using these samples would theoretically be based on material with unaltered N and C mineralization patterns. Furthermore, the added value of rapid analysis by NIR to agricultural laboratories would not be diminished by the cost of drying and grinding equipment or the time involved in sample preparation. We also compared the results of NIR calibration and changes in nitrogen chemical composition on samples receiving no drying (ND), vacuum drying in a desiccator box (VD), or freeze drying (FD).

**Materials and Methods:**

Poultry litter samples representing a wide range of chemical and physical properties (Table 4.1) were obtained from the University of Georgia Soil, Plant, and Water Laboratory (Athens, Ga.). The samples were randomly selected from litters submitted by clients of the lab and stored for less than a week at <4° C until prepared for analysis by grinding for approximately 30 seconds in a food/spice grinder (Asia Kitchen Machine, Sumeet Centre Inc, Toronto, ON) to pass through a 5 mm sieve. A volume of sample approximately 0.7 L was stored frozen in a resealable plastic bag prior to and between analyses. Unless specifically noted below, all analyses were performed on samples that received no drying (ND). Freeze dried (FD) samples were prepared using a Labconco Freezone 4.5 benchtop

lyophilizer (Labconco, Kansas City, Mo.) which required between 36 to 48 hours for samples to reach constant weight. Vacuum dried samples (VD) were prepared by placing 5-10 g in aluminum weigh pans in an airtight desiccator with granular desiccant and applying vacuum pressure until constant weight was achieved (10 days). Total water content was measured gravimetrically after drying at 65°C for 16 hours.

TN and TC were measured by combustion (Nelson and Sommers, 1982) using the LECO CNS 2000 (LECO Corp., St. Joseph, MI). $NH_4$-N and $NO_3$-N were extracted from samples by placing 1 g in a conical flask with 100 mL 1M KCl and shaking on an orbital shaker for 1 hr. $NH_4$-N was measured in filtered extracts by gas diffusion conductance (Carlson, 1978) using a Timberline TL2800 ammonia analyzer (Timberline Instruments, Boulder, CO.). $NO_3$-N in filtered extracts was determined by the Griess-Ilosvay technique (Keeney and Nelson, 1982) on an ALPKEM RFA 300 auto analyzer (OI Analytical; College Station, TX). PAN was calculated as the sum of $NH_4$-N and $NO_3$-N. ON was calculated as TN - PAN. UAN was measured by the method of Mowrer et al. (2013) using a Hewlitt-Packard series 1100 HPLC instrument with degasser and autosampler (Hewlett-Packard, Palo Alto, CA.). WSON was extracted by shaking a 0.5g sample in conical flasks with 100 mL deionized water on an orbital shaker for 30 minutes. After settling, an aliquot was carefully pipetted from the midpoint of the solution to avoid suspended particles. Ten mL of this aliquot was digested by the Kjeldhal method. $NH_4$-N in the WSON extract and Kjeldahl digest were measured by gas diffusion conductance as previously described. WSON was then calculated as the difference between Kjeldahl-N and $NH_4$-N in the extracts.

Two incubation studies were performed to measure PMN in PL. **Incubation study #1**: PMN was measured directly on fifty PL samples incubated with a Bonifay series soil (Table 4.2) by mixing 0.2 g ND PL with 10 g soil adjusted to a water content 80% of that at a water potential of -0.01 MPa in 50-mL plastic conical centrifuge tubes. The tubes were placed in racks of 24 lightly covered with aluminum foil in an incubator at 30°C for 100 days. All samples and the control soil were incubated in duplicate. Water content was monitored for gravimetric loss and the mass of water replaced on a daily basis. The soils were extracted without drying after 100 days using 50 mL 2 M KCl and measured for total mineral

N ($NH_4$-N + $NO_3$-N) by gas diffusion conductance with zinc reduction (Mansell et al., 2000). PMN was calculated by subtracting mineral N at 100 days in a control sample (soil only) plus initial mineral N (0 days) in the PL sample from the mineral in soils receiving PL at 100 days as per Qafoku et al. (2001).

**Incubation study #2:** ND PL was mixed with 50 g of an Orangeburg series soil (Table 4. 2) at a rate equivalent to 200 mg ON $kg^{-1}$ soil. The soil water content was again adjusted to 80% of that at a water potential of -0.01 MPa for this soil and placed in a specimen cup with the lid removed. The specimen cups were placed inside 1-L wide mouth glass jars with screw top lids. To prevent sample desiccation, 0.5 cm depth of deionized water was placed in the bottom of the jar. To trap and measure volatilized ammonia, 30 mL 0.1 N HCl in a 50-mL conical bottom centrifuge tube was placed inside the jar adjacent to the specimen cups containing the soil. To remove respired $CO_2$ and maintain an aerobic environment, 30 mL 1 N NaOH in a 50-mL conical bottom centrifuge tube was also placed inside the jar adjacent to the specimen cups. The lids were screwed on tightly and were removed for 1 minute in a fume in an ammonia free laboratory once a day for the first week and every 2 to 3 days for the remaining 93 days of the incubation period. Due to space constraints, the 102 jars containing 50 samples and control in duplicate were incubated on the same lab bench at room temperature (normal range 20-25°C) rather than in a temperature controlled incubator. PMN in incubation #2 was measured as in incubation #1 with the modification that the $NH_4$-N trapped by the HCl was analyzed by gas diffusion conductance and added to the PMN in each experimental unit. NIR scanning was performed on a FOSS NIRSystems 6500 instrument (FOSS North America, Eden Prairie, MN) which uses a combination of silicon and lead sulphide detectors. Samples were placed in ring cups and scanned using wavelengths from 750 to 2498 nm. Second derivative treatments of the spectra were found to produce better results than first derivative and so were used throughout the study. TN, $NH_4$-N, and UAN were chosen as test parameters for three drying treatments: no drying (ND), freeze-drying (FD), and vacuum drying (VD). For the ND treatment, TN and $NH_4$-N were measured on as received samples. For the treatments FD and VD, samples were reanalyzed following the drying process for TN and $NH_4$-N. UAN was not reanalyzed, but was recalculated based on the water removed from the dried samples. Three samples with values below 200

mg $kg^{-1}$ UAN were omitted from the calibration calculations in the software as the NIR technique is not suited to detecting low concentrations (Workman, Jr., 2001). Additionally, coefficients of determination ($r^2$) can be artificially inflated while relative differences from the model at the lowest range of concentrations also increase. Compositional outliers were removed from the model on the basis that the difference between predicted and laboratory measured values exceeded three standard deviations from the mean difference (Reeves, 2001).Analysis of the NIR spectra was performed by the software package WINISI WINSCAN v1.50 (FOSS North America, Eden Prairie, MN).

**Results**

**Drying Method:**

Fifty samples were scanned using NIR to test the effect of variable 'as received' θ of PL samples on the development of calibration equations. The variable θ appeared to cause some interference in the process of developing calibrations as evidenced by the higher coefficients of determination and lower standard errors of prediction (SEP) and corrected standard errors of prediction (SEP(C)) for the FD and VD treatments. For the calibrations for TN (Figure 4.1) based on ND, FD, and VD samples; $r^2$ = (0.899, 0.964, 0.929), SEP = (2179.191, 1594.950, 2122.84), and SEP(C) = (2196.905, 1605.433, 2138.223) respectively. However, one compositional outlier was identified and removed from the FD set which substantially reduced the prediction error. Calibrations for $NH_4$-N indicated more interference from variable water content than for TN. The results presented in Figure 4.2 show values for $r^2$ (0.752, 0.964, 0.973), SEP (597.298, 317.793, 236.466), and SEP(C) (604.048, 320.901, 238.867) for the ND, FD, and VD sets respectively. Five compositional outliers were removed from the ND set and two were removed from the FD set. The lower coefficient of determination ($r^2$) and the larger errors of prediction may be a function of the differences in response for dissolved and solid (anhydrous) forms of $NH_4/NH_3$. The principle of NIR analysis relies on patterns of detector response at wavelengths related to molecular bond vibrations induced by absorption of NIR energy of specific frequencies. Vibrational frequencies of molecules are affected by dissociation in aqueous solution ($NH_4^{1+} + OH^{1-} \leftrightarrow NH_3^0 + H_2O$) and by complexation with anionic species (e.g. $Cl^{1-}$, $NO_3^{1-}$, $PO_4^{3-}$, etc.) (Workman, Jr., 2001). Removal of water

from the PL samples has a positive effect on the ease of developing calibrations for $NH_4$-N, which could be partially attributable to the reduction in dissolved species. The results of calibrations for TN and $NH_4$-N developed on the dried sample sets are similar to those reported in previous studies using dried samples (Reeves, 2001, Fujiwara and Murakami, 2007, Chen et al., 2009). The calibration for UAN in these samples was most successful for the ND treatment (Figure 4.3). Nine compositional outliers were identified and removed from the ND treatment while eight each were removed from the sets receiving some drying. Values for $r^2$ were (0.893, 0.807, 0.8015), SEP (434.493, 880.766, 891.204), and SEP(C) (438.485, 892.280, 902.854) for the ND, FD, and VD treatments respectively. This indicates that some alteration of the UAN contents of these samples occurred during both FD and VD drying treatments.

Directly measured changes in TN and $NH_4$-N (Figure 4.4) following drying suggest that, while NIR calibrations may be improved on these samples, the chemical composition has been altered to a degree that produces an analytical result unrelated to that of the originally submitted material. Multiple comparisons analysis using Tukey's HSD (R Development Core Team, 2013) on the lab derived results for the 50 samples used in the drying study show that the mean concentrations of TN for the ND treatment differ from the FD and VD treatments at >95% confidence level with adjusted p values of 0.03137 and 0.000112 respectively. The means of the $NH_4$-N contents for ND differ from the means of the FD and VD treatments at a >99% confidence level with adjusted p values of 0.008061 and 0.0000004 respectively. Average changes in TN concentrations following drying were 11% (FD) and 20% (VD), while average changes in $NH_4$-N concentrations were 22% (FD) and 39% (VD). Differences between the drying treatments FD and VD were significant at the 95% level for the $NH_4$-N concentrations indicating that freeze drying caused less net loss of ammonium than vacuum drying. These observations emphasized to us the importance of avoiding the drying of PL samples prior to analysis for nitrogen. However, this study clearly demonstrated the potential for NIR as an analytic tool for samples with 'as received' $\theta$. This allows agricultural laboratories to measure N forms in PL without altering the chemical composition of the samples prior to analysis.

**NIR calibration with variable water content:**

Sets of fifty samples are rarely enough to develop a routine calibration equation for NIR analysis (Workman, Jr., 2001). We felt that by pursuing an expansion of the calibration sets used for TN, $NH_4$-N, UAN, and other forms of N identified as important to the prediction of PAN and PMN, we would be able to observe improvements to the models and the errors of prediction for calibrations based on samples with 'as received' θ. In all, we attempted calibrations for TN, $NH_4$-N, UAN, $NO_3$-N, WSON, PMN, TC, and finally θ so that concentrations could be expressed on a dry weight basis when needed. One hundred poultry litter samples were measured for all parameters except WSON (63 samples) and UAN (118 samples), and PMN (97 samples). An image featuring overlaid spectra for 118 samples used in this study are presented in Figure 4.5. As previously discussed, samples with concentrations less than 200 mg $kg^{-1}$ N were omitted from the $NO_3$-N (35 samples) and UAN (2 samples) models. Compositional outliers were also removed as described previously. We identified and removed 3, 1, 1, 10, 4, 8, 6, and 0 compositional outliers from the TN, $NH_4$-N, UAN, $NO_3$-N, WSON, PMN, TC, and θ calibrations respectively. Summary statistics for the measured values on the PL samples used in this study are presented on a dry weight basis in Table 4.1. Distributions of the data for each parameter are shown in Figure 4.6. Units of measurement for all parameters in NIR calibration equations are mg $kg^{-1}$ 'as received' PL except for θ (g $H_2O$ 100 $g^{-1}$ 'as received' PL).

For TN in PL (Figure 4.7), expansion of the calibration set to 100 samples did not improve the coefficient of determination ($r^2_{50}$ = 0.899, $r^2_{100}$ = 0.896). The SEP and SEP(C) for TN were lowered somewhat to 2026.177 and 2036.694. For $NH_4$-N in PL (Figure 4.8), the calibration $r^2$ value increased to 0.795 while the SEP and SEP(C) increased marginally (613.368 and 616.349 respectively). The $r^2$ value for UAN in PL calibration increased somewhat to 0.909 from 0.893 (Figure 4.9) while the SEP and SEP(C) increased considerably to 517.621 and 519.51 respectively. Expansion of the calibration set from 50 to 100 samples did not result in improved calibration models for TN, $NH_4$-N, and UAN as was hypothesized. However, the increase in calibration set size with its accompanying increase in spectral variation did not result in poorer models either. The increase in spectral variation within the calibration

set is expected to increase the robustness of the equation when predicting routine samples and should be tested over time.

The calibration for $NO_3$-N in PL samples of variable θ resulted in a successful model with an $r^2$ value of 0.926 and SEP and SEP(C) of 282.791 and 285.398 respectively (Figure 4.10). It should be noted that the distribution of values for $NO_3$-N deviates sharply from normal. Such a high density of lower values often results in more favorable coefficients of determination. ON, as calculated by subtracting the measured mineral forms $NH_4$-N and $NO_3$-N from TN, was used to develop a calibration equation with an $r^2$ value of 0.821, SEP of 2191.34, and SEP(C) of 2198.624 (Figure 4.11). The lower predictive value of the $NH_4$-N equation, the relatively low concentrations of $NO_3$-N in PL, and the non-normal distribution of the $NO_3$-N data set combine to suggest that predicting ON and calculating total mineral N by subtracting ON from TN may be a better strategy than attempting to predict $NH_4$-N in PL samples of 'as received' θ.

A calibration equation for WSON (Figure 4.12), previously described as a useful index for predicting PMN in PL (Gordillo and Cabrera, 1997a; Qafoku et al., 2001), was developed that suggested strong potential with an $r^2$ value of 0.897, SEP of 1144.648, and SEP(C) of 1152.827. For directly measured PMN, the calibration equation was slightly more successful than that developed by Qafoku et al. (2001). Those authors reported an $r^2$ value of 0.82 using 60 freeze-dried and finely ground PL samples. In this study, the equation showed strong potential for use of NIR in predicting PMN (Figure 4.13) in 97 'as received' PL samples with an $r^2$ of 0.842, SEP of 882.866, and SEP(C) of 887.106. The results of both mineralization experiments were combined to produce this equation, and very low and/or negative values were included due to the importance of predicting when N mineralization will not occur, or even when net immobilization of N may occur. Finally, TC was included in this study for its importance to certain predictive models for PMN and θ was included for its usefulness in calculating results on a dry matter basis. The calibration for TC (Figure 4.14) produced an $r^2$ value of 0.926, SEP of 0.9656.280, and SEP(C) of 9706.443. The calibration for θ (Figure 4.15) produced an $r^2$ value of 0.996, SEP of 0.456, and SEP(C) of 0.460. This last equation was not expanded beyond 45 samples due to the

historical success of calibrating for $H_2O$ in PL and other materials (Workman, Jr., 2001; Tasistro et al, 2004), the strength of the equation with such a small set, and its indirect relationship to the purpose of the study.

Due to the success of these equations, we also investigated the potential for NIR calibration on two different combinations of measured N forms, WSN (WSON + $NH_4$-N + $NO_3$-N) and total PAN after 100 days ($PMN_{100}$ + $PAN_i$). The attempted calibration for WSN was considered unsuccessful ($r^2$ = 0.610). The data for $PAN_{100}$, however, produced a stronger calibration equation than for PMN alone ($r^2$ = 0.888; SEP = 829.268; SEP(C) = 832.269) (Figure 4.16). This is an interesting result for a useful parameter, and the improvement in the prediction equation is suspected to arise from the ease of identifying PLS relationships with the larger concentrations resulting from the summed N forms.

The results of this study are the first to show systematic development of NIR calibration equations for N forms important to crop fertilization using minimally prepared (no drying, briefly processed) PL samples of varied composition (peanut hull, sawdust, pine shaving) and source (i.e. broiler, layer, etc.). The weakest of the calibration equations we developed ($NH_4$-N; $r^2$ = 0.795) was still strong enough to suggest potential for routine use in agricultural labs with NIR capability. Components of PAN and PMN as well as N forms and indices related to the prediction of PMN have been calibrated here by NIR with enough success to challenge the convention that samples must be dried and finely ground prior to analysis by NIR for this purpose. Furthermore, the evidence provided here and elsewhere (Mahimairaja et al., 1990; Wood and Hall, 1991) regarding the loss of nitrogen incurred by drying PL samples for storage and analysis deserve the attention of laboratories performing analyses on PL samples for any form of nitrogen by any method.

**Incubation studies**

Two incubation experiments were performed on poultry litters received by the University of Georgia Soil Plant and Water Testing Laboratory. In the first experiment, fifty litters were incubated with a Bonifay series soil (Table 4.2) collected from the Coastal Plain region. Total N in the samples used in incubation study 1 averaged 40.8 g $kg^{-1}$ PL (dry weight). Averaged initial values of ON, UAN, $NH_4$-N,

and $NO_3$-N for the samples used in this study were 33.4, 4.3, 7.9, and 0.46 g $kg^{-1}$ PL (dry weight) respectively. At the end of the one hundred day incubation period, PMN for these samples incubated in a Bonifay series soil averaged 5.2 g $kg^{-1}$ PL (dry weight). This mass of nitrogen is equivalent to 21.3% of the ON initially present in the samples (18.3% of the TN). The total PAN measured at the end of one hundred days ($PAN_{100} = PAN_i + PMN_{100}$) averaged 36.1% of the TN.

We felt that these values were low in comparison to previously published studies. The Bonifay soil used in this experiment, although typical of those under cultivation in the Coastal Plains of the Southeastern United States, was high in sand content and low in cation exchange capacity (CEC). We suspected that the results may have been affected by volatilization of ammonia which was not accounted for. A second incubation experiment was designed to prevent the loss of measurable ammonia. An Orangeburg series soil, also typical of the Coastal Plain but with higher clay content and CEC, was chosen for the second study. Total N in the samples used in incubation study 2 averaged 38.7 g $kg^{-1}$ PL (dry weight). ON, UAN, $NH_4$-N, and $NO_3$-N averaged 32.2, 3.5, 5.2, and 1.4 g $kg^{-1}$ PL (dry weight) respectively. At one hundred days, the PMN from PL samples incubated with an Orangeburg soil averaged 4.76 g $kg^{-1}$ PL (dry weight). This amount was equivalent to 9.0 % of the initial total nitrogen and 14.5% of the initial organic nitrogen. Total PAN at the end of 100 days was equivalent to 29.2% of the initial total nitrogen.

The results of the second study confirmed the validity of those from the first incubation experiment. Measured ammonium from the acidified traps averaged less than 1% of the total mineralized N in the second incubation study and so was considered to be of similarly low importance to the first study. Although our results were unexpectedly low in comparison to other incubation studies, there were statistically significant differences between the individual studies in this work. The percentage of $ON_i$ mineralized and the percentage of $TN_i$ available to plants at the end of one hundred days between the studies were statistically different at the 99% confidence level as determined by a two sample t-test (R Development Core Team, 2013). The two experiments differed by soil type, temperature, and litter characteristics. There was no significant difference in TN or ON for the litter samples used in the first

and second incubation studies at 95% confidence level. It is unlikely that the temperature difference in the second study had a significant effect over 100 days. One possible explanation for the difference in measured mineralized nitrogen may be the difference in sand contents between the two soils. Soils with higher sand content have been observed to mineralize nitrogen more rapidly than those with a smaller particle size distribution (Gordillo and Cabrera, 1997b; Preusch et al., 2002; Sistani et al., 2008).

Combined statistics for the two studies are presented in Table 4.1. The average PMN for litters used in these studies averaged 6109 mg N $kg^{-1}$ PL (dry weight). This result is approximately 19% of the initial ON and 16% of the initial TN. This result is far lower than the 50% to 60% mineralization of organic N often published when using fresh poultry litters and/or manures (Bitzer and Sims, 1988; Gordillo and Cabrera, 1997a, and Qafoku et al., 2001). Composted litters have been reported to mineralize less nitrogen than fresh litters (Tyson and Cabrera, 1993; Hartz et al., 2000; Preusch et al., 2002). Cooperband et al. (2002), working with litters of various ages, noted that corn (*Zea mays*) yield and N uptake was greater with raw (fresh) Pl than composted. These authors also reported that additions of fully composted PL (15 months) resulted in greater yields and N uptake than with immature composts (1 and 4 months).

Total PAN after 100 days of incubation averaged approximately 33% of the initial TN present in the samples. It is known that many of the samples used in the combined incubation studies were stored for varied time periods under different conditions. The data regarding durations and conditions of storage was not available but the results presented here are somewhat higher than those associated with composted litters and manures. Storage of PL between removal from the poultry production facility and application to pasture or crop is very common. It is very likely that typical poultry litter storage practices such as covering with a tarp or ‘stacking’ under an open-walled structure allows for volatilization of ammonia and elevated temperatures that change the profile of organic N forms without the intention or result of proper composting. If this is the case, estimates of fertilizer N value of PL used in common agriculture practice should be revised down from common research derived predictions of approximately 50% of TN based on fresh PL samples freeze dried and finely pulverized for experiments. It is also

possible that some immobilization of N occurred following mineralization. Sistani et al. (2008) reported the occurrence of re-immobilization of mineralized N in aerobic incubation studies and Hartz et al. (2000) found that immobilization was more pronounced in composted than fresh manures. Both incubation studies reported here relied on one endpoint measurement at 100 days and so any data regarding mineralization and immobilization over time are not available. Additionally, we extracted the incubated soils with PL for mineral N promptly at 100 days without freezing or drying to avoid further alterations that may have affected conclusions drawn in previous studies.

Based on the results of the incubation experiments, we investigated previously published relationships between PMN and various organic N forms and/or indices. We were unable to reproduce the results of any previous studies by other authors with regard to relationships between PMN and indices such as WSN (Ruiz-Diaz), WSON (Qafoku et al., 2001), or UAN (Fujiwara, 2007). Furthermore, multiple linear regression analysis failed to provide any convenient equations for estimation of PMN based on combinations of more easily measured components of PL such as that of Gordillo and Cabrera (1994) who found strong linear relationships with TN, UAN, TN+UAN, and TN+C:N ratio. Qafoku et al. (2001) reported a strong linear relationship between PMN and WSON. Fujiwara et al. (2007) found a surprisingly close linear relationship in poultry manure compost between PMN and uric acid (r2 = 0.99999) and PMN and TN (r2 = 0.948). Ruiz-Diaz et al. (2008) suggested that WSN was related to total PAN at the end of their incubation study. All such relationships were investigated and found to be extremely weak ($r^2 << 0.500$) for the samples used in our studies. It may be that immobilization of mineralized N during the incubation experiments has obfuscated the relationship between PMN and certain organic N pools. However, the success in calibrating NIR for PMN and total PAN after 100 days indicates that there may be no convenient substitute for direct measurement of mineralizable N. Building a calibration equation based on a larger set of samples followed by validation of the equation using a

sufficiently large set of incubated samples is time consuming, but the benefit of rapid analysis by NIR and prediction of fertilizer N value on 'real world' samples is significant.

**Conclusions**

We found that 97 PL samples received by the UGA Soil, Plant, and Water Laboratory and intended for land application to pastures and row crops mineralized approximately 19% of the initial organic N after 100 days of incubation with soils typical of the Southeastern U.S. Coastal Plain region. These litter samples closely represent the N fertilizer value of those used as soil amendments for crop production as compared to the litters collected fresh from poultry houses used in many previous studies. Our results suggest that the litters used in our studies, which varied in age, may provide an N fertilizer value closer to 33% of the total N as opposed to the 50-60% predicted by agricultural and State Extension laboratories. We suggest that the drying and grinding of PL (or any animal waste) samples prior to routine analysis for N fertilizer equivalence recommendations should be avoided. Direct measurement of total N and $NH_4N$ and indirect evidence on uric acid changes following drying of PL indicate significant alteration of these chemical properties.

This study has demonstrated that drying is unnecessary in producing strong NIR calibrations on for TN, $NH_4N$, UAN, $NO_3N$, WSON, PMN, TC, and $\theta_g$. However, it should be noted that the NIR calibration for $NH_4N$ was much improved when samples were dried, and post-dried $NH_4N$ measurements used for calibration. However, this study failed to reproduce any convenient relationships between PMN and rapidly measured chemical properties of PL such as those reported by other investigators. As a consequence, we questioned the role of particle size and pre-drying of PL samples in the mineralization of organic N in soil incubations. Also, we identified a need to investigate the effect of time in current stacking and storage practices on the release of N from PL.

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# TABLES

Table 4.1: Summary statistics for poultry litter samples used in this study. Number of samples measured for each parameter appear on bottom row.

| | TN | ON | $NH_4$-N | $NO_3$-N | UAN | WSON | PMN | TC | θ |
|---|---|---|---|---|---|---|---|---|---|
| | mg $kg^{-1}$ PL (dry weight basis) | | | | | | | | g 100 $g^{-1}$ PL (as received) |
| Mean | 38956 | 32637 | 5965 | 921 | 3890 | 10862 | 6109 | 372512 | 31.4 |
| Median | 40514 | 33364 | 5627 | 474 | 3580 | 10898 | 6167 | 381861 | 29.9 |
| Min | 14119 | 12124 | 119 | ND* | 15 | 427 | -2131 | 212640 | 16.7 |
| Max | 60344 | 54886 | 16535 | 5793 | 17910 | 23753 | 16606 | 447173 | 56.5 |
| Std Dev | 8966 | 7710 | 2666 | 1281 | 3044 | 5217 | 3262 | 39652 | 8.4 |
| No. Samples | 100 | 100 | 100 | 100 | 118 | 63 | 97 | 100 | 100 |

* ND – Not Detected

Table 4.2: Selected properties of a Bonifay series and an Orangeburg series soil used in two 100 day incubation experiments with 97 poultry litter samples. The soils are typical of those under row crop production in the Southeastern U.S. Coastal Plain region.

| Soil | $\theta_g$* | | Texture | | | Total C | Total N | LBC† | CEC†† |
|---|---|---|---|---|---|---|---|---|---|
| | -0.01 MPa | -0.033 MPa | Sand | Silt | Clay | | | | |
| | $g\ g^{-1}$ | | % | | | $mg\ kg^{-1}$ | | $mg\ CaCO_3\ kg^{-1}$ | $cmol\ kg^{-1}$ |
| Bonifay | 0.129 | 0.076 | 84.03 | 4.76 | 11.21 | 3456 | 480 | 208.2 | 0.86 |
| Orangeburg | 0.203 | 0.148 | 69.53 | 8.86 | 21.61 | 3016 | 233 | 226.3 | 1.75 |

* Water Potential
† Lime Buffer Capacity
†† Cation Exchange Capacity

# FIGURES

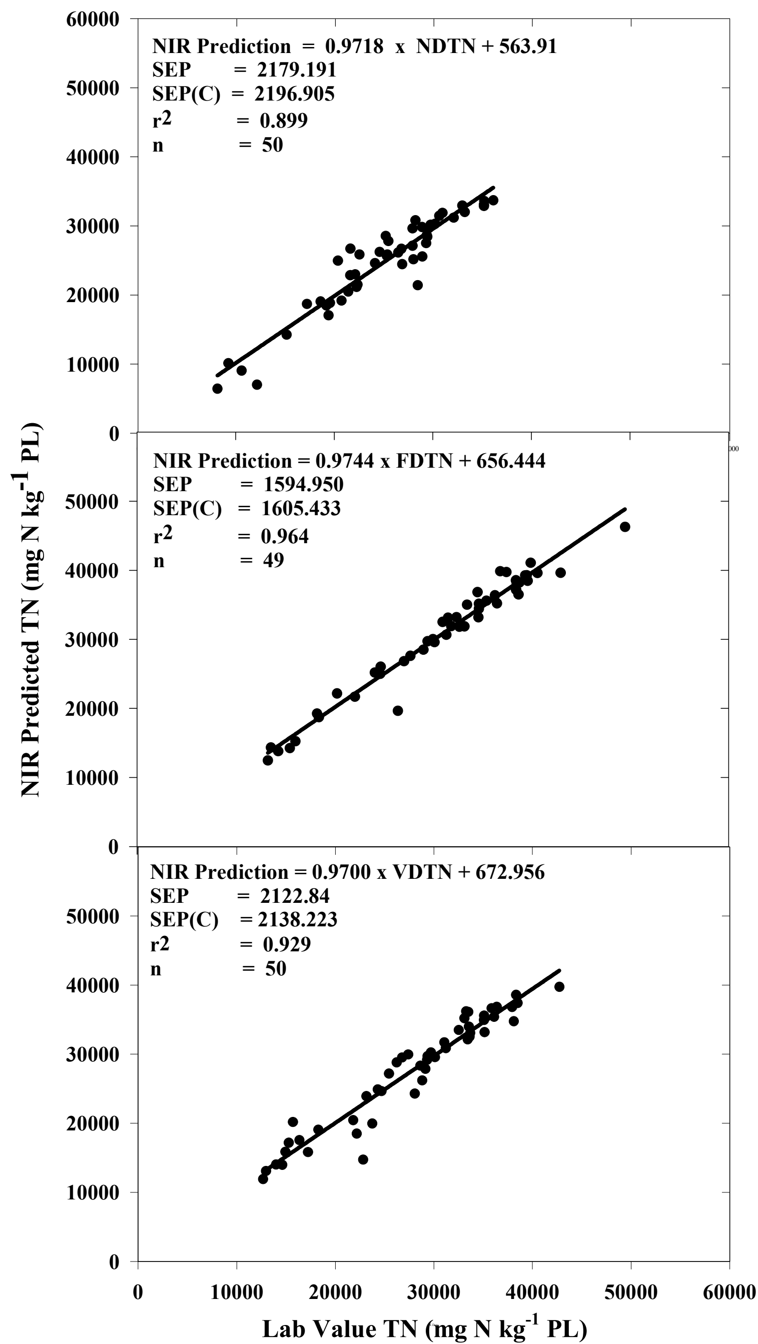


Figure 4.1: Near infrared reflectance (NIR) calibrations for total nitrogen in poultry litter by drying treatment (Treatments ND = no dry, FD = freeze dry, and VD = vacuum dry from top to bottom).

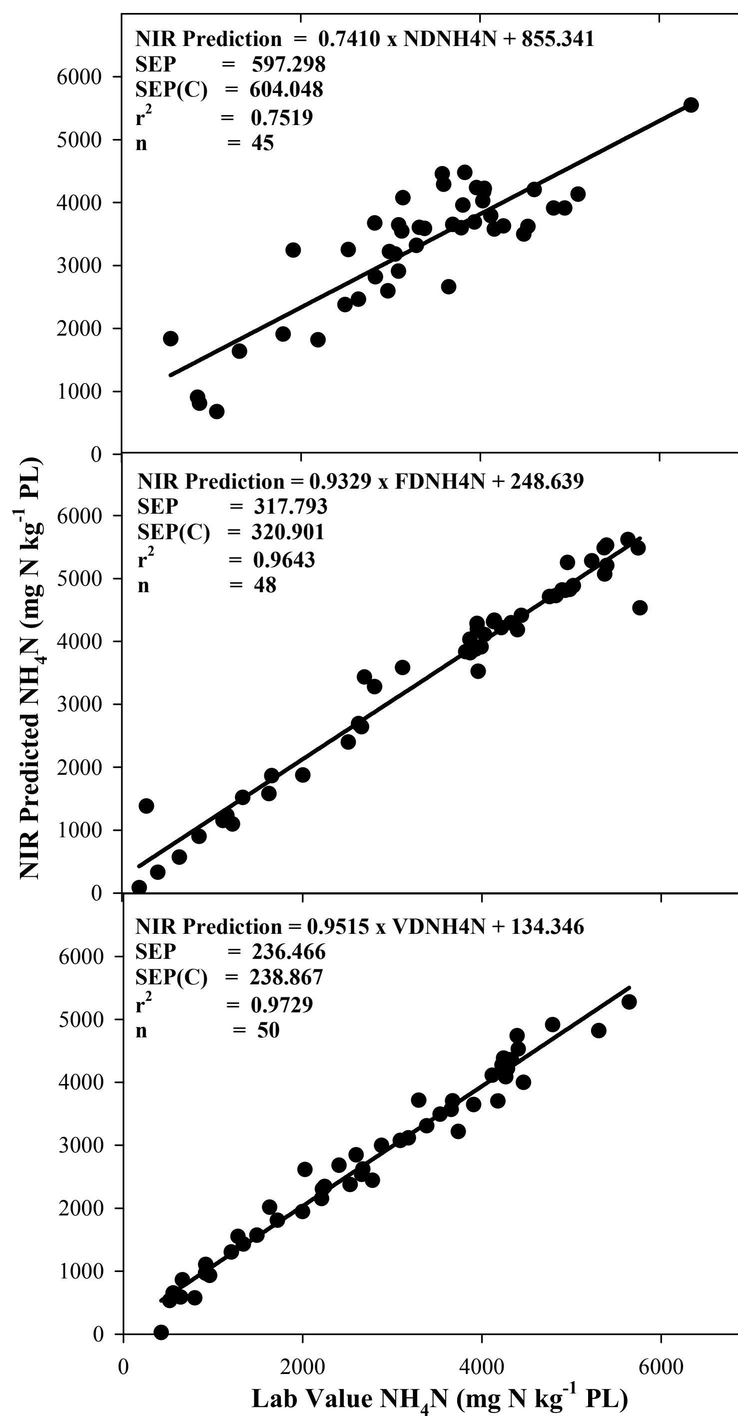


Figure 4.2: Near infrared reflectance (NIR) calibrations for ammonium nitrogen in poultry litter by drying treatment (Treatments ND = no dry, FD = freeze dry, and VD = vacuum dry from top to bottom).

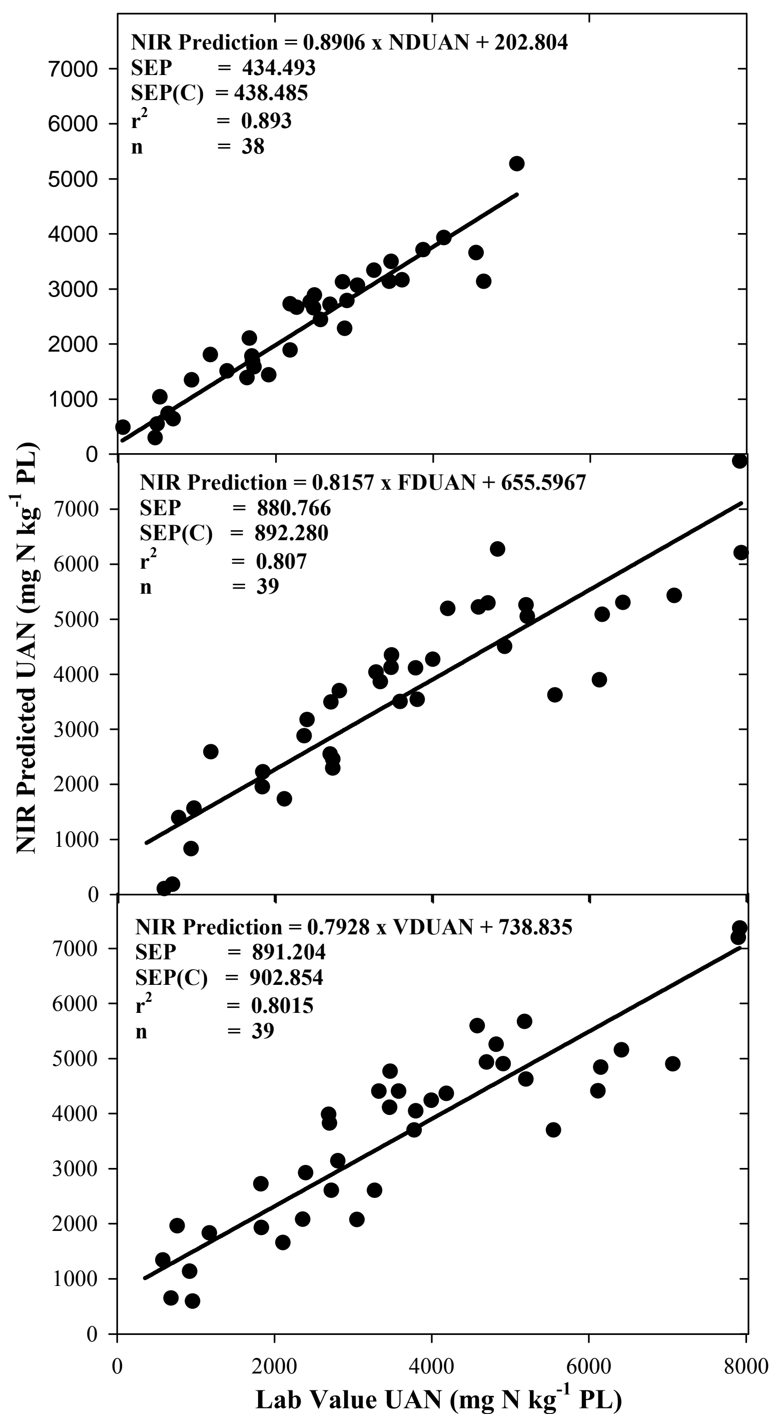


Figure 4.3: Near infrared reflectance (NIR) calibrations for uric acid nitrogen in poultry litter by drying treatment (Treatments ND = no dry, FD = freeze dry, and VD = vacuum dry from top to bottom).

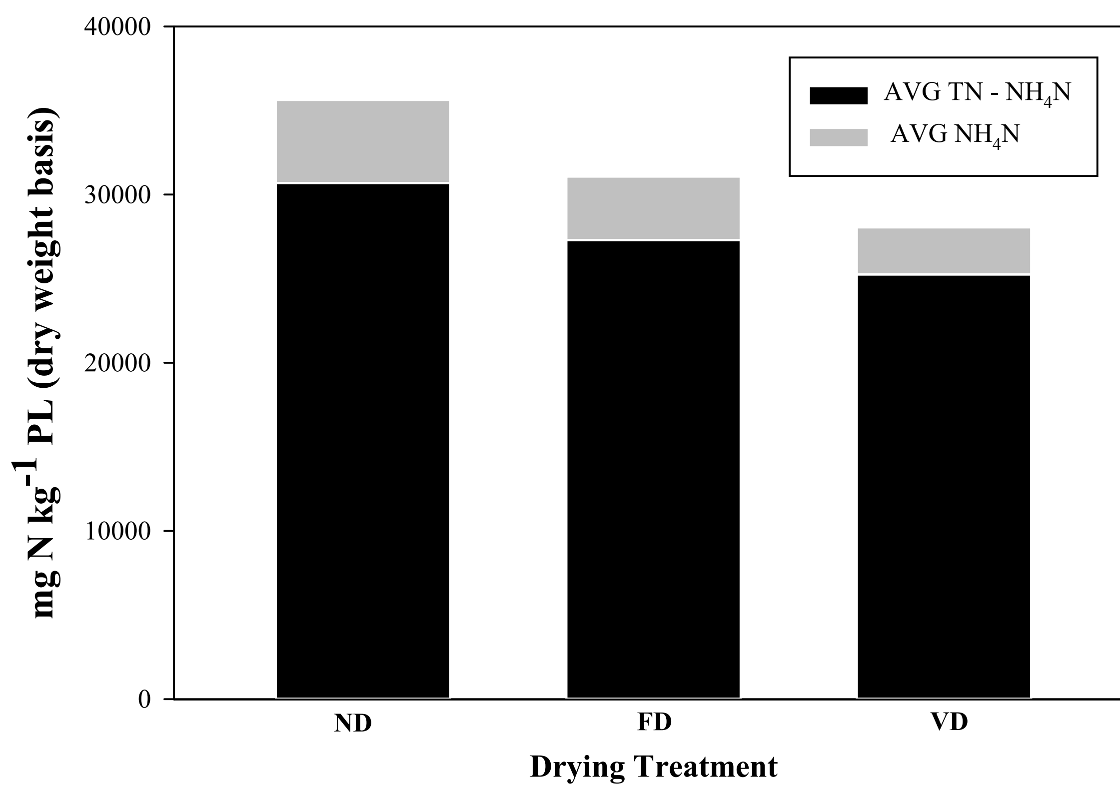


Figure 4.4: Changes in laboratory determined values of total nitrogen and ammonium nitrogen following freeze drying (FD) and vacuum drying (VD) of 50 poultry litter samples. ND = no drying.

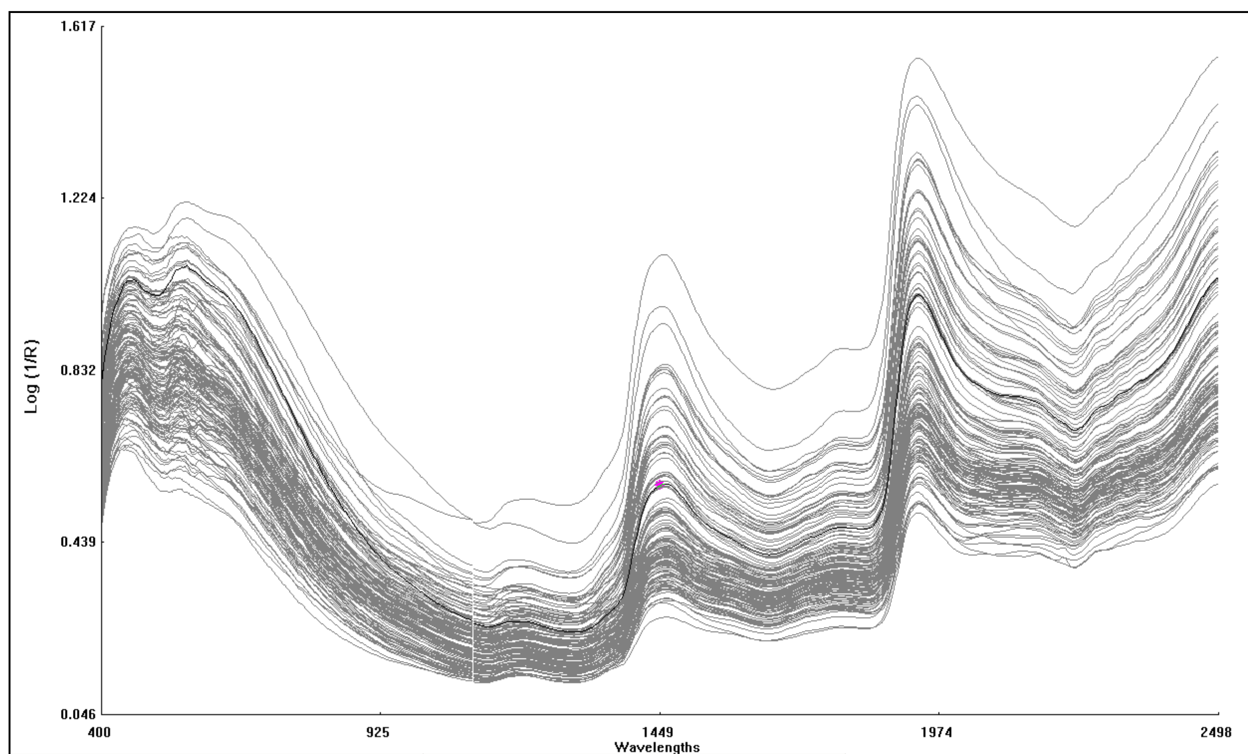


Figure 4.5: Spectra from 118 near infrared reflectance (NIR) scans of poultry litter samples used for NIR calibration. Wavelength (nm) on x-axis. Log of the inverse of reflectance on the y-axis.

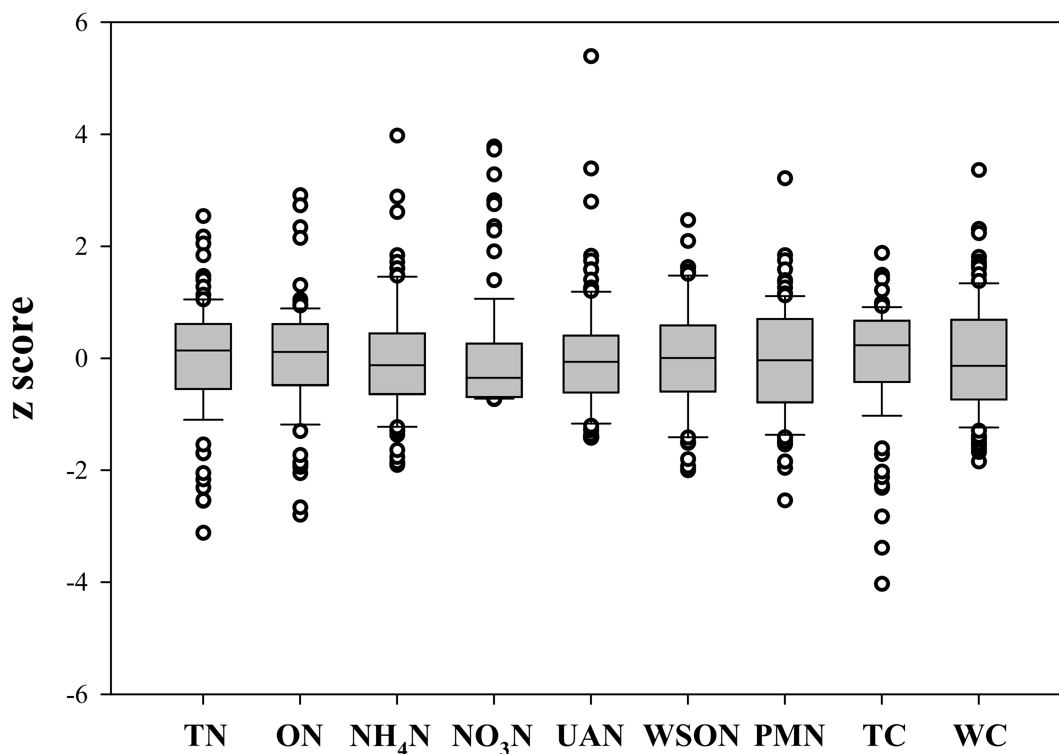


Figure 4.6: Box plot of standardized distributions for the chemical parameters of the poultry litter samples used to develop near iinfrared reflectance (NIR) calibrations in this study. Parameters on x-axis. TN = total nitrogen. ON = organic nitrogen. NH4N = ammonium nitrogen. NO3N = nitrate nitrogen. UAN = uric acid nitrogen. WSON = water soluble organic nitrogen. PMN = potentially mineralizable nitrogen. TC = total carbon. θ = water content. Standard score (z-score) on y-axis.

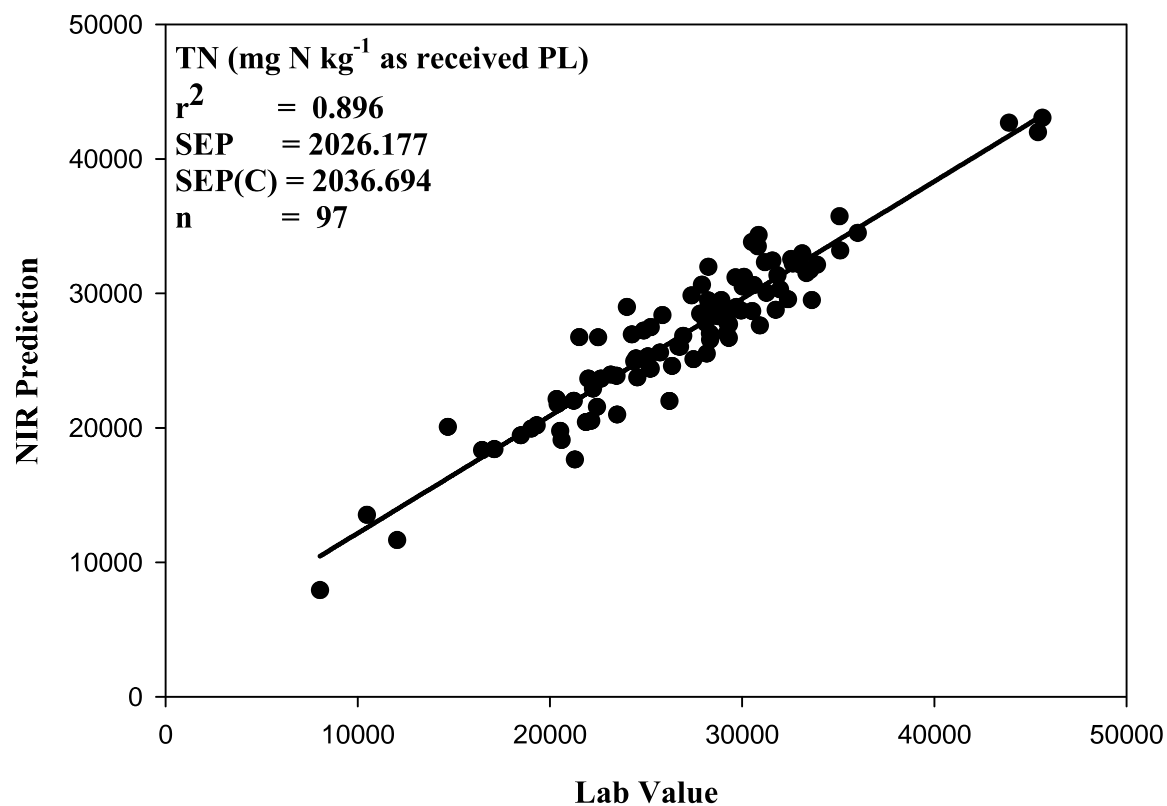


Figure 4.7: Near infrared reflectance (NIR) calibration for total nitrogen in poultry litter measured at 'as received' water content basis. Laboratory derived value (combustion nitrogen) on x-axis. NIR prediction on y-axis.

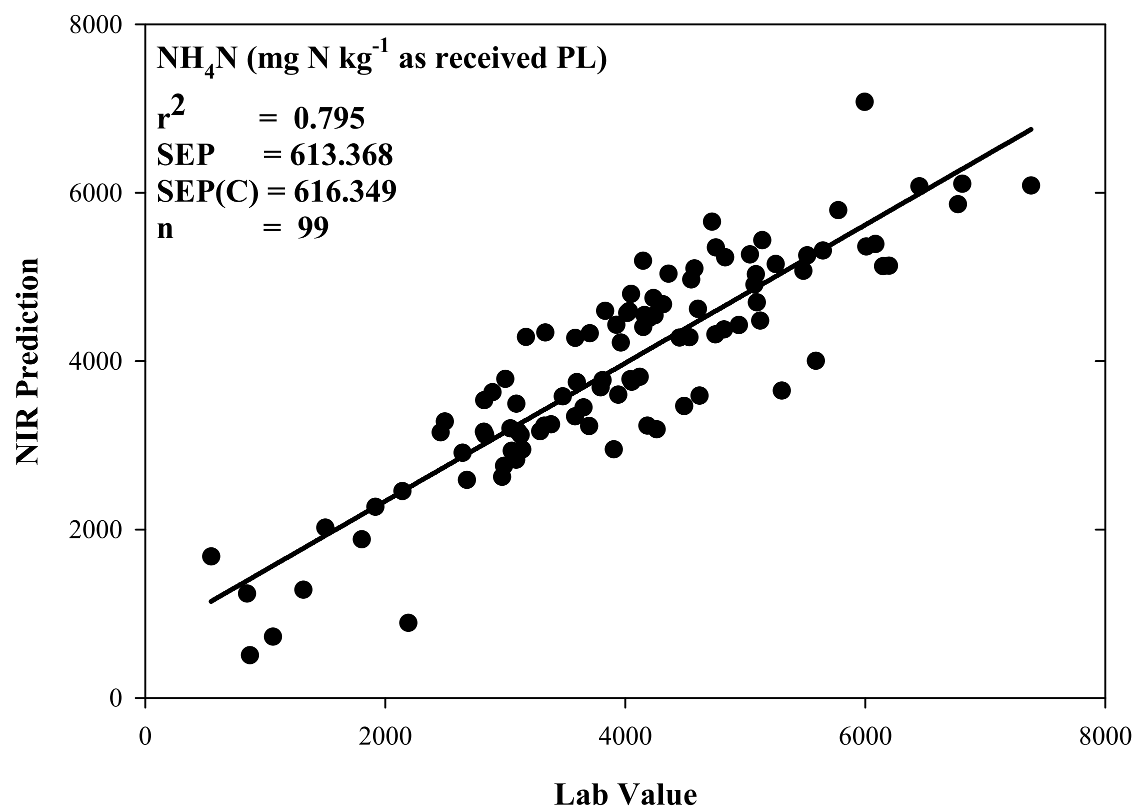


Figure 4.8: Near infrared reflectance (NIR) calibration for ammonium nitrogen in poultry litter measured at 'as received' water content basis. Laboratory derived value (gas-diffusion conductance) on x-axis. NIR prediction on y-axis.

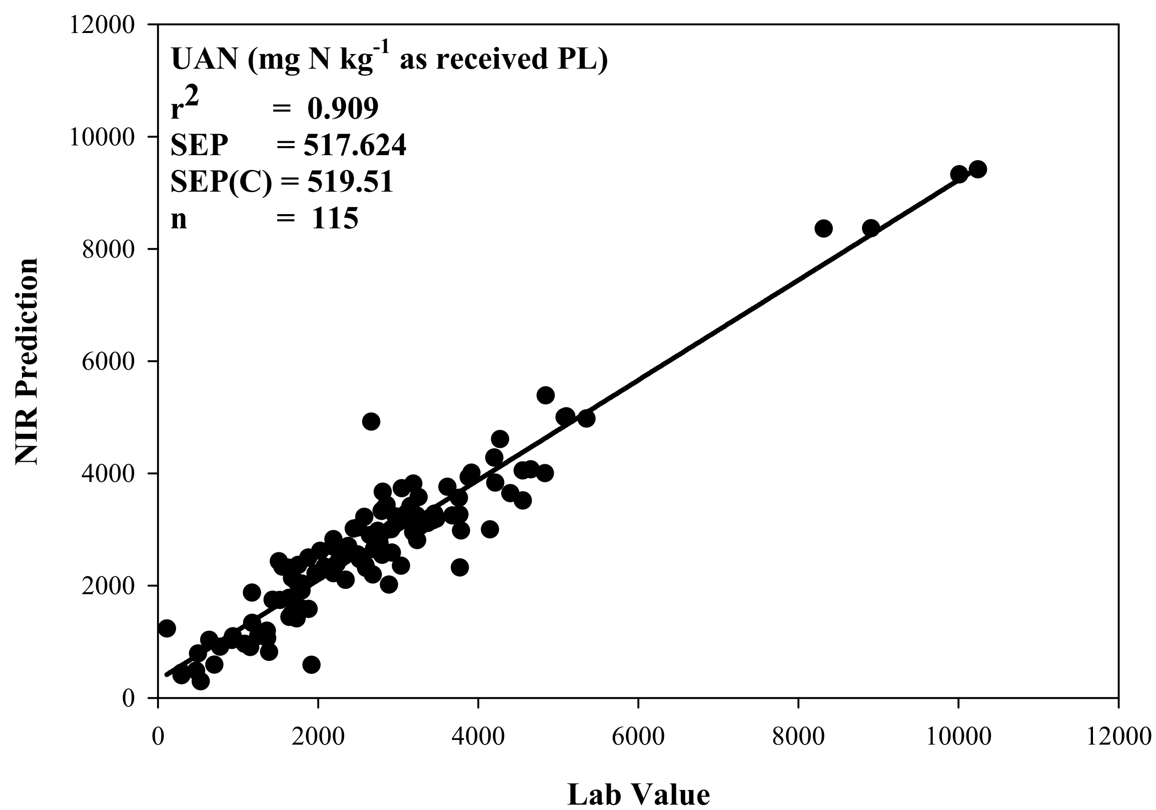


Figure 4.9: Near infrared reflectance (NIR) calibration results for uric acid nitrogen in poultry litter measured at 'as received' water content basis. Laboratory derived value (high pressure liquid chromatography (HPLC uv/vis)) on x-axis. NIR prediction on y-axis.

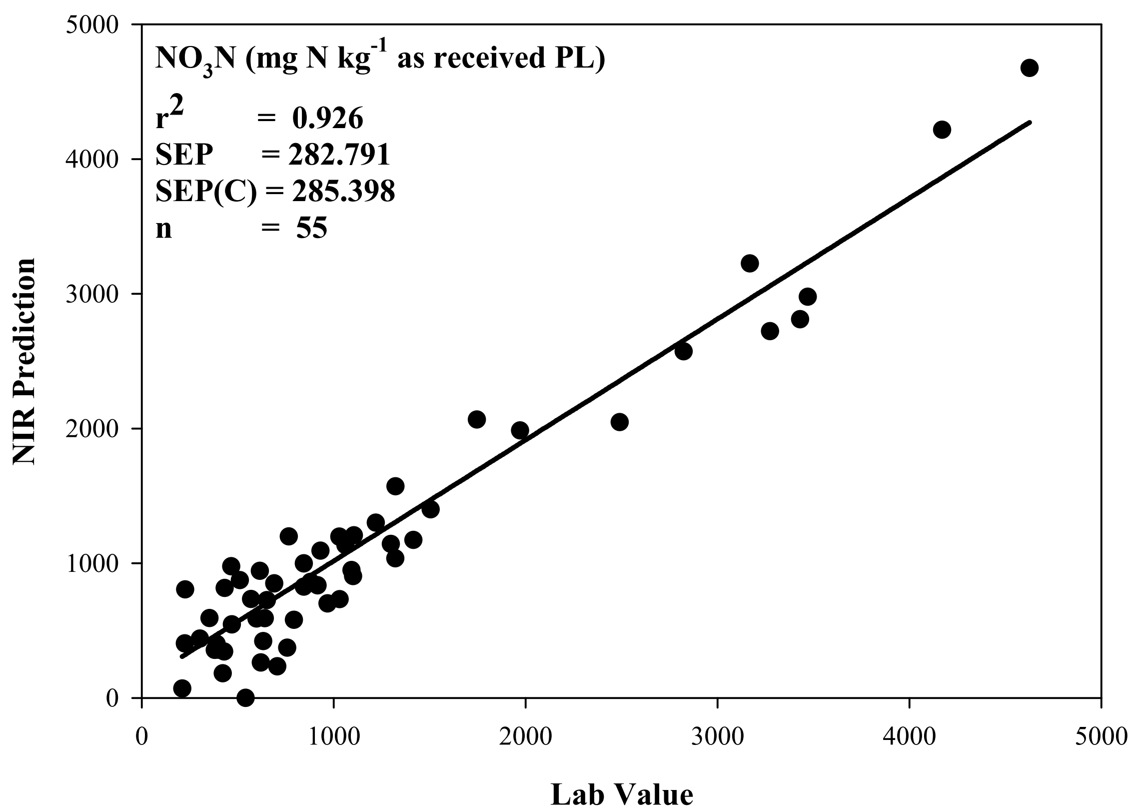


Figure 4.10: Near infrared reflectance (NIR) calibration results for nitrate nitrogen in poultry litter measured at 'as received' water content basis. Laboratory derived value (colorimetry / Greiss-Ilosvay reaction) on x-axis. NIR prediction on y-axis.

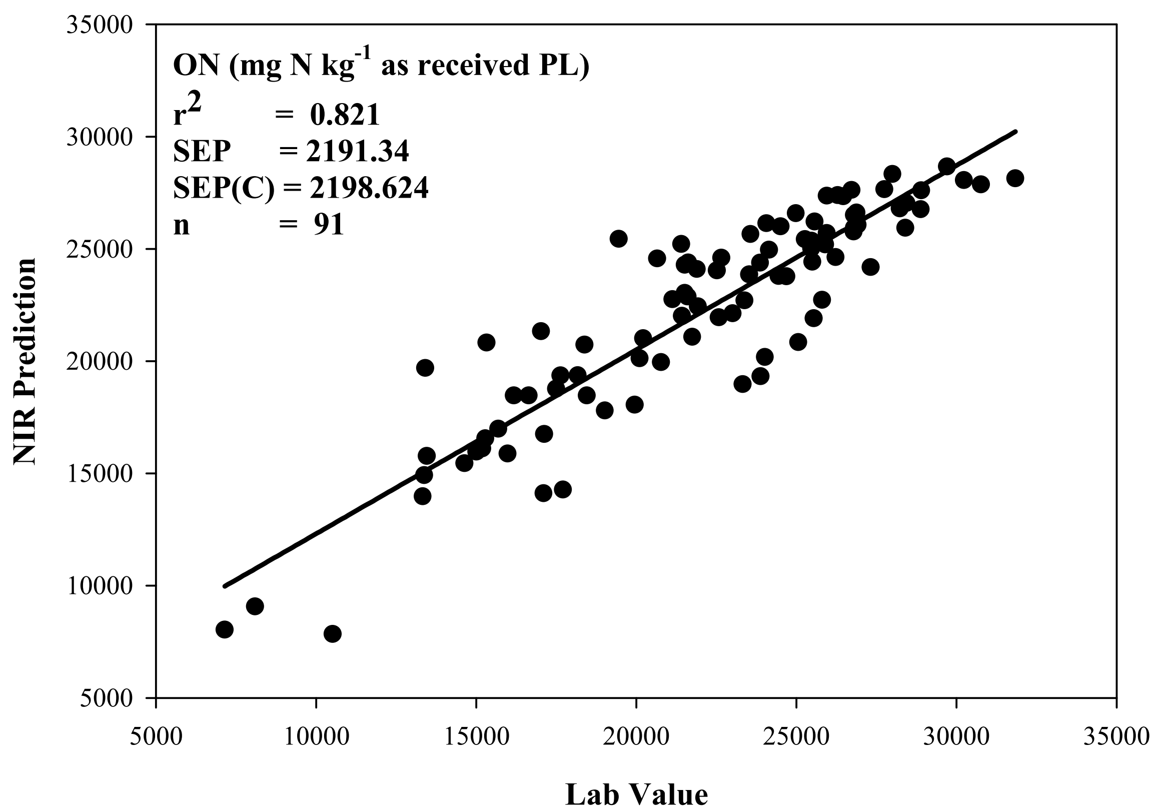


Figure 4.11: Near infrared reflectance (NIR) calibration results for organic nitrogen in poultry litter measured at 'as received' water content basis. Laboratory derived value (calculated as ON = TN - $NO_3$-N - $NH_4$-N) on x-axis. NIR prediction on y-axis.

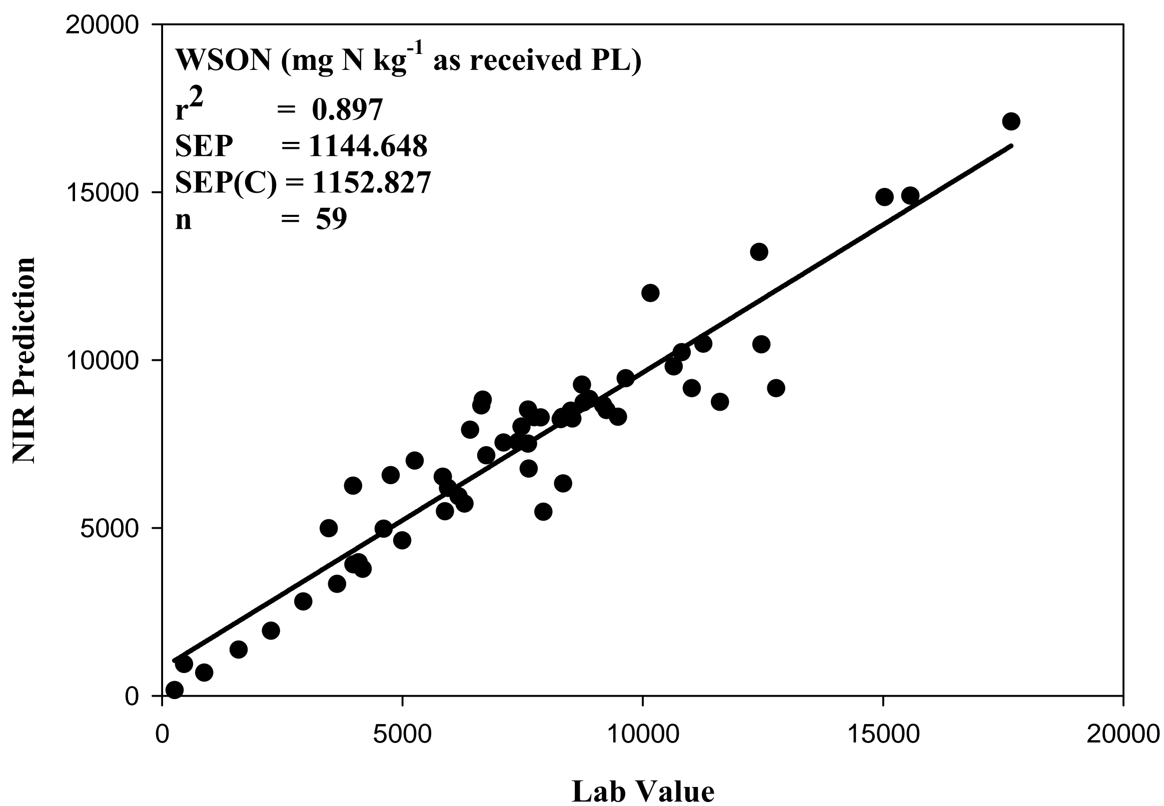


Figure 4.12: Near infrared reflectance (NIR) calibration results for water soluble organic nitrogen in poultry litter measured at 'as received' water content basis. Laboratory derived value (Kjehldal nitrogen - ammonium nitrogen) on x-axis. NIR prediction on y-axis.

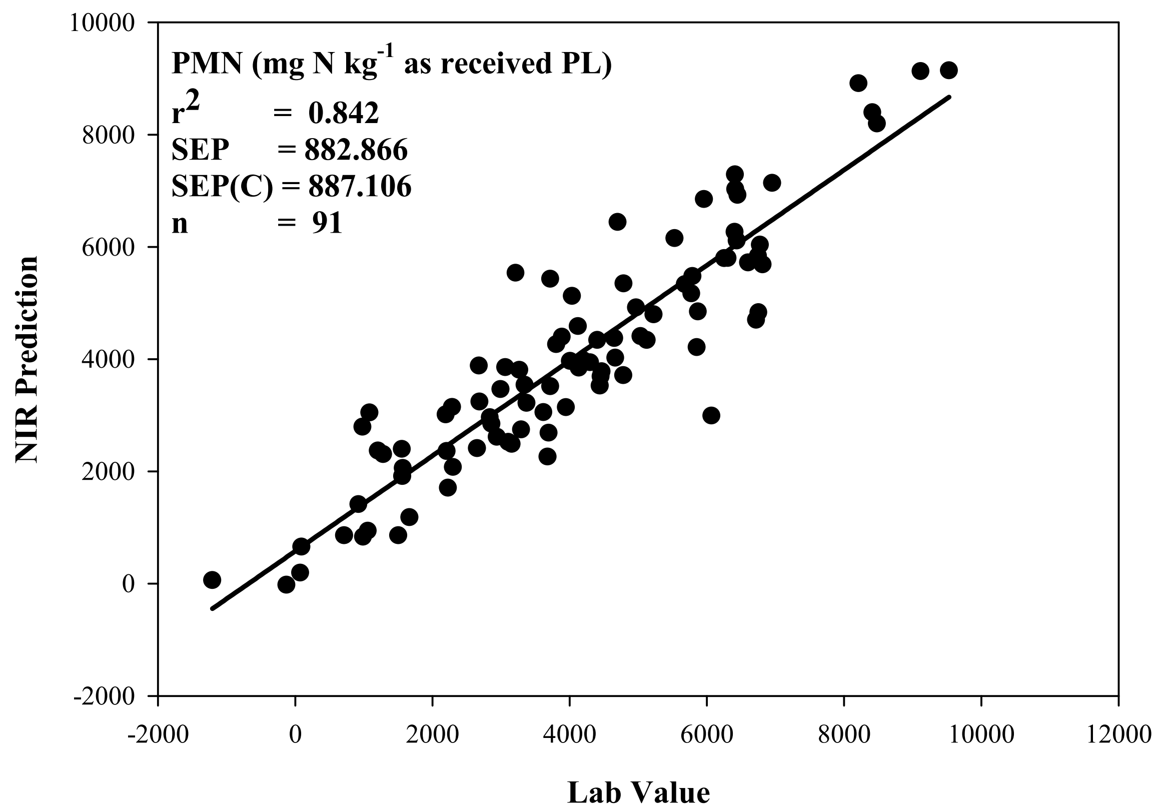


Figure 4.13: Near infrared reflectance (NIR) calibration results for potentially mineralizable nitrogen in poultry litter measured at 'as received' water content basis. Laboratory derived value (total mineral nitrogen - initial mineral nitrogen following 100 day incubation) on x-axis. NIR prediction on y-axis.

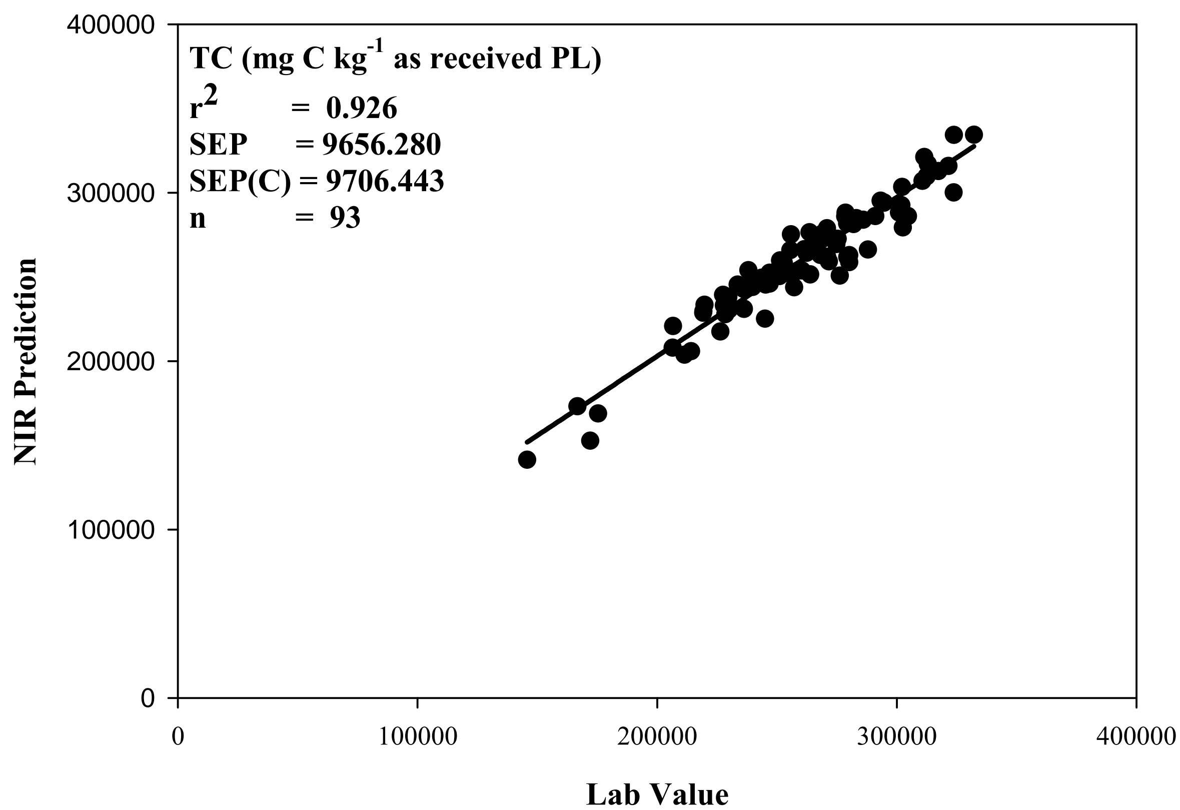


Figure 4.14: Near infrared reflectance (NIR) calibration results for total carbon in poultry litter measured at ‘as received’ water content basis. Laboratory derived value (combustion carbon) on x-axis. NIR prediction on y-axis.

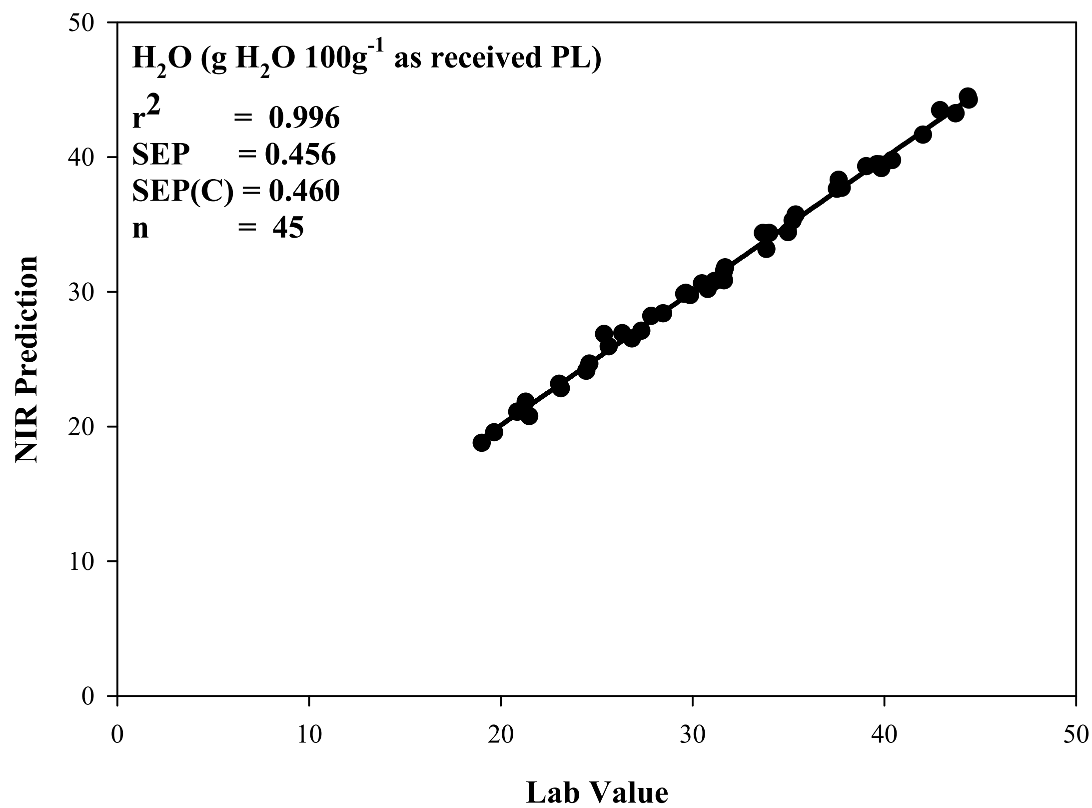


Figure 4.15: Near infrared reflectance (NIR) calibration results for water content in poultry litter measured at 'as received' water content basis. Laboratory derived value (gravimetric loss) on x-axis. NIR prediction on y-axis.

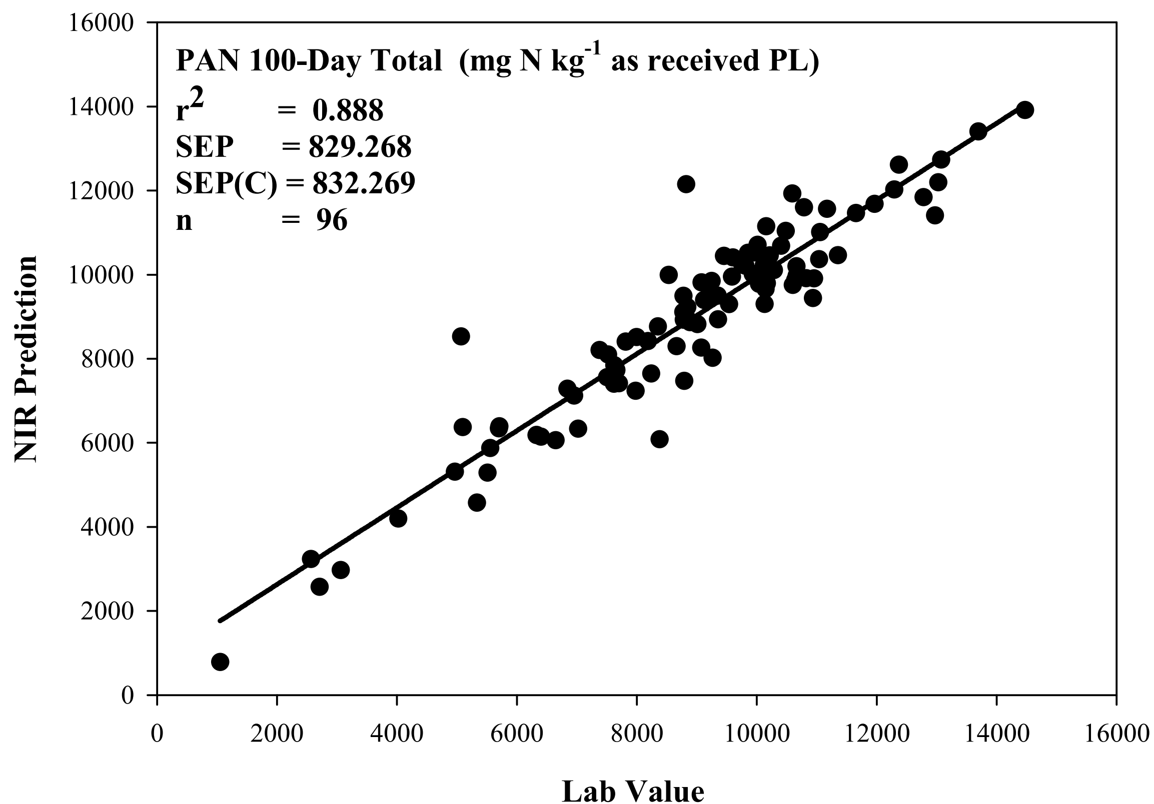


Figure 4.16: Near infrared reflectance (NIR) calibration results for total plant available nitrogen after 100 days in poultry litter measured at 'as received' water content basis. Laboratory derived value (calculated as plant avalailable nitrogen at 100 days - initial plant available nitrogen - potentially mineralizable nitrogen at 100 days ($PAN_{100} = PAN_i + PMN_{100}$)) on x-axis. NIR prediction on y-axis.

# CHAPTER 5

# NITROGEN IN STORED POULTRY LITTER: URIC ACID AND XANTHINE[3]

[3]Mowrer, J. 2014. To be submitted to Agricultural Systems.

Abstract

Laboratory incubations of four broiler litter (BL) samples were carried out to investigate the effect of water content on the decay of uric acid nitrogen (UAN) and xanthine nitrogen (XN). Three water contents investigated were 'as received' or native water content, 600 g, and 750 g $H_20$ $kg^{-1}$ BL (dry weight). UAN and XN in the samples fluctuated (increased and decreased) over the first 10 days in these samples followed by a decrease in concentration over the next 28 days. XN concentrations were at all samplings less than UAN by a factor of at least five. Natural logarithms of the measurements for UAN and XN at each time point were used to fit linear equations. The slopes of the linear equations fit to the data from 16 sampling points over 38 days were compared. UAN decayed more rapidly in all four samples with increasing water content (θ) and XN decayed more rapidly in two of four samples. A second study showed that there were significant effects in one of three samples on the decay rate of UAN with additions of flue gas desulfurized (FGD) gypsum or alum at a water content of 750 g $H_20$ $kg^{-1}$ BL. The decay rate of XN was not significantly affected. Finally, a simple two-point sampling study on the effect of water potential designed to eliminate the noisy effect of early time fluctuations on regression for the estimation of decay rates showed a strong relationship between the rate of UAN and XN decay over 28 days as a function of water potential (ψ): $UAN_{28days} = 0.0054 \times \psi + 0.1010$ ($r^2 = 0.9987$) and $XN_{28days} = 0.0066 \times \psi + 0.1101$ ($r^2 = 0.9285$).

Abbreviations: UAN = uric acid nitrogen, XN = xanthine nitrogen, ψ = water potential, θ = water content.

## Introduction

Broiler litter (BL) from poultry houses is an important source of nitrogen (N) for crop and pasture production (Mitchell and Tu, 2005; Bolan et al., 2010; Dunkley et al., 2011). BL is composed mainly of avian fecal matter, bedding material (e.g. pine shavings, sawdust, peanut hulls), feathers, and wasted feed. It can supply both mineral N (ammonium and nitrate), which is immediately available for plant uptake; and organic N, which will mineralize to release N during the crop or pasture growing season (Sistani et al., 2008; Ruiz-Diaz et al., 2008, Ruiz-Diaz et al., 2012). The chemical composition, relative concentrations of N-containing compounds, and N-mineralization patterns of BL are highly variable, and depend on the source and age of the litter and environmental conditions (Bitzer and Sims, 1988; Tyson and Cabrera, 1993; Preusch et al., 2002).

The nitrogen in fresh avian feces is composed of approximately 70% uric acid and 30% undigested protein (Groot Koerkamp, 1994). Uric acid is the final product in the degradation of the purine nucleotide guanine through the intermediate compound xanthine (Yuan et al., 1999). Its formation from the deamination of the nucleoside adenosine through the intermediate compounds inosine, hypoxanthine and xanthine has also been described (Xi et al., 2000). From the moment of deposition, the initial forms of N present in the feces begin a process of chemical transformation. Under the right conditions, uric acid in BL will be mineralized relatively rapidly through the compounds allantoin, allantoic acid, ureidoglycolate, and urea to ammonia (Figure 5.1). Ammonia from BL may be further oxidized to nitrite and nitrate via nitrification (Pote et al., 2002); or it may be released from BL and enter the atmosphere through volatilization. Such volatilization and accumulation of gaseous ammonia in the production facility can be detrimental to bird health (Carlile 1984; Moore et al., 1996; Ritz et al., 2004). In the area surrounding the facility, it can also cause substantial undesirable odors (Wheeler et al., 2006). Ultimately, though, the reduction in total N reduces the economic value of litter as a source of fertilizer N (Marshall et al., 1998).

In practice, BL is frequently collected from production facilities and stockpiled or otherwise stored for some period of time prior to land application. During storage, as in composting, organic and

mineral N forms continue to change (Hartz et al., 2000; Preusch et al., 2002). As mineralization proceeds, more N may volatilize and some organic N may be transformed into compounds that resist mineralization. The result is a material that contains supplies of plant available nitrogen (PAN) and PMN that continue to decrease with time. The initial uric acid and total mineralized organic nitrogen composition of poultry litter and manure collected fresh from production facilities are reported by Gordillo and Cabrera (1997), Qafoku et al. (2001), and Ruiz-Diaz (2008). Composted BL or manure has been found to contain less total N, uric acid N (UAN), and PMN than fresh (Tyson and Cabrera, 1993; Hartz et al., 2000; Preusch et al., 2002; Fujiwara and Murakami, 2007). However, little is known about the specific chemical changes that occur with time in BL that is stockpiled or otherwise stored without formal composting with respect to most of the compounds indicated in Figure 5.1.

Rothrock et al. (2010) measured uric acid in a single incubated BL sample treated with poultry litter chemical amendments at 0, 2, 4, and 6 weeks. Initial uric acid concentrations in the samples used in this study ranged from 1.3 to 1.6 g N $kg^{-1}$ BL (dry weight basis). These authors reported a decline in UAN in all treatments incubated at 25°C over the 6-week period with final concentrations ranging from 0.03 to 0.47 g UAN $kg^{-1}$ BL. Bao et al. (2008) measured allantoin-N concentrations in six composted poultry, swine, and dairy manures at 0, 1, 2, 4, 6, 8, and 10 weeks. Initial allantoin-N in two poultry manures was 8.77 and 11.14 g $kg^{-1}$ and declined to 5.5 and 6.6 g $kg^{-1}$ respectively. The study performed by Bao et al., (2008) suggests that allantoin-N in fresh poultry manure may average close to 30% of the total N. UAN may average 10% and 20 % of the total N in stored and fresh BL respectively (Gordillo and Cabrera, 1997; Mowrer et al., 2013). We could find no data in the existing literature on relative xanthine-N (XN) concentrations in BL.

No study has examined the decay patterns of xanthine and uric acid in multiple BL samples over time to our knowledge. We sought to investigate the changes in UAN and XN concentrations in samples of BL with varied chemical composition over time. Because water content ($\theta$) is a highly variable field condition in stored litters, we chose to study these changes in the context of variable $\theta$. A second study was performed to investigate what effect additions of two common BL chemical amendments, alum and

gypsum, may have on the decay patterns of these two compounds. Finally, we investigated the potential development of a predictor of UAN and XN decay in BL as a function of water potential ($\psi$).

**Materials and Methods:**

**Chemicals:**

Standards for uric acid (98%) and xanthine (98%) were purchased from Acros Organics (Thermo Fisher Scientific, Waltham, Ma.). Flue-gas desulfurized (FGD) gypsum was obtained from a power plant in Indiana, U.S.A. Alum (aluminum sulfate) standard ground was obtained from Delta Chemical Corporation (Baltimore, Md., U.S.A.).

**Broiler Litter:**

BL samples were obtained from routine submissions to the University of Georgia Soil Plant and Water Laboratory (Athens, Ga.). The samples were ground for approximately 30 s to roughly homogenize using a spice grinder (Asia Kitchen Machine, Sumeet Centre Inc., Toronto, ON).

**Litter Analyses:**

BL pH was measured using a standard benchtop pH meter in a 1:5 stirred supension of BL to deionized water. Specific conductance (SC) was measured using an electrical conductivity probe inserted into a stirred suspension of 1g BL in 100 mL deionized water. Total nitrogen was measured by combustion on a LECO Trumac instrument (LECO Corporation, St. Joseph, Mi.). We used ICP-AES to measure K, Al, Ca, S, Cu, Zn, Na, and Cl following closed-vessel microwave assisted digestion of 0.25 g BL in 10 mL concentrated $HNO_3$. Uric acid and xanthine from BL were extracted and analyzed by the method of Mowrer et al. (2013). The equivalent of 2 g dry weight BL was extracted with 500 mL of 0.1 mol $L^{-1}$ sodium acetate trihydrate (Thermo Fisher Scientific, Waltham, Ma.) by placing in a 60°C water bath for 2 h and filtered into a 1.8-mL target vial using a 0.45-μm polystyrene filter fitted to a Luer tip syringe. Separation was achieved by use of the ODS-2 hypersyl column (Thermo Fisher Scientific, Waltham, Ma.) as solid phase and 0.1 mol $L^{-1}$ potassium phosphate monobasic (Thermo Fisher Scientific, Waltham, Ma.) as mobile phase at a flow rate of 1 mL $min^{-1}$. Sample injection volume was 20 µL. Detection of the analytes was made at a wavelength of 200 nm using the Agilent 1260 Infinity series

HPLC analyzer with UV/VIS capability (Agilent Technologies, Santa Clara, Ca.). Water potential (ψ) was measured using the WP4C Dewpoint Potentiometer (Decagon Devices Inc., Logan, UT). For that purpose, 10 g of BL (dry weight) was weighed into 1-L plastic resealable bag. One sample was retained at initial or native water content (θ). The remaining samples were adjusted to several θ values in the range of 600 to 2000 g $H_2O$ $kg^{-1}$ litter and placed in a refrigerator (<4.0°C) to equilibrate for at least 24 h. Approximately 2 g BL was placed in sampling cup for each θ and ψ was measured in replicates of three. Water content was determined by placing 5 to 10 g BL in an oven at 65°C for 48 h and expressed as g $H_20$ $kg^{-1}$ PL on a dry weight basis. All other concentrations are also expressed on a dry weight basis throughout. The relationship between water potential and water content was described with the following power equation (Eq 1.):

$$-\psi = a \times \theta^{b} \tag{1}$$

where a (MPa kg BL $g^{-1}$ $H_20$) is a multiplicative term and θ is water content (g $H_20$ $kg^{-1}$ BL) raised to the power b. Water potential curve equation (Eq. 1) parameters were calculated using the nls procedure for non-linear least squares in the open source statistical analysis software R (R Development Core Team, 2013).

**Water Content Study:**

Four BL samples of differing chemical composition were incubated at 30°C for 45 d at three θ (native or 'as received' θ, 600 g $H_2O$ $kg^{-1}$ BL, and 750 g $H_2O$ $kg^{-1}$ BL on a dry-weight basis) in 500-mL wide mouth, screw cap plastic bottles. Duplicate subsamples were taken at 0, 1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 16, 18, 27, 34, and 38 d. Each experimental unit was aerated at intervals not exceeding 48 h by opening the bottles for 1 min in a ventilation hood.

Changes in substrate concentration with time were modeled by fitting a linear equation of the type y = mx + b to the natural log of the UAN and XN measurements at each time point (Eq. 2)

$$\ln(C) = \ln(C_0) - kt \tag{2}$$

where $C_0$ is the initial concentration, C is the concentration at time = t, and k is slope. This equation is mathematically related to the first-order chemical rate equation (Eq 3.) used by Bao et al. (2008) to compare differences between samples.

$$C = C_0 e^{-kt} \quad (3)$$

Our approach has several advantages over that of Bao et al. (2008). Linear slopes representing decay rates can be related to the first-order rate equation if so desired, yet no assumptions are required concerning the reaction mechanism(s). Logarithmic transformation of such data reduces potential heteroscedasticity that can contribute to error in both model parameter estimation and in drawing conclusions from data analysis (Petersen-Øverleir, 2004). Finally, equation parameters for linear models can be easily compared for statistically significant differences between treatments. Following natural log transformation of the measurements for UAN and XN in this study, data were analyzed using the REG and GLM procedures using the SAS software (SAS, 2014). Results for slope estimation and standard error were reported at a significance level of $\alpha = 0.05$.

**Chemical Amendment Study:**

FGD gypsum and alum were added at a rate of 100 g and 200 g $kg^{-1}$ to three of the above BL samples adjusted $\theta = 750$ g $H_2O$ $kg^{-1}$ BL (dry weight). Samples were incubated at 30°C, aerated and sub-sampled as above at 0, 3, 6, 9, 13, 15, and 19 d. The results of this experiment were compared against the same sample incubated at $\theta = 750$ g $H_2O$ $kg^{-1}$ in the previous study as control. Sampling dates were as closely matched as possible for this comparison. Data for the control samples were analyzed at 0, 3, 6, 9, 12, 16, and 18 d. Observations for all other time points were omitted for the purpose of this study. As in the previous study, measurements were transformed by taking the natural log and data were analyzed using the REG and GLM procedures using the SAS software (SAS, 2014). Results for slope estimation and standard error were reported ($\alpha = 0.05$).

**Water Potential (28-day) Study:**

Two g (dry weight equivalent) from ten BL samples of differing chemical composition were incubated in duplicate for 28 d in 125-mL plastic bottles adjusted to the water potentials at -5, -7 -9 -11

and -13 MPa. Each bottle was aerated at intervals not exceeding 48 hours for the entire period and destructively sampled at the end of 28 d for the analysis of UAN and XN. Slopes (k) representing the decay of UAN and XN for each treatment between the two time points (0 and 28 days) were calculated from Eq. 2. Linear regression was used via the REG procedure in the SAS software (SAS, 2014) to estimate the relationship between the collection of slopes for each sample over the range of $\psi$ values studied. Results were reported as two-point k values for UAN and XN decay in each of ten samples at each value of $\psi$. The results of linear regression of the means of all 2-point k values for UAN and XN decay vs. their respective $\psi$ values are also reported.

**Results:**

**Water Content Study:**

Selected chemical properties of the BL samples used are provided in Table 5.1, and parameters for the equation describing water potential as a function of water content are shown in Table 5.2. Initial UAN contents in the samples were 2064, 2630, 1633, and 556 mg UAN $kg^{-1}$ BL (dry weight) for samples 1, 2, 3, and 4, respectively. These concentrations are in the range reported previously (Qafoku et al., 2001; Rothrock et al. 2010, Mowrer et al., 2013). Over 38 d, UAN in these four litters was observed to follow a general pattern marked by an increase in UAN between 1 and 8 d of incubation followed by a decline in concentration (Table 5.3). This held true in all water content treatments. Rothrock et al. (2010) and Bao et al. (2008) also observed measurable increases in uric acid from litter and allantoin from manure respectively during incubation. The sampling times in our study were placed more densely than in previous work and so captured a more detailed pattern of fluctuation occurring in the concentration of UAN with time. This result, unexpected and previously undescribed, suggests a robust micro-ecology of bacterial and fungal organisms rapidly equilibrating populations to the conditions of water and temperature set for the incubation treatments. It is impossible from the results to deduce the source of the N responsible for increases in the UAN pool. It is proposed here that these increases are confined to one of two processes: continued purine catabolism or incorporation of previously mineralized N into nucleic material. In either case, the idea that uric acid in BL represents an inceptive pool of N that only

undergoes decay is challenged by the observations in this study. Uric acid represents reactant and product (Fig. 5.1), therefore increases can be expected under certain conditions of storage.

XN from the BL samples extracted and measured simultaneously with UAN followed the same general pattern (Table 5.4). However, XN concentrations were much lower than UAN in all treatments at all sampling periods. Initial XN in samples 1 through 4 was 632, 339, 212, and 89 mg $kg^{-1}$ BL (dry weight). Initial UAN:XN ratios for the samples in the same order were 3.3:1, 7.8:1, 7.7:1 and 6.3:1. Final UAN:XN ratios (where XN was measurable) ranged from 11:1 to 83:1, suggesting that xanthine is more readily catabolized in BL than uric acid regardless of water content.

The results of linear regression to estimate the slope (k) from Eq.2 for UAN and XN are provided in Table 5.5. Data for θ and ψ are also provided. Rates of decay of UAN from the 'as received' water content treatment litters (range of 172 to 438 g $H_2O$ $kg^{-1}$ BL) averaged 0.0076 with a range of 0.0033 to 0.0110 mg $day^{-1}$. Rates of UAN decay for the 600 and 750 g $H_2O$ $kg^{-1}$ BL ranged from 0.0299 to 0.0681 (mean = 0.0516) and 0.0063 to 0.1191 (mean = 0.0578) $day^{-1}$ each. The very low value of the rate constant for the highest θ treatment for sample BL1 is anomalous to the other samples in this treatment group. The pattern of decay observed for this particular sample/treatment shows a minimum concentration achieved at day 12 followed by an increase to above initial levels for the remaining period of incubation. All other samples at the 750 g $H_2O$ $kg^{-1}$ BL treatment show substantial declines in UAN concentration beyond the $16^{th}$ day of sampling. The slopes (k) for the decay of XN at the 'as received' θ treatment ranged from 0.0152 to 0.0763 (mean = 0.0449 $day^{-1}$). For the 600 and 750 g $H_2O$ $kg^{-1}$ BL, the XN decay rates ranged from 0.0762 o 0.1161 (mean= 0.0988) and 0.0347 to 0.1308 (mean = 0.0950) $day^{-1}$ each. The means for the UAN and XN decay rates were observed to increase with increasing water content. Results of the GLM procedure (SAS, 2014) indicate significant effects of water content on the rate of UAN decay in all BL samples studied (Fig. 5.5). The effect of water content on the decay of XN in BL was significant in samples 3 and 4 only.

The results indicate that XN decays at a faster rate than UAN over 38 days. Patterns of decay in the observations (Tables 5.3 & 5.4) also show that XN is more nearly depleted during this time period

than UAN. Uric acid in the BL samples we studied persisted for longer periods of time at higher concentrations than xanthine in all samples at all θ levels.

**Chemical Amendment Study:**

FGD gypsum- and alum-amended litters were incubated to study the effect of these common chemical amendments on the pattern of UAN and XN decay in stored BL. Changes in the pH of BL following additions of FGD gypsum were less than those following additions of alum as demonstrated in Figure 5.2. Water contents were adjusted to 750 g $H_2O$ $kg^{-1}$ BL as this was the treatment that generally exhibited the greatest rates of decay for UAN and XN. We chose to investigate BL samples 2, 3, and 4 BL θ due to the anomalous results observed in BL sample 1 in the 750 g $H_2O$ $kg^{-1}$ treatment. Results for the decay of UAN and XN in amended litters are presented in Table 5.6 and Table 5.7, respectively. Slopes (k) for UAN and XN decay from Equation 2 are presented in Table 5.8.

Decay rates for UAN and XN expressed as linear slopes appear greater when calculated over 18 to 19 d when compared to 38 d. The results in this study were more influenced by the most rapid period of decay in the second week, but not the less rapid period after this when concentrations began to level off. Results of the GLM procedure (SAS, 2014) show no significant effects on UAN decay with additions of FGD gypsum or alum in BL samples 2 and 4. However, three significant groups of treatments were observed in BL sample 3. Additions of FGD gypsum at 100 and 200 g $kg^{-1}$ BL increased the rate of UAN decay while addition of alum at 200 g $kg^{-1}$ BL decreased the rate of UAN decay. Additions of chemical amendments were not found to have significant effect on the rate of XN decay in any of the samples used in this study.

**Water Potential Study:**

We chose to explore the effect of water content on UAN and XN decay in BL in a simpler fashion based on the observations of the change in UAN and XN in the water content study. A study was conducted on ten BL samples adjusted to five water potentials between -13 and -5 MPa. Water potential, rather than water content by mass, is often more closely related to microbial and enzymatic activity. Initial UAN concentrations ranged from 696 to 2643 mg $kg^{-1}$ BL (dry weight) with a mean value

of 2119 mg kg$^{-1}$ BL and initial XN concentrations ranged from 67 to 298 mg kg$^{-1}$ BL (mean = 162 mg kg$^{-1}$ BL). First-order rate constants calculated from two time points ($t_1$ = 0 days; $t_2$ = 28 days) tell us little about the rapid fluctuations occurring in the first two weeks but do provide insight on the overall decay rates for UAN and XN in BL that can be expected after approximately one month of storage under a reasonable range of water potentials for BL stored under 'field' conditions. Furthermore, the rate constants are based simply on a ratio of initial to final concentrations. The 2-point k values for each litter at each water potential are shown in Table 5.9. For three of the samples, XN was not detected in any of the treatments at 28 d and was presumed to have decayed to a concentration below the analytical detection limit 5 mg kg$^{-1}$. No k value was calculated for these samples. In a fourth BL sample used in this study, XN in one of the treatments was not detected. For this sample the slope of the change in 2-point k values was truncated to the remaining four treatments. The average 2-point first order decay constants for UAN and XN from ten different BL samples incubated at 30°C for 28 d, when plotted against water potential, exhibit a strong positive relationship (Figures 5.3 and 5.4). Linear regression equations for the relationship between these constants and water potential are: $k_{UAN}$ = 0.0054(MPa) + 0.101 ($r^2$ = 0.9987); and $k_{XN}$ = 0.0066(MPa) + 0.1101 ($r^2$ = 0.9285). Though these relationships are very strong, the standard deviation for the mean 2-point k value at each water potential is relatively high. However, there are strong relationships between the UAN and XN 2-point k values and water potential for each individual sample in this study (Table 5.9), supporting the idea that decay rates are positively related to water potential.

**Conclusions:**

Our results indicate that rates of decay of both UAN and XN from BL increase with increasing water potential. XN decays more rapidly than UAN. We also found that FGD gypsum and alum, common chemical amendments used to prevent ammonia volatilization and phosphorus runoff from BL, had a significant effect on the pattern of UAN decay in one of three samples and no effect on XN decay when added at 100 g or 200 g kg$^{-1}$ BL. Finally, we have shown there exists a potential for a predictive index for the decay of these important N compounds in BL as a function of water potential during one

month of storage. Our studies suggest that a more thorough investigation of purines and ureides in BL may provide better insight on N mineralization dynamics.

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TABLES

Table 5.1: Selected chemical properties of BL samples used in the water content study.

| PL | µs/cm | | g $kg^{-1}$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | EC | pH | N | K | Al | Ca | S | Cu | Zn | Na | Cl |
| 1 | 1138 | 8.65 | 47.6 | 29.3 | 1.21 | 27.1 | 7.0 | 0.32 | 0.38 | 5.9 | 6.7 |
| 2 | 1001 | 8.66 | 44.2 | 26.6 | 1.01 | 96.8 | 5.5 | 0.48 | 0.37 | 4.6 | 8 |
| 3 | 1006 | 8.29 | 42.9 | 24.3 | 2.64 | 19.8 | 5.5 | 0.20 | 0.28 | 5.5 | 9 |
| 4 | 717 | 8.62 | 31.5 | 19.4 | 0.95 | 57.2 | 5.6 | 0.25 | 0.31 | 3.8 | 3.5 |

Table 5.2: Parameters for the water potential equation of the form $-\psi = a \times \theta^{b}$; where $\theta$ is water content (g $H_2O$ kg-1 PL).

| PL Sample No. | a | b | RSE |
|---|---|---|---|
| 1 | 65772 | -1.413 | 0.7934 |
| 2 | 10064 | -1.101 | 0.4941 |
| 3 | 63270 | -1.423 | 0.4892 |
| 4 | 68170 | -1.506 | 0.2638 |

RSE = residual standard error of regression

Table 5.3: Changes in uric acid nitrogen in four broiler litter samples over 38 days incubated at 30°C and at three water contents.

| | PL Sample Identifier | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | 2 | | | 3 | | | 4 | | |
| | Water Content (g $H_20$ kg$^{-1}$ PL dry weight) | | | | | | | | | | | |
| Time (days) | 429 | 600 | 750 | 438 | 600 | 750 | 172 | 600 | 750 | 199 | 600 | 750 |
| 0 | 2064.1 | 2064.1 | 2064.1 | 2629.6 | 2629.6 | 2629.6 | 1633.3 | 1633.3 | 1633.3 | 555.7 | 555.7 | 555.7 |
| 1 | 2382.8 | 2173.5 | 2167.9 | 2926.0 | 3981.7 | 2680.9 | 1686.7 | 1901.0 | 1706.4 | 323.3 | 526.8 | 503.0 |
| 2 | 2323.2 | 2633.7 | 2406.4 | 3521.6 | 5208.4 | 2606.9 | 2175.4 | 1100.7 | 2104.6 | 459.0 | 415.5 | 482.1 |
| 3 | 2278.1 | 2469.4 | 2191.1 | 4943.9 | 5283.5 | 5044.9 | 1424.6 | 1600.4 | 1599.6 | 633.3 | 831.8 | 579.7 |
| 5 | 2059.8 | 2390.5 | 2211.7 | 6402.2 | 3437.6 | 4071.1 | 1792.5 | 1603.2 | 1272.8 | 405.6 | 1108.5 | 394.7 |
| 6 | 3091.3 | 2233.5 | 4882.9 | 6198.2 | 3864.6 | 3812.1 | 1829.4 | 1806.2 | 1663.9 | 466.0 | 450.3 | 35.8 |
| 7 | 2180.3 | 2620.4 | 4514.5 | 5699.4 | 3579.8 | 2790.9 | 1919.2 | 1767.6 | 1622.2 | 655.2 | 859.2 | 146.1 |
| 8 | 2331.7 | 2053.4 | 4255.0 | 5953.3 | 3423.3 | 2706.9 | 1020.2 | 2310.6 | 1307.0 | 457.4 | 629.9 | 114.0 |
| 9 | 1313.5 | 1513.0 | 1830.8 | 4980.0 | 2495.8 | 2283.5 | 1404.2 | 1242.0 | 1076.4 | 543.8 | 714.9 | 128.0 |
| 10 | 1504.8 | 1460.2 | 1829.5 | 4552.9 | 3058.3 | 946.2 | 1506.8 | 708.1 | 484.2 | 609.6 | 500.4 | 14.2 |
| 12 | 1488.1 | 1262.0 | 1632.2 | 4539.2 | 2195.9 | 1505.0 | 1503.6 | 591.0 | 278.1 | 494.8 | 789.2 | 109.7 |
| 16 | 2355.5 | 1419.6 | 2774.4 | 4005.0 | 1565.1 | 1301.4 | 1044.5 | 527.5 | 216.7 | 406.0 | 681.1 | 7.2 |
| 18 | 1593.1 | 1376.0 | 2276.9 | 4836.4 | 1825.9 | 1413.6 | 1003.9 | 218.9 | 80.0 | 405.6 | 554.0 | 70.9 |
| 27 | 1190.0 | 1696.3 | 2006.5 | 4473.8 | 1172.6 | 972.2 | 1874.9 | 321.6 | 113.9 | 441.7 | 119.3 | 64.3 |
| 34 | 2268.5 | 719.8 | 1896.2 | 3407.6 | 1000.8 | 883.2 | 1555.9 | 243.2 | 58.3 | 474.3 | 78.6 | 40.0 |
| 38 | 2114.8 | 772.8 | 2300.2 | 2010.4 | 837.1 | 969.7 | 841.2 | 161.8 | 24.4 | 426.3 | 65.1 | 42.4 |

mg Uric Acid N kg$^{-1}$ PL (dry weight basis)

Table 5.4: Change in xanthine nitrogen in four broiler litter samples over 38 days incubated at 30°C and at three water contents.

| | PL Sample Identifier | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | 2 | | | 3 | | | 4 | | |
| | Water Content (g $H_2$0 kg$^{-1}$ PL dry weight) | | | | | | | | | | | |
| Time (days) | 429 | 600 | 750 | 438 | 600 | 750 | 172 | 600 | 750 | 199 | 600 | 750 |
| 0 | 631.7 | 631.7 | 631.7 | 338.7 | 338.7 | 338.7 | 212.0 | 212.0 | 212.0 | 88.9 | 88.9 | 88.9 |
| 1 | 656.2 | 686.8 | 638.8 | 346.9 | 455.6 | 369.5 | 92.5 | 180.2 | 183.0 | 42.6 | 142.4 | 183.8 |
| 2 | 576.5 | 767.5 | 720.0 | 391.8 | 524.5 | 336.1 | 240.7 | 250.4 | 428.0 | 89.4 | 122.6 | 190.2 |
| 3 | 531.0 | 746.1 | 593.9 | 587.4 | 439.2 | 451.7 | 180.4 | 386.7 | 341.9 | 40.9 | 143.0 | 116.9 |
| 5 | 543.3 | 716.5 | 576.5 | 719.1 | 219.1 | 229.4 | 183.8 | 390.1 | 183.2 | 84.1 | 332.2 | 106.2 |
| 6 | 757.8 | 633.7 | 1230.3 | 747.8 | 228.5 | 203.1 | 112.5 | 104.8 | 56.9 | 89.3 | 235.5 | 47.5 |
| 7 | 582.1 | 714.9 | 1186.4 | 662.6 | 262.6 | 126.4 | 97.1 | 86.4 | 49.5 | 41.0 | 171.2 | 31.6 |
| 8 | 644.4 | 541.3 | 1124.1 | 690.2 | 189.2 | 143.9 | 43.7 | 105.7 | 46.1 | 99.1 | 254.9 | 56.1 |
| 9 | 440.4 | 388.3 | 638.0 | 285.3 | 61.8 | 26.2 | 86.5 | 33.6 | 40.3 | 32.6 | 153.1 | 33.5 |
| 10 | 463.6 | 321.6 | 620.7 | 439.0 | 126.9 | 81.4 | 204.1 | 64.5 | 52.6 | 74.7 | 265.7 | 48.0 |
| 12 | 139.1 | 43.1 | 203.9 | 284.5 | 47.8 | 25.5 | 220.7 | 26.4 | 52.9 | 34.2 | 154.6 | 35.4 |
| 16 | 306.0 | 39.8 | 380.6 | 206.0 | 41.4 | 22.4 | 103.7 | 44.4 | 28.7 | 39.7 | 80.0 | 24.2 |
| 18 | 269.4 | 75.5 | 406.1 | 161.3 | 34.3 | 23.3 | 109.6 | 26.4 | 34.0 | 33.4 | 90.1 | 19.6 |
| 27 | 171.5 | 57.5 | 371.9 | 107.1 | 30.6 | 30.2 | 187.9 | 29.6 | 27.8 | 68.1 | 22.1 | 8.9 |
| 34 | 208.4 | 22.6 | 239.4 | 38.2 | 5.4 | 5.7 | 78.1 | 6.4 | 3.7 | 14.5 | 2.5 | 0.0 |
| 38 | 106.6 | 10.3 | 221.3 | 33.1 | 10.1 | 19.0 | 68.5 | 14.5 | 2.1 | 9.6 | 2.2 | 0.0 |

mg Xanthine N kg$^{-1}$ PL (dry weight)

Table 5.5: Water content, water potential, results for slope estimates, and associated standard errors from linear regression of the type ln(C) = ln($C_0$) - kt for uric acid nitrogen (UAN) and xanthine nitrogen (XN) decay in four incubated broiler litters (BL) adjusted to three water contents (θ).

| Sample | θ * | ψ** | UAN slope (k)† | standard error | XN slope (k)† | standard error |
|---|---|---|---|---|---|---|
| BL1 | 429 | -13.11 | 0.00554[a] | 0.0058 | 0.04558[a] | 0.0074 |
| | 600 | -6.79 | 0.02991[b] | 0.0046 | 0.07616[a] | 0.0261 |
| | 750 | -5.19 | 0.00634[a] | 0.0075 | 0.03474[a] | 0.0091 |
| BL2 | 438 | -12.65 | 0.01048[a] | 0.0070 | 0.07632[a] | 0.0093 |
| | 600 | -8.91 | 0.04541[b] | 0.0048 | 0.11358[a] | 0.0107 |
| | 750 | -6.33 | 0.03932[b] | 0.0079 | 0.09936[a] | 0.0165 |
| BL3 | 172 | -41.82 | 0.01096[a] | 0.0056 | 0.01521[a] | 0.0108 |
| | 600 | -6.66 | 0.06806[b] | 0.0090 | 0.08941[b] | 0.0139 |
| | 750 | -5.12 | 0.11905[c] | 0.0107 | 0.11507[b] | 0.0129 |
| BL4 | 199 | -23.56 | 0.00325[a] | 0.0043 | 0.04259[a] | 0.0105 |
| | 600 | -3.46 | 0.06286[b] | 0.0105 | 0.11614[b] | 0.0164 |
| | 750 | -2.93 | 0.06653[b] | 0.0252 | 0.13081[b] | 0.0093 |

* g $H_2O$ $kg^{-1}$ BL (dry weight)

** MPa

† mg N $kg^{-1}$ BL (dry weight) ; Letters indicate significantly different groups of treatments within a sample as determined by the general linear model procedure in SAS.

Table 5.6: Changes in uric acid nitrogen in three broiler litter samples adjusted to a water content of 750 g $H_20$ kg$^{-1}$ PL, amended with two rates of FGD gypsum or alum and incubated at 30°C for 19 days .

| | PL Sample Identifier | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | | | 3 | | | | 4 | | | |
| | (g amendment kg-1 PL dry weight) | | | | | | | | | | | |
| Time (days) | 100g | 200g | 100a | 200a | 100g | 200g | 100a | 200a | 100g | 200g | 100a | 200a |
| 0 | 2629.6 | 2629.6 | 2629.6 | 2629.6 | 1633.3 | 1633.3 | 1633.3 | 1633.3 | 555.7 | 555.7 | 555.7 | 555.7 |
| 3 | 1587.5 | 2667.7 | 3101.4 | 2383.0 | 1368.6 | 1166.3 | 1364.8 | 1013.9 | 172.1 | 140.2 | 293.2 | 209.1 |
| 6 | 2230.1 | 703.8 | 1406.3 | 1285.4 | 519.3 | 79.3 | 577.1 | 224.3 | 174.1 | 167.9 | 488.6 | 110.1 |
| 9 | 1404.1 | 1436.7 | 3178.7 | 2630.7 | 637.6 | 124.4 | 1530.3 | 790.3 | 24.2 | 5.1 | 209.5 | 49.7 |
| 13 | 277.6 | 148.3 | 101.9 | 75.0 | 153.1 | 17.6 | 81.0 | 271.4 | 5.1 | 5.1 | 93.7 | 20.3 |
| 15 | 884.5 | 607.3 | 343.9 | 269.2 | 143.1 | 27.4 | 146.4 | 550.2 | 16.1 | 13.5 | 36.1 | 5.1 |
| 19 | 1155.2 | 747.0 | 249.2 | 303.0 | 25.1 | 13.0 | 118.6 | 396.0 | 10.9 | 7.9 | 14.8 | 10.0 |

mg Uric Acid N kg$^{-1}$ PL (dry weight basis)
g = FGD gypsum
a = alum

Table 5.7: Changes in xanthine nitrogen in three broiler litter samples adjusted to a water content of 750 g $H_20$ $kg^{-1}$ PL, amended with two rates of FGD gypsum or alum and incubated at 30°C for 19 days.

| | PL Sample Identifier | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | | | 3 | | | | 4 | | | |
| | (g amendment kg-1 PL dry weight) | | | | | | | | | | | |
| Time (days) | 100g | 200g | 100a | 200a | 100g | 200g | 100a | 200a | 100g | 200g | 100a | 200a |
| 0 | 338.7 | 338.7 | 338.7 | 338.7 | 212.0 | 212.0 | 212.0 | 212.0 | 88.9 | 88.9 | 88.9 | 88.9 |
| 3 | 172.4 | 209.2 | 325.2 | 214.8 | 82.8 | 69.4 | 124.4 | 62.0 | 85.8 | 74.0 | 122.2 | 94.5 |
| 6 | 96.7 | 44.3 | 247.3 | 122.2 | 18.5 | 12.9 | 95.4 | 71.2 | 43.4 | 30.7 | 139.0 | 25.6 |
| 9 | 115.4 | 48.9 | 122.4 | 84.8 | 24.3 | 17.7 | 119.7 | 101.1 | 10.8 | 7.0 | 32.2 | 11.3 |
| 13 | 21.7 | 37.5 | 12.6 | 7.7 | 8.7 | 8.6 | 13.6 | 74.6 | 5.6 | 4.2 | 12.2 | 5.2 |
| 15 | 45.1 | 42.4 | 51.4 | 74.3 | 17.9 | 21.3 | 9.6 | 78.6 | 18.3 | 10.3 | 9.3 | 8.1 |
| 19 | 27.3 | 32.2 | 25.3 | 47.8 | <5.0 | <5.0 | <5.0 | <5.0 | 9.7 | 5.7 | 18.0 | 15.8 |

mg Xanthine N $kg^{-1}$ PL (dry weight)
g = FGD gypsum
a = alum

Table 5.8: Results for slope estimates and associated standard errors from linear regression of the type $\ln(C) = \ln(C_0) - kt$ for uric acid nitrogen (UAN) and xanthine nitrogen (XN) decay in three incubated broiler litters (BL) amended either flue gas desulfurized (FGD) gypsum or alum at rates of 100 and 200g $kg^{-1}$ BL.

| Sample | Treatment* | UAN slope† | standard error | XN slope† | standard error |
|---|---|---|---|---|---|
| BL2 | no amendment | 0.06440[a] | 0.2000 | 0.18697[a] | 0.0419 |
| | 100FGD | 0.06827[a] | 0.0388 | 0.13524[a] | 0.0262 |
| | 200FGD | 0.09779[a] | 0.0499 | 0.11772[a] | 0.0320 |
| | 100Alum | 0.16543[a] | 0.0553 | 0.16808[a] | 0.0423 |
| | 200Alum | 0.16048[a] | 0.0593 | 0.12672[a] | 0.0573 |
| BL3 | no amendment | 0.17187[a] | 0.0276 | 0.12310[a] | 0.0311 |
| | 100FGD | 0.21039[b] | 0.0262 | 0.17244[a] | 0.0346 |
| | 200FGD | 0.26887[b] | 0.0446 | 0.16047[a] | 0.0442 |
| | 100Alum | 0.16617[a] | 0.0452 | 0.20960[a] | 0.0313 |
| | 200Alum | 0.06191[c] | 0.0389 | 0.12414[a] | 0.0532 |
| BL4 | no amendment | 0.16654[a] | 0.0719 | 0.09257[a] | 0.0154 |
| | 100FGD | 0.23057[a] | 0.0502 | 0.13572[a] | 0.0402 |
| | 200FGD | 0.23878[a] | 0.0701 | 0.16203[a] | 0.0394 |
| | 100Alum | 0.19078[a] | 0.0291 | 0.14063[a] | 0.0394 |
| | 200Alum | 0.23849[a] | 0.0318 | 0.13256[a] | 0.0453 |

* g chemical amendment $kg^{-1}$ BL (dry weight).
† mg N $kg^{-1}$ BL (dry weight) ; Letters indicate significantly different groups of treatments within a sample as determined by the general linear model procedure in SAS.

Table 5.9: Two-point calculated rates for the decay of UAN and XN and their means for ten BL samples adjusted to 5 water potentials (ψ). $C_0$ = initial concentration. Slope = slope of linear regression ($k_\psi$ = slope(ψ) + intercept). $r^2$ = correlation coefficient for linear regression.

| | BL Sample Identifier | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BL Water Potential (MPa) | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | mean |
| | Calculated UAN 2-point decay rates (k) | | | | | | | | | | |
| -13 | 0.0479 | 0.0441 | 0.0467 | 0.027 | 0.0347 | 0.0144 | 0.0129 | 0.0643 | 0.0086 | 0.0026 | 0.0303 |
| -11 | 0.056 | 0.0633 | 0.0577 | 0.0326 | 0.0429 | 0.0289 | 0.0194 | 0.08 | 0.0241 | 0.0173 | 0.0422 |
| -9 | 0.044 | 0.0667 | 0.0737 | 0.0375 | 0.0465 | 0.0493 | 0.0419 | 0.0881 | 0.0279 | 0.0379 | 0.0513 |
| -7 | 0.0669 | 0.0711 | 0.076 | 0.0407 | 0.0544 | 0.032 | 0.0917 | 0.0842 | 0.0513 | 0.0623 | 0.0631 |
| -5 | 0.0845 | 0.0751 | 0.0773 | 0.1012 | 0.0498 | 0.0533 | 0.0857 | 0.0938 | 0.0655 | 0.0548 | 0.0741 |
| $C_0$ | 2643 | 773 | 865 | 6843 | 696 | 1552 | 2615 | 1670 | 1762 | 1771 | |
| slope | 0.0042 | 0.0035 | 0.004 | 0.0078 | 0.0021 | 0.004 | 0.0109 | 0.0032 | 0.007 | 0.0075 | |
| $r^2$ | 0.6638 | 0.844 | 0.868 | 0.667 | 0.782 | 0.6491 | 0.881 | 0.802 | 0.9647 | 0.89 | |

| | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Calculated XN 2-point decay rates (k) | | | | | | | | | | |
| -13 | 0.0567 | 0.0193 | ND | 0.0018 | ND | 0.0394 | 0.037 | ND | -0.0099 | 0.0086 | 0.0218 |
| -11 | 0.0598 | 0.0397 | ND | 0.0142 | ND | 0.0634 | 0.0503 | ND | 0.0294 | 0.0327 | 0.0413 |
| -9 | 0.0652 | 0.0353 | ND | 0.0543 | ND | 0.069 | 0.0664 | ND | 0.0094 | 0.0382 | 0.0482 |
| -7 | 0.0889 | 0.053 | ND | 0.1195 | ND | 0.0836 | 0.0891 | ND | 0.0367 | 0.0382 | 0.0727 |
| -5 | 0.1026 | 0.0406 | ND | ND | ND | 0.0947 | 0.1135 | ND | 0.0401 | 0.0387 | 0.0717 |
| $C_0$ | 298 | 86 | 67 | 258 | 132 | 206 | 177 | 95 | 173 | 125 | |
| slope | 0.006 | 0.0028 | | 0.0197 | | 0.0065 | 0.0116 | | 0.0054 | 0.003 | |
| $r^2$ | 0.9044 | 0.5285 | | 0.9173 | | 0.9634 | 0.834 | | 0.6501 | 0.6466 | |

ND = Not detected

FIGURES

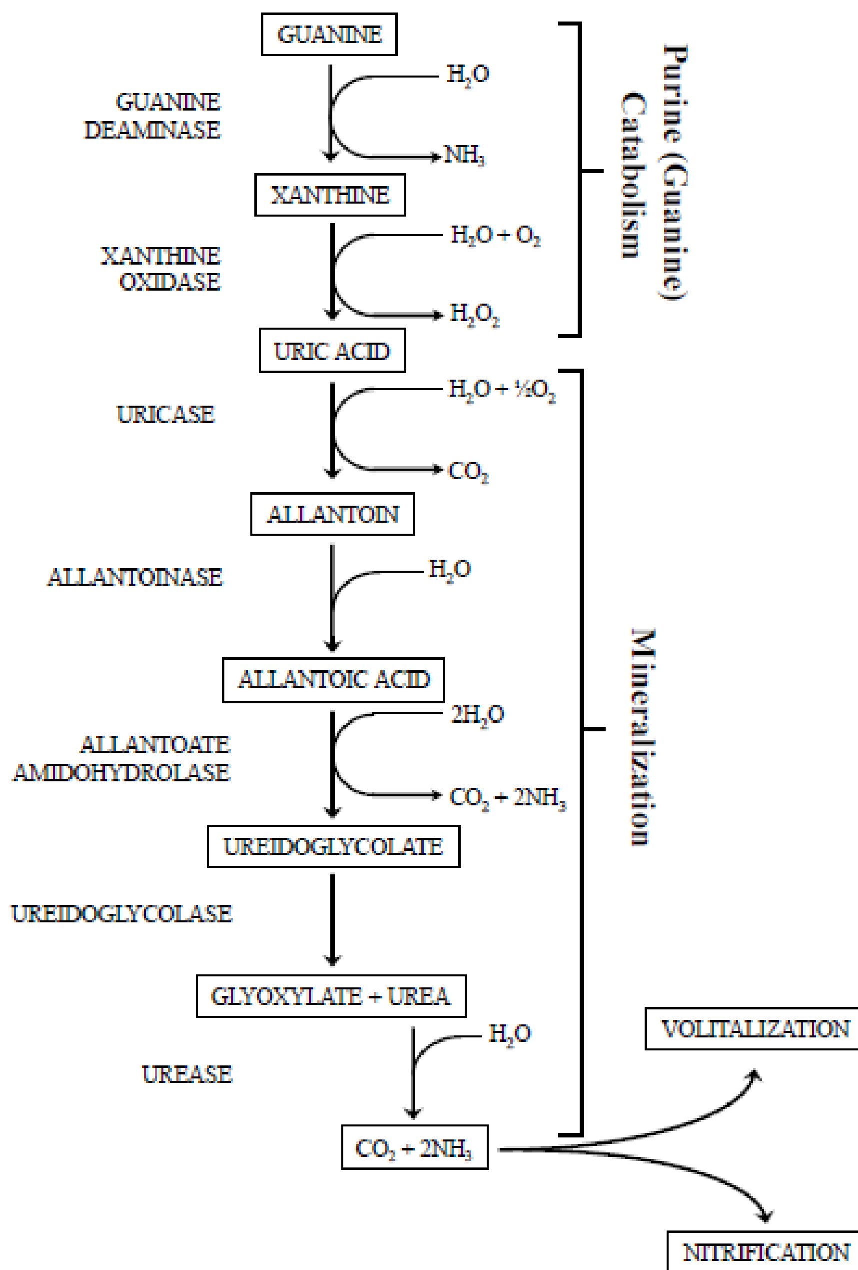


Figure 5.1: Simplified Purine (Guanine) Catabolysis – Nitrogen Mineralization pathway. Modified from Carlile (1984) and Yuan et al. (1999).

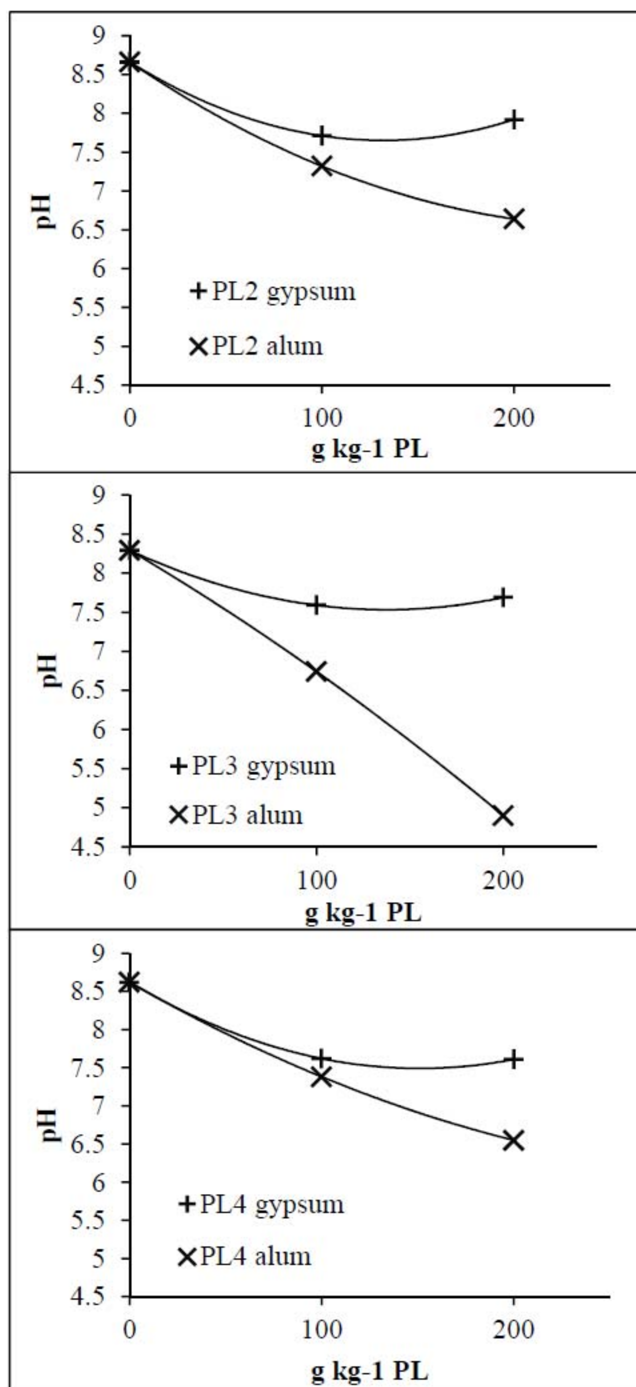


Figure 5.2: Effect of flue-gas desulfurized (FGD) gypsum or alum additions at 100 and 200 g $kg^{-1}$ broiler litter (BL) on BL pH.

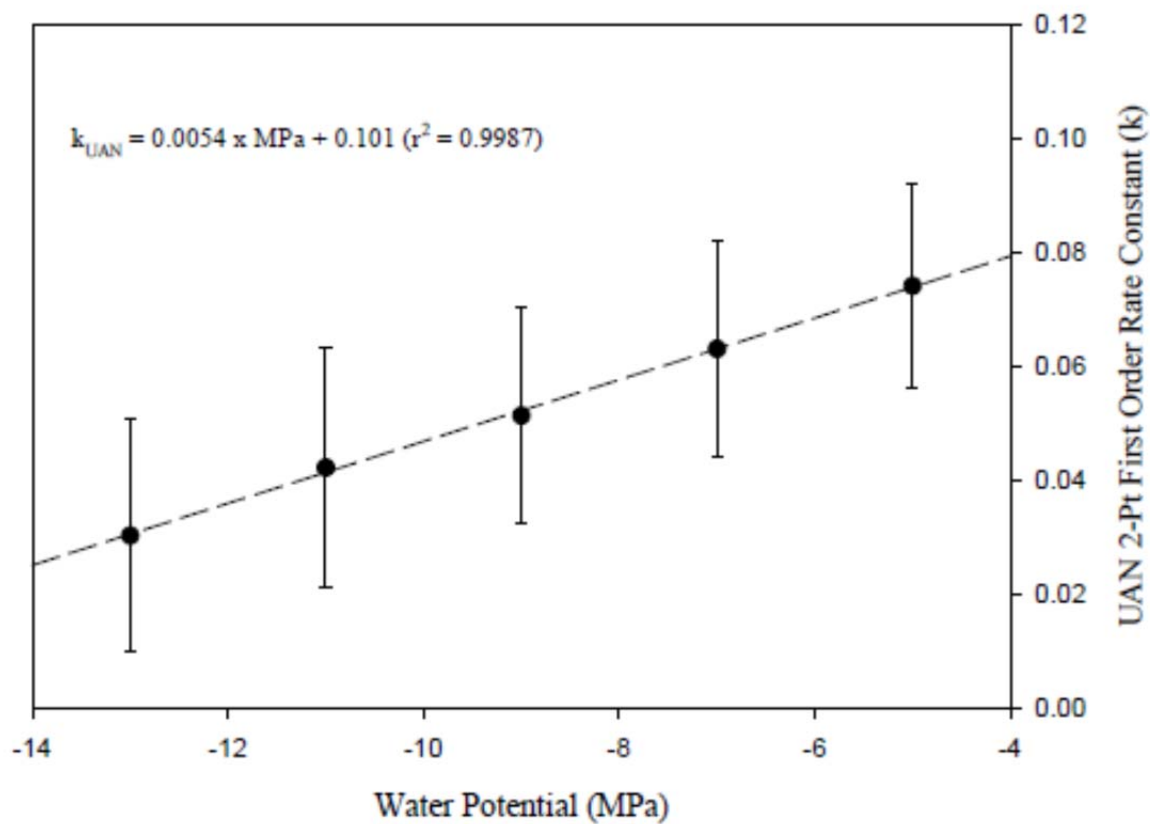


Figure 5.3: Linear regression of two-point calculated decay rate means as a function of water potential (ψ; MPa) for UAN. Error bars = +/- 1 standard deviation.

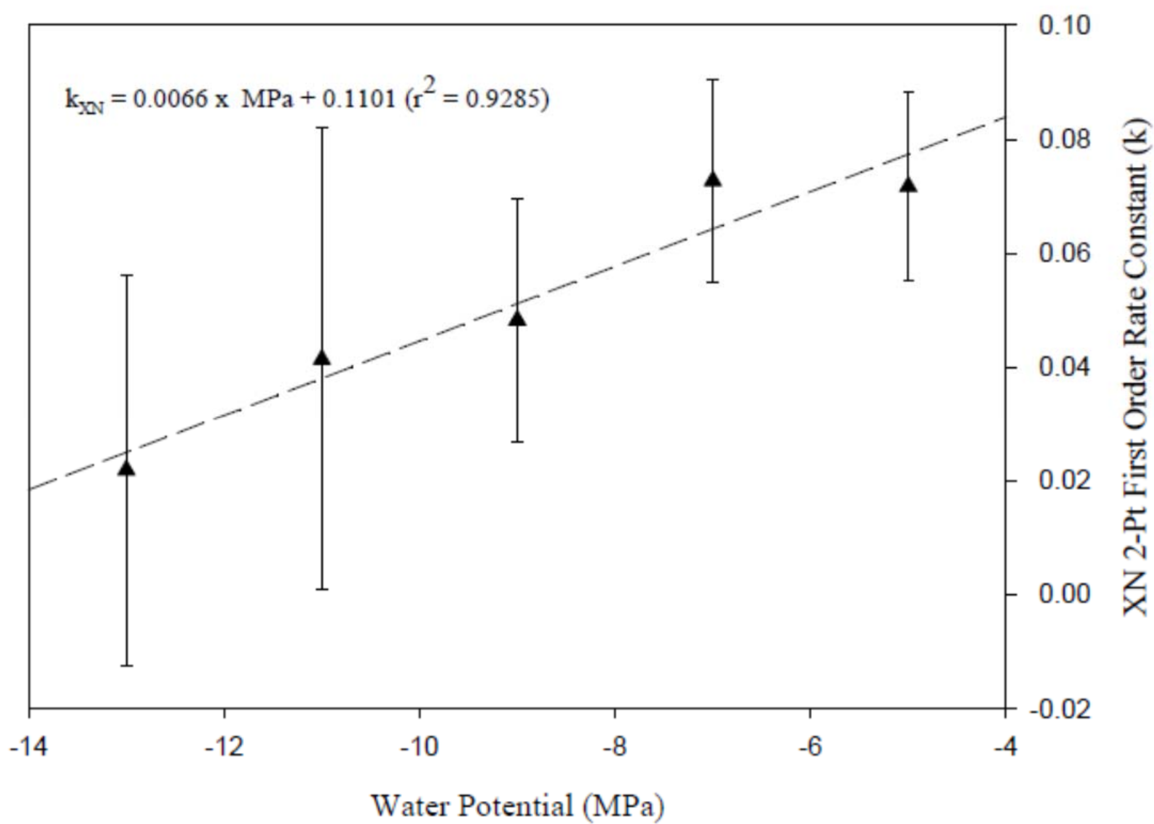


Figure 5.4 Linear regression of two-point calculated decay rate means as a function of water potential (ψ; MPa) for XN. Error bars = +/- 1 standard deviation.

# CONCLUSION

The focus of this work has been to contribute to our understanding of the details of the purine catabolysis - N mineralization pathway as it pertains to the release of mineral N from poultry litter for the purpose of crop fertilization. There is an established economic value for PL in the production of agricultural commodities arising largely from its nitrogen content. Producers and users of this material have an expectation that approximately half of the total N contained within will become available to a given crop. However, this expectation is based upon conclusions drawn from a pool of highly variable research results. Actual results in the field frequently deviate from prediction. The idea has been put forward in this work that the deviation between laboratory prediction and outcome in the field can be reduced by placing emphasis on the details of the purine catabolysis - N mineralization pathway.

This work has produced an improved method for the measurement of uric acid by HPLC UV/VIS detection. The major improvement comes in the form of an extractant demonstrated to completely solubilize the uric acid from PL without causing rapid degradation of the analyte. This quality was absolutely necessary for using measurements from the lab to calibrate NIR for UAN in PL. This work has also contributed a considerable amount to the body of research concerned with the use of NIR in measuring N forms in PL. Direct comparisons between NIR calibrations developed using dried samples and samples of ‘as received’ water content has shown that drying PL is unnecessary for the success of this process. Drying PL samples prior to analysis causes severe loss and transformation of N forms that measurement afterward will produce a result unrelated to the original material. If the central thesis of this work - that attention to individual organic N compounds is the key to improvement - then drying PL samples should never be part of any procedure for determining any form of N. NIR calibrations using PL samples with ‘as received’ $\theta$ were successful for several forms of N identified in previous studies as being

important to predicting N release for crop production. Strong potential has been demonstrated for the rapid analysis by NIR of total N, $NO_3$-N, $NH_4$-N, UAN, Organic N, PMN, $PAN_{100}$, and WSON in PL.

This work has raised a question concerning the difference between the types of PL samples used in research studies and the types of PL material used in practice. If most end-users of this PL are receiving a material that has been stacked or otherwise stored for some period of time prior to land application; are the results of research using fresh collected PL generating realistic expectations of N release? Results of two incubation experiments in this dissertation suggest that the total amount of N mineralized from the organic fraction is less in stored samples than in fresh samples, and that this amount of PMN falls somewhere between the expectations for fresh and composted PL. Both the range and the mean of PMN when expressed as percent of organic N mineralized or percent of total N are lower than those reported in studies using fresh PL. These results support the assertion that a change in the way predictions are based is necessary.

Finally, the research presented here has made inroads in understanding the decay patterns of two of the compounds in the purine catabolysis - N mineralization pathway: uric acid and xanthine. Rate constants for first-order models fit to the decay patterns of these compounds have been shown to increase with increasing water potential in the range of -13 to -5 MPa. As more of these compounds are studied and their relative concentrations and rates of decay are revealed, a better understanding of the process of N release from PL will unfold. It is expected that this improvement in our understanding of this process, coupled with the rapid analysis and turnaround of results by NIR, will lead to practical improvements in the certainty of expectations for end users of PL.

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