GYPSUM EFFECTS ON BROILER LITTER

by

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(Under the Direction of Miguel Cabrera)

ABSTRACT

The poultry industry in the USA produced about 8.8 million broilers (*Gallus gallus domesticus*) in 2014 and generated an estimated 12 million Mg of broiler litter, a mixture of bird excreta, feathers, and bedding material. This byproduct is commonly used as fertilizer for crops and forages because it contains several macro and micro nutrients. In the broiler-rearing houses, ammonia (NH_3) volatilization from broiler litter impairs bird health, decreases the fertilizer value of litter, and negatively impacts the environment. Gypsum has been proposed as a litter amendment due to its hygroscopic nature, but reports of NH_3 abatement vary, and the mechanism responsible for NH_3 reduction is not well understood. Laboratory studies were conducted to evaluate the effect of adding 20 or 40% flue-gas desulfurization gypsum (FGG) to broiler litter on litter water content, urea-degrading bacteria (UDB) and nitrogen (N) mineralization in litter, and to identify the mechanism responsible for reductions in NH_3 loss. Results show that FGG can absorb moisture from litter, thereby increasing matric and osmotic stress, which led to a 38 to 71 % decrease in UDB. The stress encountered by microorganisms led to a 9.9 to 10.6 % increase in N mineralization possibly due to an increase in urease activity that ranged from 27 to 41 %. Amending litter with FGG also decreased litter pH by 0.09 to 0.84 pH units, with a consequent 18 to 28% decrease in NH_3 volatilization. Experiments were conducted to better

understand the mechanism responsible for pH suppression showed that the addition of gypsum to litter decreased pH immediately due to the precipitation of calcium carbonate ($CaCO_3$) from gypsum-derived calcium and litter bicarbonate. As urea was hydrolyzed in gypsum-amended litter, additional $CaCO_3$ precipitated and buffered against large increases in pH that accompany urea hydrolysis.

INDEX WORDS: Ammonia, volatilization, nitrogen, broiler litter, gypsum, flue-gas desulfurization gypsum, urea-degrading bacteria, $CaCO_3$, surface-application

EFFECTS OF GYPSUM ADDITION TO BROILER LITTER

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CHAPTER 1

LITERATURE REVIEW

Broiler Production

The United States (US) poultry industry is the largest in the world and was valued at more than $44.1 billion in 2013 (USDA, 2014). The poultry industry is generally divided into three segments: broilers, turkeys, and eggs. Broiler production accounts for 65% of the poultry industry, and is the single largest segment of meat production in the US, producing more than 8.6 billion birds annually (USDA 2014). Broilers are classified as chickens less than 13 weeks old that are raised specifically for meat. Before the developments of broilers, chickens were typically used to produce both eggs and meat. During the 1920s and 1930s, broilers were developed for the sole purpose of meat production, and made-up a small portion of the total poultry industry. By the 1950s, broilers became the dominate source of chicken meat. Since this time, US per capita consumption of broiler meat has steadily increased from 13.6 kg in 1965 to 37.8 kg in 2013 (USDA, 2014).

Modern broiler production is a vertically integrated industry in which companies, known as integrators, contract with independent farms or "growers" to raise broilers to maturity. In 1997, there were approximately 48 companies that controlled broiler production, and 15 of these companies controlled 77% of total industry production (Thorton, 1997). Vertical integration provides a relatively stable market for growers, and reduces production cost for the integrators.

In this system, integrator companies own the hatcheries, feed mills, broiler chickens, and processing plants. The growers are responsible for providing housing facilities, labor, and equipment that is used during the grow-out period. In order to reduce transportation costs, integrators contract grower facilities that are located close to feed mills and processing plants. This system has led to the creation of the Broiler Belt in the southern and eastern US where more than 90% of total US production occurs. Georgia, Alabama, and Arkansas are the top three producing states in the nation, and account for almost 40% of total US production. Georgia ranks first among these states producing more than 1.3 billion birds worth more than $4 billion annually (USDA, 2014).

A large portion of Georgia's poultry economy relies on broiler production, and income that is generated by this segment is highly dependent on the health and productivity of the birds. More specifically, income received by growers is based on the performance of their flocks, and they receive bonuses if their flocks' performance is greater than that of the company's average performance. Therefore, growers have an economic incentive to provide optimal conditions that allow birds to achieve their genetic potential.

A major concern for growers is the volatilization of ammonia (NH_3) from the mixture of bedding material and broiler excretion that covers the floor of the rearing facility. NH_3 is considered the most harmful gas in broiler facilities (Carlile, 1984), and it is well documented that the in-house accumulation of volatile NH_3 can decrease bird health and performance (Dawkins et al., 2004; Yahav, 2004; Moore et al., 1999). Broiler respiratory health is impaired when in-house NH_3 levels exceed 20 mg L^{-1} (Anderson et al., 1964), and decreased body weight and feed efficiency occurs when NH_3 concentrations are 25-50 mg L^{-1} (Miles et al., 2002; Miles et al., 2004). Once NH_3 levels are greater than 50-75 mg L^{-1}, broilers are at risk of corneal

ulceration and blindness (Miles et al., 2006). To ensure the safety of broilers, as well as agricultural workers, it is suggested that NH_3 levels do not exceed 50 mg L^{-1}.

The term broiler litter is used to describe the mixture of bedding material, wasted feed, excretion, and feathers that covers the floor of broiler houses (Tasistro et al., 2004; Kelley et al., 1996). Locally available materials such as straw, wood shavings, saw dust, peanut hulls, rice hulls and other absorbent materials are used as bedding material (Miles et al., 2011; Grimes et al., 2006; Malone and Gedamu, 1995). Sand has also been investigated as a bedding material but sand weighs more than pine shavings, and more land would be needed when disposing of sand litter (Bowers et al., 2003). Bedding material is generally used for multiple flocks before growers perform a complete clean-out of the house (Chamblee and Todd, 2002; Malone, 1992). Frequent decaking and total clean-outs are suggested because litter that accumulates over multiple flocks generally has a greater potential for NH_3 volatilization (Loch et al., 2011) as well as increased concentrations of pathogenic microorganisms and nutrients (Kelley et al., 1996; Chamblee and Todd, 2002).

Total broiler litter production per flock varies in the literature due to differences in moisture content, end weight of birds, flock-to-flock variations, and intervals between litter removal (Malone, 1992). Typically, each broiler excretes 1.13 kg of fresh manure that has a water content of approximately 750 g kg^{-1} wet litter (Vest, Merka, and Segars, 1994). Once fresh manure is mixed with bedding material and spilt feed, it is estimated that approxiamtely 1.0 to 2.0 Mg of litter is generated per 1,000 broilers per flock (Chamblee and Todd 2002; NRAES 1999; Malone 1992). Furthermore, Sharpley et al. (2009) estimates that between 105 to 135 Mg of litter is produced per broiler house annually. The US poultry industry produces 10.2 million Mg of litter annually (Dunkley et al., 2010), and the states of Georgia, Arkansas, and Alabama

account for approximately 50% of total litter production (Sharpley et al., 2009; Dunkley et al., 2010; Mitchell and Tyson, 2001). Specifically, the state of Georgia is the largest producer of poultry litter generating about 2 million Mg of poultry litter annually (Dunkley et al., 2010).

Broiler excretion accounts for a substantial portion of the material in broiler litter. Broilers digest 85 to 90% of the dry matter in the feed (NRC, 1994), and excrete approximately 55% of the total nitrogen (N) consumed (Bolan et al., 2010). Nitrogen in broiler litter is in the form of complex organic-N, labile organic-N, and inorganic-N (Diaz et al., 2008; Sharpley and Smith, 1995; Sims and Wolf, 1994). Uric acid and urea are the most common forms of labile organic-N in broiler litter, and represent 70-80% of the total N content (Nahm, 2003; Kelleher et al., 2002). Approximately 50-80 % of N in broiler litter is converted to NH_3 depending on the pH of the litter (Sims and Wolf, 1994; Ritz et al., 2004). Due to the size of the poultry industry and the amount of N excreted by broiler chickens, the poultry industry is responsible for a significant portion of US NH_3 emissions. In fact, it is estimated that 27.5 % (664,238 Mg) of NH_3 emmissions from US domestic animals comes from the poultry industry (USEPA 2004), while 16% of United Kingdom (UK) NH_3 emmissions comes from poultry farms (Misselbrook, 2000). More specifically, in the southeastern US, broiler production releases 33-43% of the agricultural NH_3 emissions (Stephen and Aneja, 2008; Aneja et al., 2003).

Nitrogen Mineralization

Nitrogen mineralization in broiler litter is a microbially mediated enzymatic reation in which organic-N is decomposed to form ammoniacal-N and carbon dioxide (CO_2). Uric acid is the main form of N in fresh chicken excretion, and accounts for 70% of the total N in poultry manure (Groot Koerkamp, 1994). Mineralization of N in broiler litter begins when uricase,

produced by uric acid-degrading (uricolytic) microorganisms, converts 1 mole of uric acid to 2 moles of urea (Equation 1). Each mole of urea is then hydrolyzed by the urease enzyme that is produced by urea-degrading (ureolytic) microorganisms to form 2 moles of NH_3 (Equation 2) (Rothrock et al., 2010).

$$C_5H_4N_4O_3 \text{ (uric acid)} + O_2 + 4H_2O \xrightarrow[Uricase]{} \quad [1]$$

$$2(NH_2)_2CO \text{ (urea)} + C_2H_2O_3 \text{ (glyoxylic acid)} + H_2O_2 + CO_2$$

$$(NH_2)_2CO \text{ (urea)} + H_2O \xrightarrow{Urease} 2NH_3 + CO_2 \quad [2]$$

Urease activity is considered the limiting factor for N mineralization and subsequent NH_3 volatilization (Nahm, 2003). The urease enzyme is an intracellular enzyme (Hammes et al., 2003; Klose and Tabatabai, 2000; Gianfreda et al., 1994) that is produced by ureolytic bacteria and fungi (Rothrock et al., 2008). Ureolytic microorganisms are widespread in the environment (Burbank et al., 2012; Rothrock et al., 2008; Fujita et al., 2008; Hammes et al., 2003), and can potentially represent 17-30% of the soil bacterial community (Lloyd and Scheaffe, 1973). Furthermore, many ureolytic microorganisms produce significant amounts of intracellular urease. For example, urease can represent ~10% of *Helicobacter pylori* total cell protein content (Bauerfeind et a., 1997), and close to 1% of *Bacillus pasteurii* dry cell weight (Braissant et al., 2002).

Bacterial urease is the dominant form of urease in environments where there is considerable amounts of urea present (Barua et al., 2012). Broiler litter can contain large amounts urea, and cultured ureolytic bacteria from poultry litter include members of the genera *Micrococcus*, *Corynebacterium*, and *Alcaligenes* (Schefferle, 1965). A more recent survey of

ureolytic microorganisms in broiler litter, using molecular techniques, identified and quantified a novel group of poultry litter urease producers (PLUPs) that represent 0.1 to 3.1% of the total microbial population (Rothrock et al., 2008; 2010). Broiler litter ureolytic bacteria were found to be the dominate urease producers, and were quantified at $49.6 \pm 12.0 \times 10^7$ cells g^{-1} compared to ureolytic fungi at $0.02 \pm 0.00 \times 10^6$ cells g^{-1}. Results from this work show that mineralization of urea can be delayed when the ureolytic bacteria to fungi ratio is less than 2.0. Therefore, the suppression of ureolytic bacteria should be considerd when examing methods to reduce NH_3 volatiliation.

Urease Enzyme Activity

There are many different urease enzymes, but there is a basic trimeric structure with three catalytic centers that is conserved in all. The two most commonly studied bacterial urease enzymes are associated with *H. pylori* and *Klebsiella aerogenes*. Most bacterial ureases are heteropolymeric, and have three distinct types of subunits: UreA, UreB, and UreC (UreABC collectively) (Lam et al., 2010). The active site of urease contains two nickel (Ni) atoms that are bridged by a carboxylated residue and a solvent molecule. The first nickel atom is associated with two histidine residues and a terminal water molecule. The second nickel atom is associated with two histidine residues, an aspartate residue, and a terminal solvent. There are also two additional histidine residues that may play a role in substrate binding. Urease's activity is dependent on the intracellular Ni_2^+ levels. If excess ions accumulate, Ni_2^+ will inhibit growth and exhibit toxic effects by interfering with protein–metal binding and catalysis (Muller et al., 2011).

Urea hydrolysis is dependent on the movement of urea to the urease enzyme and the activity of the urease enzyme (Kissel and Cabrera, 1988). The movement of urea to the urease

enzyme is controlled by diffusion and mass flow of water while urease activity is affected by urea concentrations, pH, and temperature (Kissel and Cabrera, 1988). Cabrera and Kissel (1984) found that soil urease activity is affected by urea concentrations due to low and high affinity reactions of the urease enzyme. Urease activity was greatest at 12M urea-N, but quickly declined as urea concentrations approached 16M urea-N. Furthermore, these two different reaction affinities cause the effect of pH on urea hydrolysis to be dependent on urea concentrations (Rachhpal-Singh and Nye, 1984). It should be noted that the optimum pH for the enzyme can be as high as 9, which falls within the range of broiler litter pH. The effect of temperature on urea hydrolysis can be described using a linear relationship (Vlek and Carter, 1983), but is more accurately defined by the Arrhenius equation (Kissel and Cabrera 1988; Gould et al., 1973).

Urease activity is also affected by moisture content and water activity (Kissel and Cabrera, 1988). It has been proposed that water potential, rather than gravimetric water content, should be used to accurately describe the effect of water on urease activity (Kissel and Cabrera, 1988; Myers, 1974). Fluctuations in water availability can cause the lysis of ureolytic microbial cells and the release of intracellular urease (Rachhpal-Singh and Nye, 1984). Extracellular urease can then bind with clay and humic substances that stabilize and protect the enzyme from degradation (Ciurli et al., 1999; Sylvia et al., 1998), and can lead to an increase in overall urease activity (Klose and Tabatabai, 2000). Optimum urease activity can be achieved at a soil water potential of -20 kPa, and activity decreases slightly as water potentials approach saturation (Kissel and Cabrera, 1988). In addition, urease activity is impacted more by dry conditions than by water potentials approaching saturation. This is likely due to limited movement of urea toward the enzyme via mass flow and diffusion, as well as limited microbial activity. Based on

these results, amendments that alter water activity and cause microbial cell lysis could potenially increase urease hydrolysis.

NH_3 Volatilization from Broiler Litter

NH_3 volatilization from broiler litter occurs during all stages of manure handling (USEPA, 2004). Nicholson et al. (2004) and Moore et al. (2011) calculated a NH_3 balance for broiler litter that quantified losses from housing, storage and land spreading, and found that the greatest losses of NH_3 occur in poultry facilities and during land application. These studies showed that 28 to 62% of NH_3 emission from broiler litter occurs during housing. Ventilation (Elliott and Collins, 1982), dietary changes (Hong et al., 2012; Ferguson et al., 1998), and litter amendments (Cook et al., 2011; Kim and Patterson, 2003; de Oliveira et al., 2003; Moore et al., 1996) are the most prominent methods used to reduce NH_3 formation and volatilization in broiler houses. Ventilation systems are commonly used to reduce NH_3 concentrations in broiler facilities, but this is not an ideal remediation strategy for growers due to energy costs and environmental concerns. NH_3 that is emitted to the environment contributes to particulate matter formation, acid rain deposition, and soil acidity (Heald et al., 2012; Galloway et al., 2002; Ap Simon et al., 1987).

To limit the use of ventilation systems, the poultry industry has adopted litter management practices that decrease uricolytic and ureolytic activity, as well as alter litter parameters that favor the retention of NH_3 as NH_4^+. Strategies aimed at reducing NH_3 volatilization should focus on manipulating conditions that favor NH_3 volatilization. Temperature, pH, and water content have been implicated as the most prominent factors affecting NH_3 volatilization (Carr et al., 1990; Elliott and Collins, 1982). Temperatures in broiler

houses initially range from 32 to 35°C and is reduced to 22°C by the third week of the grow-out period (Nicholson et al., 2004; Elwinger and Svensson 1996). Temperatures in broiler houses are regulated to optimize broiler productivity, and are generally not considered when growers develop strategies to reduce NH_3 volatilization. Furthermore, during the summer months, ventilation rates are increased to maintain target temperatures, and this can lead to greater NH_3 losses (Nicholson et al., 2004). Therefore, NH_3 abatement strategies should focus on manipulating pH and water activity to reduce ureolytic activity.

Litter amendments such as acidifiers, chemical absorbents and chemical/biological inhibitors have become the most widely accepted method for reducing NH_3 volatilization in broiler facilities (Timmons and Harter-Dennis et al., 2011; Cook et al., 2011; Loch et al., 2011; Ritz et al., 2004; Moore et al., 2003; Kithome et al., 1999). Acidifiers, such as aluminum sulfate (alum), and sodium bisulfate, have emerged as the most common litter amendments due to their ability to decrease litter pH below 7 (Rothrock et al., 2010; Choi et al., 2008), which favors the formation and retention of non-volatile NH_4^+ (Kim & Choi, 2009). NH_3 volatilization is reduced when litter pH is below 7, and increases as the pH increases to 8 or more (Reece et al., 1979). Litter amendments that decrease litter pH or prevent pH increase from urea hydrolysis have successfully reduced NH_3 losses (DeLaune et al., 2004; Oliveira et al., 2003; Moore et al., 1996). Furthermore, recent research suggests that acidifiers also reduce NH_3 volatilization by creating an enivornment that decreases the presence of ureolytic bacteria that are known to facilitate nitrogen mineralization (Rothrock et al., 2010). Controlling the pH of litter for the duration of a flock is difficult (Lacey et al., 2004), and reapplication is often needed to maintain adequate atmospheric NH_3 concentrations. In addition, the cost of acidifying amendments can range from $220 to $460 per Mg (Shah et al., 2006; McWard and Taylor, 2000; Moore et al.,

1999) depending on the type of acidifier used and transportation cost. For these reasons, the poultry industry is actively exploring non-acidifying litter amendments that reduce NH_3 volatilization.

Coufal et al. (2006) suggested that litter moisture and temperature are the most important factors affecting NH_3 volatilization due to the fact that litter pH is genereally greater than 8. Water content can influence the diffusion and convection tranfer of gaseous NH_3 (Ni, 1999), as well as alter microbial activity that is associated with NH_3 volatilization (Murakami et al., 2011; Groot Koerkamp, 1998). Water content of litter can vary depending on the bedding material, ventilation rates, and drinking systems (Da Borso and Chiumenti 1999; Elwinger and Svensson 1996; Tucker and Walker 1992). Furthermore, litter moisture throughout the house is not uniform due to the placement of feeders and waters. Water leaks and concentrated deffecation in these areas of the house causes the formation of "caked litter" that generally has a water content greater than 0.35 g H_2O g^{-1} (Malone et al., 1992). Caked litter accounts for 30 to 40% of total litter production (NRAES, 1999; Malone et al., 1992), and growers typically remove caked litter between flocks and leave the remaining litter for subsequent flocks. Compared to bedding material alone and litter from total cleanout, litter cake exhibits the greatest potential for N mineralization (Watts et al., 2012), which can lead to increased NH_3 volatilization.

Many studies have found that NH_3 volatilization is positively correlated with water content (Ferguson et al., 1998; Cabrera and Chang, 1994; Carr et al., 1990; Elliott and Collins, 1982), while other researchers suggest that NH_3 emissions are sensitive to moisture content (Miles et al., 2011; Carr et al., 1990; Valentine, 1964), and suggest an optimal range of water content for NH_3 emissions. Liu et al. (2007) examined the effect of moisture on NH_3 emissions from poultry litter, and found that greater water contents increased total ammoniacal-nitrogen,

yet suppressed NH_3 evolution for a short time. The suppression of NH_3 volatilization was attributed to limited gas diffusion out of the litter due to water films that cover the surface of litter particles. After 7-14 days, NH_3 emissions began to increase and this evolution of NH_3 was correlated with increased water content. Miles et al. (2011) also investigated the effect of water content on NH_3 volatilization and found that there is a critical moisture content that can maximize NH_3 loss. The optimum range for water content in this study was between 37.4 to 51.1 %. Miles et al. (2011) acknowledged the activity of microorganisms at these critical water contents, and cited Groot Koerkamp (1998) to support their hypothesis. Groot Koerkamp (1998) suggests that there is an optimum range of water contents for microorganisms and NH_3 volatilization. NH_3 evolution and microbial activity can be divided into four levels based on water content: 0-5 % moisture results in microbial destruction and a sharp increase in NH_3 emission equal to half the maximum rate, 5 - 37% moisture gradually increases NH_3 volatilization, but microbial growth is limited, 37 -56% moisture results in maximum NH_3 volatilization and optimum microbial growth, and >56% moisture decreases NH_3 volatilization. Carr et al. (1990) recommends limiting moisture levels below 30% to achieve minimum NH_3 loss. Furthermore, Murakami et al. (2011) showed that total N can be increased in poultry litter by limiting water content to approximately 35%. At this water content, there is a lower conversion of uric acid to urea compared to litters with water content of 55% or greater. They hypothesized that the reduction in uric acid conversion is due to reduced microbial activity at low water contents.

Research by Chowdhury et al. (2011) and Manzoni et al. (2012) further supports the hypothesis of Groot Koerkamp (1998) in which reduced microbial activity occurs at low water contents. Chowdhury et al. (2011) conducted a soil experiment to test the effect of osmotic and

matric stress on bacteria and fungi. This study demonstrated that soil respiration decreased with decreasing water potential, and matrix stress had a greater effect on microbial activity compared to osmotic stress. Matric stress was attributed to less diffusion of substrate, and this reduced microbial synthesis of osmolytes that are needed to maintain cell wall integrity during times of stress. Results of this work also showed that fungi are less tolerant to decreasing osmotic stress compared to bacteria, and are more tolerant to decreasing water content. Authors also acknowledged that decreasing soil water content increases salt concentrations in solution. More recent work by Manzoni et al. (2012) found that biological activity ceases in mineral soils at -14 MPa and at -36 MPa for surface-applied poultry litter. Authors hypothesized that the reduction of microbial activity in litter decreases due to a dehydration effect rather than diffusion effect. Payne et al. (2007) also showed that a decrease in water activity reduces the survival of *Salmonella* in litter produced by the turkey industry, while an increase in water activity facilitates *Salmonella* growth.

Gypsum

Gypsum ($CaSO_4$) is a geologic mineral that is found on almost all continents. Gypsum deposits occur in areas where evaporate beds formed from the evaporation of seawater, and is one of the first minerals to precipitate. Gypsum is generally found in the hydrated form ($CaSO_4 \cdot 2H_2O$), and has a solubility of 2.5 g L^{-1} in water. Gypsum is an ionic compound that can take up water from the atmosphere, as well as from materials with which it is in contact. Ionic compounds are hygroscopic because anions, such as sulfates, can form strong hydrogen bonds with water allowing water molecules to diffuse into the structure and become trapped there. Moisture can be liberated from this structure at high temperatures (110 - 150°C), which are not typically observed in poultry litter.

Gypsum has been used as a building material since the construction of the Egyptian pyramids. In the 18th century, gypsum received renewed interest when large deposits were found in France, and scientists of the time created what is known as "Plaster of Paris" by heating the material to remove structural water. Gypsum was also used as a soil conditioner in France to improve crop yield, and was later introduced to America by Benjamin Franklin in the late 1700s. The majority of gypsum today is used in the construction industry to make wallboard for commercial and residential buildings.

Tesarek et al. (2006) examined the basic mechanical, hydric and thermal properties of flue-gas desulfurization gypsum (FGG) as they relate to the construction industry. Even though these results are intended for wall-board construction, their findings are of interest when considering water and water vapor transport characteristics of the material. FGG has a negative native water potential, and the addition of water sharply increases the water potential. Results of this work show that water and water vapor transport of FGG are quite high; indicating the potential for water redistribution when mixed with another material. Furthermore, there is a large hysteresis of adsorption – desorption process that allows for greater water holding capacity.

In addition to natural gypsum, synthetic gypsum is created as an industrial waste product from the neutralization of sulfuric acid with Ca-alkaline material. Due to its high purity and cost of disposal, synthetic gypsum is generally marketed to the wallboard industry and agriculture. Gypsum from industrial processes usually contains trace impurities, and the concentration of these elements varies depending on the gypsum source (Miller, 1995).

The most common sources of synthetic gypsum come from the extraction of phosphorus from phosphate rock and the desulfurization of flue gas from coal-fired power plants (Miller, 1995). Phosphogypsum (PG) is formed when phosphate rock is treated with sulfuric acid, and

this material is 85-95% pure $CaSO_4$ with a pH of 4.8 - 5.6 (May and Sweeney, 1983). Impurities in this form of gypsum include residual phosphorus, fluorine and quartz sand. In the US, phosphorus fertilizer processing creates 45 million Mg of PG a year (Miller and Sumner, 1997), and the use of this material has been restricted by the United States Environmental Protection Agency (USEPA) due to the radioactivity of the ^{238}U decay series. Flue-gas desulfurized gypsum is synthesized when limestone-forced oxidation scrubbers remove sulfur dioxide from flue-gas steam produced from the combustion of coal (Laperche and Bigham, 2002), and it is a nearly pure source of $CaSO_4$, containing 0.2 - 0.5 % Mg and less than 1% fly ash. Approximately 0.1 Mg of FGG is produced for each Mg of coal burned (Miller, 1995). The American Coal Ash Association (ACAA) has observed a steady increase in FGG production over the past ten years. In 2008, 17,755,000 Mg of FGG was produced, of which 58% was used in wallboard manufacturing and 2% in agriculture (ACAA, 2010). As more coal burning plants are constructed and equipped with FGD systems, FGD-gypsum production is expected to double in the next ten years, likely lowering the already low-cost of FGG.

Gypsum as a Litter Amendment

Oliveira et al. 2003 published research that examines the effect of gypsum on NH_3 volatilization from poultry litter. They analyzed dry matter content, pH, and NH_3 volatilization from poultry litter treated with different litter additives. These additives included aluminum sulfate ($Al_2(SO_4)_3$•14 H_2O), gypsum, and hydrated lime ($Ca(OH)_2$). In this experiment, poultry litter treated with gypsum had the lowest NH_3 volatilization after 42 days compared to other treatments. The reduction in NH_3 volatilization from poultry litter treated with gypsum was attributed to a lower pH. Gypsum reduced litter pH from 8.04 to 6.97, while alum addition resulted in a pH of 7.07. Authors hypothesized that gypsum's high capacity to absorb moisture

reduced the activity of ureolytic microorganisms that facilitate NH_3 volatilization. Oliveira et al. (2003) also suggested that NH_3 volatilization was limited by the chemical reaction between nitrogen compounds and gypsum that is cited in Bruno et al. (1999) and developed by Teuscher and Adler (1965) (Equation 3).

$$(NH_4)_2CO_3 + CaSO_4 \text{ — } \rightarrow (NH_4)_2SO_4 + CaCO_3 \qquad \text{Eq. [3]}$$

Oliveira et al. (2003) followed the previously mentioned work with another study examining the effect of chemical conditioners on poultry litter quality (Oliveira et al., 2004). This research is very similar in nature (e.g. experimental design, sampling, analytical techniques) to Oliveira et al. (2003), but provides very different results for the effects of gypsum on poultry litter. In this study, researchers used three consecutive flocks to study the effects of alum, gypsum, superphosphate, and hydrated lime on NH_3 volatilization. Similar to the previous experiment, there was no statistical difference between the treatments for dry matter, but gypsum treatments did have the greatest amount of dry matter after the three flocks. The pH of poultry litter amended with gypsum resulted in values of 8.22, 8.12, and 7.50 for flocks 1, 2, and 3 respectively, and these values are greater than those found in Oliveira et al. (2003). Furthermore, the pH of poultry litter amended with gypsum was lower than that of poultry litter alone, but these values were not found to be statistically different. Likewise, the addition of gypsum did reduce NH_3 volatilization compared to the control, but was not statistically significant. Alum was the most efficient amendment at reducing NH_3 losses. The pH values in this publication for alum treatments are lower than in the previous publication, and are likely responsible for the reduction in NH_3 volatilization. It should be strongly considered that all of the amendments, except for gypsum, were added to poultry litter before each of the flocks. Gypsum was only added to the bedding before the entry of the first flock.

More recent work by Loch et al. (2011) examined the quality of poultry litter submitted to different treatments for five consecutive flocks. Moisture content, pH, density and volatilized NH_3 were measured for this study. One aspect of this paper that is an improvement from the previous two studies is that litter was sampled at day 21 and 42 instead of at day 42 alone. Results of this publication showed that gypsum application decreases the moisture content of litter compared to the control, but these values were not significantly different due to conservative statistical analysis that employed a cut-off of $p < 0.02$. Literature that uses gypsum as a poultry litter amendment generally does not show any significant difference in terms of moisture content after treatment. This is partially due to the fact that the addition of gypsum does not cause water to evaporate from the broiler litter mixture. Instead, there is a redistribution of water from poultry litter particles to gypsum, which affects the amount of water available to microorganisms. Research by Wyatt and Goodman (1992) found that the percentage of moisture in litter amended with refined gypsum was significantly lower than other treatments that used wood shavings as a bedding material. However, based on weight, gypsum amended litter contained equal or more water compared to other treatments. These findings accurately reflect gypsum's ability to absorb water from other materials and the atmosphere. The addition of gypsum did reduce litter pH for the first, second, and fourth flocks after 42 days. It should be noted that a dehydrated form of gypsum was used in this experiment ($CaSO_4 \cdot 0.5\ H_2O$), and this material was only applied at the beginning of the first flock while alum was added before each flock entered. After five flocks, NH_3 losses from litter amended with alum and gypsum were not significantly different from each other, suggesting that gypsum has a longer lasting effect on NH_3 volatilization than alum.

Sampaio et al. (1999) is the only known study that examines microbial populations in conjunction with the release of NH_3 from poultry amended with gypsum. In this experiment, poultry litter was amended with gypsum plaster, and treatments consisted of 10, 20, 30, and 40% gypsum, as well as split applications of these rates. Gypsum was added to the bedding material before the birds arrived, and then again on day 25 for treatments that were to receive a split application. Treatments that received a split application of gypsum were treated with half of the prescribed amount before the birds arrived, and the other half was applied on day 25 (5+5, 10+10, 15+15, and 20+20). Overall, results from this study showed that the addition of gypsum to poultry litter can reduce bacterial populations and NH_3 volatilization. After 25 days, NH_3 volatilization and bacteria decreased with increasing application rates, and the greatest reduction was achieved with a split-application of 20+20%. After 49 days, NH_3 volatilization from the 20, 30, and 40% single-application treatments did not differ and were all significantly less than the control. The split application of 15+15% and 20+20% gypsum resulted in the lowest levels of NH_3 volatilization and bacteria. It should be noted that this research used Plate Count Agar for bacteria enumeration and quantification, which grows a variety of bacteria, and is not specific to ureolytic bacteria. A urea agar, such as Christensen agar, would have been more appropriate for this study.

Based on the literature presented, gypsum's ability to reduce NH_3 volatilization varies among studies, and the mechanism responsible for decreasing NH_3 losses has not been determined. Many authors hypothesized that gypsum's high capacity to absorb moisture reduced the activity of ureolytic microorganisms that facilitate NH_3 volatilization; however, there are no studies that quantify urea-degrading bacteria in gypsum-amended litter. Authors have also hypothesized that NH_3 volatilization is limited by a chemical reaction (Equation 3) between

nitrogen compounds and gypsum as described by Teuscher and Adler (1965). The reaction developed by Teuscher and Adler (1965) suggests that NH_3 abatement is due to the formation of ammonium sulfate ($(NH_4)_2SO_4$), but this reaction is questionable due to the solubility of $(NH_4)_2SO_4$. It has also been proposed that the reduction in NH_3 loss from gypsum-amended litter could be caused by a decrease in litter pH, although the mechanism responsible for litter pH suppression has not been identified. This hypothesis is in agreement with Fenn et al. (1981) who observed that the addition of soluble Ca salts reduced NH_3 volatilization from surface-applied urea. The authors attributed these results to the formation of $CaCO_3$ and to a decrease in soil pH. The formation of $CaCO_3$ and the resulting decrease in pH should be further investigated as a mechanism responsible for NH_3 abatement in broiler litter amended with gypsum.

It is well recognized that microbial species can precipitate mineral carbonates through metabolic processes (Cahal et al., 2011; Hammes et al., 2003; Knorre and Krumbein, 2000; Fujita et al., 2000). There are two known examples of heterotrophic microbial activity that cause carbonate precipitation: sulphate reduction (Peckman et al., 1999) and urea hydrolysis (Wright 1999). Carbonates can precipitate by sulfate reduction when organic matter is consumed by sulfate reducing bacteria in the presence of gypsum ($CaSO_4$) (Equations 4 and 5) (Peckman et al., 1999; Wright et al., 1999).

$$CaSO_4 \bullet 2H_2O \rightarrow Ca^{2+} + SO_4^{2-} + 2H_2O \qquad \text{Eq. [4]}$$

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \qquad \text{Eq. [5]}$$

The second known pathway for microbial induced carbonate precipitation begins with the degradation of urea as described by Kissel et al. (1988; Equations 6-8). In equation 6, the hydrolysis of urea produces NH_4^+, bicarbonate (HCO_3^-), and an increase in pH due to the

consumption of H^+. This increase in pH favors the volatilization of NH_4^+ as NH_3 as well as the deprotonation of bicarbonate (Equation 7). In the presence of calcium (Ca^{2+}), CO_3^{2-} will precipitate as $CaCO_3$ as described by Koelliker and Kissel (1988) (Equation 8). The precipitation of $CaCO_3$ will favor the consumption of HCO_3^{2-}. The consumption of HCO_3^{2-} will release H^+ that can buffer against an increase in pH that is responsible for increased NH_3 losses.

$$CO(NH_2)_2 + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO_3^- \quad \text{Eq. [6]}$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \quad \text{Eq. [7]}$$

$$Ca^{+2} + CO_3^{2-} \rightarrow CaCO_3 \quad \text{Eq. [8]}$$

The negative charge of microbial cells and the functional groups of the cell wall can bind Ca^{2+} making ureolytic cells nucleation sites for $CaCO_3$ formation. Calcite crystal morphology has been found to be strain specific, and the presence of 30 mM calcium can actually increase some ureolytic isolates' urease activity up to 10-fold (Hammes et al., 2003). Furthermore, the rate of calcite formation is directly related to the rate of urea hydrolysis (Fujita et al., 2000). Therefore, we hypothesize that amending broiler litter with a soluble source of Ca^{2+} can increase N mineralization and favor the formation of NH_4^+ by increasing urease activity and buffering against large increases in pH.

$CaCl_2$ is a soluble Ca^{2+} salt that has previously been used to reduce NH_3 losses from acidic soils (Fenn et al., 1981) and poultry manure (Kithome et al., 1999). Kithome et al., (1999) examined the effect of two calcium salts on nitrogen losses during composting of poultry manure. In this study, the application of 9 and 20% calcium chloride ($CaCl_2$) decreased NH_3 volatilization compared to the control, but gypsum did not have any effect on NH_3 losses. However, amending poultry litter with $CaCl_2$ and gypsum did result in significantly more NH_4^+-N than un-amended litter. Researchers suggested that the difference in the two Ca^{2+} salts was

due to the greater solubility of Cl^- salts. In addition, it was hypothesized that the lower application rate of $CaCl_2$ reduced NH_3 emission by decreasing the pH of the manure, while the 20% addition increased salt concentrations to levels that inhibited microbial activity. It should be noted that poultry manure used in this experiment had a low moisture content of 0.07 g H_2O g^{-1}, which was probably not sufficient to liberate enough Ca^{2+} from $CaSO_4$ to decrease NH_3 losses. Witter (1991) also successfully used $CaCl_2$ to reduce NH_3 volatilization from chicken slurry, and attributed these results to the reaction outlined in Fenn et al. (1981) in which the precipitation of CO_3^{2-} as $CaCO_3$ buffers against an increase in pH. This reduction in pH favors the retention of NH_3 as NH_4^+.

Land Application of Broiler Litter and Gypsum

Land application of poultry litter can be viewed as a disposal strategy, as well as a fertlizer practice. Land application of broiler litter has been shown to increase soil total carbon, microbial biomass, extractable soil phosphorus, potassium, soil cation exchange capacity, and the stability of soil aggregate (Adeli et al., 2010), but land area used for disposal is limited, and application rates can often exceed crop nutrient requirements (Moore, 1998). Over application of litter can lead to enviromental contamination that includes: leaching of nitrogen in subsurface drainage (Bitzer and Sims, 1988), contamination of surface water with phoshorus (Franklin et al., 2007), greenhouse gas emission (Sistani et al., 2011), and increased metal inputs (Gupta and Charles, 1999). Excessive application of poultry litter can also increase the presence of pesticide residues, pharmaceuticals, endocrine disrupting compounds, and microorganisms in the environment (Bolan et al., 2010).

Poultry litter nutient content can vary depending on litter management and the number of flocks reared on the litter, but typically litter has a fertilizer grade of 3-3-2 of which 60% of the nitrogen (N), 80-100% of the phosphorus (P), and 100% of the potassium (K) are available to plants (Dunkley et al., 2010). In 2008, 10.2 million Mg of poultry litter was produced in the US (Dunkley et al., 2010), and it was estimated to contain 2.2 million Mg of nitrogen, 0.7 million Mg of phosphorus, and 1.4 million Mg of potassium (McDonald et al., 2009). In addition to N, P, and K, poultry litter also contains significant levels of secondary plant nutrients such as Ca, magnesium (Mg) , and sulfur (S) as well as trace elements that include copper (Cu), zinc (Zn), and molybdenum (Mo). Secondary and trace elements orginate from the birds diet where they are used as feed additives to help build bones, maintan osmotic balance, and egg production (Goff 2006; Preusch et al., 2002; Dong et al., 2001), and poultry litter is known to contain more Ca, Mg, and S than other manures (Azeez and Van Averbeke, 2010).

Due to broiler litter's rich nutrient content, it is often purchased by farmers in broiler producing states as an inexpensice source of plant nutrients. Broiler litter in Alabama is estimated to have an average nutrient value of $35.60 per Mg, but due to market constraints, litter typically sales for $10 per Mg (Adhikari et al., 2002). Furthermore, broiler litter in Georgia is estimated to have a fertlizer value of approximately $80 (Ritz and Merka, 2009), and farmers are able to purchase this litter for $10 to $50 per Mg depending on transportation and handling cost (Dunkley et al., 2010). Transportation cost is a major limiting factor when farmers consider using litter as a fertilizer, and many states have initiated incentive programs that provide payment for transporting litter out of areas where broiler production is concentrated (Mullen et al., 2011).

Litter application rates are generally calculated to meet crop N requirements, but establising application rates can be problematic due to variability in intitial N content and losses through NH_3 volatilization and NO_3^- leaching. Variations in initial N content is determined by number of flocks raised on the litter, litter managemet, diet, and storage methods (Rothrock et al., 2010; Bowers et al., 2003; Barker 1990; O'Dell et al., 1960), and can not be controlled by farmers receiving litter. NH_3 losses from surface-applied manure is influenced by environental factors that include soil moisture (Agehara and Warncke, 2005; Cabrera and Vervoort, 1998), temperature (Sims,1986), wind speed (Nathan and Malzer, 1993; Sommer et al.1991), and relative humidity (Cabrera et al. 2010; Weaver and Meijerhof, 1991). Many laboratory experiments have been conducted to study NH_3 volatilization from surface-applied litter and losses in these studies ranged from 0.5% to 31% of the total N applied (Cassity-Duffey et al., 2015; Mishra et al., 2013; Doydora et al., 2011; Brinson et al., 1994; Cabrera and Chiang 1994). The majority of the NH_3 loss occurs in the first 7 days after application, and can represent approximately 9 -21% of the total N applied (Brinson et al., 1994).

Litter application rates that are calculated to meet crop N requirements can result in P accumulation in soil (Sharpley et al., 1993, 1991). Phosphorus is the primary nutrient responsible for fresh water eutrophication (Carpenter et al., 1998), and concentrations in run-off typically increase as application rates and soil test phosphorus increase (Sharpley and Kleinman, 2003; Pote et al., 1999). Total P in broiler litter ranges from 0.3 to 2.4% of the dry matter (Bolan et al., 2010), and approximately 67% is present as solid-phase organic P and the remainder is inorganic P (Sharpley et al., 2004; Sharpley and Smith, 1995). Phosphorus from poultry litter can be adsorbed by soil particles or dissolved in rainwater, which can be carried to nearby surface waters in run-off. Vonies et al. (2001) found that total loss of orthophosphate from soil

treated with poultry litter was four times greater than from a field receiving commercial fertilizer. It has been suggested that litter application rates should be based on P soil test levels to reduce P in run-off, but lower application rates may cause farmers to supplement commercial fertilizers to meet crop N requirements (Sharpley et al., 1993). Litter amendments such as alum, ferrous sulfate, and ferric chloride can be used to reduce water soluble P in broiler litter by adsorbing P to metal hydroxides (Tasistro and Kissel, 2006). Gypsum can also be added to poultry litter to reduced water soluble phosphorus due to the precipitation of P as calcium phosphate (Bashan and Bashan, 2004). Tasistro and Kissel (2006) examined the effect of alum, ferrous sulfate, ferric chloride, and gypsum on P species in aqueous extracts of broiler litter. Litter amendments used in this study, except gypsum, decreased litter pH thereby favoring greater concentrations of water soluble P in litter. Furthermore, Al and Fe amendments could shift the equilibrium of soluble P by removing water soluble P from solution, subsequently leading to the release of more P in solution. In contrast, gypsum reduces water soluble P by forming various Ca-P compounds, and by co-precipitating or entrapping P during the formation of carbonates (Zhang et al., 2004; Stout et al., 1998). Compared to un-amended litter, gypsum reduced molybdate-reactive P and dissolved unreactive P by 77% and 52%, respectively. Al and Fe were more effective than gypsum at reducing molybdate-reactive P and dissolved unreactive P. However, greater amounts of these amendments are needed than are commonly applied by growers to reduce water soluble P to a minimum.

Gypsum can be applied to soils, grass buffers or used as manure amendment to reduce phosphorous run-off that occurs as erodible soils accumulate soluble phosphorous. Ca^{+2} ions from the dissolution of gypsum react with phosphorus to form calcium phosphate, which precipitates out of solution thereby reducing dissolved reactive phosphorus (Favaretto et al.,

2006). Brauer et al. (2005) demonstrated that dissolved reactive P can be reduced in run-off when gypsum is applied to soil at a rate of 2.0 Mg per acre. Furthermore, Watts and Tolbert (2009) showed that gypsum can be applied to grass buffer strips to reduce phosphorus in run-off from soils amended with poultry litter. In this experiment poultry litter was surface-applied, and gypsum was added to grass buffers at a rate of 0, 1, 3.2, and 5.6 Mg ha^{-1}. The addition of gypsum resulted in 32-40% decrease in soluble P concentrations in surface run-off, and there was no significant difference in application rates. Results of Watts and Tobert (2009) and Brauer et al. (2005) suggest that a maximum of 2 Mg per ha should be adequate to reduce P in run-off.

In addition to reducing phosphorus in run-off, gypsum is well recognized as a soil conditioner due to its ability to improve soil physical and chemical properties (Watts and Dick 2014; Chen and Dick 2011). Land application of gypsum to agronomic soils can increase soil structure (Norton et al., 1993; Radcliffe et al., 1986), reduce Al photoxicity in acidic soils (Stehouwer et al., 1999; Sumner 1995) , and improve crop yield (Chen et al., 2010; Sumner, 2007; Stehouwer et al., 1999; Stout et al., 1979). Gypsum can alleviate clay dispersion and Al toxicity in highly weathered soils due to its composition and moderate solubility. Gypsum is 200 times more soluble than traditional lime (EPRI, 2006) allowing for greater movement of Ca^{2+} down the soil profile. As gypsum dissolves, Ca^{2+} cations are released into the soil solution where they can floculate clay particles by forming cationic bridges with soil organic carbon (Bronick an Lal, 2005). 1-2 Mg per ha has proven to be a sufficent application rate to decrease clay dispersion that leds to soil crusting and poor drainage (Norton and Rhoton, 2007). It should be considererd that gypsum's effect on soil structure can be limited due to difference in soil textures. For example, Sumner et al. (1990) showed that most gypsum applied to a sandy soil

leached below 1 m after 5 years while only 50% of the gypsum was lost during this time in an Altavista soil that had a greater clay content.

In addition to improving soil structure, gypsum can improve crop yield by reducing subsoil acidity and Al toxicity. In general, there is an inverse relationship between exchangeable Ca^{2+} and Al^{+3}, and root elongation depends on available Ca^{+2} and is depressed by toxic Al^{3+} (Shainberg et al., 1989; Farina and Channon, 1988; Smyth and Cravo, 1992). Reeve and Sumner (1972) suggest that gypsum has a "self-liming effect" on highly weathered soils. In this proposed reaction, ligand exchange occurs between the added SO_4^- of gypsum and the OH^- groups of soil surfaces. It has also been suggested that Al toxicity is reduced by gypsum application via ion pair formation (Pavan et al., 1982). In this scenario SO_4^- from gypsum complex with Al forming $AlSO_4^+$. This leads to an increase in alkalinity thereby precipitating Al from solution. Reducing subsoil Al^{3+} with gypsum generally leads to increased crop yield due to greater rooting depth and lower water stress (Farina et al., 2000; Ritchey et al., 1995). Gypsum can also be used as calcium fertilizer to increase peanut yield (Tillman et al., 2010; Sumner, 2007) as well as reduce root rot of avocado trees and blossom-end rot of watermelon and tomatoes (Scott et al., 1993; Shear, 1979).

CONCLUSIONS

Broiler litter contains elevated amounts of organic nitrogen and urea-degrading bacteria that facilitate NH_3 volatilization. NH_3 volatilization from broiler litter has a negative effect on the environment and is associated with decreased broiler health. Litter amendments such as acidifiers, chemical absorbents and chemical/biological inhibitors have become the most widely accepted method for reducing NH_3 volatilization in broiler facilities. Acidifiers have emerged as

the most common litter amendments, but controlling the pH of litter for the duration of a flock is difficult, and reapplication is often needed to maintain adequate atmospheric NH_3 concentrations. Previous research suggest that the addition of gypsum to broiler litter may lower in-house NH_3 emission. However, these results vary among studies, and the mechanism responsible for decreasing NH_3 volatilization is not well understood. Two mechanisms that have not been thoroughly investigated is the effect of gypsum on urea-degrading bacteria and the buffering of litter pH by the precipitation of $CaCO_3$. Results from previous work indicate that the addition of gypsum to broiler litter reduces total bacteria concentrations, but its effect on urea-degrading bacteria and urease activity has not been examined. Furthermore, it is well recognized that microbial species can precipitate mineral carbonates through urea hydrolysis, but this reaction has not been investigated for broiler litter. Therefore, we hypothesize that gypsum can reduce NH_3volatilization by reducing the number of urea-degrading bacteria, and by buffering against large pH increases created by urea hydrolysis.

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CHAPTER 2

GYPSUM EFFECTS ON UREA-DEGRADING BACTERIA AND AMMONIA VOLATILIZATION FROM BROILER LITTER

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ABSTRACT

A major concern of the broiler industry is the volatilization of ammonia (NH_3) from the mixture of bedding material and broiler excretion that covers the floor of broiler houses. Gypsum has been proposed as a litter amendment to reduce NH_3 volatilization, but reports of NH_3 abatement vary among studies, and the mechanism responsible for decreasing NH_3 volatilization is not well understood. The primary goal of this study was to evaluate the effect of adding 20 or 40% FGG to broiler litter on electrical conductivity, ureolytic bacteria activity, NH_3 and CO_2 evolution, and N mineralization in several 21-d experiments. The addition of FGG to broiler litter increased electrical conductivity by 24 to 33%, decreased ureolytic bacteria by 48 to 57 % and increased N mineralization by 10 to 11%. The increase in N mineralization was likely due to a 27 to 41 % increase in urease activity. Also, the addition of FGG to broiler litter decreased NH_3 volatilization by 18 to 28% apparently because $CaCO_3$ precipitation buffered against an increase in pH and resulted in pH values that were 0.09 to 0.49 units lower than the pH of un-amended broiler litter.

INTRODUCTION

The United States (US) poultry industry accounts for 28% (664,238 Mg) of the national livestock ammonia (NH_3) emissions (USEPA, 2004). Specifically, in the southeastern US, broiler production is the largest segment of the poultry industry, and it releases 33 to 43% of the agricultural NH_3 emissions (Stephen and Aneja, 2008; Aneja et al., 2003). Broilers are classified as chickens younger than 13 weeks which are raised specifically for meat. The floor of the broiler production facilities is covered with a mixture of bedding material and excretion that is rich in organic and inorganic nitrogen (N). Nitrogen in broiler litter is subject to NH_3 volatilization and represents a major concern for producers because NH_3 is considered the most harmful gas in broiler facilities (Carlile, 1984). It is well documented that the accumulation of volatile NH_3 can impair bird respiratory health (Anderson et al., 1964), decrease body weight and feed efficiency (Miles et al., 2002; Miles et al., 2004), and cause corneal ulceration and blindness. Furthermore, the NH_3 that is released to the environment by ventilation systems contributes to atmospheric particulate matter formation, acid rain deposition, and soil acidity (Heald et al., 2012; Galloway et al., 2002; Ap Simon et al., 1987).

The NH_3 in broiler manure originates from the mineralization of organic N present in broiler excretion. Mineralization of organic N to NH_3 is a microbially mediated enzymatic reaction that requires enzymes produced by uric acid-degrading and urea-degrading populations. (Rothrock et al., 2010). Uric acid and urea are the most common forms of organic N in broiler litter, and they account for 70 to 80% of the total N content (Kelleher et al., 2002; Groot Koerkamp, 1994). Uricase produced by uric acid-degrading microbes degrades uric acid to urea, and urea is then hydrolyzed by urease enzymes produced by urea-degrading microbes. Urea hydrolysis produces ammonium (NH_4^+), bicabonate (HCO_3^-), and an increase in pH (Kissel et

al., 1988). The volatilization of NH_3 is strongly influenced by pH, and it increases when litter pH is greater than 7 (Reece et al., 1979). It is estimated that approximately 50-80 % of the N in broiler litter is converted to NH_3 (Sims and Wolf, 1994; Ritz et al., 2004), and the majority (55-82%) of NH_3 losses occur in rearing facilities (Moore et al., 2011; Pain et al., 1998).

To reduce NH_3 volatilization from broiler litter, amendments such as acidifiers, chemical absorbents and chemical/biological inhibitors have been investigated (Timmons and Harter-Dennis, 2011; Cook et al., 2011; Loch et al., 2011; Kithome et al., 1999; Moore et al., 1996). Acidifiers, such as alum ($AlSO_4$) and sodium bisulfate ($NaHSO_4$), are the most common litter amendments due to their ability to lower litter pH to levels that favor the formation and retention of non-volatile NH_4^+ (Rothrock et al., 2010; Kim & Choi, 2009). Furthermore, recent research suggest that acidifiers also reduce NH_3 volatilization by creating an environment that decreases the presence of urea-degrading bacteria, which are known to facilitate N mineralization (Rothrock et al., 2010). Although acidifiers have been proven to reduce NH_3 volatilization, their use as broiler litter amendments is not always effective. This is because controlling the pH of the litter for the duration of a flock is difficult, and reapplication of acidifiers is often needed to maintain adequate atmospheric NH_3 concentrations (Lacey et al., 2004).

Gypsum ($CaSO_4 \cdot 2H_2O$) has previously been used as a soil amendment to increase infiltration (Norton, 2008; Yu et al., 2003), ameliorate subsoil acidity and Al^{3+} toxicity (Sumner, 1993; Wright et al., 1989), and reduce soluble phosphorus concentration in run-off water (Endale et al., 2014; Desutter et al., 2011; Watts & Torbert, 2009). Gypsum has also been proposed as a litter amendment to reduce NH_3 volatilization (Loch et al., 2011; Oliveira et al., 2004: 2003; Sampaio et al., 1999; Wyatt and Goodman 1992). However, results vary among studies, and the mechanism responsible for decreasing NH_3 volatilization is still not well understood. Oliveira et

al. (2003) found that the amendment of poultry litter with gypsum significantly decreased NH_3 volatilization compared to un-amended litter. In contrast, a similar experiment was conducted by Oliveira et al. (2004) in which the quality of poultry litter treated with different amendments was investigated for three consecutive flocks. In this study gypsum only reduced NH_3 volatilization during the first flock, and pH values of gypsum amended litter were not significantly different than those of un-amended litter. Witter (1991) used $CaCl_2$ to reduce NH_3 volatilization from chicken slurry, and attributed these results to the reaction outlined by Fenn et al. (1981) in which the precipitation of CO_3^{2-} as $CaCO_3$ buffered against an increase in pH. It is well recognized that bacteria species can precipitate mineral carbonates as a by-product of urea hydrolysis (Cahal et al., 2011; Hammes et al., 2003; Stocks-Fischer et al., 1999; Fujita et al., 2000), but this reaction has not been examined in poultry litter even though a group of urease producing microorganisms specific to poultry litter has been identified (Rothrock et al., 2008; 2010).

Historically, natural gypsum has been used in agricultural systems, but the addition of flue-gas desulfurization (FGD) systems to coal-burning power plants offers an alternative to mined gypsum. FGD-gypsum (FGG) is synthesized when limestone-forced oxidation removes sulfur dioxide from flue gas steam (Laperche and Bigham, 2002). The American Coal Ash Association (ACAA) has observed a steady increase in FGG production over the past ten years. In 2008, 17,755,000 tons of FGG was produced of which 58% was used in wallboard manufacturing and only 2% in agriculture (American Coal Ash Association, 2010). As more coal burning plants are equipped with FGD systems, FGG production is expected to double in the next ten years making FGD an inexpensive source of FGG.

Current information on the effect of adding FGG to broiler litter on N transformations is very limited. Therefore, the primary goal of this study was to evaluate the effect of adding 20 or

40% FGG to broiler litter on litter water content, ureolytic bacteria activity, NH_3 and CO_2 evolution, and N mineralization in several 21-d experiments. We hypothesized that FGG amendments would reduce urea-degrading bacteria and enzymatic activity thereby decreasing NH_3 losses.

MATERIALS AND METHODS

Broiler Litter and flue-gas gypsum (FGG):

Broiler litter samples (Table 2.1) used for the experiments were collected from two broiler houses in Georgia, USA. The samples were passed through a 2-mm sieve and analyzed for total C and N, pH, electrical conductivity (EC), water potential, and water content as described below. FGG was collected from a power plant in Illinois and analyzed for elemental composition using ICP-OES after acid digestion (USEPA, 1986) (Table 2.2). Water content of FGG was determined by drying at 105°C for 48 hours.

Broiler Litter Analyses

Total C and N in broiler litter was determined by dry combustion (Nelson and Sommers, 1982). pH was measured using an AB150 pH meter by Fisher Scientific in a 1:5 (litter/deionized water) ratio. EC was measured in a 1:5 (litter/dH_2O) ratio using a CDM80 Conductivity Meter (Radiometer America, Cleveland, OH). Water potential was measured using a WP4C Dewpoint Potentiameter (Decagon, Pullman, WA). Water content of the broiler litter was determined by drying at 65°C for 48 hours.

Experiment 2.1: Effect of FGG on urea-degrading bacteria and N mineralization in broiler litter

To determine the effect of FGG on ureolytic bacteria and N mineralization, broiler litter with two rates of FGG was incubated for 21 days. Treatments included: (i) broiler litter (control), (ii) broiler litter + 20% FGG, and (iii) broiler litter + 40% FGG. There were six replicates of each treatment, all arranged in a completely randomized design. Each experimental unit consisted of 125 g of broiler litter (0.63 g H_2O g^{-1}) placed in a 4-L glass container. Flue-gas desulfurization gypsum was added to designated experimental units based on broiler litter wet weight, with broiler litter + 20% FGG receiving 25 g of FGG and broiler litter + 40% FGG receiving 50 g of FGG. Flue-gas desulfurization gypsum was thoroughly mixed with broiler litter in each container. All containers were then closed and placed inside an incubator at 27°C for 21 days. Broiler litter from each container was sampled on day 1, 3, 6, 14, 17, and 21. During each sampling event 5 g of broiler litter (dry weight) was retrieved from each container to be analyzed for pH, EC, water potential , urea, inorganic nitrogen, and urea-degrading bacteria. The sample pH was measured in a 1:5 (litter/dH_2O) ratio using an AB150 pH meter (Fisher Scientific), and it was also measured in 0.01M $CaCl_2$ on day 21 to cancel any salt effect that the addition of gypsum may have on the pH probe. Urea was determined by shaking 1 g of litter with 100 mL of 1000 mg L^{-1} $AgSO_4$ for 5 min., and then filtering solution through a 0.45-µm filter. Extracts were analyzed following a colorimetric procedure (Keeney and Nelson, 1982). Ammonium and NO_3-N were determined by shaking 1 g of litter with 100 mL of 1 mol L^{-1} KCl for 45 minutes, and then filtering solution through a 0.45-µm filter. Extracts were analyzed following the colorimetric procedure described by Keeney and Nelson (1982).

The population of urea-degrading bacteria was determined using a protocol similar to that of Kim and Patterson (2003). One gram of broiler litter was diluted with 30 mL of

deionized water and shaken for 15 min. A 1-mL aliquot of poultry litter suspension was then pipetted into a dilution tube containing 9 mL of deionized water. A ten-fold dilution was repeated twice. Aliquots from each dilution tube were plated on Christensen urea agar following Miles and Mirsa (1938). After incubating the plates for 24 hours at 37°C, the colonies on each plate were quantified and results reported as urea-degrading bacteria colony-forming units (CFU) per gram of dry litter.

Experiment 2.2: Effect of FGG on carbon dioxide and NH_3 emmisions from broiler litter

To determine the effect of FGG on carbon dioxide (CO_2) and NH_3 volatilization, broiler litter with two rates of FGG was incubated for 21 days. Treatments, replications, and experimental design were the same as in the previous experiment. Each experimental unit consisted of 10 g broiler litter (0.63 g H_2O g^{-1}) placed in a 0.95-L glass container with a suspended vial containing 40 mL of 1 N H_2SO_4 for trapping NH_3. FGG was added to designated experimental units based on broiler litter wet weight, with broiler litter + 20% FGG receiving 2 g of FGG and broiler litter + 40% FGG receiving 4 g of FGG. FGG was thouroughly mixed with broiler litter in each container before incubation. All containers were then closed with screw-cap lids fitted with rubber septa, and placed inside an incubator at 27°C for 21 days. Carbon dioxide concentration in the headspace and NH_3 in the traps of each experimental unit were measured on days 1, 2, 3, 4, 5, 9, 12, 15, 18, and 21. Triplicate 3-mL air samples for CO_2 determination were collected with a syringe from the head space of each container and injected into a 2.0-mL glass sample vial. Concentrations of CO_2 were determined using a Varian Star 3600 CX Gas Chromatograph with a Varian 8200 CX Auto Sampler (Varian Analytical Instruments, Sugarland, TX). After samples for CO_2 determination were withdrawn from each experimental unit, containers were aerated and H_2SO_4 traps were changed and analyzed for NH_4-N

colorimetrically (Keeny and Nelson, 1982). The volume of air in the container was calculated by subtracting the volume occupied by the litter from the air volume of an empty container and an NH_3 trap. The rate of CO_2 production from the samples was determined by subtracting background CO_2 from the measured concentrations. Statistical analysis of CO_2 was performed by incremental fitting as described by Ellert and Bettany (1988). Data for NH_3 volatilization was calculated as cumulative loss and analyzed using a one-way analysis of variance as a completely randomized design

Experiment 2.3: Nitrogen balance for litter amended with differential rates of FGG

A third 21-day incubation was conducted to calculate a N balance for litter amended with two rates of FGG. Treatments in this experiment were the same as in the previous two experiments. There were four replications of each treatment, all arranged in a completely randomized design. Each experimental unit consisted of 16.3 g broiler litter (0.63 g H_2O g^{-1}) placed in a 0.95-L glass container with a suspended vial containing 35 mL of 0.1 N H_2SO_4 for trapping NH_3. Broiler litter + 20% FGG received 3.26 g of FGG and broiler litter + 40% FGG received 6.52 g of FGG. FGG was thouroughly mixed with broiler litter in each container before incubation. H_2SO_4 traps were changed on days 1, 2, 3, 8, 12, 16, 19, and 21. After the H_2SO_4 traps were removed on day 21, 500 mL of 1M KCl was added to each experimental unit and shaken for 45 minutes in a reciprocating shaker set at 120 oscillations per minute. Each extract was then filterered through a 0.45-μm filter, and extracts were analyzed for NH_4^+ and NO_3^- following the colorimetric procedure described by Keeney and Nelson, (1982). NH_3 from the H_2SO_4 traps and NH_4^+ and NO_3^- from the KCl extracts was calculated on a μg g^{-1} of dry litter basis and summed to calculate total nitrogen recovery.

Experiment 2.4: Effect of gypsum on urease activities in broiler litter with different water contents

In the fifth experiment, the effect of gypsum on urease activity was examined at three water contents (0.31 (Low), 0.44 (Medium), and 0.78 (High) g H_2O g $^{-1}$) over the course of five days. These particular water contents were selected based on Groot Koerkamp (1998) who suggested that maximum NH_3 volatilization and optimum microbial growth occurs between 0.37 g H_2O g^{-1} and 0.56 g H_2O g-1, and water contents outside of this range can cause limited growth due to limited water or anaerobic conditions. Broiler litter was air-dried for 24 hours, and then wetted with dH_2O to achieve desired water contents. Treatments included un-amended litter and litter + 20% reagent-grade gypsum at each of the three water contents described above. There were 36 replications of each treatment, all arranged in a completely randomized design. Each experimental unit consisted of 5 g of broiler litter (dry weight) placed in a 0.95-L container. For this experiment, 1 g of gypsum was added to treatments with gypsum and all containers were incubated at 29°C. On days 0, 1, 3, and 5, eight experimental units from each treatment were retrieved to measure the rate of urea hydrolysis. Four experimental units from each treatment received 25 mL of 1000 mg L^{-1} urea-solution while the other four experimental units did not receive urea. All experimental units were then incubated on the lab bench for 12 minutes to allow for urea hydrolysis. After 12 minutes, urea and NH_4^+-N were extracted by adding 250 mL of 2.5M KCl-100 ppm Ag_2SO_4 solution. All experimental units were then shaken for 15 minutes, and filtered through a 0.45-µm filter. Extracts were analyzed for urea and NH_4^+-N as previously described. On day 5, the pH of four additional experimental units was measured for gypsum-amended and un-amended litter by using an AB150 pH meter by Fisher Scientific in a 1:5 (litter/0.01M $CaCl_2$ solution) ratio. The initial amount of urea in the treatments that received

urea was estimated by adding the average amount of urea extracted from experimental units that did not receive urea to the average amount of urea added to the other four experimental units of the same treatment. To calculate the rate of urea hydrolysis (μg urea g^{-1} dry litter min^{-1}), the amount of urea remaining after 12 min was subtracted from the initial amount of urea.

Experiment 2.5: Effect of gypsum on pH and carbonate precipitation in broiler litter

A final experiment was conducted to measure the amount of $CaCO_3$ that precipitates from the hydrolysis of urea in the presences of gypsum. This experiment was designed using procedures similar to those of Bundy and Bremner (1972), which are used to determine inorganic carbon in soils. Treatments for this experiment included: broiler litter + 20 mg urea, broiler litter + 20 mg urea + 20% gypsum, and broiler litter alone. There were 4 replicates for each treatment, all arranged in a completely randomized design. Each experimental unit consisted of 1 g of broiler litter (0.33 g H_2O g^{-1}) mixed with 20 mL of CO_2-free dH_2O placed in a sealed 240-mL French square bottle. Reagent-grade gypsum (0.2 g) was added to broiler litter on a dry weight basis. Each bottle was equipped with a 7 mL 4M KOH trap to trap CO_2. All experimental units were placed on a rotary shaker set at 150 oscillations per minute for 48 hours. After 48 hours, experimental units were removed from the shaker, and the KOH traps were retrieved to measure the amount of CO_2 evolved from respiration and urea hydrolysis. The content of each trap was poured into a 50-mL centrifuge tube, and 9.3 mL of 1.5N $BaCl_2$ was added to each tube. All tubes were then centrifuged for 5 minutes. After centrifuging, a 5-mL aliquot was taken from each tube, and diluted with 10 mL of dH_2O. This solution was then titrated with 0.1N HCl to a pH of 8.3 to measure the amount of unreacted KOH. The amount of CO_2 trapped was calculated from the difference between initial and final amounts of KOH.

After removal of the KOH traps, the broiler litter slurry was transferred to a filtering unit with a 0.45-μm filter. Each bottle was rinsed twice with dH_2O to ensure complete transfer of broiler litter to the filtering unit. The resulting filtrate was titrated to pH 4.5 with 0.1M HCl to measure the amount of HCO_3^- and CO_3^{2-} in solution. To measure the amount of $CaCO_3$ that precipitated as a solid, the filter paper with broiler litter was retrieved from each filtering unit, and placed into a 250-mL French square bottle equipped with a 7 mL 4M KOH trap. All bottles were then sealed, and 50 mL of air was removed from each bottle using a syringe. Twenty milliliters of 2M HCl was then injected into each bottle through a septum at the top of the bottle, and all experimental units were allowed to stand on the lab bench for 24 hours. After 24 hours, the KOH traps were removed, and the contents were poured into a 50-mL centrifuge tube containing 9.3 mL of 1.5 N $BaCl_2$. After centrifuging, a 5-mL aliquot was taken from each tube and diluted with 10 mL of dH_2O. This solution was then titrated with 0.1N HCl to a pH of 8.3 to measure the unreacted KOH.

To determine the pH evolution that occurs during the first 24 hours following the addition of gypsum to broiler litter, a second study was conducted in which the pH of litter amended with and without gypsum was measured during a 24-hour incubation. Treatments for this experiment included: un-amended broiler litter and broiler litter + 20% gypsum. There were three replicates of each treatment, all arranged in a completely randomized design. Each experimental unit consisted of 1.33 g of broiler litter (0.33 g H2O g^{-1}) plus 10 mL of dH_2O placed in a 125-mL Erlenmeyer flask. Gypsum (0.2 g) was added to broiler litter treatments on a dry weight basis. 40 mL of 2.5M KCl-100 ppm Ag_2SO_4 solution was then added to each experimental unit, and pH was measured immediately using an AB150 pH meter (Fisher Scientific). The pH of all experimental units was measured after 0, 1, 2, 3, 4, and 24 hours.

To better understand the decrease in pH that was observed at time 0 in amended litter, compared to un-amended litter, in the preceding experiment, the litter pH buffering capacity and the initial amount of bicarbonate (HCO_3) in litter were determined. The litter pH buffering capacity was determined using a titrimetric method described by Cassity-Duffey et al. (2015). The initial amount of HCO_3^- in litter was determined using procedures similar to those of Bundy and Bremner (1972), which are used to determine inorganic carbon in soils. There were four replicates used to determine the initial amount of HCO_3^- in broiler litter, and each experimental unit consisted of 10 g of broiler litter (0.33 g H_2O g^{-1}) placed in a 250-mL Erlenmeyer flask with 200 mL of dH_2O. All experimental units were thoroughly mixed and filtered immediately through a 0.45-µm filter. A 20-mL aliquot of each filtrate was placed in a 250-mL square bottle that contained a 2.5 mL 0.87 N KOH trap for capturing CO_2. All bottles were then sealed, and 50 mL of air was removed from each bottle using a syringe. Next, 5 mL of 0.098 M HCl was then injected into each bottle through a septum at the top of the bottle, and all experimental units were allowed to stand on the lab bench for 24 hours. After 24 hours, the KOH traps were removed the contents poured into a 50-mL centrifuge tube, and brought to a final volume of 50 mL using dH_2O. Individual traps were then titrated according to Bundy and Bremner (1972).

Statistical Analysis

Data from all the experiments were analyzed using a one-way analysis of variance as a completely randomized design. Significant differences among treatment means were determined using Fisher's protected LSD at $p = 0.05$.

RESULTS

Experiment 2.1: Effect of FGG on urea-degrading bacteria and N mineralization in broiler litter

Amending broiler litter with 20% and 40% FGG increased water potential after the first 24 hours, and this effect was sustained throughout the experiment (Table 2.3). The addition of FGG to broiler litter also resulted in significantly different pH values throughout the experiment. At the end of the experiment, broiler litter receiving 40% FGG had the lowest water pH (8.22), followed by broiler litter + 20% FGG (8.33) (Table 2.3). The pH of all experimental units was also measured in a 0.01M $CaCl_2$ solution on day 21 to dismiss any concerns regarding the effect of electrolyte concentration on pH measurements. The pH values measured in 0.01M $CaCl_2$ were lower than pH values measured in dH_2O (Table 2.3), and the pH of litter amended with FGG was still lower than the pH of unamended litter.

Urea-degrading bacteria concentrations declined significantly following the addition of FGG to litter (Figure 2.1 A) and remained suppressed for the duration of the experiment compared to un-amended litter. The addition of 20% FGG to broiler litter also decreased urea concentrations throughout the experiment compared to un-amended litter (Figure 2.1 B), and this resulted in greater concentrations of NH_4^+-N (Figure 2.1 C). Furthermore, amending poultry litter with 40% FGG reduced urea concentrations during the experiment (Figure 2.1 B), but these values were not different from un-amended litter after 21 days. However, amending poultry litter with 40% FGG did produce more NH_4^+-N compared to un-amended litter after 21 days (Figure 2.1 C). Nitrate concentrations were very low throughout the experiment likely because the high NH_4^+-N and high pH would imply high NH_3 concentrations, which would inhibit nitrifiers. Results from experiment 2.1 showed that the addition of FGG to broiler litter decreased the pH of litter, as well as the concentration of urea-degrading bacteria. Amending

broiler litter with FGG also decreased the concentration of urea, while significantly increasing the concentration of NH_4^+ in litter.

Experiment 2.2: Effect of FGG on carbon dioxide and NH_3 emmisions from broiler litter

Amending broiler litter with 20% or 40% FGG did not have an effect on CO_2 emissions after 21 days, but FGG addition did reduce cumulative NH_3 volatilization compared to unamended litter (Figure 2.2 A and B). Carbon dioxide emissions from all treatments increased sharply to approximately 1500 ug g^{-1} d^{-1} in the first 24 hours, and declined slightly by day 3 (Figure 2.2 A). On day 5, broiler litter amended with 40% FGG had the greatest amount of CO_2 loss, but by day 9 both application rates of FGG generated less CO_2 emission than unamended litter. After day 9, there was no difference in CO_2 emission between any of the treatments.

Cumulative NH_3-N volatilization was reduced by the addition of 20% or 40% FGG during the course of the experiment (Figure 2.2 B). Approximately 47% of NH_3-N loss from un-amended litter occurred in the first two days of the experiment, while similar losses were delayed until day three for litter amended with FGG. After 21 days, cumulative NH_3-N loss from un-amended litter represented 19.01% of the total N in the litter, while cumulative losses from poultry litter amended with 20% or 40% FGG represented 14.7% and 15.3% of the total N, respectively (Figure 2.2 B).

Experiment 2.3: Nitrogen balance for litter amended with differential rates of FGG

Amending broiler litter with 20% or 40% FGG resulted in more mineralized-N than unamended litter after 21 days (Table 2.4). The majority of mineralized-N recovered in all treatments was in the form of ammonical-N (92.6-95.2%) making NO_3^--N a small portion (4.8-7.3%) of the total N recovered. Cumulative NH_3-N loss from broiler litter was reduced

throughout the experiment by the addition of 20% or 40% FGG, and amending litter with 40% FGG resulted in the least amount of cumulative NH_3-N loss after 21 days (8.43% total N). Concentrations of NH_4^+-N were also affected by the addition of FGG to broiler litter. Unamended broiler litter contained approximately 50% less NH_4^+-N than FGG-amended litter, and the amount of NH_4^+-N in litter increased with greater rates of FGG application (Table 2.4). Even though there was a significant increase in NH_4^+-N concentrations in litter amended with FGG, NO_3^--N concentrations in FGG-amended litter were not different from those found in un-amended litter on a weight basis ($\mu g\ g^{-1}$) (Table 2.4).

Experiment 2.4: Effect of gypsum on urease activities in broiler litter with different water contents

The rate of urea hydrolysis in unamended litter increased during the experiment for litter with Low (0.31) and High (0.78) water contents, while the rate of urea hydrolysis in litter with Medium (0.44 g $H_2O\ g^{-1}$) water content remained fairly constant (Figure 2.3: A-C). The increase in urea hydrolysis that was obsereved during the experiment for un-amended litter with Low and High water contents is likely due to the acclimation of urease-producing microorganisms to the new conditions that were created when dH_2O was added to broiler litter to achieve desired water contens. After wetting, broiler litter of each water content was allowed to rest on the lab bench for 72 hours before the experiment began. We assumed that this would be an adequate amount of time for microbial populations to adapt to the new conditions, but this does not appear to be true, because the rate of urea hydrolysis in un-amended litter did not begin to stabilize until after day 1 and 3 for litters with Low and High water contents, respectively.

The rate of urea hydrolysis in broiler litter with different water contents was affected by the addition of 20% reagent-grade gypsum. Differences in the rate of urea hydrolysis between un-amended litter and gypsum-amended litter with Low and High water contents were only observed in the first 24 hours following the addition of gypsum to litter (Figure 2.3: A and C). Significant increases in the rate of urea hydrolysis caused by the addition of gypsum to litter with Medium water content were only observed on days 3 and 5. By the end of the study, the slowest rates of urea hydrolysis were observed in un-amended and gypsum-amended litter with Low water content (97 ug g^{-1} $min.^{-1}$ and 96 ug g^{-1} $min.^{-1}$, respectively) as well as in un-amended litter with Medium water content (87 ug g^{-1} $min.^{-1}$) (Figure 2.3 A and B). An intermediate rate of urea hydrolysis (125 ug g^{-1} $min.^{-1}$) was observed in gypsum-amended litter with Medium water content, while the greatest rate of urea hydrolysis was measured in un-amended and gypsum-amended litter with High water content (164 ug g^{-1} $min.^{-1}$ and 151 ug g^{-1} $min.^{-1}$, respectively). Additional experimental units for each treatment were used to measure pH following urea hydrolysis on day 5. The pH for un-amended and gypsum-amended litter increased with increasing water content, and pH values for gypsum-amended litter were less than those of un-amended litter (Figure 2.4: A-C). Litter pH was also suppressed in experiment one by the addtion of gypsum, and these results agree with findings by Oliviera et al. (2003).

The increase in urea hydrolysis that was observed on day 0 following the addition of gypsum resulted in a significant increase in NH_4^+-N for all water contents (Figure 2.4: A-C). These findings are similar to results from experiment one in which NH_4^+-N in litter increased following the addition of gypsum. The concentration of NH_4^+-N in unamended and FGG-amended litter with Medium water content remained constant after day 0, while NH_4^+-N in the rest of the treatmens increased by the end of the experiment. After five days, the greatest rate of

urea hydrolysis occurred in litter with High water content, and this resulted in the greatest concentration of NH_4^+-N in litter. Amending this litter with gypsum resulted in more NH_4^+-N (9,873 ug g^{-1}) than un-amended litter (9,069 ug g^{-1}). Overall, the rate of urea hydrolysis increased with increasing water content, and greater rates of urea hydrolysis resulted in greater increases in pH. Furthermore, amending broiler litter with reagent-grade gypsum increased the rate of urea hydrolysis for all water contents, and this resulted in a greater concentration of NH_4^+-N compared to un-amended litter.

Experiment 2.5: Effect of gypsum on pH and carbonate precipitation in broiler litter

The results of our urea-C balance show that more urea-C precipitated as $CaCO_3$ in gypsum-amended litter than in un-amended litter, and this led to less HCO_3^- and CO_3^{2-} in solution (Table 2.5). A second study was conducted to identify the mechanism responsible for the suppression of pH that occurred following the addition of gypsum to broiler litter. Broiler litter used in experiments four and five had a pH buffer capacity of 200 mmol (pH unit)$^{-1}$ kg^{-1} dry litter, and a sufficient amount of HCO_3^- in solution to produce 50.6 $\pm$ 5.9 meq H^+ kg^{-1} (data not shown). Measurements of litter pH buffering capacity and HCO_3^- in solution indicate that there is an adequate amount of HCO_3^- in solution to decrease litter pH 0.25 pH units. Therefore, it seems likely that the immediate decrease in litter pH (0.23 pH units) that was observed when gypsum was added to litter (Figure 2.5) is due to the precipitation of $CaCO_3^-$ from litter HCO_3^- as described by equations 2 and 3. Overall, results from experiment five indicate that the addition of gypsum to broiler litter produces more $CaCO_3$ than in un-amended litter, and this precipitation of $CaCO_3$ buffers against increases in pH.

DISCUSSION

Several authors have hypothesized that amending broiler litter with gypsum reduces NH_3 volatilization due to a decrease in microbial activity associated with N mineralization. In the present study, amending broiler litter with FGG decreased the abundance of urea-degrading bacteria by 48 to 57 % in agreement with findings by Sampaio et al. (1999) who observed a reduction in bacterial populations when litter was amended with different rates of gypsum. The reduction in urea-degrading bacteria observed in our study coincided with a significant increase in electrical conductivity, indicating that the addition of gypsum to broiler litter induced osmotic stress on urease-producing bacteria. Increases in osmotic stress encountered by microorganisms can cause cell dehydration and increased concentrations of solutes in the cytoplasm (Sleator and Hill, 2001), which can lead to a reduction in microbial biomass and respiration (Chowdhury et al., 2011; Gennari et al., 2007; Tripathi et al., 2006). Amending broiler litter with 20% and 40% FGG did not decrease CO_2 emissions even though the addition of this material decreased the abundance of culturable urea-degrading bacteria. We suspect that CO_2 emissions from broiler litter were not affected by the decrease in urea-degrading bacteria in gypsum-amended litters because urease-producing microorganisms only represent 0.1 to 3.1% of the total microbial population in litter (Rothrock et al., 2008).

In addition to decreasing the abundance of urea-degrading bacteria in litter, amending broiler litter with FGG increased urease activity by 27 to 41% at three different water contents (Figure 2.3: A-C). These results agree with results by Hammes et al. (2003) who observed a 4- to 10-fold increase in urease activity when ureolytic bacteria isolates were placed in a urea solution containing calcium (Ca). The authors hypothesized that the increase in urease activity that was observed resulted from a detoxification response of bacteria to high Ca concentrations.

Calcium metabolism in bacterial cells requires energy in the form of adenosine triphosphate (ATP), and it has been demonstrated that numerous microorganisms can produce ATP through urea hydrolysis (Mobley et al., 1989). Increases in urease activity resulting from a detoxification response to high Ca concentrations could explain the decline in urea concentrations (Figure 2.2 B), and the 9.9 to 10.6% increase in N-mineralization that was observed in FGG-amended litter (Table 2.4).

The increase N-mineralization that was observed in FGG-amended litter did not increase NH_3 volatilization compared to un-amended litter (Table 2.4). In fact, the addition of FGG to broiler litter decreased cumulative NH_3 loss by 18 to 22% in experiment 2.2 and by 22 to 28% in experiment 2.3. We suspect that the decrease in NH_3 loss from FGG-amended litter was due to the decrease in litter pH that was observed in multiple experiments (Table 2.3, Figure 2.4, and Figure 2.5). Urea hydrolysis generally causes an increase in pH according to the following reaction (Kissel et al., 1988):

$$CO(NH_2)_2 + 2H_2O + H^+ \rightarrow NH_4^+ + HCO_3^- \qquad \text{Eq. [1]}$$

As the pH of broiler litter increases to 8.2 and above, HCO_3^- deprotonates to form CO_3^{2-} (Equation 2). In the presence of Ca^{2+}, CO_3^{2-} will precipitate as $CaCO_3^-$ (Equation 3).

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \qquad \text{Eq. [2]}$$

$$Ca^{+2} + CO_3^{2-} \rightarrow CaCO_3 \qquad \text{Eq. [3]}$$

The precipitation of $CaCO_3^-$ in equation 3 favors the consumption of HCO_3^{2-} leading to the release of additional H^+ (Equation 2) that can buffer against increases in pH.

It is well recognized that bacteria species can precipitate $CaCO_3$ as a by-product of urea hydrolysis when adequate concentrations of calcium are present (Cahal et al., 2011; Hammes et al., 2003; Stocks-Fischer et al., 1999; Fujita et al., 2000). Results of our urea-carbon balance showed that less urea-derived carbon was recovered as CO_2 and as carbonates in solution when gypsum was added to litter. These differences were due to a greater precipitation of $CaCO_3$ in gypsum-amended litter than in un-amended litter (Table 2.5), which led to a decrease in litter pH according to the reactions described above. In addition, amending broiler litter with gypsum decreased litter pH immediately due to the precipitation of $CaCO_3$ from gypsum-derived Ca and litter bicarbonate.

It should be considered that microbial carbonate precipitation generally occurs on the external surface of bacterial cells (Castanier et al., 1999), and the accumulation of $CaCO_3$ on the cell surface limits exchange of materials with the environment (Silva-Castro et al., 2015). In addition, the expulsion of H^+ from bacteria reduces the pH of the microenvironment surrounding the cell, increases intracellular pH, and reduces the proton pool available for biological processes (Norris et al., 1996). Both of the reactions mentioned are detrimental to cell survival, and could explain the reduction in the number of culturable urea-degrading bacteria that was observed in FGG-amended treatments (Figure 2.1 A)

CONCLUSIONS

Our results suggest that the addition of FGG at 20 or 40% of fresh broiler litter weight increased the osmotic stress encountered by urea-degrading bacteria. These conditions were detrimental to the survival of urease-producing bacteria, and led to an increase in N mineralization due to an increase in urease activity. As urea was hydrolyzed in gypsum-

amended litter, $CaCO_3$ precipitated and buffered against large increases in pH. The increase in urease activity coupled with a lower pH in gypsum-amended litter resulted in less cumulative NH_3 volatilization and greater concentrations of NH_4^+-N. The addition of 20% or 40% FGG to broiler litter resulted in similar amounts of urea-degrading bacteria, urea, cumulative NH_3 loss, and nitrogen mineralization after 21 days. Thus, amending broiler litter with 20% FGG is a sufficient rate to reduce NH_3 volatilization.

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TABLES AND FIGURES

Table 2.1: Initial physiochemical characteristics of broiler litter used in laboratory experiments 1-3 and 4-5.

Parameter	**Unit**	**Exp. 1-3**	**Exp. 4-5**
Water Content	g H_2O g^{-1}	0.63	0.33
Water Potential	MPa	-9.20	-15.70
pH		8.62	7.17
EC	μS cm^{-1}	26.4	17.76
Total C	ug g^{-1}	177,000	252,500
Total N	ug g^{-1}	24,500	34,400
C/N Ratio		7.22	7.34

Table 2.2: Elemental composition of Flue-gas desulfurization gypsum (FGG) used in experiments 1-3.

Element	Al	Ca	Fe	Mg	P	K	Si	S	Na
	----------	----------	----------	----------	mg kg^{-1}	----------	----------	----------	----------
	1,171	138,463	1,626	1,224	71	334	434	84,627	100

Table 2.3: Physiochemical characteristics of broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG), and broiler litter + 40% FGG (BL + 40%) after incubation at 27°C for 21 d (Experiment 2.1). FGG addition was based on the litter wet weight. Values represent the mean of six replicates.

	----------pH----------		EC	Water Potential
Treatment	**dH_2O**	**0.01M $CaCl_2$**	**µs cm^{-1}**	**-MPa**
BL	8.78 A†	8.28 A	26.78 C	10.29 B
BL + 20% FGG	8.33 B	8.11 C	33.36 B	8.58 A
BL + 40% FGG	8.22 C	8.19 B	35.65 A	8.57 A

† Within a column, means with different letters are significantly different according to Fisher's LSD at $p<0.05$.

Table 2.4: Cumulative NH_3 loss and inorganic nitrogen concentrations in broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG), and broiler litter + 40% FGG (BL + 40%) after incubation at 27°C for 21 d (Experiment 2.3). FGG addition was based on the litter wet weight. Values represent the mean of six replicates.

Treatment	NH_3-N loss µg g^{-1}	NH_4-N Litter µg g^{-1}	NO_3-N Litter µg g^{-1}	Total N recovered ug g^{-1}	Total N recovered % Total N‡
BL	2854 A†	2813 C	446 A	6117 B	24.9 B
BL + 20%	2217 B	5870 B	450 A	8537 A	34.8 A
BL + 40%	2061 B	6218 A	418 A	8698 A	35.5 A

†Within a column, means with different letters are significantly different according to Fisher's LSD at $p<0.05$.

‡ %Total N recovered = (NH_3–N loss + NH_4^+-N + NO_3–N) / Total N

Table 2.5: Distribution of urea-C evolved as CO_2, urea-C present as soluble HCO_3^- and CO_3^{2-}, and urea-C present in solid phase $CaCO_3$ in broiler litter with (0.2 g g^{-1} dry litter) or without gypsum incubated for 48 hrs at 25°C (Experiment 2.5). †

Treatment	CO_2 in traps	HCO_3^- and CO_3^{2-} in solution	$CaCO_3$ (s)	Total urea-C
	----------mg urea-C g^{-1} broiler litter----------			
BL	1.46 B‡	1.03 B	1.39 A	3.96 A
BL + Gypsum	1.03 A	0.44 A	2.11 B	3.67 A

† Values presented were corrected by subtracting values determined for un-amended litter without additional urea.

‡ Within a column, means followed by different letters are significantly different according to Fisher's LSD at $p<0.05$.

Figure 2.1: Urea-degrading bacteria, urea, and NH_4^+-N concentrations in broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG) and broiler litter + 40% FGG (BL + 40%) incubated at 27°C for 21 d. (a) Urea-degrading bacteria (UDB) concentrations in broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG), and broiler litter + 40% FGG (BL + 40%) incubated at 27°C for 21 d; (b) urea concentrations in broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG), and broiler litter + 40% FGG (BL + 40%)incubated at 27°C for 21 d; (c) NH_4^+-N concentrations in broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG), and broiler litter + 40% FGG (BL + 40%) incubated at 27°C for 21 d (Experiment 2.1). FGG addition was based on litter wet weight. Symbols represent the mean of six replicates, and error bars represent SD.

Figure 2.2: Carbon dioxide (CO_2) and NH_3 volatilization broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG), and broiler litter + 40% FGG (BL + 40%) incubated at 27°C for 21 d. a) CO_2-C emissions from broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG), and broiler litter + 40% FGG (BL + 40%)incubated at 27°C for 21 d; b) cumulative NH_3 loss from broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG), and broiler litter + 40% FGG (BL + 40%) incubated at 27°C for 21 d (Experiment 2.2). FGG addition was based on litter wet weight. Symbols represent the mean of six replicates, and error bars represent SD.

Figure 2.3: Urease activity in litter with different water contents amended with (0.2 g g^{-1} dry litter) and without gypsum incubated at 29°C for 5 d. (a) Rates of urea hydrolysis in litter with 0.31 g H_2O g^{-1}. Litter was amended with (0.2 g g^{-1} dry litter) or without gypsum, and incubated at 29°C for 5 d; (b) rates of urea hydrolysis in litter with 0.44 g H_2O g^{-1}. Litter was amended with (0.2 g g^{-1} dry litter) or without gypsum, and incubated at 29°C for 5 d; (c) rates of urea

hydrolysis in litter with 0.78 g H_2O g^{-1}. Litter was amended with (0.2 g g^{-1} dry litter) or without gypsum, and incubated at 29°C for 5 d (Experiment 2.4). All symbols represent the mean of three replicates, and error bars represent SD.

Figure 2.4: Concentrations of NH_4^+-N in litter with different water contents amended with (0.2 g g^{-1} dry litter) and without FGG incubated at 29°C for 5 d. (a) Concentration of NH_4^+-N in litter with 0.31 g H_2O g^{-1}. Litter was amended with (0.2 g g^{-1} dry litter) or without gypsum, and incubated at 29°C for 5 d; (b) concentration of NH_4^+-N in litter with 0.44 g H_2O g^{-1}. Litter was amended with (0.2 g g^{-1} dry litter) or without gypsum, and incubated at 29°C for 5 d; (c) concentration of NH_4^+-N in litter with 0.78 g H_2O g^{-1}. Litter was amended with (0.2 g g^{-1} dry litter) or without gypsum, and incubated at 29°C for 5 d (Experiment 2.4). All symbols represent the mean of three replicates, and error bars represent SD. pH was measured on day 5 after urea hydrolysis

Figure 2.5: pH increase in broiler litter with (0.2 g g^{-1} dry litter) or without gypsum, and incubated at 25°C for 24 hrs (Experiment 2.5). Symbols represent the mean of three replicates, and error bars represent SD.

Figure 2.1

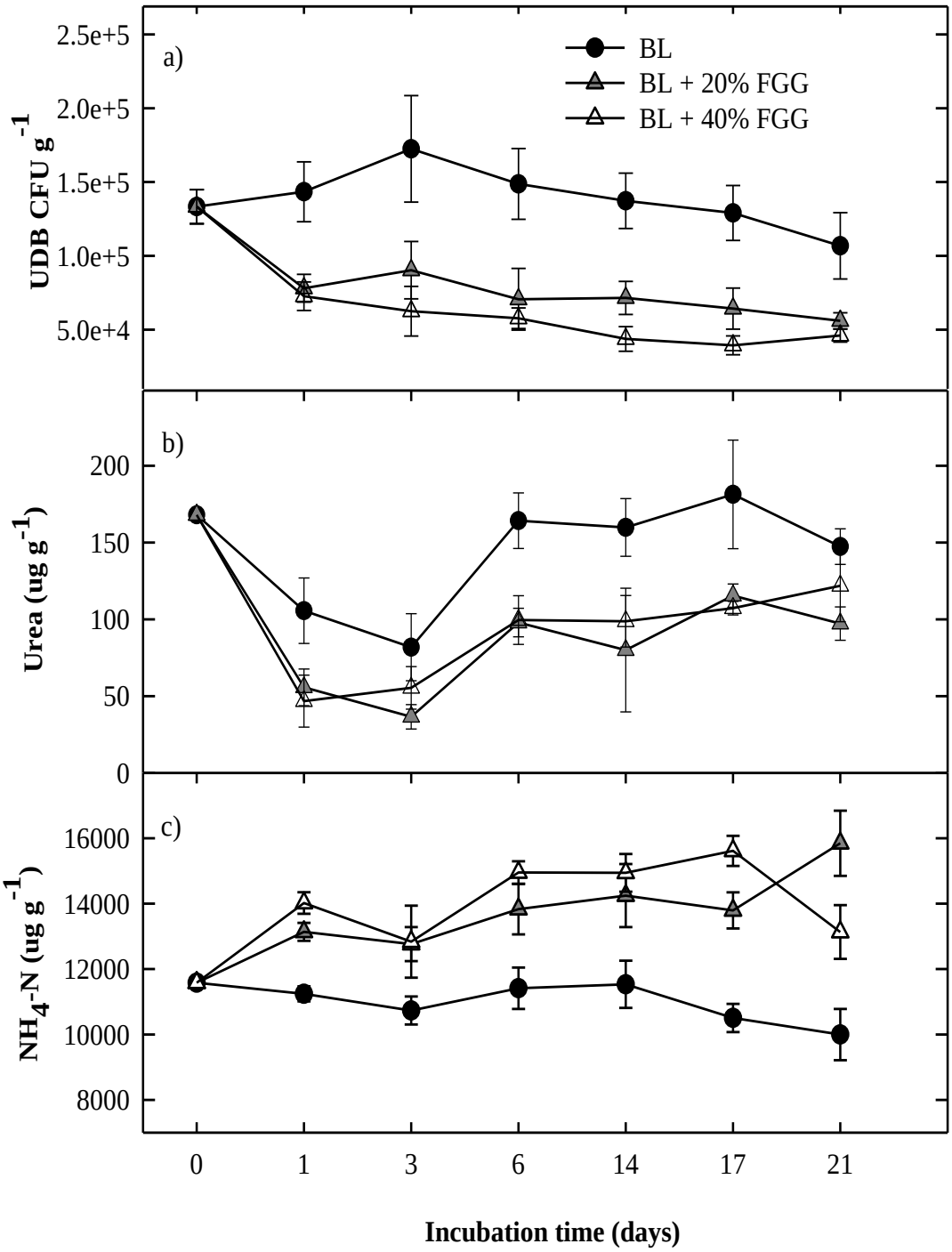

a)
b)
c)
BL
BL + 20% FGG
BL + 40% FGG
UDB CFU g -1
Urea (ug g -1)
NH4-N (ug g -1)
2.5e+5
2.0e+5
1.5e+5
1.0e+5
5.0e+4
200
150
100
50
0
16000
14000
12000
10000
8000
0
1
3
6
14
17
21
Incubation time (days)

Figure 2.2

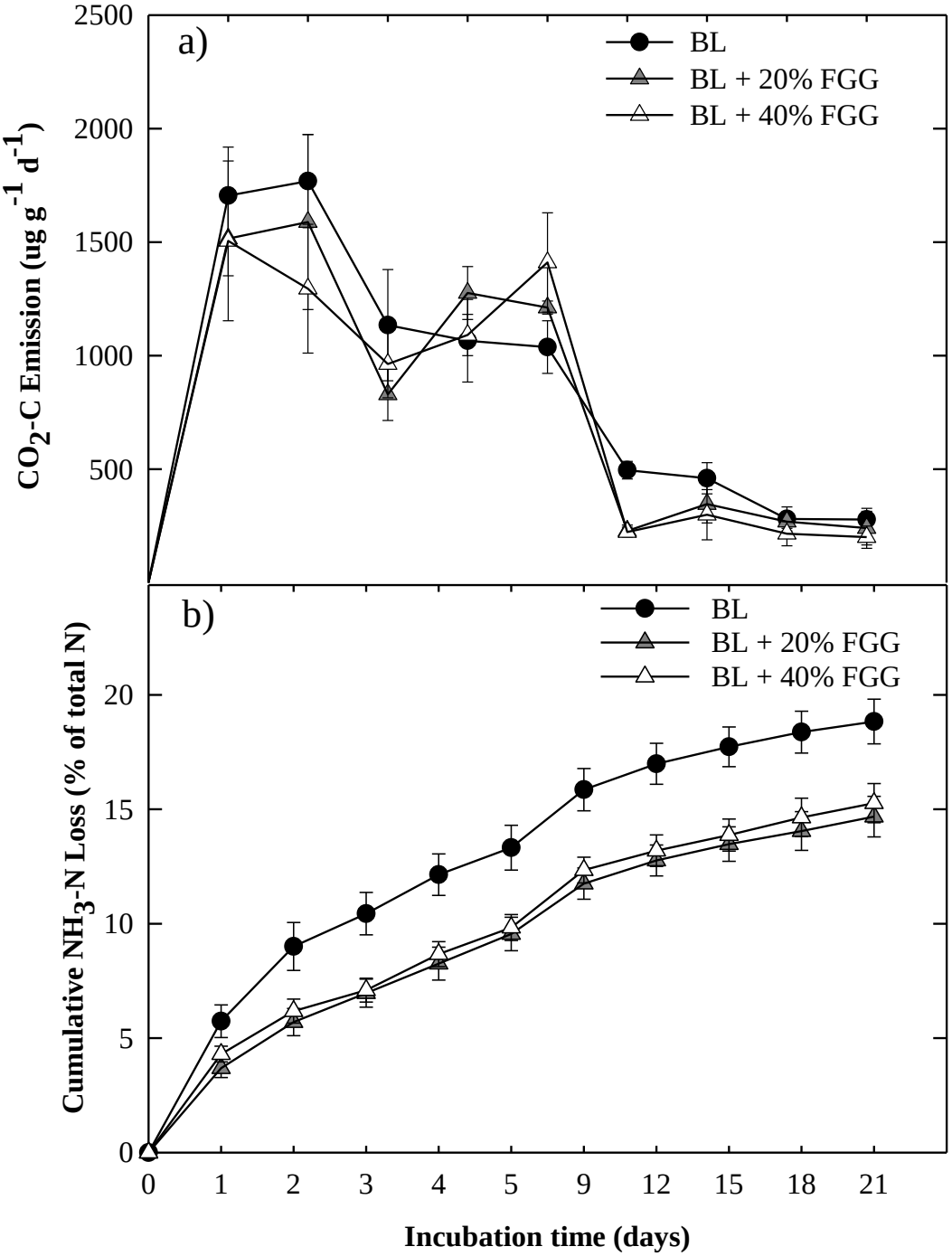

a)
BL
BL + 20% FGG
BL + 40% FGG
CO_2-C Emission (ug g^{-1} d^{-1})
2500
2000
1500
1000
500
b)
BL
BL + 20% FGG
BL + 40% FGG
Cumulative NH_3-N Loss (% of total N)
20
15
10
5
0
0
1
2
3
4
5
9
12
15
18
21
Incubation time (days)

Figure 2.3

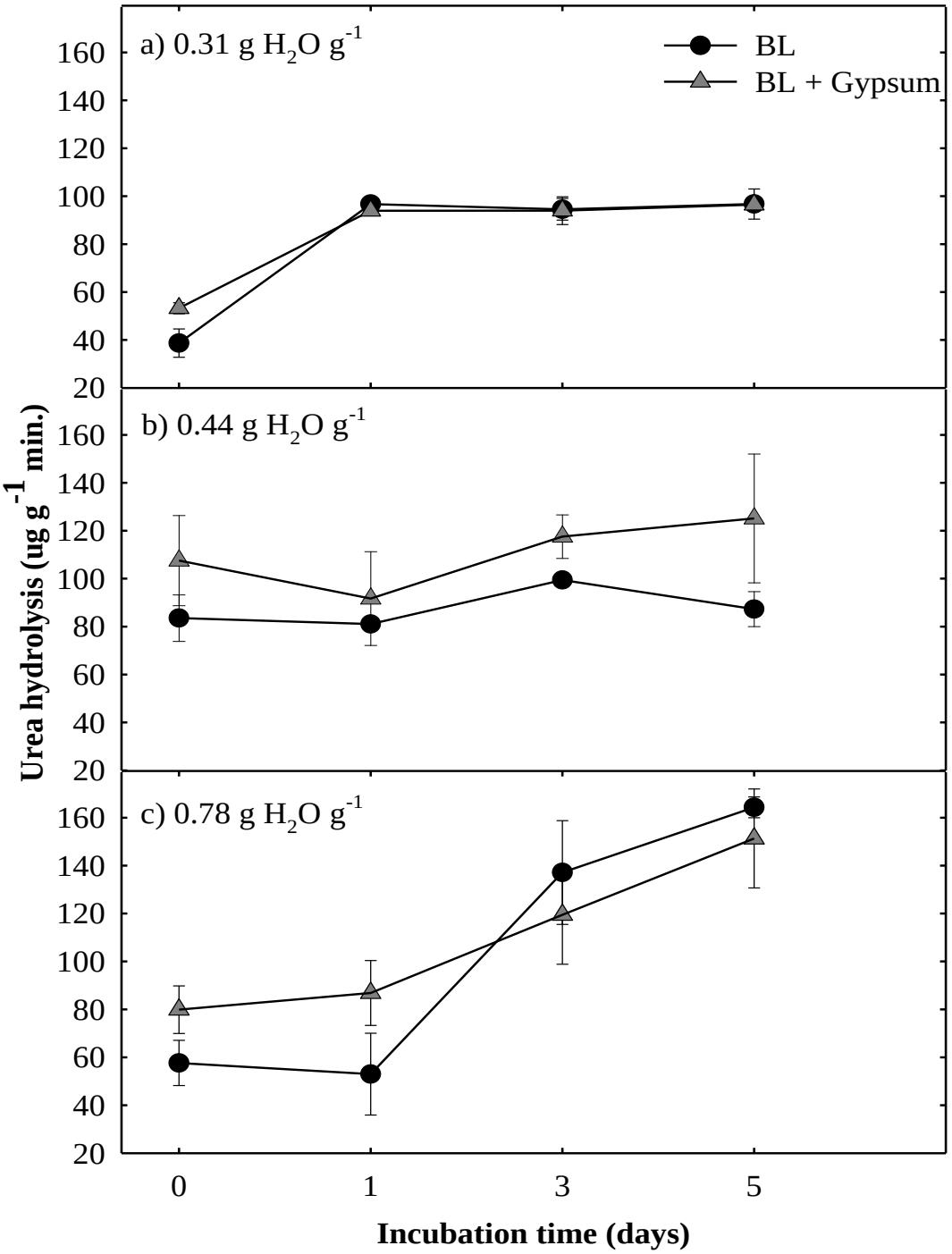

a) 0.31 g H2O g-1
b) 0.44 g H2O g-1
c) 0.78 g H2O g-1
BL
BL + Gypsum
Urea hydrolysis (ug g-1 min.)
Incubation time (days)
0
1
3
5
20
40
60
80
100
120
140
160

Figure 2.4

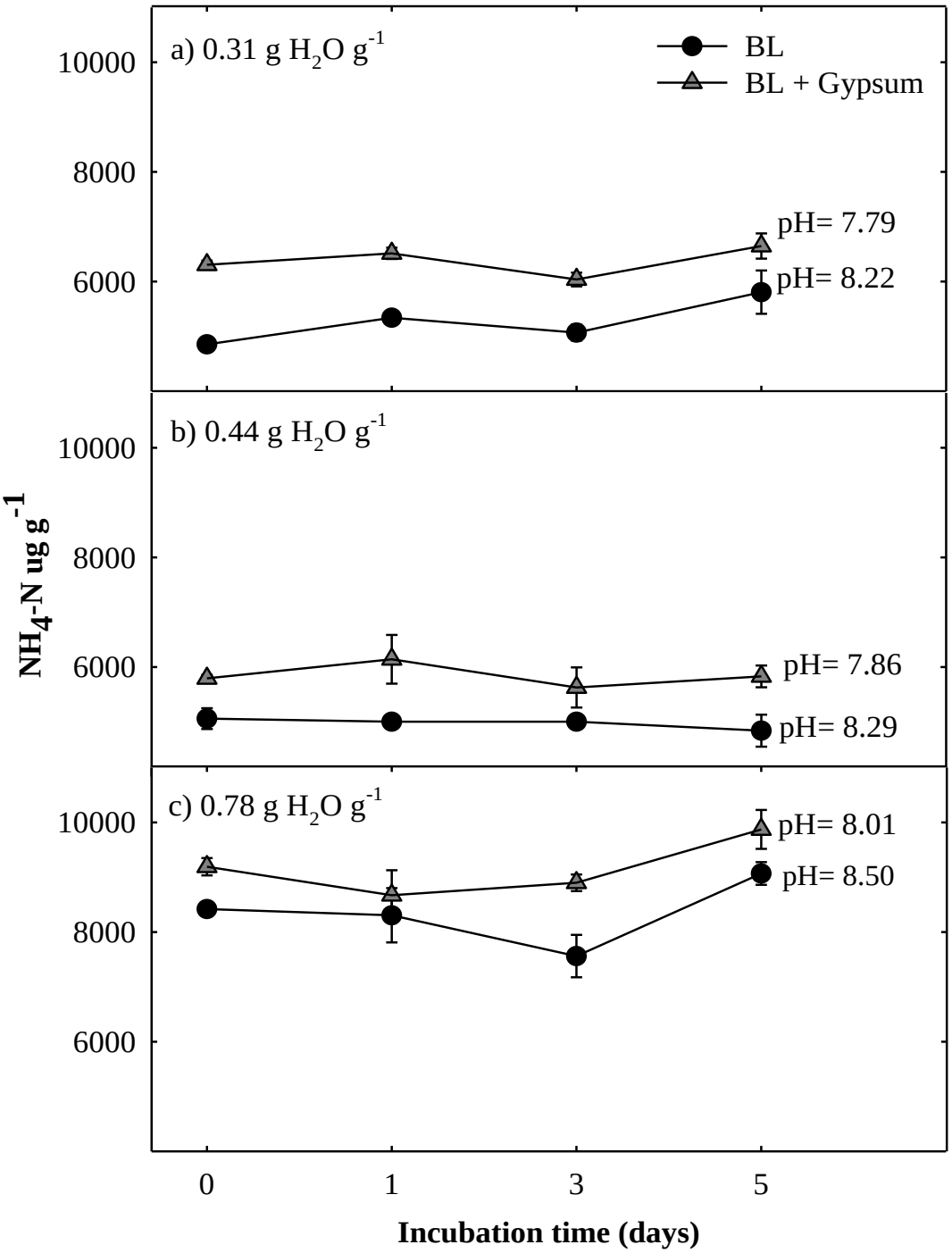
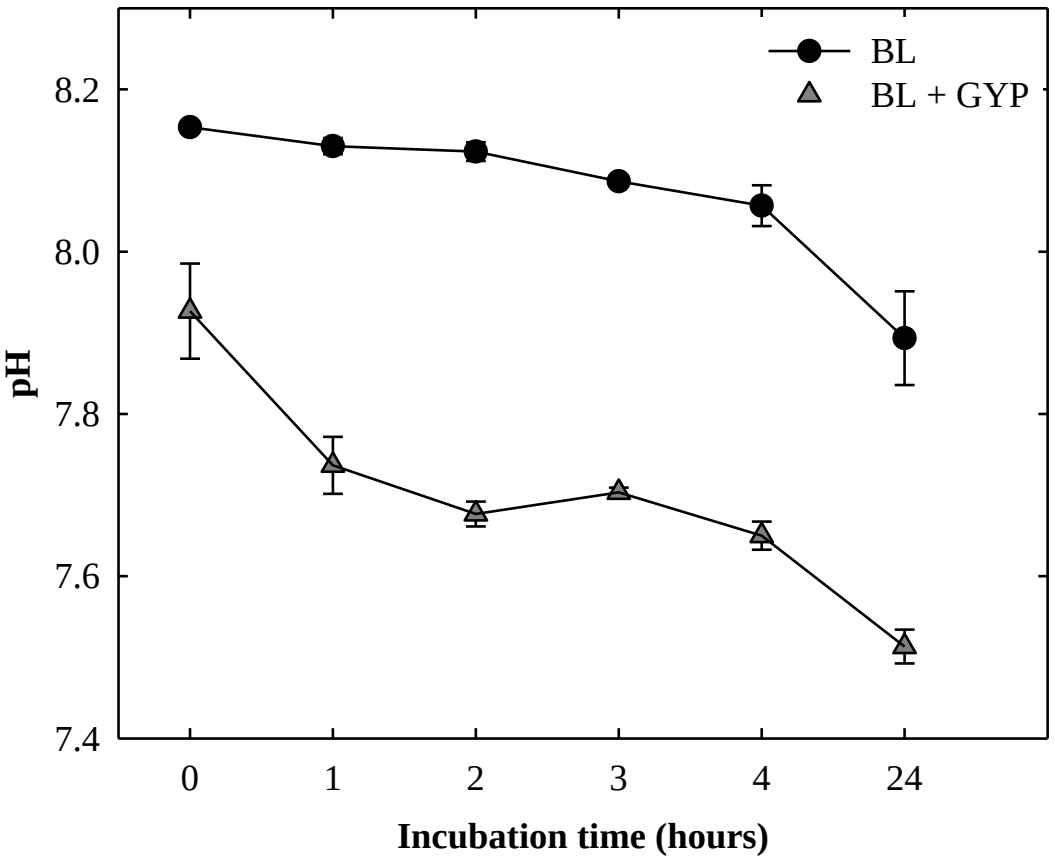

a) 0.31 g H2O g-1
BL
BL + Gypsum
pH= 7.79
pH= 8.22
b) 0.44 g H2O g-1
pH= 7.86
pH= 8.29
c) 0.78 g H2O g-1
pH= 8.01
pH= 8.50
10000
8000
6000
NH4-N ug g-1
0
1
3
5
Incubation time (days)

Figure 2.5



CHAPTER 3

GYPSUM EFFECTS ON WATER CONTENT AND NITROGEN TRANSFORMATIONS IN BROILER LITTER

[1]Burt, C.D., M.L Cabrera, and J. Rema. To be submitted to the Soil Science Society of America Journal.

ABSTRACT

Gypsum has been suggested as a broiler litter amendment due to its ability to decrease water available for microbial activity, but there are currently no studies examining the effect of litter drying by gypsum. The primary goal of this study was to evaluate gypsum's ability to absorb water from broiler litter, and to measure the corresponding urea-degrading bacteria abundance and nitrogen (N) mineralization in broiler litter. Litter and flue-gas desulfurization gypsum (FGG) were incubated together in acrylic cylinders, with limited contact between the two materials. After 48 hours, the litter water potential decreased from -9.48 MPa to -11.55 MPa, while the water potential of FGG increased from -122.43 MPa to -14.82 MPa indicating that moisture transfer from broiler litter to FGG is possible. A 10-day incubation was conducted to evaluate the drying effect of FGG on urea-degrading bacteria and N mineralization in litter. Our results showed that FGG reduced litter water content by 9 to 12%, and this drying effect reduced the abundance of urea-degrading bacteria by 42 to 71%, but this did not have a significant effect on NH_3 volatilization.

INTRODUCTION

Nitrogen (N) mineralization in broiler litter is a microbially-mediated enzymatic reaction in which organic-N-containing compounds are degraded to form ammoniacal-N and carbon dioxide (CO_2). Uric acid and urea account for 80% of the total organic-N in broiler manure (Rothrock et al., 2010; Ritz et al., 2004), and urease activity is considered the limiting factor for N-mineralization and subsequent ammonia (NH_3) volatilization (Nahm, 2003). Urease is an intracellular enzyme (Hammes et al., 2003; Klose and Tabatabai, 2000; Gianfreda et al., 1994) that is produced by ureolytic bacteria and fungi (Rothrock et al., 2008). Ureolytic microorganisms are widespread in the environment (Burbank et al., 2012; Rothrock et al., 2008; Fujita et al., 2008; Hammes et al., 2003), and can potentially represent 17-30% of the soil bacterial community (Lloyd and Scheaffe, 1973). Bacterial urease is the dominant form of urease in environments where there is considerable amounts of urea present (Barua et al., 2012), and urea-degrading bacteria have been identified as as the main source of urease in broiler litter (Rothrock et al., 2010). Results from Rothrock et al. (2010) indicate that N mineralization can be delayed if the ratio of urea-degrading bacteria to fungi is reduced to 2.0 or less. Delaying N mineralization should reduce the pool of ammoniacial-N that can be potentially lost by volatilization. Therefore, the suppression of urea-degrading bacteria should be considered as a method to reduce NH_3 volatiliation from broiler litter.

Many different litter amenments have been investigated to decrease NH_3 volatilization and microorganisms involved in N mineralization (Cook et al., 2011; Choi et al., 2008; Kim and Patterson, 2003; Pope and Cherry, 2000; Sampaio et al., 1999; Scantling et al., 1995). These litter amendments are generaly classified as acidifiers, alkaline materials, and chemical inhibitors. Acidifiers are the most common litter amendments due to their ability to decrease

litter pH below 7 (Rothrock et al., 2010; Choi et al., 2008). This reduction in pH favors the formation and retention of nonvolatile NH_4^+ (Kim & Choi, 2009), and decreases the abundance of urea-degrading bacteria that are known to facilitate N mineralization (Rothrock et al., 2010). The acidification of broiler litter also results in an increase in urease-producing fungi that are responsible for the delayed N mineralization that occurs weeks after application. Reapplication of acidifying amendments is often needed to maintain adequate atmospheric NH_3 concentrations (Lacey et al., 2004). When used as litter ammendments, chemical inhibitors can reduce NH_3 volatilization from broiler litter by inhibiting the growth of N-mineralizing microorganisms and/or reducing the activity of enzymes involved in N mineralization (Kim and Patterson, 2003; Varel, 1997). However, the elevated cost of these materials limits their use (McCrory and Hobbs, 2001).

Adsorbents have also been suggested as litter amendments due to their ability to bind heavy metals (Cook et al., 2011) and NH_3 (Ndegwa et al., 2008), and/or reduce moisture content. Aluminum-based water treatment residuals and chitosan have been investigated as adsorbents, and amending litter with these materials resulted in a decrease in N losses and in the concentration of urease-producing microorganisms (Cook et al., 2011). Clinoptiloite zeolite has also been investigated as a litter amendent due to its ability to bind volatile NH_3. However, the amount of NH_3-N fixed by this material varies among studies (Kithome et al., 1999; Amon et al., 1997; Nakaue et al., 1981). Gypsum ($CaSO_4 \bullet 2H_2O$) has also been proposed as an absorbent amendment due to its capacity to retain moisture (Loch et al., 2011; Oliveira et al., 2003; Wyatt and Goodman, 1992), and reduce soluble phosphorus concentration in run-off water (Endale et al., 2014; Desutter et al., 2011; Watts & Torbert 2009). Gypsum application rates described in the literature range from 10-40% of the litters' total weight (Mishra et al., 2013; Loch et al.,

2011; Oliveira et al., 2004: 2003; Sampiao et al., 1999), and reports of gypsum's ability to reduce moisture in broiler litter differ among studies (Mishra et al., 2013; Oliveira et al., 2004: 2003; Wyatt and Goodman, 1992).

The suggested drying effect of gypsum on litter particles should induce matric and osmotic stress on microbial populations, which may result in cell dehydration and an increased concentration of solutes in the cytoplasm (Sleator and Hill, 2001). Microorganisms can accumulate osmolytes to limit the effects of dehydration, but a reduction in water content limits the diffusion of substrates and nutrients necessary for osmolyte synthesis (Chowdhury et al., 2011). The stress imposed by low water availability and limited osmolyte synthesis can decrease bacterial biomass (Chowdhury et al., 2011) and microbial activity (Cook and Orchard, 2008) resulting in decreased C and N mineralization (Pulleman and Tietema, 1999). In poultry litter specifically, Rothrock et al. (2008) found that the concentration of urease-producing microorganisms in litter is influenced by litter water content, while Murakami et al. (2011) found that litter drying results in less uric acid mineralization and greater total nitrogen content.

The drying effect of gypsum should also increase osmotic stress on microbial populations due to greater salt concentrations in the remaining solution (Richards, 1995). Gypsum is a moderately soluble Ca-salt, and its dissolution should further increase the osmotic stress encountered by microbial populations. Increases in osmotic stress have been shown to alter the microbial community structure (Pankhurst et al., 2001: Wichern et al., 2006) and reduce the microbial biomass and respiration (Chowdhury et al., 2011; Gennari et al., 2007; Tripathi et al., 2006) as well as N mineralization (Laura, 1974). Fungi are typically more sensitive to increases in salinity and less sensitive to matric stress compared to bacteria (Chowdhury et al., 2011).

Therefore, the drying effect and the dissolution of gypsum should decrease the abundance of urea-degrading bacteria and fungi that are associated with NH_3 volatilization.

There are currently no studies that examine the effect of litter drying by gypsum on urea-degrading bacteria and NH_3 volatilization. The primary goal of this study was to evaluate gypsum's ability to absorb water from broiler litter, and to measure the corresponding urea-degrading bacteria abundance and N mineralization in broiler litter. We hypothesize that amending broiler litter with FGG will reduce water availability for microorganisms, and this will reduce urea-degrading bateria counts and NH_3 volatilization. In order to evaluate the drying effect of FGG on urea-degrading bacteria and N mineralization, we conducted a 10-day incubation in which FGG was not in direct contact with broiler litter. Moisture content, pH, inorganic-N, NH_3-N volatilization, and urea-degrading bacteria abundance were measured throughout the experiement.

METHODS AND MATERIALS

Broiler Litter and flue-gas gypsum (FGG):

Broiler litter was collected from a broiler house in Georgia, passed through a 2-mm sieve, and analyzed for total C and N, pH, water potential, and water content as described below. FGG was collected from a power plant in Illinois and analyzed for elemental composition using ICP-OES after acid digestion (USEPA, 1986). Water content of FGG was determined by drying at 105°C for 48 hours.

Broiler Litter Analyses

Total C and N in broiler litter was determined by dry combustion (Nelson and Sommers, 1982). Litter pH was measured using an AB150 pH meter by Fisher Scientific in a 1:5 (litter/deionized water) ratio. Water potential was measured using a WP4C Dewpoint Potentiameter (Decagon, Pullman, WA). Water content of the broiler litter was determined by drying at 65°C for 48 hours.

Moisture Release Curves

Moisture release curves were developed for broiler litter, broiler litter + 20% FGG, and broiler litter + 40% FGG. For that purpose, 1 kg of broiler litter was evenly divided among four Buchner funnels. A Whatman 42 filter paper was placed at the bottom of each funnel before broiler litter was added. Different amounts of deionized water (dH_2O) were subsequently added to each funnel, and broiler litter at the different water contents was placed in plastic bags for 24 hours to allow for equilibration. After 24 hours, a sample was taken from each plastic bag, and water potential and gravimetric water content were measured as previously described. A portion of litter was then removed from each bag, and amended with 20% and 40% FGG on a wet weight basis. Broiler litter + FGG was allowed to stand on the lab bench for 24 hours before water contents and water potentials were measured.

A moisture release curve was also developed for FGG. For that purpose, 1 kg of FGG was evenly divided among 10 Buchner funnles. A Whatman 42 filter paper was placed at the bottom of each funnel before FGG was added. Different amounts of dH_2O were added to FGG in each funnel, and after draining, FGG was placed in plastic bags for 24 hours to allow for

equilibration. After 24 hours, samples were taken from each plastic bag, and water content and water potential were measured.

Experiment 3.1: Movement of water from broiler litter to FGG

An experiment was performed to evaluate the movement of water from broiler litter to FGG. In this experiment, vertical columns of litter and FGG were established in acrylic cylinders (4.4 cm i.d., 10 cm long). The bottom of each cylinder was sealed with a no.10 rubber stopper, and the interior of each cylinder was partitioned into two compartments using a plastic divider. Fifteen grams of broiler litter was added to one compartment of the cylinder, while 15 g of FGG was added to the adjacent compartment. The materials were then gently packed, and the plastic divider between the materials was removed. This resulted in limited contact between the two materials. The top of each experimental unit was then sealed with a No. 10 stopper and parafilm. The cylinders were allowed to equilibrate for 48 hours. After 48 hours, broiler litter and FGG were carefully removed from the cylinders, and the water potential of both materials was measured. Seven replicates were used in this experiment.

Experiment 3.2: Effect of litter drying by FGG on N mineralization and urea-degrading bacteria concentrations in broiler litter with different water contents

An experiment was performed to evaluate the effect of particulate FGG added to broiler litter at two water contents (0.32 (Low) and 0.65 (High) g H_2O g $^{-1}$) on litter water content, NH_3 volatilization, and corresponding concentrations of urea-degrading bacteria over the course of 10 days. Broiler litter was air-dried for 24 hours, and then wetted with dH_2O to achieve desired water contents. After wetting, broiler litter of each water content was allowed to equilibrate on the lab bench for 72 hours before the experiment began. To obtain particulate FGG, 2 kg of

FGG was first passed through a 4-mm screen, and then through a 2-mm screen. FGG that did not pass through the 2-mm screen was used for the study. Particulate FGG (15 g) was weighed into Nitex mesh fabric bags (Sefar, Heiden, Switzerland). Each experimental unit consisted of a 4-L glass container that received 37.5 g broiler litter (dry weight equivalent) to form a thin layer at the bottom. Next, a Nitex mesh bag containing 0 or 15 g particulate FGG was placed on the litter layer, followed by another 37.5 broiler litter (dry weight equivalent) placed on top of the bag. Next, a vial containing 45 mL of 0.1 N H_2SO_4 was placed inside the jar for trapping NH_3. All containers were then sealed with screw-cap lids and placed inside an incubator at 29°C for 10 days. There were four replicates of each treatment (Nitex bag with or without FGG), all arranged in a completely randomized design. Broiler litter from each experimental unit was sampled on days 1, 3, 5, 7, and 10 and H_2SO_4 traps were changed on the same days and analyzed for NH_4-N colorimetrically (Keeny and Nelson, 1982). Before any litter was retrieved, the mesh bags were carefully removed from each container, and the litter was thoroughly mixed. For each sampling event, 5 g of broiler litter (wet weight) was retrieved from each container for analysis. At the end of the incubation period, mesh bags were removed from all experimental units, and the water potential of the FGG was measured using a WP4C Dewpoint Potentiameter (Decagon, Pullman, WA). Gravimetric water content was determined for each sampling event by drying litter at 65°C for 48 hours. Urea-degrading bacteria, inorganic-N concentrations and pH were measured on days 0, 5, and 10. Litter pH was measured in a 1:5 (litter/0.01 M $CaCl_2$) ratio using an AB150 pH meter (Fisher Scientific). Inorganic N (NH_4^+-N and NO_3^-N) was determined by shaking 1 g of litter with 100 mL of 1 mol L^{-1} KCl for 45 minutes, and then filtering the solution through a 0.45-µm filter. Extracts were analyzed following the colorimetric procedure described by Keeney and Nelson, (1982).

The population of urea-degrading bacteria was determined using a protocol similar to that of Kim and Patterson (2003). One gram of broiler litter was diluted with 30 mL of dH_2O and shaken for 15 min. A 1-mL aliquot of poultry litter suspension was then pipetted into a dilution tube containing 9 mL of dH_2O. A ten-fold dilution was repeated twice. Aliquots from each dilution tube were plated on Christensen urea agar following Miles and Mirsa (1938). After incubating the plates for 24 hours at 37°C, the colonies on each plate were quantified and results are reported as urea-degrading bacteria colony-forming units (CFU) per gram of dry litter.

RESULTS AND DISCUSSION

Moisture Release Curves

The moisture release curve for FGG shows a very low water potential at its native water content and a relatively high water potential (> -2 MPa) at water contents above 0.2 g g^{-1} (Fig. 3.1). In contrast, broiler litter has a water potential of -19 MPa at 0.2 g g^{-1} and a water potential lower than -5 MPa at a water content of 1 g g^{-1} (Fig. 3.1). It is probably due to the large increase observed in the water potential of FGG at water contents above 0.2 g g^{-1} that addition of FGG to broiler litter results in a large increase in water potential. Figure 3.1 clearly shows that at a given water content the water potential is much higher (less negative) in litter + FGG than in litter alone. These results suggest a transfer of water from broiler litter to FGG.

Experiment 3.1: Movement of water from broiler litter to FGG

Due to gypsum's low water potential and water vapor transport ability (Tesarek et al., 2006), we hypothesized that water from litter moves to FGG where it would not be available for ureolytic activity. In this study, broiler litter and FGG were incubated together in acrylic cylinders, with limited contact between the two materials. After 48 hours, the litter water potential decreased from -9.48 MPa to -11.55 MPa, while the water potential of FGG increased

from -122.43 MPa to -14.82 MPa. These results indicate that moisture transfer from broiler litter to FGG is possible, with this transfer likely increased when these two materials are in intimate contact with each other as when FGG is intimately mixed with broiler litter.

Experiment 3.2: Effect of litter drying by FGG on N mineralization and urea-degrading bacteria concentrations in broiler litter with different water contents

Incubating broiler litter at different water contents (0.32 (Low) and 0.65 (High) g H_2O g^{-1}) in contact with a bag containing particulate FGG at a rate equivalent to 20% of litter weight decreased litter water content, compared to litter incubated without FGG (Figure 3.2 A and B). After 10 days, particulate FGG reduced litter water content by 12 and 9% in litter with Low and High water contents, respectively. Even though there was a small difference in water content between broiler litter with and without gypsum with Low water content, the water potential of FGG incubated with litter increased from -122 MPa to -41 MPa after 10 days indicating that FGG absorbed water from the broiler litter. These results agree with the proposed drying effect of gypsum on litter particles put forth by Mishra et al. (2013). Mishra et al. (2013) observed an increase in water potential when broiler litter was amended with FGG, and they hypothesized that most of the water was associated with FGG particles and not with litter particles. However, this could not be confirmed because it was not possible to measure the individual water contents of the materials once they were mixed. In our study, FGG was placed in mesh bags to limit the dissolution of gypsum and to allow for separate measurements of litter water content and FGG water potential.

The water content of litter with an initial High water content incubated without FGG increased to 0.75 g H_2O g^{-1} by the end of the experiment (Figure 3.2 B). This increase in water content was not observed in litter with High water content incubated with 0.2 g g^{-1} FGG. The

water content of litter with High water content incubated with 0.2 g g^{-1} FGG remained fairly constant throughout the experiment, and was not different from the initial water content after 10 days. The increase in water content that was observed for litter with High water content is likely due to increased humidity in the experimental units that were located in incubator positions that experienced a slightly lower temperature. Condensation on the walls of the glass jars was only observed for experimental units containing litter with high water content, so it is possible that the mixing of litter within these containers before sampling likely caused litter wetting. It is also possible that the FGG incubated with this particular litter absorbed the additional water, and this suppressed the increase in water content that was observed in litter incubated without FGG. After 10 days, the water potential of FGG incubated with litter with High water content increased from -122 MPa to -8.06 MPa indicating that FGG absorbed more water from litter with a High water content than from litter with a Low water content.

Incubating broiler litter in contact with or without FGG did not affect the pH of litter with High water content after 10 days (Table 3.3). However, the pH of litter with Low water content incubated with FGG was less than that of litter incubated without FGG on day 10 (Table 3.3). These results agree with work by Oliveira et al. (2003) in which amending litter with gypsum decreased litter pH. Gypsum can suppress an increase in litter pH by precipitating $CaCO_3$ from bicarbonate and Ca ions in solution (Fenn et al., 1981), and this can lead to a significant increase in NH_4^+-N (Kithome et al., 1999). Although FGG was not in direct contact with litter, it is likely that some FGG escaped the mesh bags, and was mixed with the litter resulting in decreased pH values for litter incubated with FGG, compared to litter incubated without FGG. However, NH_4^+-N and NO_3^--N concentrations in all litters were not affected when litter was incubated with

FGG for 10 days (Table 3.3). These results suggest that FGG loss from the mesh bags was negligible, and had a minimal effect on litter characteristics.

Incubating broiler litter with 0.2 g g^{-1} FGG decreased concentrations of urea-degrading bacteria in both litters on day 10, compared to litter incubated without FGG (Figure 3.3 A and B). Urea-degrading bacteria concentrations in litter with Low water content incubated without FGG were constant throughout the experiment, but incubating these litters with 0.2 g g^{-1} FGG decreased urea-degrading bacteria concentrations by 42% on day 10 (Figure 3.3 A). However, the decrease in urea-degrading bacteria observed in litter with Low water content incubated with 0.2 g g^{-1} did not lead to significant decreases in NH_3-N volatilization, compared to litter without gypsum (Table 3.3) Urea-degrading bacteria concentrations in litter with High water content incubated without FGG were constant throughout the experiment, but incubating this litter with 0.2 g g^{-1} FGG decreased bacteria concentrations by 71% on day 10 (Figure 3.3 B). These results agree with findings by Sampaio et al. (1999) who observed that amending broiler litter with different rates of gypsum reduced total bacterial counts. However, these authors observed a significant decrease in NH_3-N volatilization from gypsum-amended litter compared to un-amended litter. Oliveira et al. (2003) also found a significant decrease in NH_3 volatilization from gypsum-amended litter. It should be noted, however, that the litter in our study was not in intimate contact with litter as was the case in Sampaio et al. (1999) and Oliveira et al. (2003). Our results suggest that FGG's drying effect alone can not significantly decrease NH_3-N volatilization during a 10-day incubation. Therefore, we hypothesize that the significant reductions in NH_3-N volatilization from gypsum-amended litter compared to un-amended litter observed by Sampaio et al. (1999) and Oliveira et al. (2003) were likely caused by a decrease in pH that occurs when gypsum is mixed with broiler litter.

CONCLUSIONS

Our results confirm that FGG can absorb moisture from litter particles, and this drying effect reduced the abundance of urea-degrading bacteria after 10 days, compared to litter incubated without FGG. However, the drying effect alone did not significantly affect NH_3-N loss or inorganic N concentrations in litter incubated with FGG, compared to litter incubated without FGG. Based on these results, it seems likely that the significant reduction in NH_3-N volatilization from gypsum-amended litter compared to un-amended litter observed by Sampaio et al. (1999) and Oliveira et al. (2003) resulted from a decrease in pH that occurs when gypsum is mixed with broiler litter

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TABLES AND FIGURES

Table 3.1: Initial physiochemical characteristics of broiler litter used in experiments 1 and 2.

Parameter	**Unit**	**Exp. 1**	**Exp. 2**
Water Content	g H_2O g^{-1}	0.63	0.31
Water Potential	MPa	-9.20	-10.67
pH		8.62	8.20
Total C	ug g^{-1}	177,000	272,342
Total N	ug g^{-1}	24,500	34,300
C/N Ratio		7.22	7.94

Table 3.2: Elemental composition of Flue-gas desulfurization gypsum (FGG) used in experiments 1 and 2.

Element	Al	Ca	Fe	Mg	P	K	Si	S	Na
	----------	----------	----------	----------	mg kg^{-1}	----------	----------	----------	----------
	1,171	138,463	1,626	1,224	71	334	434	84,627	100

Table 3.3: pH and inorganic-N concentrations in litter with different water contents incubated with (0.2 g g^{-1}) or without FGG after a 10-d incubation at 29°C. FGG additions were based on litter dry weight. Values represent the mean of four replicates.

Treatment	pH	NH_3-N µg g^{-1}	NH_4-N µg g^{-1}	NO_3-N µg g^{-1}	% Total N‡
0.32 g H_2O g^{-1}	8.15 A	1504 A	7736 A	680 A	28.9 A
0.32 g H_2O g^{-1} + FGG	8.01 B	1356 A	7748 A	656 A	28.5 A
0.65 g H_2O g^{-1}	8.51 A	2641 A	12311 A	43 A	43.7 A
0.65 g H_2O g^{-1} + FGG	8.48 A	1700 A	12487 A	40 A	41.5 A

† Within a column for each water content, means followed by different letters are significantly different according to Fisher's LSD at $p<0.05$.

‡ %Total N recovered = (NH_3–N loss + NH_4^+-N + NO_3–N) / Total N

Figure 3.1: Moisture release curves for FGG, broiler litter (BL), broiler litter + 20% FGG (BL + 20% FGG) and broiler litter + 40% FGG (BL + 40%). FGG addition was based on the litter wet weight. Water potential of FGG, un-amended litter, and amended broiler litter was measured using a WP4C Dewpoint Potentiameter. Litter gravimetric water content was determined by drying at 65°C for 48 hours, and gravimetric water content for FGG was determined by drying at 105°C for 48 hours.

Figure 3.2: Change in water content for broiler litter with different water contents incubated with (BL + FGG) and without gypsum (BL) incubated at 29°C for 10 d (Experiment 3.2). (a) Change in water content for litter with initial water content of 0.32 g H_2O g^{-1} incubated with (0.2 g g^{-1}) or without FGG; (b) Change in water content for litter with initial water content of 0.65 g H_2O g^{-1} incubated with (0.2 g g^{-1}) or without FGG; (c) Change in water content for litter with initial water content of 0.91 g H_2O g^{-1} amended with (0.2 g g^{-1}) or without FGG. All treatments were incubated at 29°C for 10 d. All symbols represent the mean of four replicates, and error bars represent SD.

Figure 3.3: Urea-degrading bacteria abundance in broiler litter with different water contents incubated with (BL + FGG) and without gypsum (BL) incubated at 29°C for 10 d (Experiment 3.2). a) Urea-degrading bacteria abundance in litter with initial water content of 0.32 g H_2O g^{-1} incubated with (0.2 g g^{-1}) or without FGG; (b) Urea-degrading bacteria abundance in litter with initial water content of 0.65 g H_2O g^{-1} incubated with (0.2 g g^{-1}) or without FGG; (c) Urea-degrading bacteria abundance in litter with initial water content of 0.91 g H_2O g^{-1} amended with

(0.2 g g^{-1}) or without FGG. All treatments were incubated at 29°C for 10 d. All symbols represent the mean of four replicates, and error bars represent SD.

Figure 3.1

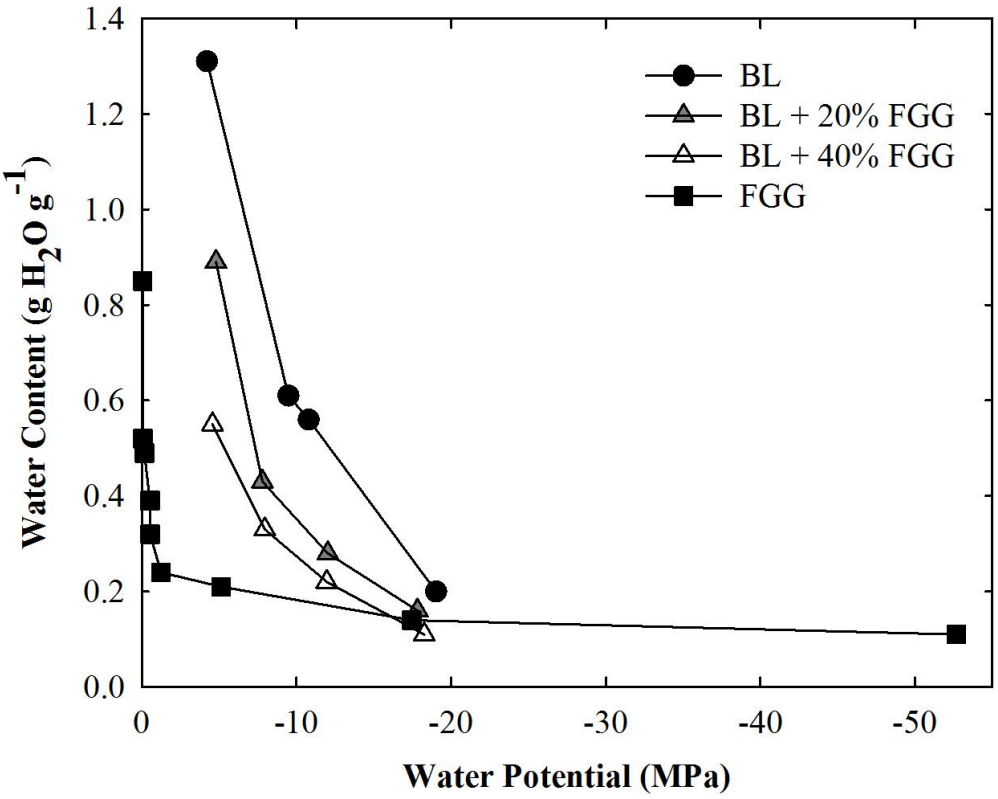

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Water Content (g H_2O g^{-1})
0
-10
-20
-30
-40
-50
Water Potential (MPa)
BL
BL + 20% FGG
BL + 40% FGG
FGG

Figure 3.2

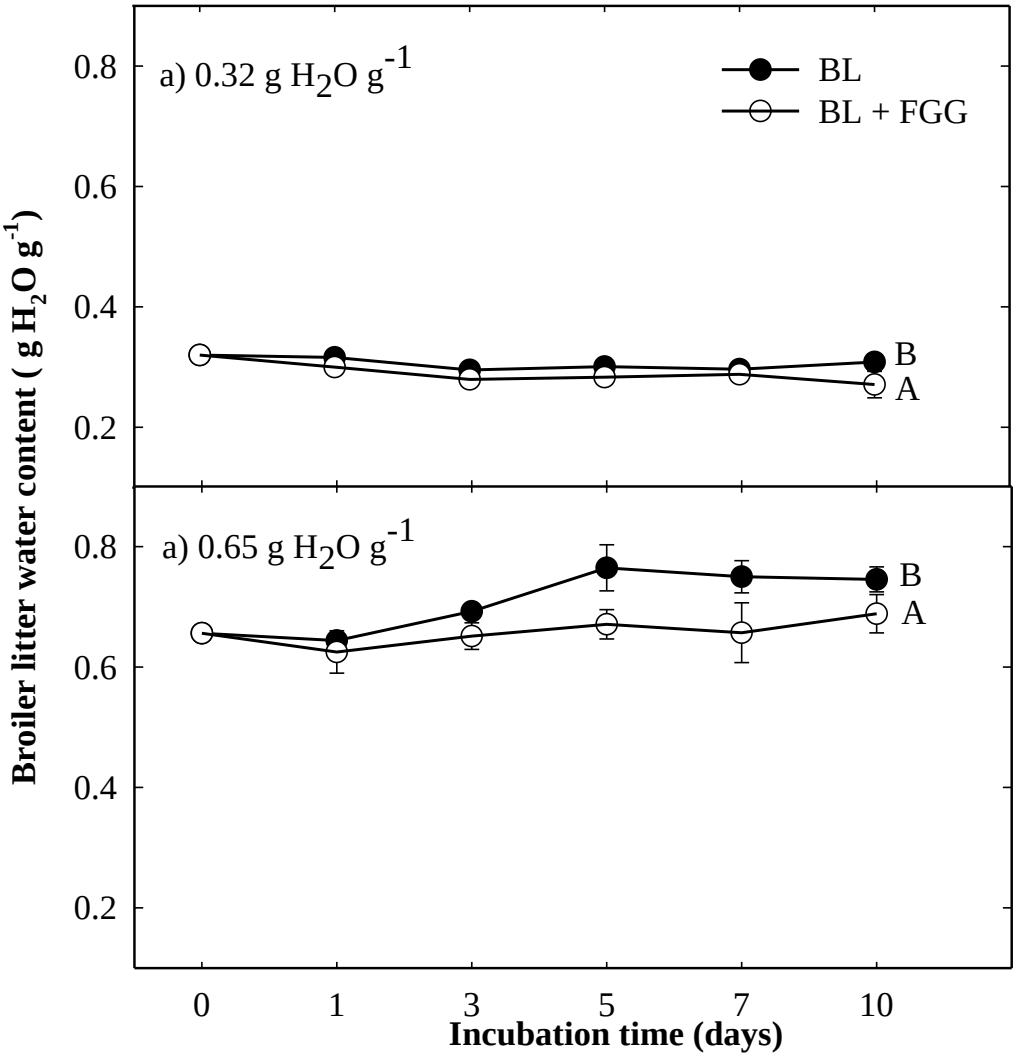
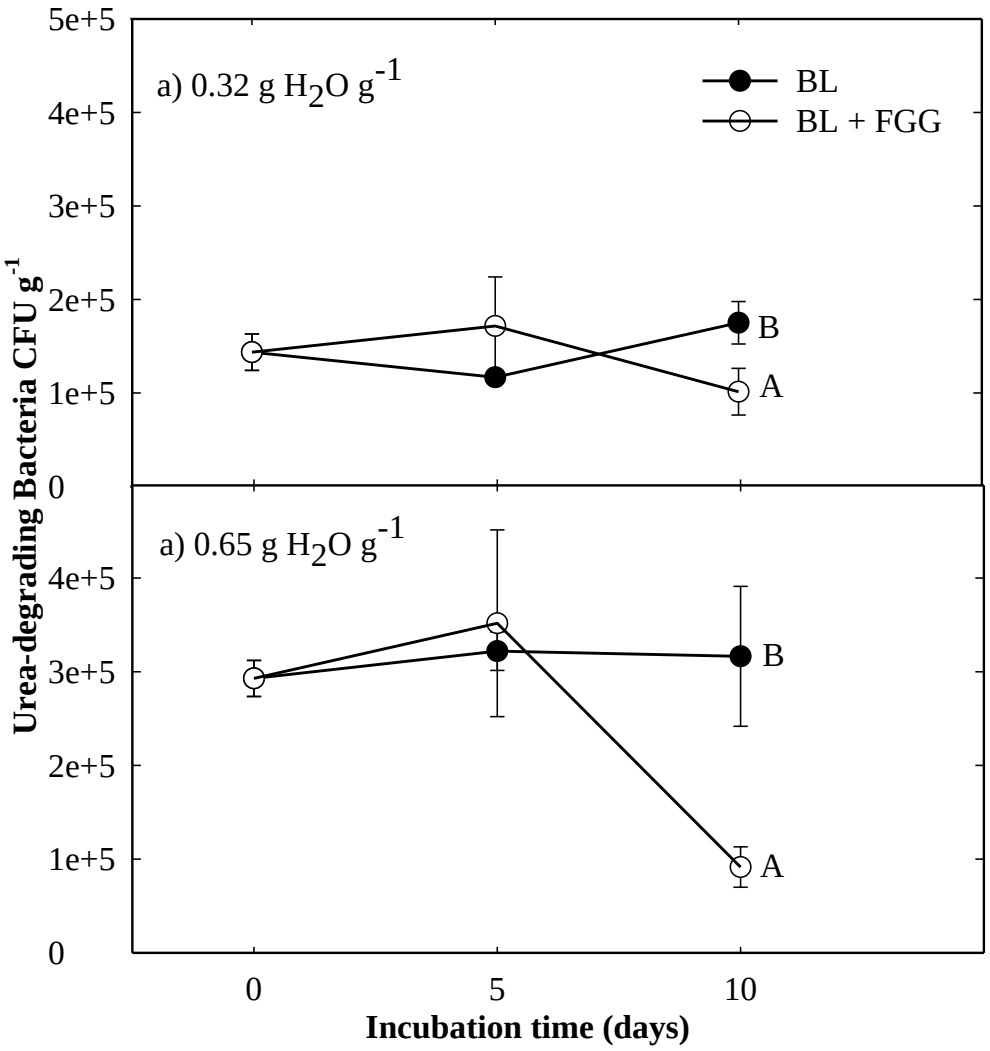

a) 0.32 g H2O g-1
BL
BL + FGG
B
A
a) 0.65 g H2O g-1
B
A
Broiler litter water content (g H2O g-1)
0.8
0.6
0.4
0.2
0
1
3
5
7
10
Incubation time (days)

Figure 3.3


a) 0.32 g H2O g-1
BL
BL + FGG
B
A
a) 0.65 g H2O g-1
B
A
Urea-degrading Bacteria CFU g-1
5e+5
4e+5
3e+5
2e+5
1e+5
0
0
5
10
Incubation time (days)

CHAPTER 4

EFFECT OF GYPSUM ON LITTER CHARACTERISTICS, NITROGEN MINERALIZATION, AND AMMONIA VOLATILIZATION FROM BROILER LITTER SURFACE-APPLIED TO SOIL

[1]Burt, C.D., M.L Cabrera, and J. Rema. To be submitted to the Soil Science Society of America Journal.

ABSTRACT

Ammonia (NH_3) volatilization from broiler litter decreases the fertilizer value of litter and negatively impacts the environment. Gypsum ($CaSO_4 \cdot 2H_2O$) has been proposed as a litter amendment to reduce NH_3 volatilization from broiler houses, but more information is needed to determine its effect on NH_3 volatilization when broiler litter is surface-applied to soil. We conducted two laboratory experiments to evaluate the effect of gypsum on urea-degrading bacteria, NH_3 volatilization, and N mineralization in broiler litter alone and in surface-applied broiler litter. In the first experiment, broiler litter amended with (0.2 g g^{-1}) or without reagent-grade gypsum or flue-gas desulfurization gypsum (FGG) was incubated alone or surface-applied to moist soil (0.13 g H_2O g^{-1}) for 15 days at 27°C. Adding gypsum to litter reduced ureolytic bacteria in litter alone, but increased ureolytic bacteria in litter surface applied to soil because of water adsorption from the soil. The adsorption of soil moisture also caused the dissolution of both types of gypsum which supressed an increase in litter pH by 0.84 to 1.09 pH units. In the second laboratory experiment, litter amended with (0.2 g g^{-1}) or without reagent-grade gypsum or FGG was surface-applied to dry soil (0.05 g H_2O g^{-1}) or moist soil (0.13 g H_2O g^{-1}) and incubated at 27°C for 14 days. Adding FGG to litter did not affect N mineralization, and decreased NH_3 volatilization by 27% only in litter applied to the surface of moist soil.

INTRODUCTION

The United States broiler industry produces 12 million Mg of broiler litter annually (Moore, 1998), and this byproduct is commonly surface-applied to soil as a disposal method and as a nutrient source for plants. The term broiler litter describes the mixture of bedding material, wasted feed, excretion, and feathers that covers the floor of broiler houses (Tasistro et al., 2004; Kelley et al., 1996). Broiler litter typically has a fertilizer grade of 3-3-2 (Nitrogen [N]-Phosphorus [P]-Potassium [K]) (Dunkley et al., 2011) and contains greater amounts of calcium (Ca), magnesium (Mg), and sulfur (S) than other types of animal waste (Azeez and Van Averbeke, 2010). In addition to supplying plant nutrients to soil, land application of broiler litter increases soil total carbon and cation exchange capacity (Adeli et al., 2010), improves soil structure (Adeli et al., 2010), and increases crop yield (McGrath et al., 2009; Tewolde et al., 2007; Sistani et al., 2004;). However, over application of litter can lead to leaching of N in subsurface drainage (Bitzer and Sims, 1988), contamination of surface water with P (Franklin et al., 2007), greenhouse gas emission (Sistani et al., 2011), and increased metal inputs (Gupta and Charles, 1999).

Litter application rates are generally calculated to meet crop N requirements, but determining application rates can be problematic due to N lost as volatile ammonia (NH_3) gas. Ammonia volatilization from broiler litter typically occurs following a microbially-mediated enzymatic reation in which organic-N in litter is decomposed to form ammoniacal-N. Uric acid and urea account for 80% of the total organic-N in broiler manure (Rothrock et al., 2010; Ritz et al., 2004), and urease activity is considered the limiting factor for N-mineralization and subsequent NH_3 volatilization (Nahm, 2003). Approximately 50-80 % of the N in broiler litter can be converted to NH_3 depending on the pH of the litter (Sims and Wolf, 1994; Ritz et al.,

2004), and it is estimated that on average 17% of all broiler litter NH_3 emissions occurs after land application (Moore et al., 2011). Several laboratory experiments have been conducted to study NH_3 volatilization from surface-applied litter, and losses in these studies ranged from 0.5% to 31% of the total N applied (Cassity-Duffey et al., 2015; Mishra et al., 2013; Doydora et al., 2011; Brinson et al., 1994; Cabrera and Chiang 1994). It has been reported that the majority of NH_3 volatilization occurs in the first 7 days after application, and can represent approximately 9 -21% of the total N applied (Brinson et al., 1994).

Ammonia volatilization from surface-applied broiler litter is a primary concern of farmers that use litter as an organic fertilizer. Ammonia volatilization decreases the fertilizer value of litter by reducing the amount of N available to plants, and negatively impacts the environment by contributing to atmospheric particulate matter formation, acid rain deposition, and soil acidity (Heald et al., 2012; Galloway et al., 2002; Ap Simon et al., 1987). Ammonia volatilization also decreases the N/P ratio in litter, and this can lead to excessive P in run-off water when litter is applied to meet crop N requirements. The decrease in N/P ratio in litter that results from NH_3 volatilization has led some researchers to suggest that litter application rates be based on P soil test levels to reduce P in run-off. However, lower litter application rates may cause farmers to supplement with commercial fertilizers to meet crop N requirements (Sharpley et al., 1993). Therefore, management strategies to reduce NH_3 volatilization from surface-applied litter are needed to maximize plant-available N and minimize environmental impacts.

Various litter amendments have been investigated to suppress NH_3 volatilization (Cook et al., 2011; DeLaune et al., 2003; Oliveira et al., 2003; Moore et al., 1995) and reduce the level of water-soluble P in litter (Tasistro and Kissel, 2006; Moore et al., 1995; Shreve et al., 1994). Gypsum ($CaSO_4 \cdot 2H_2O$) has been proposed as a litter amendment due to its ability to decrease

water-soluble P concentrations in run-off water (Endale et al., 2014; Desutter et al., 2011; Watts & Torbert 2009), but less is known about its ability to decrease NH_3 volatilization from broiler litter. Oliveira et al. (2003) amended broiler litter with 400 g kg^{-1} of agricultural gypsum, and observed a reduction in NH_3 volatilization after 42 days, compared to un-amended litter. The decrease in NH_3 volatilization from gypsum-amended litter observed by Oliveira et al. (2003) was attributed to the hygroscopic nature of gypsum and a decrease in litter pH. During a 49-day study, Sampaio et al. (1999) also observed a decrease in NH_3 volatilization from litter amended with various rates of gypsum plaster, compared to un-amended litter. Mishra et al. (2013) is the only known study that examined the effect of gypsum on NH_3 loss from surface-applied broiler litter. In this study, researchers applied broiler litter amended with and without flue-gas desulfurization gypsum (FGG) (0.25 g g^{-1}) to the surface of soil under laboratory conditions, and found that the addition of FGG to litter did not affect NH_3 volatilization. In contrast, Fenn et al. (1981) found that the addition of soluble Ca salts reduced NH_3 volatilization from acidic and calcareous soils that recieved surface-applied urea, and these results were attributed to $CaCO_3$ precipitation and soil pH depression.

Based on results from Mishra et al. (2013) and Fenn et al. (1981), additional studies are needed to better the effect of gypsum on physiochemical characteristics, abundance of urea-degrading bacteria, and N mineralization in surface-applied broiler litter. In order to evaluate the effect of gypsum on physiochemical characteristics and abundance of urea-degrading bacteria in broiler litter applied to the surface of soil, we conducted a 15-day incubation in which litter amended with and without different sources of gypsum (reagent-grade gypsum and FGG) was applied to the soil surface. We hypothesized that amending surface-applied broiler litter with different sources of gypsum would increase the water content of litter, and decrease litter pH and

the abundance of urea-degrading bacteria. A second experiment was conducted to evaluate the effect of soil moisture on N mineralization and NH_3 volatilization from surface-applied broiler litter amended with and without different sources of gypsum. We hypothesized that gypsum-amended litter would absorb more soil moisture from wet soil (0.13 g g^{-1}) than from dry soil (0.05 g g^{-1}) compared to un-amended litter. This increase in litter water content should lead to more NH_3 loss from litter applied to the surface of wet soil than from litter applied to the surface of moist soil.

METHODS AND MATERIALS

Broiler Litter and FGG for Experiments 1 and 2:

Broiler litter was collected from a broiler house in Georgia, passed through a 2-mm sieve, and analyzed for total C and N, pH, electrical conductivity (EC), water potential, and water content as described below (Table 4.1). Flue-gas gypsum was collected from a power plant in Illinois and analyzed for elemental composition using ICP-OES after acid digestion (USEPA, 1986) (Table 4.2). Water content of FGG was determined by drying at 105°C for 48 hours.

Broiler Litter Analyses

Total C and N in broiler litter was determined by dry combustion (Nelson and Sommers, 1982). pH was measured using an AB150 pH meter by Fisher Scientific in a 1:5 (litter/deionized water) ratio. EC was measured in a 1:3 (litter/dH_2O) ratio using a CDM80 Conductivity Meter (Radiometer America, Cleveland, OH). Water potential was measured using a WP4C Dewpoint Potentiameter (Decagon Devices, Inc., Pullman, WA). Water content of the broiler litter was determined by drying at 65°C for 48 hours.

Soil for Experiments 1 and 2:

Soil (Table 4.1) was collected from an area mapped as a Cecil sandy loam (fine, Kaolinitic, thermic Typic Kanhapludult) at the University of Georgia Plant Sciences farm located in Watkinsville, Georgia. There had been no N applied to the sampled area for more than five years. The collected soil was air-dried for 48 h, passed through a 2-mm sieve, and analyzed for particle size by the hydrometer method (Gee and Or, 2002). Total C and N of soil was determined by dry combustion (Nelson and Sommers, 1982). Inorganic N was determined by shaking 5 g soil with 40 mL 1 mol L^{-1}KCl for 40 min, and the extracts were analyzed following the colorimetric procedure described by Keeney and Nelson, (1982). Soil pH was measured in a 1:2 (soil/dH_2O) ratio. The water content of the air-dried soil was determined by drying three replicates of soil (10 g) at 105°C for 24 h. To achieve a soil water content comparable to that used in Mishra et al. (2013), soil was placed in Buchner funnels, and wetted with deionized water (dH_2O) to achieve a final water content of 0.13 g H_2O g^{-1}. After wetting, soil was allowed to equilibrate on the lab bench for 24 h. Water content of the soil was then confirmed by drying three soil samples at 105°C for 24 hours. Soil characteristics included 0.1 g clay g^{-1}, 0.2 g silt g^{-1}, and 0.7 g sand g^{-1}.

Experiment 1: The effect of reagent-grade gypsum and FGG on physiochemical characteristics and urea-degrading bacteria in surface-applied broiler litter

A 15-day incubation was conducted to evaluate the effect of gypsum on physiochemical characteristics and abundance of urea-degrading bacteria in broiler litter alone or broiler litter applied to the surface of soil. The treatments were as follows: litter only (L), litter + reagent gypsum (LG), litter + FGG (LF), soil only (S), soil + litter (S+L), soil + litter + reagent gypsum

(S+LG), and soil + litter + FGG (S+LF). Each treatment was replicated three times, and arranged in a completely randomized design. Experimental units consisted of an acrylic cylinder (4.4 cm i.d., 10 cm long), with its bottom end closed with a No. 10 stopper, placed inside a 0.95-L glass container with an acid trap containing 20 mL 0.1N H_2SO_4 for trapping NH_3. Treatments that included soil were prepared by gently packing 83 g of soil (0.13 g H_20 g^{-1}) into each cylinder to a depth of 3.0 cm to achieve a bulk density of 1.61 g cm^{-3}. Broiler litter (2.0 g at 0.63 g H_20 g^{-1}) with (0.4 g g^{-1}) or without reagent-grade gypsum or FGG was then placed on the soil surface for all treatments that included soil + litter. Treatments that did not include soil were prepared by placing litter at the bottom of the acrylic cylinders. All containers were then closed with screw-cap lids, and placed inside an incubator at 27°C for 15 d. Experimental units were aerated every three days. After 15 days, cylinders were removed from the glass containers, and the contents were separated and weighed. A subsample of 1 g of litter from each experimental unit was used to determine EC, pH, and the abundance of urea-degrading bacteria. Litter EC was measured in a 1:3 (litter/dH_2O) ratio using a CDM80 Conductivity Meter (Radiometer America, Cleveland, OH), and litter pH was measured in a 1:5 (litter/dH_2O) ratio using an AB150 pH meter (Fisher Scientific).

The population of urea-degrading bacteria in litter was determined using a protocol similar to that of Kim and Patterson (2003). One gram of broiler litter was diluted with 30 mL of dH_2O and shaken for 15 min. A 1-mL aliquot of poultry litter suspension was then pipetted into a dilution tube containing 9 mL of deionized water. A ten-fold dilution was repeated twice. Aliquots from each dilution tube were plated on Christensen urea agar following Miles and Mirsa (1938). After incubating the plates for 24 hours at 37°C, the colonies on each plate were

quantified and results reported as urea-degrading bacteria colony-forming units (CFU) per gram of dry litter.

Experiment 2: The effect of soil moisture on N mineralization and NH_3 volatilization from surface-applied broiler litter amended with and without different sources of gypsum

The second experiment was conducted to evaluate the effect of soil moisture (0.05 g H_2O g^{-1} vs 0.13 g H_2O g^{-1}) on N mineralization and NH_3 volatilization from surface-applied broiler litter amended with or without different sources of gypsum. The treatments were as follows: dry soil (DS)(control), dry soil + litter (DS+L), dry soil + litter + reagent gypsum (DS+LG), dry soil + litter + FGG (DS+LF), wet soil (WS, control), wet soil + litter (WS+L), wet soil + litter + reagent gypsum (WS+LG), and wet soil + litter + FGG (WS+LF). Each treatment was replicated four times, and replicates were arranged in a completely randomized design. Experimental units consisted of an acrylic cylinder (4.4 cm i.d., 10 cm long), with its bottom end closed with a No. 10 stopper, placed inside a 0.95-L glass container with an acid trap containing 33 mL 0.1N H_2SO_4 for trapping NH_3. Soil (73 g dry weight basis) was gently packed into each cylinder to a depth of 3.0 cm to achieve a bulk density of 1.60 g cm^{-3}. Broiler litter (2.0 g at 0.63 g H_20 g^{-1}) with (0.4 g g^{-1}) or without reagent-gypsum and FGG was then placed on the soil surface for all treatments. All containers were then closed with screw-cap lids, and placed inside an incubator at 27°C for 14 d. Experimental units were aerated every three days, and acid traps were weighed and changed during this time. After the H_2SO_4 traps were removed on day 14, 800 mL of 1 mol L^{-1} KCl was added to each experimental unit, and shaken for 45 minutes in a reciprocating shaker set at 120 oscillations per minute. Each extract was then filtered through a 0.45-μm filter, and extracts were analyzed for NH_4^+ and NO_3^- following the colorimetric procedure described by

Keeney and Nelson, (1982). Ammonia from the H_2SO_4 traps and NH_4^+ and NO_3^- from the KCl extracts were calculated on a μg g^{-1} of dry litter basis and summed to calculate total N recovery.

RESULTS AND DISCUSSION

Experiment 1: Effect of reagent-grade gypsum and FGG on physiochemical characteristics and urea-degrading bacteria in broiler litter alone and in surface-applied broiler litter.

The addition of reagent-grade gypsum or FGG (0.2 g g^{-1}) to litter only (no soil) decreased the water content of litter compared to un-amended litter. This decrease in water content (expressed on a litter weight basis) was due to a dilution of litter moisture by the addition of gypsum (Table 4.3). Conversely, the addition of FGG to litter increased the water content of broiler litterwhen litter was applied to the surface of moist soil. Our results are similar to results by Mishra et al. (2013) who observed an increase in litter water content when FGG-amended litter was applied to the surface of moist soil (0.13 g H_2O g^{-1}). These authors did not observe a decrease in NH_3 volatilization from litter amended with FGG compared to un-amended litter, and these results were attributed to the adsorption of soil moisture by FGG, which eliminated the litter-drying effect of gypsum. The litter drying effect of gypsum has been proposed as a mechanism to reduce the activity of bacteria associated with the production of ammoniacal N. Our results showed that amending broiler litter with reagent-grade gypsum and FGG decreases the abundance of urea-degrading bacteria when litter is not in contact with moist soil (Table 4.3). However, surface-applying broiler litter amended with different sources of gypsum to the surface of moist soil actually increased the abundance of urea-degrading bacteria in litter due to the adsorption of soil moisture by gypsum (Table 4.3). The decrease in pH for gypsum-amended litter observed in our study agrees with results of Fenn et al. (1981) who observed that the

addition of soluble Ca salts reduced NH_3 volatilization from acidic and calcareous soils that received surface-applied urea. Thse authors attributed the decrease in pH to the precipitation of $CaCO_3$ which buffers against pH increases caused by urea hydrolysis, as shown below:

$$CO(NH_2)_2 + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO_3^- \quad \text{Eq. [1]}$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \quad \text{Eq. [2]}$$

$$Ca^{+2} + CO_3^{2-} \rightarrow CaCO_3 \quad \text{Eq. [3]}$$

The suppression of pH resulting from the precipitation of $CaCO_3$ in gypsum-amended litter is dependent on the amount of Ca ions in solution. It has been demonstrated that analytical grade-gypsum is more soluble than FGG due to greater surface area and less $CaCO_3$ impurity (Bolan et al., 1991), and these characteristics explain why the addition of reagent-grade gypsum to broiler litter led to greater litter EC and a greater decrease in litter pH, compared to litter amended with FGG (Table 4.3).

Experiment 2: Effect of soil moisture on N mineralization and NH_3 volatilization from surface-applied broiler litter amended with and without different sources of gypsum

Cumulative NH_3 loss and amount of mineralized-N recovered from surface-applied broiler litter amended with orwithout different sources of gypsum was not affected by soil water content (0.05 g H_2O g^{-1} vs 0.13 g H_2O g^{-1}) after 14 d (Table 4.5). Sistani et al. (2011) also observed no difference in N mineralization when litter was applied to soil with different moisture regime. However, soil water content did have an effect on the form of N recovered. Applying broiler litter amended with orwithout different sources of gypsum to the surface of dry soil (0.05 g H_2O g^{-1}) resulted in a greater concentration of NH_4^+-N in litter (Table 4.4), compared to litter applied to the surface of wet soil (0.13 g H_2O g^{-1}). In contrast, applying litter amended with

orwithout different sources of gypsum to the surface of wet soil (-0.08 MPa [0.8 bar]) resulted in a greater concentration of NO_3^--N in litter (Table 4.4), compared to litter applied to the surface of dry soil (-109 MPa [1090 bar]). Our results agree with results by Miller and Johnson (1964) who observed that increasing the water content of soil increased nitirification. These authors found that the maximum rate of nitrification occurred in the range of 0.5-0.15 bar of tension, and soil tensions >15 bars signicantly decreased nitrification.

Based on results by Mishra et al. (2013), we initially hypothesized that gypsum-amended litter would absorb more soil moisture from wet soil (0.13 g g^{-1}) than from dry soil (0.05 g g^{-1}) compared to un-amended litter. This increase in litter water content would negate the litter drying effect of FGG that has been proposed as a mechansism for reducing NH_3 loss from broiler litter. In our study, the addition of reagent-grade gypsum and FGG to broiler litter did not decrease NH_3 volatilization from litter applied to the surface of dry soil compared to un-amended litter (Table 4.4, Figure 4.1 A). However, the addition of FGG to broiler litter did decrease NH_3 volatilization from litter applied to the surface of moist soil, compared to un-amended litter and litter amended with reagent-grade gypsum (Table 4.4, Figure 4.1 A). Although the difference in mineralized-N recovered from FGG-amended litter was not statistically different from than that of litter amended with reagent-grade gypsum, this slight difference in N mineralization may explain why there was more cumulative NH_3 volatiliztion from litter amended with reagent-grade gypsum, compared to litter amended with FGG. Our results from experiment two do not agree with results of Mishra et al. (2013) who observed that the addition of FGG to broiler did not have an effect on NH_3 volatilization from litter applied to the surface of moist soil (0.13 g g^{-1}). The authors attributed these results to the adsorption of soil moisture by FGG, which eliminated the litter-drying effect of gypsum. Our results suggest that water absorbed by litter

from moist soil caused a greater dissolution of FGG compared to FGG-amended litter applied to the surface of dry soil, and this decreased NH_3 volatilization due to a decrease in litter pH. This hypothesis agrees with results by Fenn et al. (1981) who observed that the addition of soluble Ca salts reduced NH_3 volatilization from acidic and calcareous soils that received surface-applied urea. The decrease in NH_3 volatilization was attributed to the precipitation of $CaCO_3$ which buffered against an increase in pH.

CONCLUSIONS

Gypsum has been proposed as a litter amendment due to its ability to decrease water-soluble P concentrations in run-off water but less is known about its ability to decrease NH_3 volatilization from surface-applied broiler litter. Results from Exp. 1 suggest that FGG-amended litter absorbs more water from moist soil than un-amended litter. This increase in litter water content increased the abundance of urea-degrading bacteria. The absorption of soil moisture also led to the dissolution of FGG which supressed an increase in pH likely due to the precipitation of $CaCO_3$. Results from Exp. 2 suggest that soil water content does not have an effect on NH_3 volatilization or the amount of mineralized-N recovered from surface-applied broiler litter. . The addition of FGG to broiler litter reduced NH_3 volatilization from litter applied to the surface of moist soil but did not have an effect on NH_3 volatilization when litter was applied to dry soil. Overall, results from both experiments indicate that the addition of FGG to broiler litter can reduce NH_3 volatilization when litter is applied to the surface of moist soil.

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TABLES AND FIGURES

Table 4.1: Initial physiochemical characteristics of broiler litter used for Exp. 1 and 2.

Variable	**Unit**	**Litter**	**Soil**
Moisture Content	g H_2O g^{-1}	0.62	0.05
Water Potential	MPa	-9.2	-108.9
pH		8.6	5.33
EC	µS cm^{-1}	26.4	---
Total C	mg kg^{-1}	177,000	8,200
Total N	mg kg^{-1}	24,500	600
C/N Ratio		7.2	13.7

Table 4.2: Elemental composition of Flue-gas desulfurization gypsum (FGG) used in Exp. 1 and 2.

Element	Al	Ca	Fe	Mg	P	K	Si	S	Na
	----------mg kg^{-1}----------								
	1,171	138,463	1,626	1,224	71	334	434	84,627	100

Table 4.3: Physiochemical characteristics and abundance of urea-degrading bacteria in broiler litter amended with (0.2 g g^{-1}) or without different sources of gypsum after incubation at 27°C for 15 d. Gypsum addition was based on the litter wet weight (Experiment 1). Values represent the mean of three replicates.

Treatment	**Water content (g H_2O g^{-1})**	**pH**	**EC (ms/cm)**	**UDB log CFU**
Litter treatments				
L	0.58 B†	8.08 C	13.7 A	5.5 B
LG	0.50 A	7.57 A	14.9 B	0 A
LF	0.51 A	7.81 B	14.4 B	0 A
Litter + soil treatments				
S+L	0.65 A	8.92 C	2.5 A	7.0 A
S+LG	0.70 A	7.83 A	3.8 C	7.6 B
S+LF	0.87 B	8.08 B	3.0 B	7.6 B

L = litter; LG = litter + gypsum; LF = litter + FGG; S+L = soil + litter; S+LG = soil + LG; S+LF = soil + LF

†Within a column for *Litter treatments* or for *Litter + soil treatments*, means followed by the same letter are not significantly different according to Fisher's LSD at $p<0.05$.

Table 4.4: Nitrogen recovered from surface-applied broiler litter amended with (0.2 g g^{-1}) or without different sources of gypsum after incubation at 27°C for 14 d (Experiment 2). Values represent the mean of four replicates.

Treatment	NH_3-N loss	NH_4-N Litter	NO_3-N Litter	Total N recovered	
	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	% Total N‡
Dry Soil Treatments					
DS+L	865 Aa†	7112 Ab	3537 Aa	11515 Aa	47.0 Aa
DS+LG	925 Aa	7632 Ab	3391 Aa	11948 Aa	48.8 Aa
DS+LF	820 Aa	7047 Ab	3196 Aa	11063 Aa	45.2 Aa
Wet Soil Treatments					
WS+L	1126 Ba	3022 Aa	7064 Ab	11212 Aa	45.8 Aa
WS+LG	1136 Ba	3510 Aa	6836 Ab	11482 Aa	46.9 Aa
WS+LF	824 Aa	3087 Aa	6852 Ab	10763 Aa	43.9 Aa

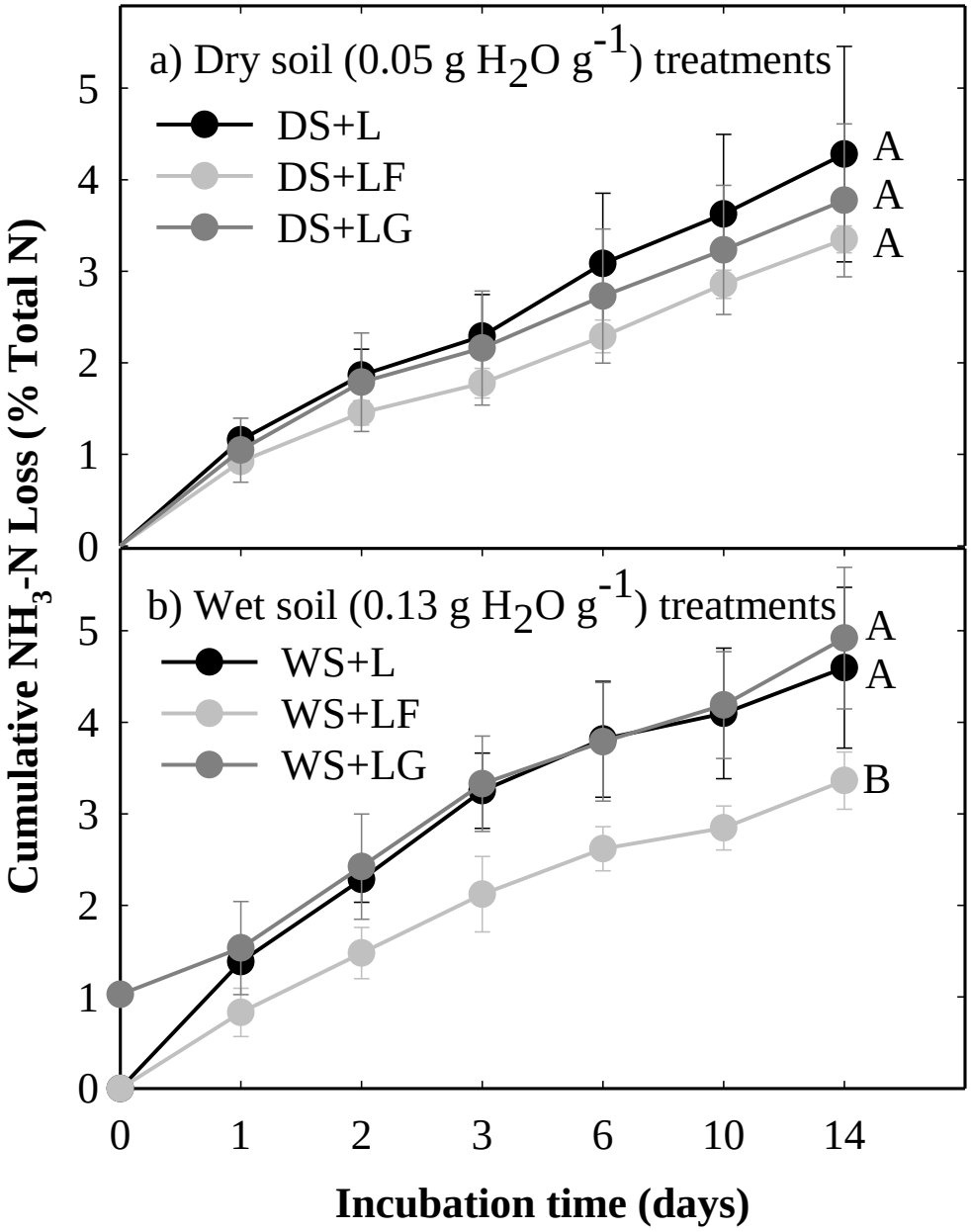
DS+ L = dry soil + litter; DS + LG = dry soil + gypsum-amended litter; DS+LF = dry soil + FGG-amended litter; WS+L = wet soil + litter; WS+LG = wet soil + gypsum-amended litter; WS+LF = wet soil + FGG-amended litter

†Within a column for Dry Soil Treatments or Wet Soil Treatments, means with the same upper case letter are not significantly different according to Fisher's LSD at $p<0.05$. Lower case letters are for comparisons of means between the same treatment in wet or dry soil (DS+L vs WS+L; DS+LG vs WS+LG; DS+LF vs WS+LF); different letters indicate a significant difference according to Fisher's LSD at $p<0.05$.

‡ %Total N recovered = (NH_3–N loss + NH_4^+-N + NO_3–N) / Total N

Figure 4.1: Cumulative NH_3-N loss from broiler litter amended with and without different sources of gypsum incubated on the surface of dry or wet soil incubated at 27°C for 14 d. (a) Cumulative NH_3-N loss from broiler litter amended with (0.2 g g^{-1}) or without different sources of gypsum incubated on the surface of dry soil at 27°C for 14 d (Experiment 2). (b) cumulative NH_3-N loss from broiler litter amended with (0.2 g g^{-1}) or without different sources of gypsum incubated on the surface of wet soil at 27°C for 14 d. All symbols represent the mean of four replicates, and error bars represent SD.

Figure 4.1.


a) Dry soil (0.05 g H_2O g^{-1}) treatments
DS+L
DS+LF
DS+LG
A
A
A
b) Wet soil (0.13 g H_2O g^{-1}) treatments
WS+L
WS+LF
WS+LG
A
A
B
Cumulative NH_3-N Loss (% Total N)
0
1
2
3
4
5
0
1
2
3
6
10
14
Incubation time (days)

CONCLUSION

The United States poultry accounts for 28% of the national livestock ammonia (NH_3) emission. Specifically, in the southeastern US, broiler production is the largest segment of the poultry industry, and it releases 33 to 43% of the agricultural NH_3 emissions. Broilers are classified as chickens younger than 13 weeks that are raised specifically for meat. The floor of the broiler production facilities is covered with a mixture of bedding material and bird excreta that is rich in organic and inorganic nitrogen (N). Nitrogen in broiler litter is subject to NH_3 volatilization and represents a major concern for producers because NH_3 is considered the most harmful gas in broiler facilities (Carlile 1984). NH_3 volatilization also decreases the fertilizer value of litter and negatively impacts the environment. Litter amendments such as acidifiers, chemical absorbents, and chemical/biological inhibitors have become the most widely accepted method for reducing NH_3 volatilization in broiler facilities.

Gypsum has been proposed as a litter amendment due to its hygroscopic nature, but reports of NH_3 abatement vary, and the mechanism responsible for NH_3 reduction is not well understood. Results from Chapter 2 showed that the addition of flue-gas desulfurization gypsum (FGG) to broiler litter decreased the availability of water for ureolytic activity and increased salt concentrations in solution. These conditions were detrimental to the survival of urease-producing bacteria, and led to increased N mineralization due to an increase in urease activity. Amending litter with FGG suppressed litter pH, and this decrease in pH reduced the amount of NH_3 volatilization and increased the concentration of ammonium (NH_4^+) in litter. A series of experiments was conducted to better understand the mechanism responsible for pH suppression. The addition of gypsum to litter decreased pH immediately due to the precipitation of calcium carbonate ($CaCO_3$) from gypsum-derived calcium and litter bicarbonate. Furthermore, as urea

was hydrolyzed in gypsum-amended litter, additional $CaCO_3$ precipitated and buffered against large increases in pH that accompany urea hydrolysis. The addition of 20% or 40% FGG to broiler litter resulted in similar amounts of urea-degrading bacteria, urea, cumulative NH_3 loss, and nitrogen mineralization in 21 days.

Results from Chapter 3 confirm that FGG absorbs moisture from litter particles, and this drying effect reduced the abundance of urea-degrading bacteria after 10 days compared to litter incubated without FGG. However, the drying effect alone did not significantly affect NH_3 loss or inorganic N concentrations in litter incubated in limited contact with FGG, compared to litter incubated without FGG. Based on these results, it seems likely that the significant reduction in NH_3 volatilization from gypsum-amended litter compared to un-amended litter observed in other studies resulted from a decrease in pH that occurs when gypsum is intimately mixed with broiler litter.

Results from Chapter 4 showed that FGG-amended litter absorbs more water from moist soil than un-amended litter. This increase in litter water content increased the abundance of urea-degrading bacteria. The absorption of soil moisture also led to the dissolution of FGG which supressed an increase in pH likely due to the precipitation of $CaCO_3$. A second experiment conducted to evaluate the effect of soil moisture on N mineralization and NH_3 volatilization from surface-applied broiler litter amended with orwithout different sources of gypsum. We hypothesized that gypsum-amended litter would absorb more soil moisture from wet soil (0.13 g g^{-1}) than from dry soil (0.05 g g^{-1}), and this increase in litter water content would negate the litter drying effect of FGG that has been proposed as a mechansism for reducing NH_3 loss from broiler litter. Our results showed that soil water content did not have an effect on NH_3 volatilization or the amount of mineralized-N recovered from surface-applied broiler litter. .

However, the addition of FGG to broiler litter reduced NH_3 volatilization from litter applied to the surface of moist soil but did not have an effect on NH_3 volatilization when litter was applied to dry soil.

The results presented here show that gypsum absorbs moisture from litter, thereby decreasing the availability of water for urea-degrading bacteria. The decrease in water availability induces osmotic stress on urea-degrading bacteria, and leads to an increase in N mineralization. The addition of gypsum to broiler litter also causes an immediate decrease in litter pH due to the precipitation of $CaCO_3$ from gypsum-derived calcium and litter bicarbonate. As urea is hydrolyzed in gypsum-amended litter, additional $CaCO_3$ precipitates and buffers against large increases in pH that accompanies urea hydrolysis. The decrease in litter pH that is observed in gypsum-amended litter reduces NH_3 volatilization compared to un-amended litter. Furthermore, the addition of FGG reduced NH_3 volatilization from litter applied to the surface of moist soil but did not have an effect on NH_3 volatilization when litter was applied to dry soil. These results suggest that water absorbed by FGG-amended litter from moist soil causes a greater dissolution of FGG compared to FGG-amended litter applied to the surface of dry soil. The greater dissolution of FGG suppresses large increases in litter pH that are known to facilitate NH_3 losses.