GYPSUM EFFECTS ON BROILER LITTER

by

CHRISTOPHER DANIEL BURT

(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

by

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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.

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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

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.

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 $p = 0.05$.

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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

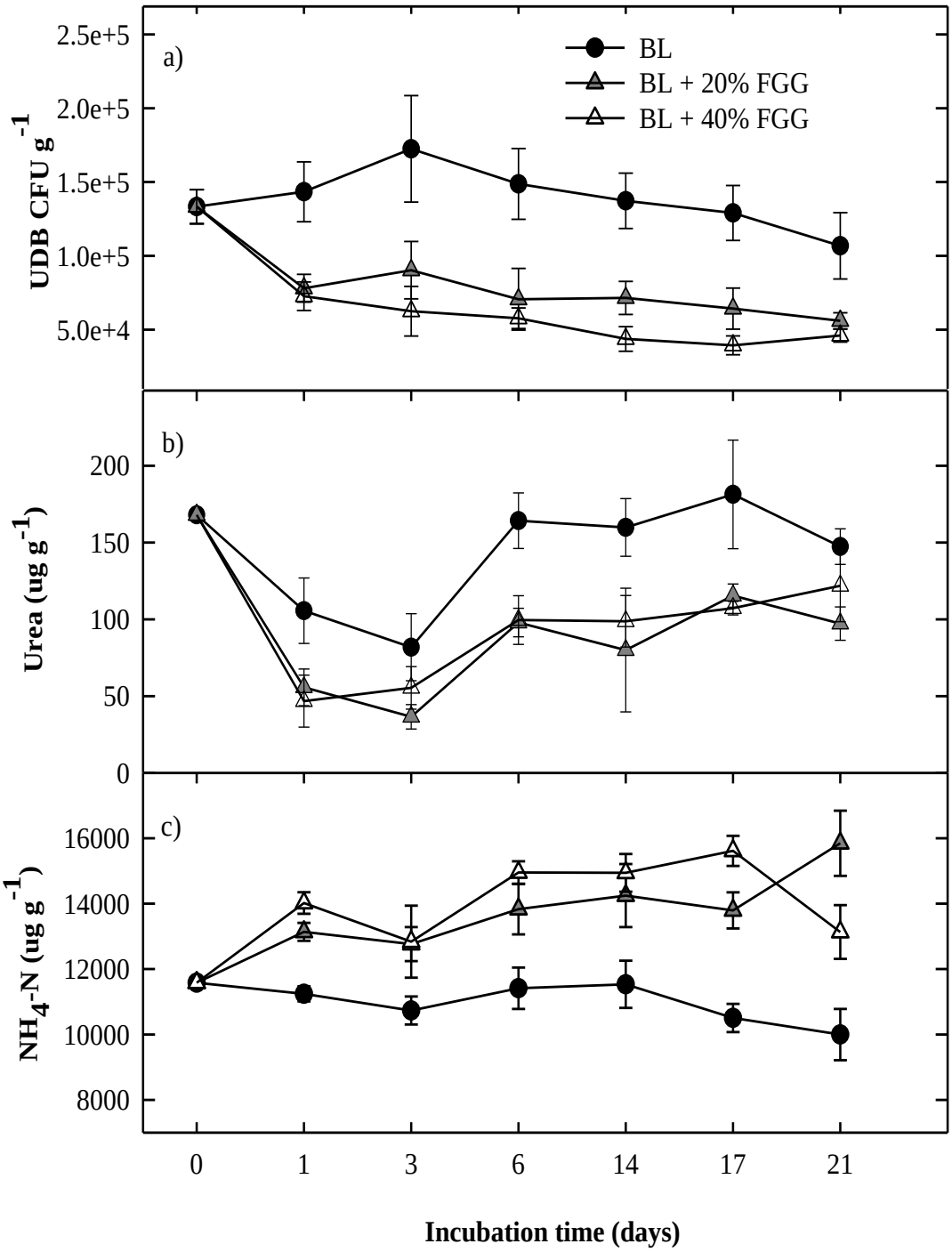

a)
b)
c)
BL
BL + 20% FGG
BL + 40% FGG
UDB CFU g -1
Urea (ug g -1)
NH4-N (ug g -1)
2.5e+5
2.0e+5
1.5e+5
1.0e+5
5.0e+4
200
150
100
50
0
16000
14000
12000
10000
8000
0
1
3
6
14
17
21
Incubation time (days)

Figure 2.2

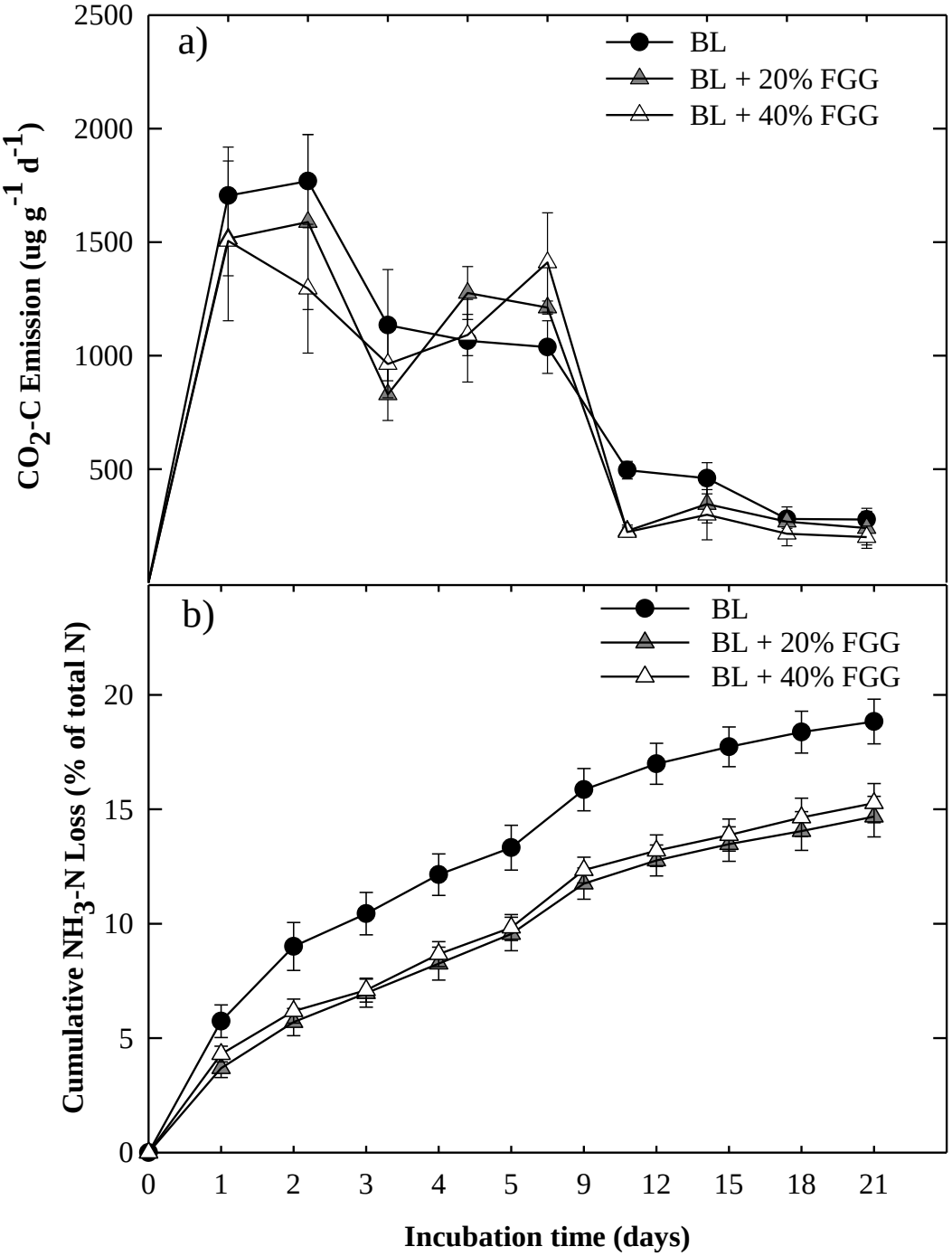

a)
BL
BL + 20% FGG
BL + 40% FGG
CO_2-C Emission (ug g^{-1} d^{-1})
2500
2000
1500
1000
500
b)
BL
BL + 20% FGG
BL + 40% FGG
Cumulative NH_3-N Loss (% of total N)
20
15
10
5
0
0
1
2
3
4
5
9
12
15
18
21
Incubation time (days)

Figure 2.3

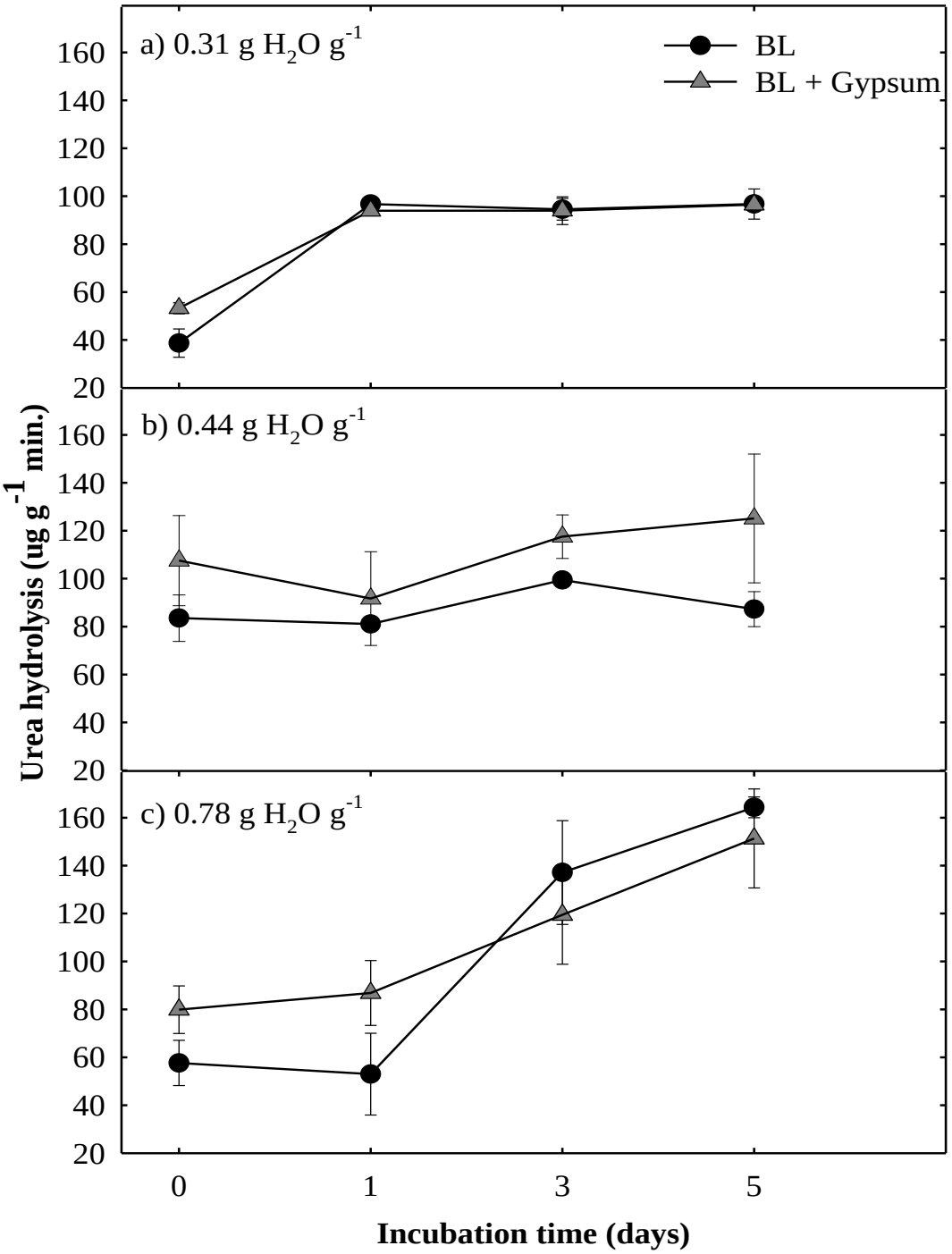

a) 0.31 g H2O g-1
b) 0.44 g H2O g-1
c) 0.78 g H2O g-1
BL
BL + Gypsum
Urea hydrolysis (ug g-1 min.)
Incubation time (days)
0
1
3
5
20
40
60
80
100
120
140
160

Figure 2.4

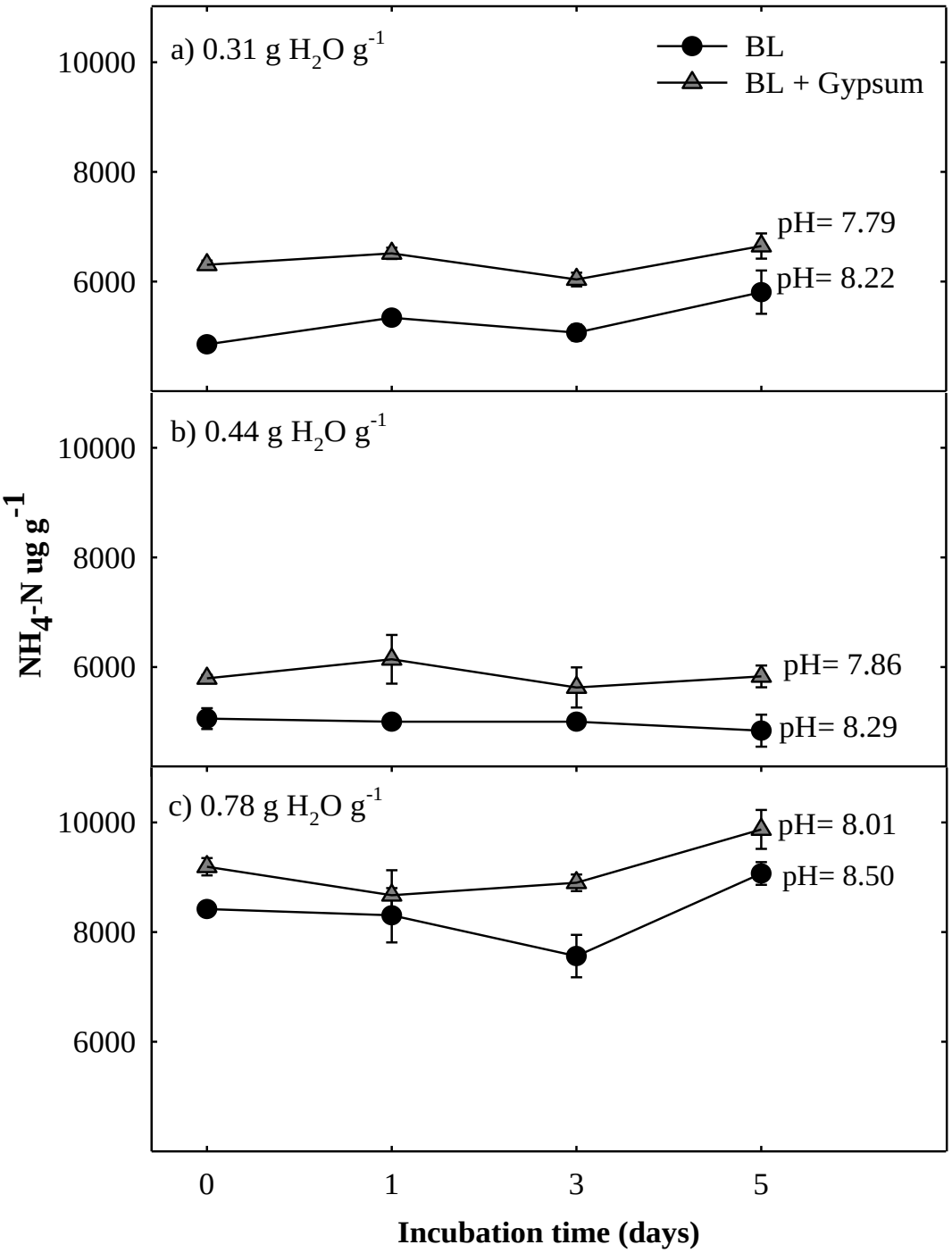
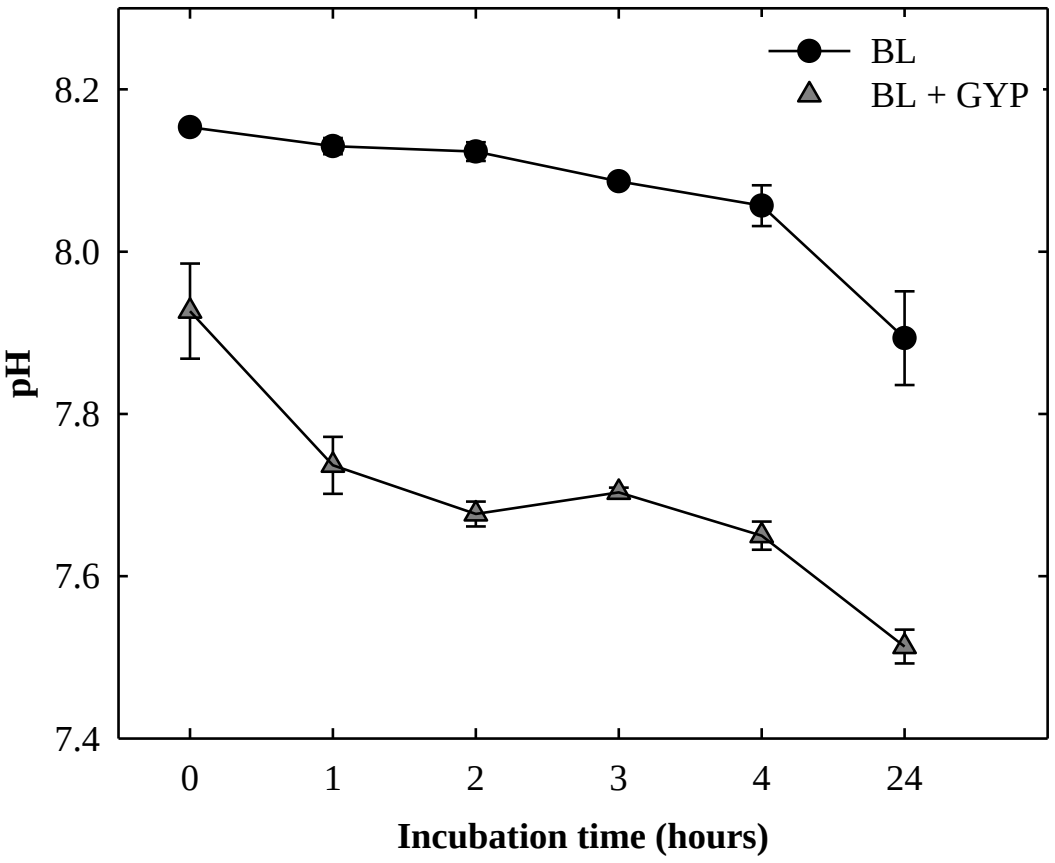

a) 0.31 g H2O g-1
BL
BL + Gypsum
pH= 7.79
pH= 8.22
b) 0.44 g H2O g-1
pH= 7.86
pH= 8.29
c) 0.78 g H2O g-1
pH= 8.01
pH= 8.50
10000
8000
6000
NH4-N ug g-1
0
1
3
5
Incubation time (days)

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

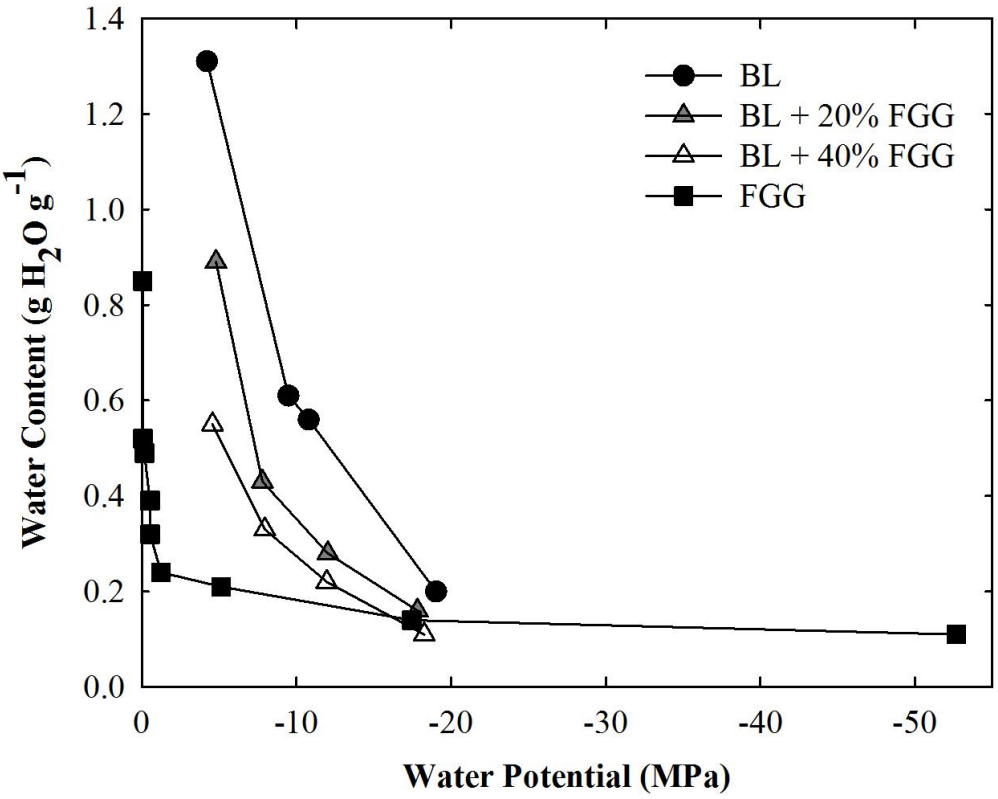

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Water Content (g H_2O g^{-1})
0
-10
-20
-30
-40
-50
Water Potential (MPa)
BL
BL + 20% FGG
BL + 40% FGG
FGG

Figure 3.2

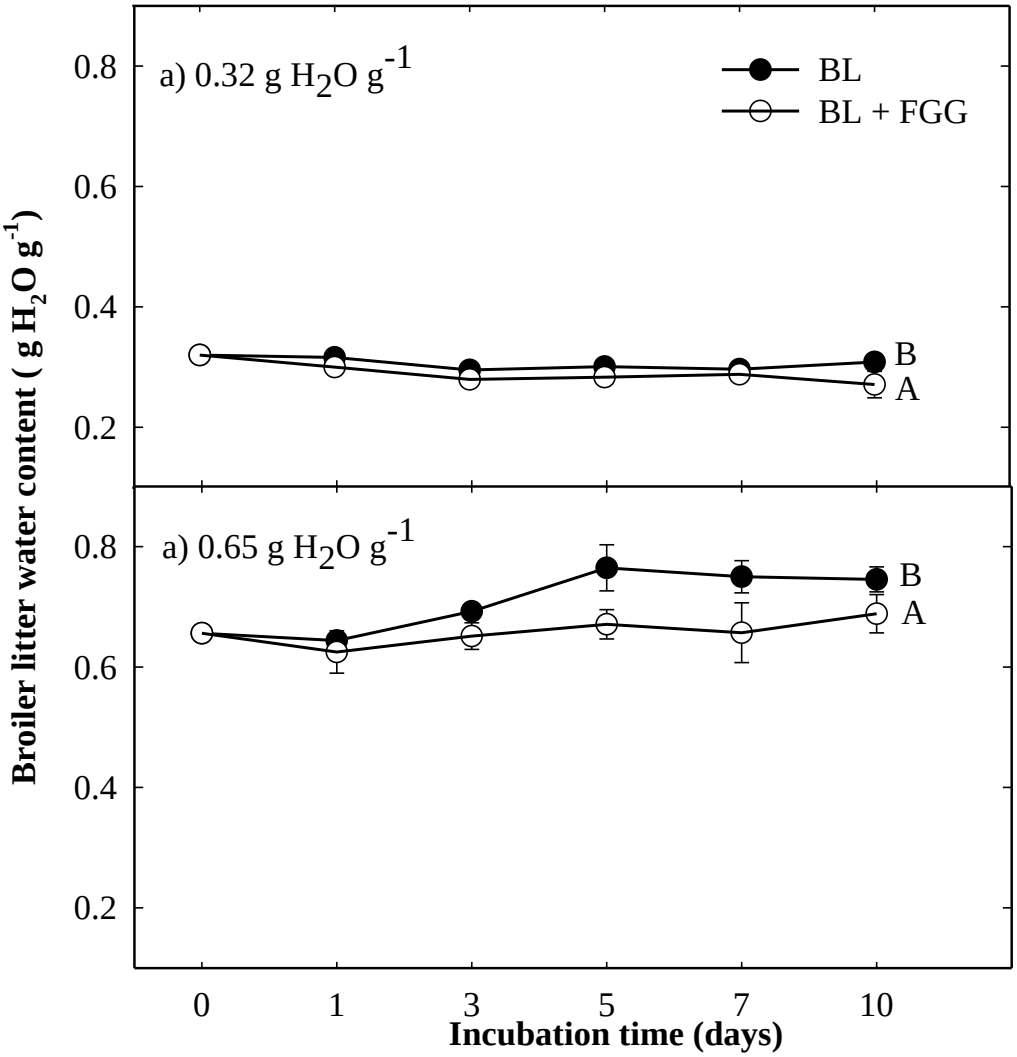
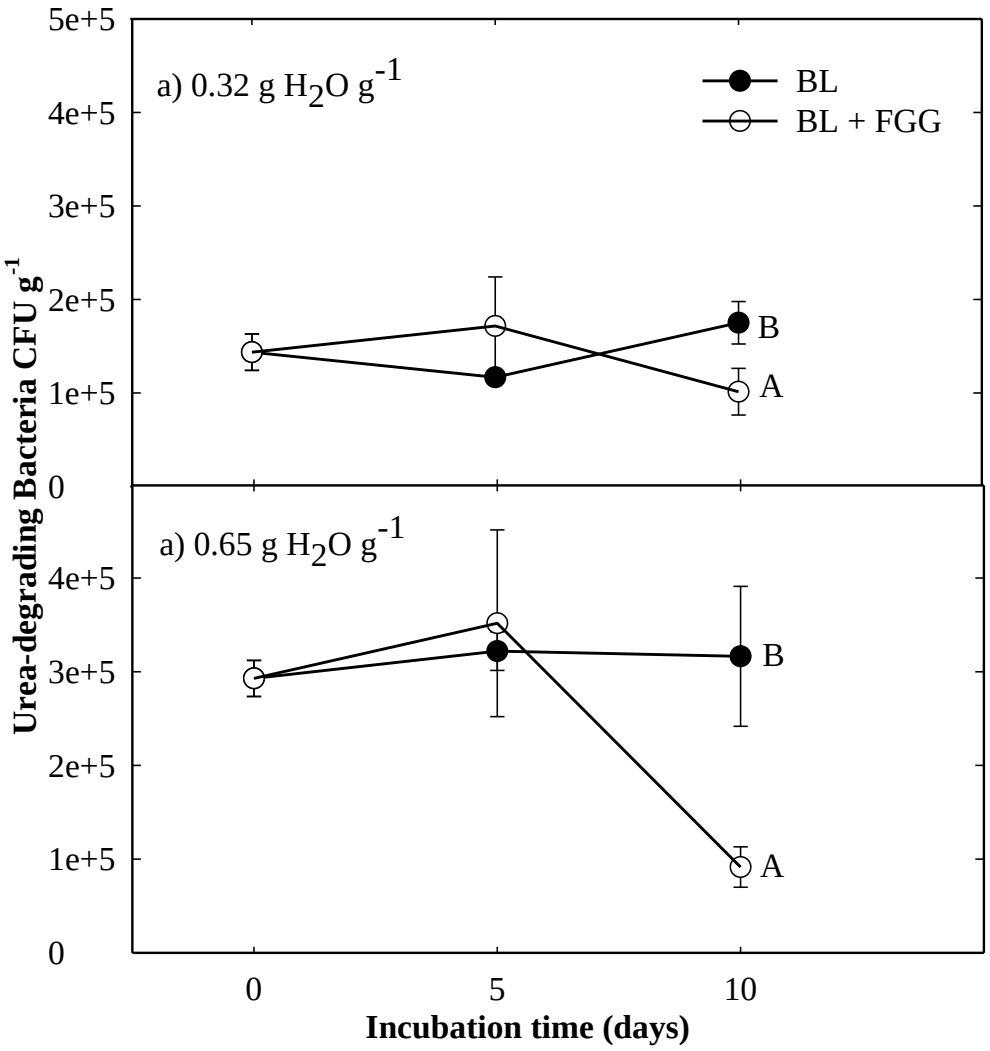

a) 0.32 g H2O g-1
BL
BL + FGG
B
A
a) 0.65 g H2O g-1
B
A
Broiler litter water content (g H2O g-1)
0.8
0.6
0.4
0.2
0
1
3
5
7
10
Incubation time (days)

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

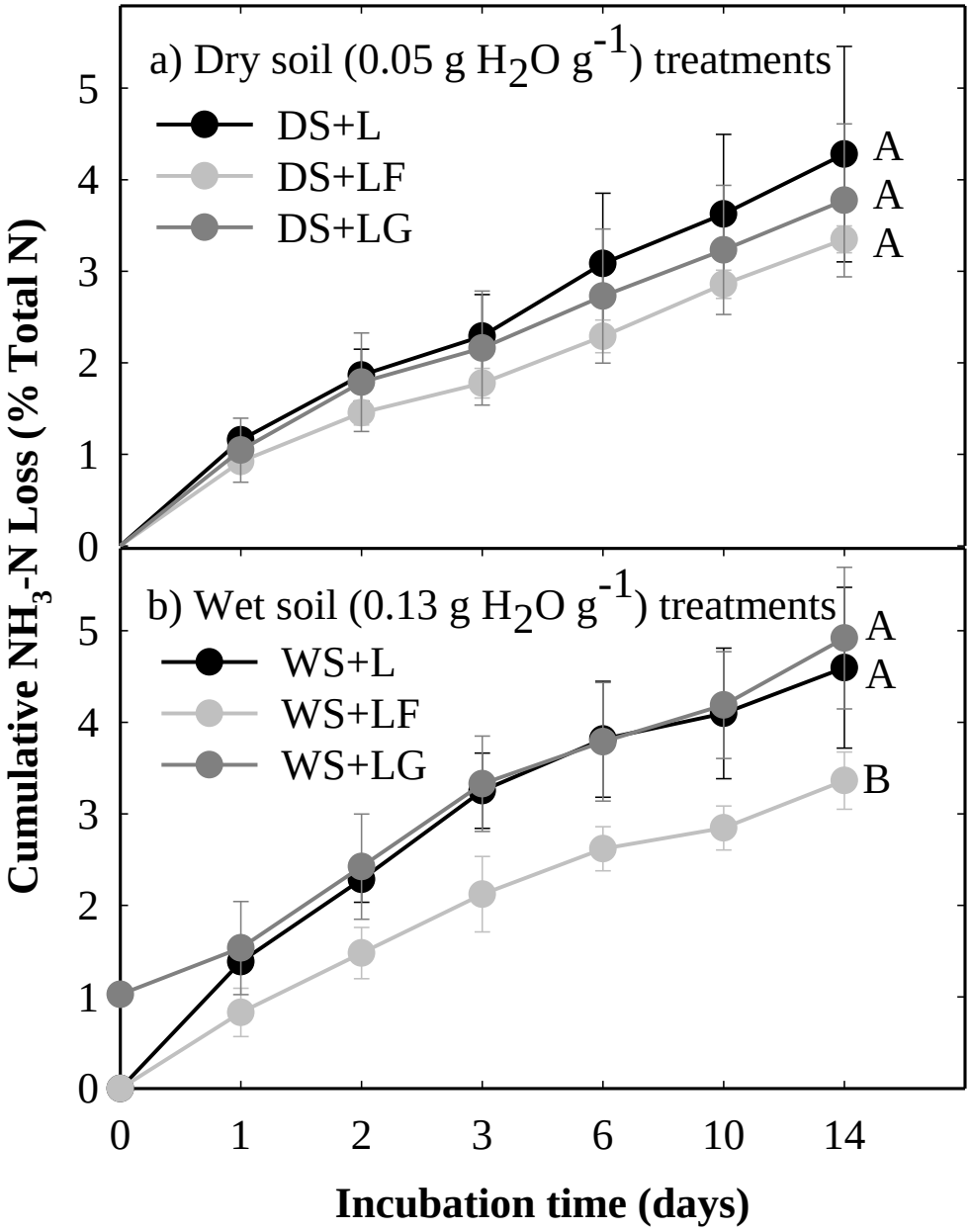
DS+ L = dry soil + litter; DS + LG = dry soil + gypsum-amended litter; DS+LF = dry soil + FGG-amended litter; WS+L = wet soil + litter; WS+LG = wet soil + gypsum-amended litter; WS+LF = wet soil + FGG-amended litter

†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.