Reducing Ammonia Volatilization and *Escherichia coli* from Broiler Litter Using Multiple Applications of Sodium Bisulfate

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ABSTRACT

Ammonia (NH_3) emissions from animal manure, such as poultry litter, can cause air quality problems. These emissions also result in excessive nitrogen (N) loading into aquatic environments which can lead to water quality problems where N is the limiting nutrient for eutrophication, such as the Chesapeake Bay. Poultry litter treatment (PLT, sodium bisulfate) is an acidic amendment that is applied to litter in poultry houses to decrease NH_3 emissions, but currently it can only be applied before birds are placed in the houses. This project analyzed the effect of multiple PLT applications on litter properties and NH_3 release in a controlled and field environment. Volatility chambers with acid traps were used to compare multiple, single, and no applications of PLT to poultry litter. Both single and reapplied PLT caused a greater moisture content and lower pH in litter. Additionally, the *E.coli* in litter was decreased significantly with both single and reapplied PLT. After 15 days, NH_3 released from litter treated with reapplied PLT was significantly less than litter with both single and no applications. Furthermore, NH_4^+-N content of litter was greatest in litter treated with reapplied PLT increasing its fertilizer value. The efficacy of a new farm scale system capable of applying several additions of PLT to poultry litter throughout the growth of a flock was also evaluated. Though litter pH, *E.coli*, and NH_3 volatilization were temporarily decreased with PLT application, the overhead reapplication auger system is not recommended at this time due to moisture and corrosion problems.

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CHAPTER 1: INTRODUCTION

Volatilization from intensive poultry and livestock production farms is the largest contributor to atmospheric ammonia (NH_3) because of the significant amounts of animal waste produced (Campbell-Nelson, 2009; Pinder et al., 2007; Walker et al., 2000). Elevated concentrations of NH_3 have a negative impact on the health of farmers and animals, particularly in confined poultry facilities where concentrations can exceed the EPA-recommended maximum of 25 mg/L (ppm) (Aneja et al., 2008; Charles and Payne, 1966; Homidan et al., 2003; Miles et al., 2004; Reece et al., 1981). The release of NH_3 into the atmosphere from animal operations may also have negative environmental impacts on processes outside farming operations, such as eutrophication and soil acidification (Aneja et al., 2008; Campbell-Nelson, 2009; Paerl and Fogel, 1994; Williams et al., 1999). In addition, NH_3 can raise the NO_x presence thereby lowering the pH of water and contributing to acid rain production (Delaune et al., 2004).

Identifying solutions for decreasing NH_3 levels in broiler houses continues to be a priority for safety, health, and production efficiency. Broiler facility temperature, humidity, ventilation, and air quality can be manipulated to maximize production while minimizing the known impacts of concentrated NH_3. Adjustments to broiler bedding composition and litter amendment application can both reduce NH_3 emissions and increase fertilizer potential with retention of nitrogen (N) in the litter (Miles et al., 2011). Several litter amendments are available commercially to increase the retention of N in litter as ammonium (NH_4^+), which is non-volatile, thereby decreasing NH_3 emissions. This litter can then serve as a source of N and other nutrients for crops.

For this study, an acid amendment reapplication system was evaluated for NH_3 emission reduction on a large-scale broiler operation in the Shenandoah Valley. Reapplication is defined as applying chemical amendment prior to each flock and twice mid-flock. Reapplication-amended litter was subsequently evaluated for *Escherichia coli* levels. Volatility chambers were used for litter amendment comparison in a controlled environment.

OBJECTIVES

The overall objective of this study was to assess the suitability of an acidic reapplication system for the mitigation of NH_3 emissions from broiler houses. The individual study objectives were:

1. To demonstrate, quantify, and compare a reapplication technology that reduce NH_3 emissions from poultry litter.
2. Evaluate and quantify a PLT re-application delivery system in whole house trials to decrease ammonia release and *E.coli* presence in litter
3. Evaluate and quantify PLT reapplication effects on ammonia release, litter properties, and *E.coli* presence in litter using temperature controlled cabinets

CHAPTER 2: LITERATURE REVIEW

Nitrogen and Ammonia Chemistry

Nitrogen (N) is a non-metal element essential to life that comprises 78% of the earth's atmosphere. It is a colorless, odorless, and tasteless gas that occurs naturally as N_2. The N cycle describes the biogeochemistry of N chemical forms, which allows it to be utilized in physical and chemical processes to support life.

Various chemical forms of N can be found in the growth, development, and decomposition of an organism's life cycle in both terrestrial and aquatic environments. These include ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), nitric oxide (NO), and inorganic N gas (N_2) (Vitousek et al., 1997). The phases of the N cycle that produce these chemical forms are fixation, ammonification, assimilation, nitrification, and denitrification.

During fixation, the N_2 molecule is split and recombines with hydrogen to form ammonia gas (NH_3) or with oxygen to produce nitrate (NO_3^-) or nitrite (NO_2^-). The N_2 molecule can be split by biological or industrial means, by lightning, or by fossil fuel combustion. Biological N fixation (Reaction 3.1) relies on the enzyme nitrogenase produced by anaerobic bacteria and accounts for 67% of all NH_3 production (Harvey and Havelka, 1975).

$$N_2 + 8\,H^+ + 8\,e^- \rightarrow 2\,NH_3 + H_2 \qquad \text{(Reaction 3.1)}$$

Industrial N fixation (Haber-Bosch process) accounts for another 30% of NH_3 production (Smith et al., 2004). Industrial fixation, lightning strikes, and fossil fuel combustion can result in nitrogen oxide production (Chapin et al., 2002). The production of this N-containing greenhouse gas has 298 times more impact per unit weight and greater global warming potential than that of carbon dioxide (CO_2), according to the EPA (EPA-2, 2010). Nitrogen oxides and NH_3 can also be byproducts of fertilizer storage, manufacturing and use, biomass burning, cattle, feedlots, and industrial sources (Chapin et al., 2002).

Assimilation is the next phase of the N cycle; this describes how organisms obtain N. Plants are able to incorporate NH_3, NH_4^+, and NO_3^- from soils and rhizobia into biological tissues and use these molecules to create amino acids. Some bacteria use inorganic forms of N as the sole source of N; however, most animals obtain N by ingesting amino acids from plants and bacteria in the food chain.

Additionally, ammonification or mineralization is a process that describes the conversion of organic N to the plant-usable form NH_4^+ by bacteria or fungi. The NH_4^+ ion is created when NH_3 (a weak base) reacts with proton donors, as depicted in Reaction 2.2:

$$H^+ + NH_3 \longrightarrow NH_4^+ \qquad \text{(Reaction 2.2)}$$

Free NH_3 is in equilibrium with NH_4^+. This equilibrium is shown in Reactions 2.3 and 2.4. This equilibrium would tend to move toward the left at a lower pH and to the right at a higher pH. The concentration relationship of free NH_3 and NH_4^+ ions can be represented by the ionization constant (Reaction 2.5).

$$NH_4^+ (aq) \longleftrightarrow NH_3 (aq) + H^+(aq) \qquad \text{(Reaction 2.3)}$$

$$NH_3 (aq) \longleftrightarrow NH_3 (g) \qquad \text{(Reaction 2.4)}$$

$$K_a = \frac{[NH_3 (aq)]\,[H^+ (aq)]}{[NH_4^+ (aq)]} \qquad \text{(Reaction 2.5)}$$

When NH_3 is dissolved in water, a small amount of NH_3 is converted to NH_4^+, depending on the pH of the water (Reaction 2.6).

$H^+ + NH_3 \rightleftharpoons NH_4^+$ (Reaction 2.6)

At high pH, the equilibrium of this equation will shift to the left due to low hydrogen ion concentration. Ammonium can be converted back to NH_3 by the addition of a base.

Finally, nitrification describes the process where NH_4^+ is converted to NO_2^-, primarily by bacteria (Reaction 2.7 and 2.8).

$2\ NH_4^+ + 3\ O_2 \xrightarrow{(\textit{Nitrosomonas})} 2\ NO_2^- + 2\ H_2O + 4\ H^+$ (Reaction 2.7)

$2\ NO_2^- + O_2 \xrightarrow{(\textit{Nitrobacter})} 2\ NO_3^-$ (Reaction 2.8)

Nitrobacter oxidizes NO_2^- into NO_3^-, which is the form used by plants. Excess nitrates in groundwater and freshwater can cause health problems in human infants, such as blue baby syndrome, and lead to eutrophication.

As previously stated, the nitrogen cycle provides pathways of N in various forms. The mass balance principle states the total input of N is equal to the sum of N recovered in agricultural products and N lost in the air, soil, and water (Schrøder, 1985). More N in the cycle may alter the equilibria of various pathways, possibly resulting in deleterious environmental or health effects. For example, human interventions such as intensive agricultural practices result in higher and more harmful concentrations of atmospheric NH_3, which lead to subsequent health effects such as vitamin A shortages, decreased oxygen-carrying capacity in the blood, and cancer. (Pearson and Stewart, 1993; Simon et al., 1987).

Eutrophication

Eutrophication, which is an increase in algal growth in water bodies, is expedited as the result of excessive nutrient pollution into surface waters and leads to a decrease in water quality

(Schuurkes, 1986). Phosphates and nitrates stemming from both deposition and runoff are the largest contributors (Nolon, 1995; Schrøder, 1985). Cloudy, green, or red waters are indicators of eutrophication; algal death and the consequent decay can deplete oxygen, causing anoxic dead zones where aquatic life cannot survive (Colorado-State, 2008). For example, excess N loading into the Chesapeake Bay in the last few decades has resulted in decreased water quality due to eutrophication (Jaworski et al., 1997).

Excess N and P loading into aquatic environments often originates from agricultural lands (Carpenter et al., 1998). Improper fertilization by manures and synthetic nutrients can exceed crop need and leach from fields where crops are grown. In addition, livestock operations can become complicated when considering regulations and management strategies due to feed inputs and manure production being unpredictable (Beegle et al., 2000). Nutrient Management Plans (NMPs) have been put in place to limit N and P loss. NMPs are utilized for proper crop nutrition, soil productivity, and quality, and to minimize the movement of N and P to waterways (Beegle et al., 2000). For some landowners, NMPs are voluntary; however in cases involving biosolids, poultry manure, many other confined animal feeding operations (CAFOs), state-owned lands, and government programs, landowners are required to use NMPs (Parry, 1998). A Certified Nutrient Management Planner must prepare the NMPs (Sorisio, 2003). These plans are accepted for three years unless conditions or management changes. NMPs are just one strategy for N input reduction in order to improve environmental quality and are important in areas such as Virginia, Maryland, and Delaware where there are many CAFOs.

Soil and Water Acidification

Air pollutants deposited from emissions of fossil fuels, combustion, and agricultural production can lead to the acidification of freshwater and soils (Nilsson, 1988). Deposition can occur by both wet and dry processes, and the deposits may contain elevated H^+ content, making them acidic. The NO_x and SO_x produced by the aforementioned processes are negatively charged and not in themselves acidic; however, they are associated with protons than can be released after transformations occur. Transformations are typically mediated by bacteria (Jury et al., 1987).

When NH_3 reaches the soil surface and reacts with water, it is converted to NH_4^+, which will then dissociate or nitrify into NO^{2-} or NO^{3-} by nitrobacter (Reaction 2.7). Hydrogen ions (H^+) released from the dissociation are then retained on colloids after leaching of other cations. This reaction releases H^+, thereby acidifying the soil (Colorado-State, 2008). Soil acidification occurs by the retention of H^+ on colloids aided by the leaching of bases such as calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and sodium (Na^+). Hydrogen is held more strongly than other cations (except Al^{3+}), and is therefore less likely to leach. The cation exchange capacity (CEC) is a measure of the negative charge of the soil. The percent of base cations, such as K, Ca, Mg, Na, that occupy the CEC make up the base saturation. It is the maximum amount of cations that a soil can hold at a particular pH and that is available for exchange. As the soil becomes more acidic, the pH decreases along with the CEC. In addition, soil acidity can lead to Al^{3+} toxicity. This occurs with reduced base cation concentrations due to leaching. Soil acidification of agricultural fields can be problematic, particularly where NH_4^+ fertilizers have been applied or acid precipitation occurs. Nutrient availability, crop growth, and microbial activity can be decreased as soils acidify (Novak, 2009).

Soil acidification has been linked to microbial activity reduction, toxic metal release, decreased crop and plant growth, and nutrient efficiencies. Additionally, fresh water bodies are negatively affected. Waters acidify following the increase of H^+ and Al^{3+} inputs from runoff (Krug, 1983). This pH reduction in water is harmful to organisms and often results in die-off as well as reproductive problems, particularly for those organisms that are sensitive to habitat alterations.

Monitoring agricultural N loss including emissions and manure use can be beneficial in determining a new N input reduction strategy to improve soil and aquatic environments (Boesch et al., 2001).

Ammonia Volatilization from Poultry Manure

Poultry production in the United States is an important part of agriculture, and poultry products are included in many citizens' diets (USDA. 2013). Birds are mostly raised in closed buildings, which increases production efficiency and minimizes labor costs. In areas where production is concentrated because of proximity to processing plants, NH_3 volatilization from waste is a cause for concern (Moore et al., 1995).

In poultry houses, NH_3 is formed through mineralization and decomposition of the uric acid ($C_5H_4N_4O_3$) present in poultry manure (O'dell et al., 1960). Mineralization involves a series of reactions that convert substances from organic to inorganic forms and can be affected by bird diet, house conditions, and manure management (Reaction 2.9).

$$C_5H_4N_4O_3 + O_2 + 4H_2O \xrightarrow{\text{Uricase, allantoinase, allantoicase}} 2(NH_2)_2CO + C_2H_2O_3 + H_2O_2 + CO_2$$

$$(NH_2)_2CO + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2 \qquad \text{(Reaction 2.9)}$$

During mineralization, uric acid is broken down to allantoin ($C_4H_6N_4O_3$) by the enzyme uricase. Next, allantoin is converted to either urea [$(NH_2)_2CO$] or glyoxalic acid ($C_2H_2O_3$). Finally, the urea produced is broken down into NH_3 and CO_2 by the enzyme urease (Kim et al., 2009). This process is dependent on pH, moisture, and temperature. In fact, this reaction is most effective at a temperature of 30-40 °C and a pH of 10.5 (Bongaerts and Vogels, 1979; Vlek and Carter, 1983).). With increased conversion of urea to NH_3 , greater amounts of NH_3 are lost to the atmosphere, particularly near the surface of a substrate (Grant and Brandon, 2004).

Excessive input of man-made fertilizers containing N and urea onto agricultural fields can lead to high rates of volatilization if applied in the wrong conditions (Pearson and Stewart, 1993). Course-textured and moist soils, and windy conditions, typically increase NH_3 losses (Grant and Brandon, 2004). Ammonia volatilization increases with increasing pH, moisture content, wind speed, and temperature (Delaune et al., 2004; Reddy et al., 1979). Under more acidic conditions, NH_3 reacts with hydrogen ions to form NH_4^+ (Reaction 2.6), whereas the opposite occurs in basic conditions as shown in Reaction 2.3 (Blake and Hess, 2001).

Impact of Concentrated Ammonia from Broiler Production

Negative physical and environmental effects stemming from concentrated broiler production can be minimalized by utilizing best management practices (BMPs) (Cestti, 2003). Best Management Practices have the potential to address nonpoint source pollution from animal agriculture, even though it is difficult to predict the NH_3 concentrations that are deposited by intensive agriculture (Pearson and Stewart, 1993). Legislation requiring concentrated poultry farms to have a nutrient management plan can address odor problems, emissions, and nutrient runoff problems for confined animal operations (Cabrera et al., 2000; Edwards and Daniel, 1992; Simpson, 1998; Sims and Coale, 2002).

Ammonia gas is hazardous in high concentrations, and according to the Agency for Toxic Substances and Disease Registry (ATSDR) ToxGuide™, livestock or the farmers who raise them, can be exposed to excessive and harmful levels of NH_3 through inhalation. In fact, the U. S. Occupational Safety and Health Administration (OSHA) has set a 15-minute exposure limit for gaseous NH_3 of 35 mg/L (ppm) in the air and an 8-hour exposure limit of 25 mg/L (ppm) (Rothrock et al., 2010). Regardless, exposure is unavoidable in some occupations. For example, broiler house NH_3 levels have the potential to exceed 100 mg/L (ppm) during the winter months, when ventilation is reduced to conserve heat, and manure storage pits can emit concentrations as high as 500 mg/L (ppm) (Patterson, 2002; Pitts et al., 1998).

Human Health

Though there are many environmental concerns with poultry house $NH_{3,}$ the health of farm workers and citizens in close proximity to broiler house operations is a top priority (Moore et al., 1996). Indeed, NH_3 that contributes to the formation of particulates has a broader public implication, whereas gaseous NH_3 is more harmful for agricultural workers (Colorado-State, 2008). Particulate matter less than 2.5 microns in diameter (PM2.5) is a large contributor to human health concerns involving broiler house NH_3 emissions (Gay and Knowlton, 2005). Atmospheric fine particulate NH_3 reduces visibility, leads to climate change, and adversely affects human health (Pathak et al., 2009; Speizer, 1989). When NH_3 from broiler house emissions reacts with water vapor, it dissolves and reacts with anions, forming fine particles. These anions typically come from combustion and are usually seen near locations where NH_3 gas undergoes chemical reactions with urban emissions such as SO_4 and NO_3 (Reaction 2.11) (Mensink and Deutsch, 2008; Van Breemen et al., 1982). In addition to combustion, industrial processes also contribute to PM2.5, which can include dust, dirt, smoke, and water droplets. It has a lifetime of 15 days in the atmosphere, therefore allowing it to travel further than gaseous NH_3 which has a lifetime of around 24 hours and is often deposited near the source (Krupa, 2003).

$$2NH_3 + H_2SO_4 \longrightarrow (NH_4)_2SO_4 \qquad \text{(Reaction 2.11)}$$

$$NH_3 + HNO_3 \longrightarrow NH_4NO_3$$

Ammonia gas is a hydrophilic base that has the potential to injure the respiratory tract when in contact with water. When contact occurs, ammonium hydroxide (NH_4OH) dissolves in the mucus membranes and hydrolyzes, resulting in high pH and respiratory damage. In addition, when in fine particulate form, NH_3 is taken in via respiration and travels to lung tissue, causing respiratory ailments. The type of particulate matter delivered to the lungs depends on the size, shape, and density of the particles being inhaled; therefore, smaller particles are able to travel further into the respiratory system (Subramaniam et al., 2003).

According to the American Conference for Governmental Industrial Hygienists (ACGIH), short-term NH_3 exposure of 50–150 mg/L (ppm) can cause eye, throat, and skin irritation, whereas NH_3 exposure >150 mg/L (ppm) can cause pulmonary edema (Colorado-State, 2008; De Boer et al., 1991; Merchant et al., 2002). Death can occur at an exposure concentration of 500–5,000 mg/L (ppm). Concentrations high enough to cause death after a short exposure time are not typically found with livestock operations; however, concentrations above the limit can occur in closed manure storage facilities that do not have proper ventilation (Merchant et al., 2002). Over time, NH_3 damages a farmer's sense of smell, preventing the detection of low NH_3

levels. The 8-hour exposure limit of 25 mg/L (ppm) set by OSHA is intended to protect from chronic effects, and the 15-minute short-term limit at 35 mg/L (ppm) aims to reduce irritant effects.

Broiler Health

Broiler health is important because farmers often get paid according to total weight before slaughter. Lower bird weights leading to production profit decline is a concern for farmers (Alloui et al., 2013; Blake and Hess, 2001; Moore et al., 1999; Moore et al., 2000; Rothrock et al., 2010). Sellable broilers come from minimizing death and keeping them healthy. Broilers may contract diseases that render them unsellable or harm the security of the surrounding operations. If the birds contract certain diseases, or if biosecurity is broken, whole flocks must sometimes be disposed of, as they cannot be sold in stores (Sims, 2008).

The categories of diseases affecting broilers are infectious diseases, parasitic diseases, behavioral diseases, and metabolic/nutritional diseases. One example of an infectious disease is avian tuberculosis caused by *Mycobacterium avium*, which may be contracted as a result of poor feed and management of broilers. Transmission occurs by ingestion, excretion, and inhalation, and the bacterium is difficult to eliminate due to its resistance to environmental changes (Dhama et al., 2011). Signs of contraction in a flock include weight loss, decreased appetite, death, lameness, and diarrhea. Tuberculosis has a high mortality in birds and is one of several common diseases in broilers (Dhama et al., 2011).

Infections by parasites cause parasitic diseases in which the organism cannot live without its host nutrients. Worms or lice are examples of this type of disease, and although they may not kill the broiler they inhabit, the host can become sick, underweight, and undernourished (Pickworth and Morishita, 2003).

When present in high quantities, NH_3 has many negative effects on broiler health such as blindness, respiratory infection, liver problems, increased blood pH, and even death (Carlile, 1984; Kling and Quarles, 1974). Ammonia concentrations as low as 25 mg L^{-1} produced in broiler houses can decrease the health and performance of birds (Alloui et al., 2013; Carlile, 1984; Parsons, 2006; Rothrock et al., 2010; Yahav, 2004). A decrease in bird weight has been reported at an NH_3 concentration of 25 to 50 mg/L (ppm). For a flock of 25,000 birds, this could mean a cumulative decrease of 7,750 lbs in weight, leading to significant economic loss (Kentucky, 2010). Young birds are typically more susceptible to problems within the first 28 days in broiler houses due to their rapid growth rates and undeveloped immune systems (Reece et al., 1980).

Impact of Concentrated Ammonia in the Environment

The National Academy of Science has reported a need to collect emissions data from animal operations due to the environmental effects. Poultry production is reportedly responsible for 10–27% of total NH_3 emissions in the U.S. (Battye et al., 1994). Additionally, atmospheric deposition accounts for 6–8% of total N delivered to the Chesapeake Bay with NH_3 making up 33% of that N (EPA-1, 2010).

The NH_3 volatilization from the mass amount of broiler litter produced can pose a problem for water and air quality surrounding large-scale operations (Moore et al., 1999; Rothrock et al., 2010; Zaman and Blennerhassett, 2010). For instance, the runoff and leaching effects of poultry litter utilized as a fertilizer, and the atmospheric deposition of N as NH_3, are major sources of air and water quality degradation (Choi and Moore, 2008). The excess N results in eutrophication, which significantly decreases water quality (Keeney and Hatfield, 2008; Schuurkes, 1986).

Broiler production modifications to reduce ammonia volatilization

Broiler Feed

Feed efficiency is calculated by dividing intake by weight gain and is an important part of broiler house feeding and management programs (Willems, 2012). It can be a good indicator of flock performance and allow alterations to be made to reach maximum weight. Factors such as feed content, temperature, ventilation, and other house management activities can all have an effect on feed efficiency (Willems, 2012). For example, feed intake increases after the brooding period because the birds need to use feed for body heat. In colder temperatures, feed intake also increases in order to maintain a healthy internal body temperature.

There are three stages of broiler feeding: starter, grower, and finisher. Each stage is formulated for the specific stage in the broiler growth cycle to maximize the weight and health of birds until processing (Saleh et al., 1997). Starter diets typically feed broilers up to three weeks old and have more antibiotics, nutrients, and energy content. The next stages add more fat and vitamin content in order to facilitate survival under broiler house conditions. Broilers are raised for 42–56 days until a processing weight of approximately 2.72 kg (6lbs) is reached, and the feed typically represents 60–75% of the total production costs. Feed ingredients can include corn and soybean meal, fish or meat meals, calcium and phosphorous, salts, lipids, antibiotics, and antiparasitics. Additives and ingredients can be altered and vary by price and content (Saleh et al., 1997). The N concentration in poultry feed is typically based on the total amino acid content, and there is usually an overconsumption of non-essential amino acids in order to meet requirements for broilers (Ritz et al., 2004). Broilers are capable of excreting two-thirds of the N consumed. Additionally, the salt content of the feed leads to more water consumption and subsequently wetter litter from waste, allowing for greater volatilization potential (Nahm, 2005). Changes such as reduction of crude protein and addition of probiotics can be made to the broiler diet to decrease NH_3 emissions (Aneja et al., 2008; Chang and Chen, 2003).

Broiler House Environment

Broiler house management is an important task that allows farmers to maximize weight production while maintaining low operation costs. It is typical that adjustments be made throughout and between flocks to maximize profit. Adjustments can involve bedding material, cleanout procedures, ventilation management, and feed (Kao et al., 2011; Miles, 2008). Poultry bedding can be a variety of low-cost, high-absorbent materials such as peanut hulls, wood shavings, and saw dust (Edwards and Daniel, 1993). Once bird excrement, feathers, water, and feed become mixed with bedding, it is referred to as litter (Edwards and Daniel, 1993; Espinoza et al., 2007; Funderburg, 2009). House floors are initially covered with bedding and reused until it is removed as litter for fertilizer (Kelley et al., 1998). The top hardened manure layer of litter that develops during a flock is referred to as cake. Decaking is done between flocks in order to remove parts of litter with more than 35% moisture that have the potential to increase NH_3 levels in-house (typically near waterers). Decaking litter between each flock is a common practice that is cheaper than complete cleanout after every flock, yet still can decrease litter moisture. Because the underlying bedding material is retained for several flocks, total house cleanout is done only every 3–5 years (Blake and Hess, 2001). Over time, bacteria will accumulate in the litter leading to more rapid hydrolysis and breakdown of uric acid and subsequently higher NH_3 volatilization. Therefore, it is important when decaking to not stir up moist or underlying litter because it can lead to higher NH_3 concentrations (Blake and Hess, 2001; Espinoza et al., 2007). Because of the vast amount of manure produced annually, litter management is important for environmental quality surrounding agricultural operations (Dick et al., 1998).

Poultry production involves seven steps, including a breeder flock, pullet farm, breeder house, hatchery, broiler farm, processing, and distribution (Martinez, 1999). Vertical integration of these stages improves efficiency and makes it necessary to have each part in close proximity. Closeness minimizes transportation costs and allows for better overall control and management from companies (Doye et al., 1996).

Over the past 50 years, poultry production and consumption has increased 4% per year in the United States. Furthermore, more poultry is produced and consumed than beef or pork (Dumas et al., 2011; American Meat Institute, 2011). This increases not only waste products but also agricultural crop production for feed use and manure disposal as fertilizer.

Poultry houses are often split in half by a curtain. One-half is designated as the brood end where the chicks stay for around 2 weeks until the farmer decides they are big enough to open the whole house. The brood end of a house is typically the only side that receives chemical litter treatment (Shah et al., 2006). By only applying treatment to half the house, money is saved and initial NH_3 levels are kept at a reasonable level as heat is increased for chicks. In addition, birds are smaller, so the amendment lasts longer than when birds are larger due to the volume of feces. Removal of birds typically occurs within 6–7 weeks of placement when birds reach a marketable weight of 7 pounds (Gates et al., 2008).

Litter age, NH_3 volatilization, and N content are positively correlated (Mahimairaja et al., 1994). As vapor pressure and temperature rise in broiler houses, NH_3 solubility decreases, thereby increasing the amount of NH_3 emitted (Gustafsson, 1987). Poultry litter has a low carbon to N ratio that allows for more NH_3 release (Delaune et al., 2004). The moisture of the litter is important in hydrolysis reactions and will affect the conversion of uric acid to NH_3 (Sims and Wolf, 1994). As seen in Reaction 2.12, litter moisture can affect the conversion rate from Urea to NH_4^+. As moisture levels increase, NH_3 generation in litter can occur (Liu et al., 2007). For example 1.4 times more NH_3 is released at 24°C (75°F) with 25% moisture than with 20% moisture.

$$CO(NH_2)_2 + H^+ + 2H_2O \longrightarrow 2NH_4^+ + HCO_3^- \qquad \text{(Reaction 2.12)}$$

Bacterial growth and hydrolysis are optimal at 30–40 °C. This temperature range also allows for maximal conversion of uric acid to NH_3. Pre-heating the broiler house for 24–48 hours before chicks arrive can decrease NH_3 concentrations by lowering moisture levels in the litter (Ritz et al., 2004). Over half of the total N produced by broilers is lost to the atmosphere while the litter is still in-house; therefore, in-house treatments are important (Moore et al., 2013).

Several emission mitigation practices have been used for large-scale animal operations, including pre-/post-excretion treatments (Burns, 2010; Choi and Moore, 2008; Shah et al., 2006). Increased air circulation is correlated with better weight and survival (Weaver and Meijerhof, 1991). This circulation is a major contributor to decreased litter moisture and caking, presumably improving the ability of the litter to absorb or trap uric acid or NH_3 in a solid form before NH_3 volatilization occurs. Combining chemical amendments with air circulation is a more cost-effective alternative than solely using ventilation for air quality control (Moore et al., 1995).

Poultry House Ventilation

Ventilation helps to control broiler house air quality. Ventilation for poultry houses reduces in-house NH_3 levels by bringing in clean air from outside the house (Gay and Knowlton, 2005). It can be beneficial in reducing moisture, NH_3, disease organisms, and heat during the summer months (Moore, 1986).

Ventilation depends on the season, type of building, floor, number and age of broilers, and waste handling system (Moore, 1986). Natural ventilation provides a cheaper way for air circulation through inlet and outlet points but is hard to control and not very effective in the absence of wind (Doye et al., 2010). Natural ventilation relies on wind and the thermal buoyancy of broilers, which contributes to warm air rising (Moore, 1986). Two main types of mechanical ventilation systems exist for poultry houses: pressure and exhaust systems. A pressure system relies on a fan blowing air through inlet openings into a building. This creates a positive indoor pressure and forces air out of the house through outlet openings (Donald et al., 2000). An exhaust system involves fans that expel air from inside the building and lower the atmospheric pressure inside the broiler house. These types of ventilation help with temperature consistency, heat, dust, and moisture removal, as well as facilitate oxygen circulation and expel NH_3 out of the house to improve broiler health and survival rate (Bucklin et al., 2009). The air flow volume of a fan varies with the speed and size of the fans that are present. Tunnel ventilation is a common type of exhaust system. In this system, exhaust fans are located on one end of a poultry house and inlet openings are placed on the opposite end. Air is drawn in and down the house, and the flow velocity at bird level is kept fairly constant; this effectively cools birds while lowering in-house NH_3 and dust (Bucklin et al., 2009).

Ammonia levels in-house can increase due to inappropriate ventilation practices (Blake and Hess, 2001). Emission rates of NH_3 depend on the air velocity above the source. When the ventilation rate decreases in-house, the NH_3 concentration present tends to increase. In order to reduce moisture levels and remove NH_3 from the house, continuous airflow is suggested. Humidity levels from manure, drinkers, and bird exhalation at 45% or below in broiler houses is correlated with lower NH_3 levels, better weights, and improved survival (Weaver and Meijerhof, 1991). During summers, when ventilation is increased to reduce in-house temperatures, the emission rates of NH_3 from broiler houses increase (Gustafsson et al., 1996). As emissions increase, the in-house NH_3 concentrations decrease due to circulation of clean air (Kavolelis, 2003). It is not cost-effective to continuously run fans during winter months due to fans drawing out the heat needed to warm the birds and the need to use heaters to re-heat the house; therefore, NH_3 levels tend to be higher at that time (Ritz et al., 2009).

Litter pH

Due to financial restraints and minimizing the need for waste disposal, farmers no longer completely clean out houses between each flock of birds (Ritz et al., 2014). This reduces the waste produced and lowers bedding costs; however, aged litter can have negative health effects on birds and harm the surrounding environment. As litter ages, the likelihood of health effects due to NH_3 increases (Ritz et al., 2014). The pH of litter in broiler houses is positively correlated with NH_3 concentration and should be kept below 7 in order to reduce volatilization. Litter pH typically ranges from 8–10 and is difficult to control throughout the production cycle of the flock (Blake and Hess, 2001). Enzymatic reactions are a large component of NH_3 production; at more acidic pH, the enzyme urease is less likely to convert urea to NH_3 gas. Therefore, lowering the pH with acid application results in decreased NH_3emissions (Madrid et al., 2012). Acid amendments lower pH and convert NH_3 gas to NH_4^+ ions, increasing the N content in litter and thereby its potential fertilizer efficacy (Blake and Hess, 2001).

Litter amendments

A major component of NH_3 control is house management. Alterations in feed content, between-flock procedures, ventilation, temperature, and humidity control are all important for the health and productivity of birds (Casey et. al, 2006). Even so, NH_3 concentrations can become high and other strategies are needed to supplement management.

Litter amendments have been used for odor and NH_3 control (Blake and Hess, 2001). In order to choose the best litter treatment, a grower should consider his goals. Treatments are preferential based on the goals of improving litter composition for use as a fertilizer, use of BMPs to decrease odors and emissions, or decreasing foodborne pathogens (Blake and Hess, 2001). Technologies should be used on litter before its removal to decrease in-house emissions (Moore, 2010). Moisture, brooding, lighting programs, ambient temperature, strain type, and ventilation management are some important components in deciding which litter amendment should be used (Blake and Hess, 2001). The need for ventilation and energy use can decrease with the use of litter amendments; this proves cost-beneficial in most cases (Parsons, 2006). A common BMP is the use of chemical additives on litter. Several types of litter amendments exist for NH_3 control including acidifiers, alkaline materials, adsorbers, inhibitors, and microbial treatments, with acidifiers and alkaline materials being the most common (Parsons, 2006).

Adsorbers

Adsorbers are naturally occurring minerals that have a tendency to adsorb NH_3, however they are neither economically feasible nor practical (Blake and Hess, 2001). Zeolinite is a mineral that has been added to poultry manure during composting and has shown to affectively decrease NH_3 loss, potentially improving the applicability of the treated litter as fertilizer (Li et al., 2008). Similarly, acidified clay materials have similar effects to using an acid chemical amendment (McWard and Taylor, 2000). Though positive results have been obtained with adsorbers, the labor, cost, and maintenance involved is not ideal, and these are not used in large-scale operations.

Inhibitors

Inhibitors slow the conversion of NH_3 by inhibiting enzymes and microorganisms. Because of the ubiquitous presence of urea in poultry facilities, inhibitors must be reapplied often to prevent enzyme and microbial repopulation (Shah et al., 2006). For example, *N*-(*n*-Butyl) thiophosphorictriamide (NBPT) slows conversion of urea to NH_3, however very litter research has been done on broiler litter (Singh et al., 2009). Additionally, urease inhibitors are not readily available on the market for broiler houses.

Microbial and Enzymatic Treatments

Microbial and enzymatic treatments are another alternative litter treatment. These applications provide an environment that is ideal for the conversion of uric acid and urea to NH_3 (Shah et al., 2006). The reaction occurs very quickly, and houses must be subsequently ventilated. Ventilation will rid the facility of the produced NH_3 and lead to an overall in-house decrease. The costs of ventilation increase and the risk of failure can harm the health of birds and workers because NH_3 concentrations can become extremely high. Though this method has the potential to provide better indoor air quality, the emissions of harmful NH_3 will increase and degrade the air quality outside the house (Blake and Hess, 2001). The concern with surrounding air and water quality makes the application of microbial and enzymatic treatments less than ideal.

Alkaline Materials

Similarly, alkaline materials are applied between flocks and convert NH_4 to NH_3 by keeping the pH above 7. This conversion, combined with ventilation practices to remove NH_3 from the inside, decreases in-house NH_3 and is beneficial to bird health and performance (Shah et al., 2006). However, this practice increases NH_3 emissions from houses, degrading the quality of surrounding air and water. Because N is released in the form of NH_3, the potential fertilizer value of the litter decreases. Typically, this type of additive is less likely to be used than acidifiers (Parsons, 2006).

Acidifiers

Dry acid applications are the most commonly used litter amendment. They have been proven to reduce emissions from poultry houses by lowering pH and inhibiting microbial activity through rapid pH change. In addition, acidifiers trap more N as NH_4^+, which has more value as a fertilizer after removal (Aneja et al., 2008; Choi and Moore, 2008; Madrid et al., 2012; Parsons, 2006; Rothrock et al., 2010). The pH reduction in litter achieved through the use of acid amendments and proper ventilation practices is the most ideal management practice in large-scale broiler operations. Several acidifiers are available and can be chosen based on individual management practices.

Common acidifying litter amendments

Al^+ Clear

Alum (Al^+ clear) is a poultry litter amendment comprised of aluminum sulfate ($Al_2(SO_4)_3 \cdot 14H_2O$) (Blake and Hess, 2001; Rothrock et al., 2010). It is marketed in both liquid and dry forms and is an acidifier due to the hydrogen ions produced when it is dissolved (Reaction 2.13) (Moore et al., 2000). The hydrogen ions react with NH_3 to produce non-volatile NH_4^+, which then reacts with SO_4^{2-} yielding $(NH4)_2SO4$ (a water-soluble fertilizer) (Moore et al., 2000). Alum has been proven to decrease NH_3 emissions, heavy metal runoff, and phosphorous runoff from agricultural lands while increasing bird performance (Blake and Hess, 2001; Delaune et al., 2004; Moore et al., 1999; Moore et al., 2000; Moore et al., 1996; Parsons, 2006; Sims and Luka-Mccafferty, 2002). The NH_4^+ present in litter through Alum treatment has been shown to double the N concentration in litter, thereby increasing its fertilizer value (Delaune et al., 2004; Moore et al., 2000). It is applied once before flock arrival at a rate of 250 lbs/1000 ft^2. Common acidifiers competing with Alum are PLT and Klasp.

$$Al_2(SO_4)_3 \cdot 14H_2O + 6H_2O \longleftrightarrow 2Al(OH)_3 + 6H^+ + 3SO_4^{2-} + 14H_2O \qquad \text{(Reaction 2.13)}$$

$$6NH_3 + 6H^+ \longleftrightarrow 6NH_4^+$$

$$6NH_4^+ + 3SO_4^{2-} \longleftrightarrow 3(NH_4)_2SO_4$$

Klasp

The poultry litter amendment Klasp is a dry granular form of ferric sulfate [$Fe_2(SO_4)_3 \cdot 9H_2O$] used as a BMP in broiler houses. Klasp is an acidifying, pre-flock amendment capable of decreasing NH_3 production by partially suspending the microbial breakdown of urea. Notably, it creates 38% more acidity per pound than PLT (Best Veterinary Solutions, 2013). In addition, it has a dust-free application and is claimed to be less corrosive than other acidifiers. Moisture in poultry litter reacts with Klasp and not humidity in the air; this allows it to be more efficient for volatilization control from broiler house floors. The product states a drier litter base will be present after continued use of Klasp (Reeder and Kemira, 2011). Because microbial activity is moisture- and temperature-dependent, it has the potential to decrease not only emissions from broiler houses, but also diseases contracted by broilers through litter. It reacts with litter moisture and is not temperature-dependent (Best Veterinary Solutions, 2013). Application of Klasp should occur 2-5 days prior to flock arrival at a rate of 488 kg/m^2 (100 $lb/1000\ ft^2$) to allow reaction and activation time. It is a fairly new acidifier and studies involving Klasp as an additive to litter are lacking, particularly in comparison to other treatments in a volatility chamber.

Poultry Litter Treatment

PLT is an acidifier used in a variety of livestock operations and industrial, agricultural, and food industries. It is composed of sodium bisulfate ($NaHSO_4$) and is a common pH reducer in broiler houses. In broiler operations, PLT is typically applied once directly on the brood end alone, before broilers are released into the house, at a rate of 244 kg/m^2 (50 $lbs/1000\ ft^2$) (Blake and Hess, 2001; Jones-Hamilton, 2010; Tasistro et al., 2008). As seen in Reaction 2.14, treatment lowers the pH, converts NH_3 to NH_4^+, and finally forms ammonium sulfate [$(NH_4)_2SO_4$] (Blake and Hess, 2001). This reaction is non-reversible; therefore N is not released from litter after conversion occurs. The acidifying and NH_3-binding properties of PLT also lead to bacterial population reduction in litter. In fact, studies show PLT decreases *Salmonella*, *Campylobacter* populations, beetle populations, house NH_3 levels, litter pH, and fuel usage while increasing bird performance (Blake and Hess, 2001).

$$2\ NaHSO_4 + 2NH_4OH \longrightarrow (NH_4)_2SO4 + Na_2SO_4 + 2H_2O \qquad \text{(Reaction 2.14)}$$

When broilers are present, PLT is safe to apply allowing for application variability. In fact, PLT is limited only by the quantity applied to litter (Johnson and Murphy, 2008). This acidifier has hygroscopic properties and performs best in houses with lower humidity; therefore, a combination of litter amendment, ventilation, and temperature control yields the best results (Kentucky, 2010).

PLT is not effective indefinitely; as birds continue to excrete waste, the pH will begin to rise. If application is repeated throughout the cycle of the flock, it is expected that the pH will be maintained at a point that retains N in the NH_4^+ form (<7) and decreases NH_3 volatilization from litter (Blake and Hess, 2001). In addition to decreasing volatility, holding N in litter can be more desirable for fertilizer use (Blake and Hess, 2001). Some research has been done on the reapplication of PLT; however, controlled and large-scale studies are still needed to determine applicability. In addition, a procedure or mechanism for mid-flock application has yet to be marketed.

Alternative solutions to ammonia control

Ammonia Scrubber Systems

Acid scrubbers provide an alternative to in-house NH_3 treatments by treating emitted air for the external environment (Melse et al., 2009). They are typically attached to exhaust fans and provide no benefit to bird health or in-house air quality. Scrubbers that remove NH_3 and particulate matter through water and acidic reactions have been beneficial to environmental air quality surrounding broiler houses in the past (Melse et al., 2011). The idea is to clean the poultry house air immediately after it exits the house.

The two main types of scrubbers include 1) acid scrubbers and 2) bio-scrubbers (biotrickling filters) (Melse and Ogink, 2005). In an acid scrubber, a weak acid solution with a pH of 2–4 is continuously circulated over the scrubber, producing a waterfall through which exhaust air exits the house. Sulfuric acid is commonly used in an acid scrubber system; however, other acids can be used. Ammonia will react with the acid solution and form a salt bi-product that is retained by the system. As a result, NH_3 is converted to non-volatile NH_4^+ (Moore, 2010). When the pH exceeds 6, new acid is brought in. The old acid can then be re-processed or used as a source of N in fertilizers. In bio-scrubbers, bacteria convert NH_3 into nitrite and nitrate. They also have higher odor-removing potential and are designed to provide maximum contact between the gas and liquid streams (McGahan et al., 2002; Moore, 2010). The excess particulate matter leaving broiler houses should be considered when using scrubbers. Exhaust air should go through a dust-removal stage followed by an NH_3-removal stage. Scrubber designs were initially problematic and had several technical difficulties. Because of the technical problems and high cost of maintenance and production, design improvements have been considered (Ullman et al., 2004). Although scrubber systems are an effective treatment for NH_3 emission control, dust reduction, and odor removal, further research and design is needed (Ullman et al., 2004).

Filters

Dust, feathers, and other materials exiting broiler house fans can contribute to air quality problems and NH_3 contributions to PM 2.5. Filters can provide a cheaper alternative to having a scrubber in place. In fact, filters have been shown to decrease dust by up to 50%. Even so, clogging becomes a problem, and their maintenance is time-consuming for farmers (Ullman et al., 2004). It is suggested that a series of filters be used to decrease filter changes; however, with the massive amount of dust and feathers present, they are still not commonly used in large-scale operations.

Post-flock Litter Management

Replacing the litter for each new flock is proven to drastically improve NH_3 concentrations in house; however, building up litter over time is a common practice in today’s broiler houses because of the high cost of complete cleanout after every flock. Therefore, other strategies must now be incorporated to maximize litter use over several flocks (Wheeler et al., 2008). In addition, bacterial and beetle presence in drastically increased in retained litter. The downtime between flocks should be efficiently planned for NH_3 removal by ventilation, decaking, litter treatments, or other strategies.

In addition to removing cake from litter between flocks, farmers can also utilize windrowing. Windrowing is a composting practice that allows bacteria in litter to generate enough heat to destroy some pathogens and enzymes (Barker et al., 2013). In fact, in-house composting of litter has been shown to generate temperatures of 130–140°F within two days of initiation. In-house windrow composting is an economically feasible practice that is beneficial for reducing NH_3 concentrations, extending litter life, and providing disease control; however, it can require specialized equipment, is time-consuming, and decreases N content of litter which is not desirable when it is used as a fertilizer (AAFRD, 2005; Barker et al., 2013; Shi et al., 1999).

Measuring ammonia

Several methods exist for measuring the amount of NH_3 in a system. A drager tube is a gas detection tube that can be used in broiler houses to measure the concentration of NH_3 in one area. It is used by standing in one spot, pulling the end of a tube (like a syringe), and reading the color change in the tube for an output. This has a one-time use and is not as accurate as gas analysis of a sample, but it is a quick and effective way to obtain an approximate NH_3 concentration in the air (Ni and Heber, 2001).

Absorption spectroscopy is useful for gas samples and measures how a sample absorbs light at different wavelengths (Simonescu, 2012). Infrared spectroscopy has the advantage of not requiring physical collection of air samples as it automatically analyzes them even without a human present. It can be run over long periods of time allowing for treatment vs. time interaction results. Person-to-person variations in results are lower due to the absence of physical sample collection, chemical inputs (such as standards), or interpretations of results because it provides a number and no interpretation is needed. Disadvantages of this analyzer include initial setup (filters, backgrounds, references, temperature-controlled area), cost (equipment, maintenance, electricity), the need for outlets, limited portability, as well as its fragile and sensitive nature. A common type of infrared spectroscopy is Fourier transform infrared (FTIR) in which a mathematical process is needed to convert raw data into a readable spectrum (Ni and Herber, 2001).

Use of litter as fertilizer

There are 25 elements essential to life including carbon (C), hydrogen (H), oxygen (O), N, and phosphorous (P). Nitrogen is particularly important when considering vegetation growth. Nitrogen is a mobile, macronutrient for crops to grow and develop (Abdel-Ghani et al., 2013). Nitrogen additions have been used for many years and are essential for good crop yields for non-legumes (Keeney and Hatfield, 2008). Different levels of N can be required to optimize corn growth and yield depending on field placement (Scharf, 2001; Scharf et al., 2002; Schmitt and Randall, 1994; Spargo et al., 2009; Yin et al., 2012). Nitrogen application rates for corn are based on yield expectations (Kyveryga and Blackmer, 2012; Spargo et al., 2009; Yin et al., 2012). There are several N sources available for crop management, including granular urea, urea-ammonium nitrate solutions, anhydrous ammonia (NH_3 [AA]), and various manures (Kyveryga and Blackmer, 2012).

Soil tests should be performed in order to develop a proper nutrient management plan, however in Virginia soil tests are typically not performed for N due to its mobility. Deficiencies in N can be determined by leaf concentration analysis (Blackmer and Schepers, 1994)**.** Fertilizers are commonly used to prevent deficiencies in soils and are an important nutrient source for vegetation. The requirement for N is a major input cost for farmers; therefore, it can be beneficial to use litter as an N source. Therefore retaining N in litter by the use of chemical additives rather than allowing it to be lost to volatilization is important.

The prices of synthetic fertilizers have been increasing, and less expensive replacements are being found (Keeney and Hatfield, 2008; Lindsey et al., 2013). Manure applications on crops can be an important source for recycled nutrients (Shah et al., 2012). They provide yields that are at least equal to, and in some cases greater than, synthetic fertilizers (Eghball and Power, 1999). Poultry manure in particular can provide nutritional benefits to crop systems (Funderburg, 2009; Ruiz et al., 2012).

Poultry manure utilized as fertilizer can be a more cost-effective alternative to synthetic fertilizer because the major associated cost is that of transportation (Funderburg, 2009). The overall integrity of the soil (biological, chemical, and physical) can be improved by poultry manure applications due to it being nutrient and organic rich (Ruiz et al., 2012). Manure from poultry has a higher by-weight N content than other manures (Ruiz et al., 2012). This N is present in the NH_4^+ and organic forms (Nahm, 2005). Litter amendments that acidify NH_3 have been proven to yield a higher N content than that of untreated litter (Li et al., 2013). Poultry litter has an unpredictable amount of essential nutrients, but can be used as a source for organic matter (Dick et al., 1998; Espinoza et al., 2007; Funderburg, 2009). The organic N in the litter must be converted by bacteria first before it can be used by crops. In addition, some of the NH_4^+ is lost due to volatilization, and the organic compounds can run off due to rain or leaching below root levels (Funderburg, 2009; Rothrock et al., 2010). As a result, it is thought that only about 50% of the N in a ton of broiler litter is actually used by the plants and is only available in the season during which it is applied. The availability depends on such variations as climate, flock management, and storage. For example uncovered manure stored for longer periods of time will have less N content than manure that is covered or used immediately. This is due to atmospheric NH_3 loss (Zhang et al., 2002). Chemical litter treatment use can allow more N to be held as NH_4^+ in the litter, increasing its fertilizer value. Studies have found that there is very little residual N remaining in the soil after a year if surface applied (Diaz and Sawyer, 2008; Gaskin et al., 2007; Nicholson et al., 1999). Therefore, it is often recommended that litter be injected or incorporated and applied annually depending on phosphorous (P) presence in soils (Dick et al., 1998; Espinoza et al., 2007). The N that is not immediately available from manure is a potential long-term N source for plants (Bergstrom and Kirchmann, 1999; Cusick et al., 2006).

Litter microbiology

Due to litter reuse over several flocks, poultry house environments have a buildup of disease-causing microorganisms (Dumas et al., 2011). The microorganisms in litter include both human and bird pathogens as well as those with antibiotic resistance genomes (Dumas et al., 2011). In the past, total litter bacteria have been shown to range from 10^{10}–10^{11} colony-forming units (cfu) per gram of litter (Williams, 2012). Litter with more moisture, typically near waterers, have been shown to have three times more bacteria than that in litter from less moist areas of a broiler house (Dumas et al., 2011). This moisture/bacteria relationship provides a reason for NH_3 levels being higher with increasing moisture (Williams, 2012). Areas near waterers typically have moisture levels of 43–67%, while other parts of the house such as near side walls typically have moisture levels of 10–25% (Dumas et al., 2011).

Research on poultry litter microorganism communities is difficult. When sampling litter for analysis, a centimeter in distance between samples can change the results drastically. Large houses can reach 183 m (600 ft) in length, allowing for extreme sample variability. Samples can also change with bird age, type, and management practices (Otutumi et al., 2012). As birds get older, more waste is produced, thereby allowing for a greater microorganism presence. In addition, feed additives, litter amendments, and cleanout practices all cause microbial community variation (Enticknap et al., 2006; Lu et al., 2003). The diversity and complexity of these communities makes it difficult to have exact transferable results to every poultry production facility; however, an overall observation of management changes and the subsequent bacterial results can be beneficial (Williams, 2012).

Several human pathogens exist in poultry litter; this is a cause for concern regarding water and food security. *Salmonella, E. coli,* and *Listeria monocytogenes* all have the potential to contaminate produce and are commonly found in foodborne outbreaks. Acidifiers, windrowing, and lower moisture levels have been effective in the past in reducing bacterial numbers in litter (Williams, 2012).

E. coli

Application of manure to agricultural lands can both supply nutrients to crops and provide an effective way to dispose of waste. This disposal has the potential of increasing fecal contamination in food and waterways (Soupir et al., 2006). *E. coli* is a common fecal bacterial indicator due to its ubiquitous presence. Colonies in poultry manure are hard to pinpoint due to variations in house management and individual broilers (Lu et al., 2003). *E. coli* is a gram-negative, rod-shaped, facultative anaerobe that causes several diseases in poultry and is subsequently harmful to human health (Gingerich, 2011; Williams, 2012). *E. coli* comprises approximately 12.5% of the total bacterial population in poultry litter (Williams, 2012). Problems in poultry arise as secondary infections to respiratory distress from NH_3 inhalation. Respiratory tracts can normally remove threats with ciliated epithelial cells, however when respiratory distress occurs, those cells do not function correctly and allow *E. coli* infection. Reduced ventilation and increased pH, moisture, and dust counts can all raise infection potential (Gingerich, 2011; Pope and Cherry, 2000). This bacterium can linger in litter after birds have been removed. Colonies have been found 120 days after flock removal from litter and after 2 months in soils (Ekperigin, 2000). Poultry Litter Treatment application has been proven to lower colony-forming units from 5,742 cfu/mL to 3,582 cfu/mL in litter and significantly lower colonies in the intestinal tract (Pope and Cherry, 2000). Use of acidifiers as an effective *E. coli* reduction measure should be further studied.

Salmonella

Salmonella is a rod-shaped, gram-negative, facultative anaerobe that belongs to the same family as *E. coli.* It is found in animals and causes food poisoning post-ingestion. This bacterium causes gastrointestinal stress and can be transferred readily between animals and humans. Poultry litter can be contaminated with *Salmonella* and spread rapidly. Further, the pathogen can become attached to feathers and feet and arrive in plants without being detected. This becomes a food safety problem when birds enter the processing stage and a water quality problem when litter is used as fertilizer (Kim et al., 2012; Payne et al., 2007). Therefore, it is a joint effort between growers and processing plants to maintain food security. Proper house management techniques, spraying birds with disinfectants and proper washing can benefit bacterial reduction.

Listeria monocytogenes

L. monocytogenes is a gram-positive facultative anaerobe that causes foodborne illness in humans. This pathogen also can cause meningitis in babies and is the third leading cause of death among foodborne pathogens (CDC, 2013). Composting can increase the temperature enough to inactivate this pathogen before field application. However, outbreaks can cause economic losses in all aspects of the industry; therefore, proper biosecurity measures should be taken to avoid transfer (Jemmi and Stephan, 2006; Paniel, 2010).

Summary

1. Broiler house NH_3 emissions can negatively impact the environment, human health, bird health, and productivity. BMPs for NH_3 reduction are needed.

2. Acidifying litter amendments can be used to decrease NH_3 emissions and are applied once directly before flock arrival on the brood end of a house. Amendments are no longer effective as birds continue to excrete; therefore several applications during a flock may be beneficial.

3. Poultry litter contains bacteria, some of which can be pathogenic. *E. coli* can cause disease in humans and has the potential to damage food security and water quality. Single applications of acid amendments are capable of temporarily decreasing pathogen levels.

4. PLT is an acidifier that is safe to apply while birds are present. Development and evaluation of a treatment reapplication system could be beneficial in NH_3 and *E. coli* reduction.

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CHAPTER 3: EVALUATION OF A SODIUM BISULFATE (PLT) REAPPLICATION SYSTEM IN A COMMERCIAL BROILER HOUSE

Abstract: Ammonia (NH_3) emissions from animal manure, such as poultry litter, can cause air quality problems. Poultry litter treatment (PLT, sodium bisulfate) is an acidic amendment that is currently applied to litter in poultry houses to decrease NH_3 emissions, but currently it can only be applied before birds are placed in the houses. The objectives of this paper were to analyze effects of a new overhead auger and spinner system that is capable of applying several additions of PLT to poultry litter on NH_3 concentrations in the air and litter properties. Ammonia concentration at the exhaust fans were measured and as well as litter properties in two commercial broiler houses, one with the reapplication PLT system and one without. During treatment application, the reapplication house had a significantly lower NH_3 concentration of 9.80 mg/L when compared to control house concentration of 17.41 mg/L (p=.028). Two hours after application, PLT no longer decreased NH_3 concentrations significantly. Similarly, reapplication of PLT reduced litter pH from 8.50 immediately before, to 7.35 immediately following application. However by the end of the flock the litter pH had increased to 8.56 and was not significantly different than the control. Litter moisture increased with reapplications of PLT by 10% relative to the control house after 6 PLT applications. NH_4-N in litter was significantly greater after 3 flocks in the reapplication house (p=.003). After the first flock, corrosion was noticed on some of the metal heaters and metal feed lines close to the PLT spinners, and this became worse with time despite efforts to cover affected equipment during PLT spreading. Though some positives came from this study, the overhead reapplication auger system was time consuming, and damaging to equipment and facilities.

Keywords: *Broilers, Emissions, Volatility, PLT, Sodium Bisulfate*

Introduction

Volatilization from intensive poultry and livestock production farms is the largest contributor to atmospheric ammonia (NH_3) (Pinder et al., 2007; Walker et al., 2000). High concentrations of NH_3 in the air can have negative human health and environmental impacts, particularly for confined poultry facilities where concentrations can exceed the EPA recommended maximum of 25 mg/L (ppm) (Aneja et al., 2008; Charles and Payne, 1966; Homidan et al., 2003; Miles et al., 2004; Reece et al., 1981; Stokstad, 2014).

Water and air quality degradation resulting from broiler house NH_3 emissions is of particular concern (Moore et al., 1999; Rothrock et al., 2010; Zaman and Blennerhassett, 2010). For example, nitrogen (N) from agricultural emissions is directly related to eutrophication resulting in algal blooms and fish kills causing a loss of habitat, particularly in the Chesapeake Bay (Aneja et al., 2008; Campbell-Nelson and Herbert, 2009; Chesapeake Bay Foundation, 2014; Paerl and Fogel, 1994; Williams et al., 1999). Also concerning for air quality is that NH_3 is a precursor for atmospheric fine particulate matter <2.5µm (PM 2.5). This PM 2.5 has a lifetime of 15 days allowing it to travel further than gaseous NH_3 (Krupa, 2003). Particulate matter reduces visibility, leads to climate change, and adversely affects human health by causing respiratory ailments due to its small size (Pathak et al., 2009; Speizer, 1989; Subramaniam et al., 2003). The PM 2.5 is typically seen near locations where NH_3 gas undergoes chemical reactions with urban emissions like NO_X and SO_X (Van Breemen et al., 1982).

Due to these negative effects, solutions for decreasing NH_3 in broiler houses continue to be a priority for safety, health, and poultry production efficiency. Variability of management, house environment, and birds make NH_3 emissions from broiler houses difficult to predict and monitor (Pearson and Stewart, 1993). The knowledge that NH_3 volatilization increases with increasing litter pH, moisture content, wind speed, and temperature can provide opportunities to better manage poultry facilities thereby reaching both environmental, health, and production goals (Delaune et al., 2004; Reddy et al., 1979).

The above factors affect NH_3 release in broiler houses by providing ideal or non-ideal environments for enzymes present in poultry manure to mineralize and decompose uric acid ($C_5H_4N_4O_3$) excreted by birds to urea ($CO(NH_2)_2$) and finally ammonium (NH_4^+) (O'dell et al., 1960) (Reaction 3.1). The solid NH_4^+ is converted to gaseous NH_3 as the pH of litter is raised from continued bird excretions. Moisture and temperature of litter are important in this process and are positively correlated with NH_3 release (Sims and Wolf, 1994).

$$CO(NH_2)_2 + H^+ + 2H_2O \longrightarrow 2NH_4^+ + HCO_3^- \qquad \text{(Reaction 3.1)}$$

Temperature and moisture can be partially controlled with proper ventilation (Gay and Knowlton, 2009). Ventilation moves air through poultry houses cooling birds and litter while releasing heat and moisture from the building. Several types of ventilation exist and management depends on season, type of building, floor, number and age of broilers, and waste handling system (Moore, 1986). The most widely used type of ventilation is an exhaust system which involves fans that expel air from inside the building in turn creating a lower than atmospheric pressure inside the broiler house thereby pushing out heat and moisture. Under this category is tunnel ventilation which uses exhaust fans located on one end of a poultry house and inlet openings are placed on the opposite end. Air is drawn in and down the house keeping velocity at bird level fairly constant and effectively cools birds while lowering in house NH_3, litter moisture, and dust (Bucklin et al., 1998). Though it is effective and necessary to ventilate, energy use can greatly increase particularly during winter months. It is not cost effective to continuously run fans during winter months due to fans drawing out heat needed to warm the birds and the need to use heaters to re-heat the house. Therefore, due to decreased ventilation, NH_3 concentrations tend to be higher during winter months (Ritz et al., 2009). In cases such as this, litter management becomes an even greater priority.

In addition to supplementing ventilation, litter management can increase fertilizer potential of litter with retention of N as NH_4^+; which is nonvolatile (Blake and Hess, 2001; Choi and Moore, 2008; Miles et al., 2011). By keeping N nonvolatile with cheap absorbent bedding material and chemical amendments, NH_3 emissions can be decreased. Several litter amendments are available commercially and are preferential based on economics, increasing composition of litter as a fertilizer, use as a Best Management Practice (BMP) to decrease odors and NH_3 emissions, or decreasing foodborne pathogens (Blake and Hess, 2001).

Acidifiers are the most common type of litter amendments. The pH of litter in broiler houses is positively correlated with NH_3 concentration and typically ranges from 8-10, therefore acidifying litter can lower NH_3 emissions (Blake and Hess, 2001; Parsons, 2006). Dry acid applications to bedding or litter lower the pH and thus decrease NH_3 release with two modes of action (Aneja et al., 2008; Madrid et al., 2012; Parsons, 2006; Rothrock et al., 2010). First it decreases urea enzymatic degradation, second it keeps NH_3 as NH_4^+ which is a solid rather than a gas (Madrid et al., 2012). Though neither can be completely replaced by the other, amendments can be cost beneficial by lowering the need for ventilation and heating.

The commonly used acid treatments in the poultry industry include Klasp ($Fe_2(SO_4)_3$), Al Clear ($Al_2(SO_4)_3$), and Poultry Litter Treatment (PLT , $NaHSO_4$). These treatments are generally applied once per flock, to the brood end before bird placement. For example, PLT is typically applied once directly before broilers are released in house at a rate of 24.4 kg/100 m^2 (50 lb/1000 sq ft) (Blake and Hess, 1997; Jones-Hamilton, 2010; Tasistro et al., 2008). The treatment lowers the pH and converts NH_3 to NH_4^+ and finally forms $(NH_4)_2SO_4$ (Blake and Hess, 1997). In addition to effects on NH_3, PLT has other benefits such as decreases to fuel usage, *Salmonella*, *Campylobacter* and beetle populations, while increasing bird performance (Blake and Hess, 2001).

Chemical treatments are not effective infinitely, as birds continue to excrete, the pH and subsequent NH_3 volatilization will rise. Due to acid chemical treatments being applied with a tractor when poultry houses are empty of birds, reapplication during flocks to keep NH_3 at bay is not possible. However, if an effective method for reapplication could be developed, it would be expected that the pH could be kept at a point that keeps N in the NH_4^+ form (Blake and Hess, 1997). An overhead PLT reapplication system has been evaluated on a small scale, however other locations and large production farms have not been investigated (Li et al., 2013). Therefore, the objective of this project was to assess the ability of this system to function on a large scale broiler operation in Virginia by observing the effects on NH_3 concentration and litter properties.

Materials and Methods

House Management

Two identical broiler houses were selected in the Shenandoah Valley of Virginia. Houses were 19 x 192 m (60 x 600 ft), equipped with 1.4 m (54”) choretime galvanized hyflo sidewall fans (6 each side), and two end wall 1.4 m (54”) choretime galvanized hyflo tunnel exhaust fans. Fan run time was based on temperature, humidity and NH_3 levels and was identical in both houses throughout the experiment. Cool cells measuring 30.48 m were on each side wall on the brood end of both houses.

A total of five flocks were observed. Data was collected for two flocks pre-cleanout from January 2013 - May 2013 (named flock 1 and 2), one flock directly after cleanout (flock 3) and three flocks post cleanout (named flocks 4, 5 and 6) from September 2013 - May 2014. The study was initially begun on three-year-old litter with unknown past ventilation, temperature, and bird management, although the farmer used the same management standards for both houses. Data was collected for two flocks grown on the old bed of litter and house management in the two houses was identical during that time. It was then deemed necessary by the farmer to start Flock 3 with fresh bedding in both houses, so both houses were completely cleaned out prior to Flock 3. Complete cleanout consists of removing all the previous litter and bringing in new bedding. Bedding material consisted of 50% peanut hulls and 50% wood chips and was initially applied 7.62 cm (3 in) deep. After bedding was spread, chicks were brought in and kept on the brood end where the chicks stayed for two weeks until the farmer decided they were big enough to open the whole house. Broilers were raised to approximately 3.4kg (7.5 pounds) over 56 days. Before each new flock, the top layer of litter (cake) was removed. Other than PLT reapplication, both houses received the same house management regardless of in-house variation of temperature or NH_3 levels. Flock 3 had the basic pre flock PLT treatment at 24.4 kg/100m^2 (50 lb/1000 sq ft) in both houses, but no additional applications were done in order to confirm both houses being used were under the same management. Flock 4 begins PLT reapplication post cleanout.

Treatments

Treatments were assigned randomly. House 1 was designated as the control house and received the industry standard 24.4 kg/100m^2 (50 lbs/1000 sq ft) on the brood end 24- hours before chick arrival. House 2, the PLT reapplication house, had an initial application of 24.4 kg/100 m^2 on the brood end 24- hours before chick arrival, and two whole-house reapplications of 24.4 kg/100 m^2 at week 3 and 5. An over-head auger reapplication system was utilized for the whole-house application (Fig. 3.1.).

For each reapplication, twenty-five 23 kg (50-lb) bags of PLT were placed one at a time in each hopper outside the house for an application rate of 24.4 kg/100 m^2. As shown in Fig. 4.1, two separate augers carried the PLT into the house and split it into “T”s. As the PLT reached each spinner position, it dropped through a hole in the auger pipe onto the rotating surface, spreading the chemical in a circular fashion. Spinners were attached below the auger. After application, two 18.93 liter (5-gallon) buckets of feed were run through the system for cleaning of the auger from residual PLT and provide oil for the system.

Data acquisition

The concentration of NH_3 exiting both poultry houses was continuously measured using a 600 Fourier Transfer Infrared (FTIR) spectrometer (California Analytical Instruments Inc., Orange, CA). Star samplers delivered air samples from four end wall fans (two from each house) via tubing to a switchbox by vacuum pumps (Fig. 3.2). Ambient air just outside the control room was also measured for comparison. The switch box was utilized to alternate samples with purge air in six minute intervals so only one line was run through the FTIR at a time. Before reaching the switch box, samples were run through two Whatman GF/D 90 mm glass microfibre filters

(GE Healthcare) and one 47 mm 1.0 µm pore size PTFE membrane filter (Pall Life Sciences) (Fig. 4.3) to remove particulate matter. The filters were located in a constructed wooden cabinet inside each house (Fig. 3.2). After air samples were processed by the FTIR, information was transferred and recorded automatically to a computer using the OPUS program to (Bruker Optics Inc., Billerica, MA). Equipment was located in a small "control room" attached to the PLT reapplication house (Fig. 3.2).

Due to data acquisition issues with the FTIR, mainly caused by power fluctuations and software glitches, NH_3 concentrations (mg/L) were not measured continuously throughout the study. Flock 2 and flock 4 (4 PLT reapplications) were compared for PLT reapplication effects on NH_3 concentrations during the four hours before PLT reapplications, during the applications, and two hours immediately post application.

Litter Sampling and Analysis

Litter samples were collected at 2.54 cm (1 in.) in depth. Three subsamples were collected and mixed to make one sample. Samples were collected around waterers, feeders, and sidewalls from both the brood and opposite ends of houses. Six samples per house (from 18 subsamples) were placed in plastic bags. These representative litter samples were taken for flocks 1-6 before flock arrival, after flock removal, as well as immediately before and after PLT application from the PLT reapplication house. In addition, litter samples at representative points were collected before flock arrival, after flock removal, and during the PLT application from the control house. Samples were placed in plastic bags, labeled appropriately, and placed in a refrigerator until analysis was done no more than 48 hours later (Ritz et al., 2009). Moisture content was determined by percent change in weight after drying litter at 105 ^{o}C for 24 hours (Peters et al., 2003). Litter pH was analyzed with a 2:1 deionized water to litter ratio and pH meter (Senyondo, 2013). Litter moisture % and pH were determined for flocks 1-6. Litter was analyzed for NH_4-N for flocks 4-6 post cleanout using a 1:10 2*M* KCl extraction, shaken for 30 minutes. Extractions were then allowed to settle and filtered through Whatman #42 filters. Filtered samples were analyzed using the salicylate method by Lachat analysis for NH_4-N (Lachat Instraments, 1992). Finally, Total-N for flocks 4-6 post cleanout was determined using vario Max C/N-Analyzer by combustion (Elementar Americas, Mt. Laurel, NJ). Analysis was done on an “As Is Basis”.

Statistical Analysis

For statistical analysis samples were separated by house and not identified based on positioning within house. A completely randomized design was used for this study with each house as an experimental unit. Paired t tests were done using JMP Pro 10 statistical package (SAS Institute, 2012). Statements of statistical significance were accepted at $p \leq 0.05$. The statistical model was $y_{ij} = \square + \square_i + e_{j(i)}$ where:

- y_{ij} is the dependent variable (pH, Moisture, nitrogen, NH_3) on the j^{th} house with treatment *i*
- $\square\square$is the overall average of the dependent variable.
- $\square_i$ is the average effect of treatment *i* on dependent variable
- $e_{j(i)}$ is the residual effect of the j^{th} house receiving treatment *i* on a variable

Results and Discussion

Litter Moisture and pH

Flock 3 begins the first flock post-cleanout, with both houses having the same one pre-flock PLT application. Litter analysis after this flock determined the control house was not significantly different than the reapplication treatment house for litter pH, litter moisture, or litter NH_4-N content before treatments were initiated (Table 3.1).

At the conclusion of the study, after flock 6, moisture of litter was significantly greater in the reapplication house at 34.2% than both its initial 25.7% and the control house at 21.4% ($P>0.0004$) (Table 3.1). The PLT attracting more moisture into the litter is consistent with the hygroscopic properties of PLT (Sun et al., 2008). After flock 2, the farmer reported a need to increase ventilation due to NH_3 production in the PLT reapplication house, removal of more cake between each flock from the PLT reapplication house, and decreased bird performance. Excess moisture in litter produces more cake, allows for more NH_3 production, and can lead to decreased bird performance so his report was consistent with analyzed litter samples. In order to reduce moisture levels and remove NH_3 from in house, continuous air flow is suggested (Esmay, 1978). Increased air circulation is correlated with better weight, survival and decreased littler moisture and caking, presumably improving the ability of the litter to absorb or trap uric acid or NH_3 as solids before volatilization occurs (Weaver and Meijerhof, 1991). Increased moisture results observed in this study are similar to that of a previous reapplication study, where PLT reapplication increased litter moisture from 20.2 to 23.4% (Li et al., 2013).

Houses had no significant differences in pH directly preceding treatment applications in flocks 1, 2, and 4-6 (Table 3.1). On the other hand, the reapplication house showed an immediate significant drop in litter pH by at least 0.10 units in flocks 1 and 2 and at least 0.82 units in flocks 4-6 following PLT reapplication using the overhead application (Table 3.1). Additionally, in each case the drop in pH was significantly less than the litter pH in the control house ($p<0.001$) however, after each flock was removed, there was no significant difference in litter pH between the two houses indicating PLT is not effective over longer periods of time (Table 3.1). The immediate drop in litter pH following application is consistent with using an acidifier; however the amount of manure excreted every day by growing birds in the houses was too great for PLT to maintain a lower litter pH. Enzyme activity is inhibited by low pH and degradation of uric acid that leads to NH_3 volatilization increases as pH raises (Blake and Hess, 1997). Higher pH in combination with the higher moisture content of PLT reapplication, can lead to increased NH_3 emissions (Vlek and Carter, 1983). Li et al. (2013) applied PLT every two weeks to litter in a field study and reported opposing results, suggesting repeated application of PLT to litter decreases the pH until the end of the flock from 8.05 to 7.50 (Li et al., 2013). However, it should be noted the control in that study, which received no PLT reapplication treatment, also showed a decrease in litter pH at the end of the flock from 8.12 to 7.65 indicating the reduction may be due to other house management areas such as ventilation. Moreover, Li et al. (2013), completed the study on a smaller farm with 1/10 the number of birds which provides a less humid environment than what is being discussed in this paper. Lower humidity leaves litter drier and more manageable as well as extending the PLT reaction time due to less water being available to accumulate as fast, neutralizing the effects. In addition, lowering the pH of litter has the ability to increase the N content in litter and potential fertilizer efficacy (Blake and Hess, 1997).

Litter Nitrogen Concentration

Total-N is the measure of all forms of N found in the litter and can be used to assess possible runoff issues from use as a fertilizer therefore, it is beneficial to observe the effects of PLT reapplication on Total-N content of litter over time. Litter Total-N was analyzed after the first flock following cleanout (Flock 3) and the three following flocks. Following Flock 3 both houses had Total N of 31.2 g/kg, and there was no significant difference in NH_4-N, showing that there was no extraneous factor affecting litter total N, and both houses had the same baseline litter. After flock 6, which included 6 additional PLT treatments in the reapplication house, Total-N of litter was not significantly different in the control house at 38.6 g/kg when compared to 42.5 g/kg in the reapplication house. Total-N did increase after every flock in each house and may provide fertilization opportunities (Table 3.2).

After Flocks 4 and 5 there were no significant differences in NH_4-N between houses. However, after Flock 6, the reapplication house had a significantly greater litter NH_4-N content of 15.28 g/kg than the control which had 13.24 g/kg ($P=0.003$) (Table 3.2). The NH_4-N is important for plant uptake when litter is used as a fertilizer, as all NH_4-N in manure that doesn’t volatilize is considered plant available (DCR, 2014). Both Total-N and NH_4-N content of litter increased in a previous smaller scale farm and laboratory experiment suggesting reapplication of PLT can be effective at increasing possible use as a nitrogen fertilizer (Li et al., 2013).

Air Analysis

Due to instrumentation issues, limited air quality data was collected. However, complete NH_3 data collection and analysis was done one hour before PLT application, during application, and beginning two hours after application for flocks 2 and 4. Before PLT reapplications, the houses were not significantly different in NH_3 concentrations at the end wall exhaust fan (Fig. 3.4). In contrast, during the time the overhead system was utilized, the reapplication house had significantly less NH_3 (9.80 mg/L) than the control (17.41 mg/L) (P=0.028). After two hours, there was again no significant differences in mean NH_3 concentrations in the two houses. One application of PLT pre-flock has been shown to decrease NH_3 concentrations when young birds are introduced (Gholap et al., 2012). However, the lack of significant reductions by reapplication PLT suggests PLT is not effective for long periods of time on large scale operations when birds are larger.

System Review

During this study, several problems were encountered with the reapplication system.

Corrosion of Metal

The farmer had been spreading PLT before flock arrival for years using a spinner spreader connected to the back of a tractor at the recommended rate of 24.4 kg/100 m^2. During this application, the feeders were down, but the PLT bounced off and caused no corrosion of the metal. However, during this study when the re-application was performed, birds were present causing humidity to be higher due to moisture from their breath and defecation. This higher humidity led to the PLT sticking to moisture on the feed lines, and caused corrosion. When PLT was spread during reapplication it settled on metal heaters and feed lines, which caused corrosion (Fig. 3.5). The feeder flood cable made of actuator wire was also damaged and needed replacing. Plastic components such as water lines were not affected negatively.

After three flocks, metal corrosion became apparent so plastic sheets were made to fit over the heaters and secured with clothes pins. Heaters were turned off previous to covering, then raised after they were secured in order to minimize potential settling of PLT (Fig. 3.6). Coverings needed to be removed and cleaned after each application due to buildup. In addition, inverted plastic rain gutters were placed over feed lines prior to each application, then removed and cleaned following applications (Fig. 3.7). Coverings were not fully effective in preventing damage to heaters and feed lines, but did slow down corrosion greatly. Approximately three hours were spent on setup, takedown, and cleaning of these covers for each PLT reapplication. Upkeep and labor for these covers was time consuming and impractical in a non-research setting, as well as not completely halting corrosion.

Problems with Auger and Spinner Distribution System

After several applications, excessive shaking of the reapplication system occurred and the auger “kicked” in and out of the PVC pipe that housed it, before PLT was run through. Movement was severe enough to stop application and remove the auger for inspection. It was observed the most accumulation occurred towards the end of the system. It was determined that post application feed being run through the system was unable to completely clean out the PVC piping, due to visible buildup within the pipe of what appeared to be a mixture of PLT and feed. It was assumed feed was falling through the drop holes above spinners before having a chance to reach the end of the auger. The mix of feed and PLT remaining in the auger pipe was able to accumulate and draw in moisture through the drop holes in the pipe, forming a hard layer around the augers (Fig. 3.8). Brood end augers were removed from the piping and cleaned before being replaced. On the opposite end, augers were too damaged to be cleaned, so they were replaced with new ones. At this point, holes above spinners were closed with slide-able plastic covers in order for the complete length of the PVC to be cleaned with feed. They remained in place until the next application to prevent moisture from entering the PVC and combining with any feed-PLT mix left in the pipe. Slides were only removed from holes for PLT applications. The plastic slides were not tested for extended flocks due to time restraints, but during the last three reapplications they seemed effective. Spinners had to be shut off for safety reasons previous to sliding covers off/over holes. In order to reach the slides, a ladder was carried the length of the system and someone manually moved them over each hole. This process added to the length and labor of the reapplication system.

Blown Fuses

Spinners had fuses blow throughout the study. There was no order or timing of the fuses blowing, however a fuse was blown at every application. When spinners stopped working, PLT was dumped out of the holes straight down forming a large pile rather than spreading it in a circle as shown in Fig. 3.1 (Fig. 3.9). When this occurred, the system had to be stopped so the fuse could be replaced. There was no solution worked out to solve this problem, other than early detection and replacing the blown fuse. This required two people in the house while someone put in PLT bags to the hopper outside the house. One person would walk up and down the spinners looking for problems, and another stood by the shut-off switch for quick reaction. The need for three workers would be unrealistic for a farmer, or again add costs, as they often work alone.

Uneven PLT Application

The PLT must drop out of the hole and onto the center of the spinners in order to spread in even overlapping circles through the house (Fig. 3.1). Excessive shaking of the auger system due to caking on the auger described above, led to twisting of the PVC piping and the spinners that were attached to it (Fig. 3.10). Therefore, neither spinners nor holes were aligned correctly, which caused uneven application and required additional maintenance (Fig. 3.11).

Damage to Ceiling

Holes were found on the ceiling above some spinners from PLT hitting and/or becoming crusted on and eating through the material (Fig. 3.12). This was a non-metal triply material, so it was not metal corrosion as noted above. These holes had to be patched using tape to maintain ceiling integrity.

Short Circuit

Eventually, fine PLT was deposited on the electrical outlets for the auger motors. The electrical outlets were on the ceiling well away from the spinners, so initially it was thought they were safe from corrosion. However, from inspection it was obvious that corrosion had occurred. It was concluded that the auger system had ground some PLT and this formed dust that drifted rather than being granulated PLT that was thrown by the spinner. The PLT dust then settled on the outlets and reacted with humidity causing corrosion of the metal sockets and a short circuit melting the area directly above the motor (Fig. 3.13). A similar situation was also observed on an identical PLT system on the Eastern Shore, VA, where light sockets short circuited (Fig. 3.14).

Conclusions

PLT is an acidifying litter amendment that has hygroscopic properties; which are the main reasons for hardware problems. Repeat applications of PLT caused a temporary drop in litter pH after application; however, after each flock no significant treatment effects from PLT were observed. Moisture of litter was significantly increased by reapplications of PLT, which is undesirable. Reapplications were expected to significantly decrease NH_3 concentrations throughout the flock; however, due to moisture being positively correlated with NH_3, this was not the case except for during the PLT application. The overhead PLT reapplication system had several problems including blown fuses, uneven application, damage to ceiling, and short circuits. For many years, PLT has been successfully used to decrease NH_3 in poultry houses, by applying one application before birds are placed in houses. However, the greater humidity in houses while birds are present and the hygroscopic and acidic nature of PLT caused corrosion of metal fixtures when PLT was reapplied during growth of the birds using the tested application system. The concept of reapplications of litter amendments during a flock should be further studied, however this system needs modifications and improvements in order to decrease maintenance and time involvement as well as damage to equipment.

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Table 3.1. Comparisons of litter pH and moisture% throughout the study.

		pH			Moisture%		
		PLT Reapplication		Control	PLT Reapplication		Control
	Date	Before	After		Before	After	
Pre Cleanout				Flock 1			
Before flock	1/31/13	8.56a		8.23b	29.90a		36.50b
Reapplication 1	2/25/2013	8.55a	7.90b	8.60a	32.00a	28.00a	33.10a
Reapplication 2	3/8/2013	8.50a	7.86b	8.58a	41.00a	31.80b	33.30b
Between Flocks	3/26/2013	8.64a		8.64a	41.50a		31.70b
				Flock 2			
Reapplication 1	4/26/2013	8.69a	7.60b	8.77c	39.50a	37.90a	37.40a
Reapplication 2	5/9/2013	8.00a	7.81b	8.63a	25.30a	23.60a	33.90a
After Flock	5/28/2013	8.73a		8.66b	33.84a		34.82a
Post Cleanout				Flock 4			
Before flock	10/18/13	8.59a		8.60a	26.90a		28.20a
Reapplication 1	11/14/2013	8.69a	7.03b	8.69a	28.82a	28.41a	22.50b
Reapplication 2	12/3/2013	8.65a	7.56b	8.71c	26.04a	25.32a	13.38b
Between Flocks	12/21/2013	8.57a		8.57a	25.7a		23.60b
				Flock 5			
Reapplication 1	1/23/2014	8.49a	6.46b	8.63a	29.00a	29.36a	30.35a
Reapplication 2	2/20/2014	8.64a	6.56b	8.60a	29.61a	26.87a	22.78a
Between Flocks	3/7/2014	8.32a		8.65a	28.50a		26.30a
				Flock 6			
Reapplication 1	4/9/2014	8.24a	6.95b	8.24a	31.22a	29.99a	27.77a
Reapplication 2	4/25/2014	8.57a	7.75b	8.62a	28.77a	30.55a	28.20a
After Flock 6	5/15/2014	8.55a		8.51a	34.20a		21.40b

†Different letters indicate significant difference between values for pH or moisture across rows at $P \leq 0.05$.

Table 3. 2. Comparison of Total-N and NH_4-N in litter post cleanout determined on an “As Is Basis”.

	Date	Total-N		NH_4-N	
		PLT Reapplication	Control	PLT Reapplication	Control
		--------------------------------------mg/kg--------------------------------------			
After Flock 3‡	10/18/13	31.2a	31.2a	10.12a	9.09a
After Flock 4	1/10/14	30.3a	30.2a	10.52a	10.25a
After Flock 5	3/17/14	34.5a	34.1a	5.08a	4.33a
After Flock 6	5/15/14	42.5a	38.6a	15.28a	13.24b

†Different letters indicate significance across rows at $p \leq 0.05$

‡ Flock 3 was grown on fresh litter with only one pre-flock application of PLT in both houses (no reapplication).

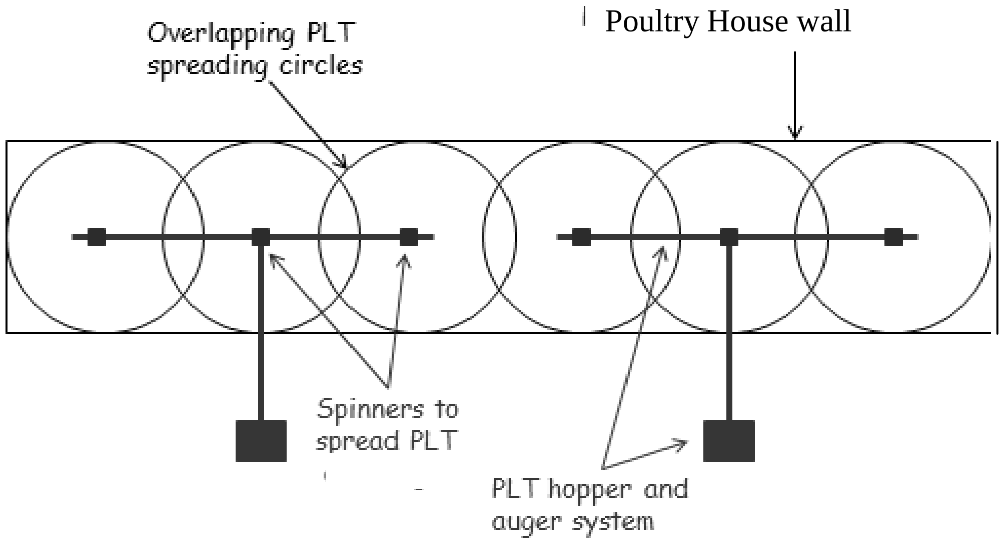

Figure 3.1. PLT reapplication schematic of the auger and spinner system installed in House 2, not drawn to scale. There were 8 spinners on each auger system for a total of 16.

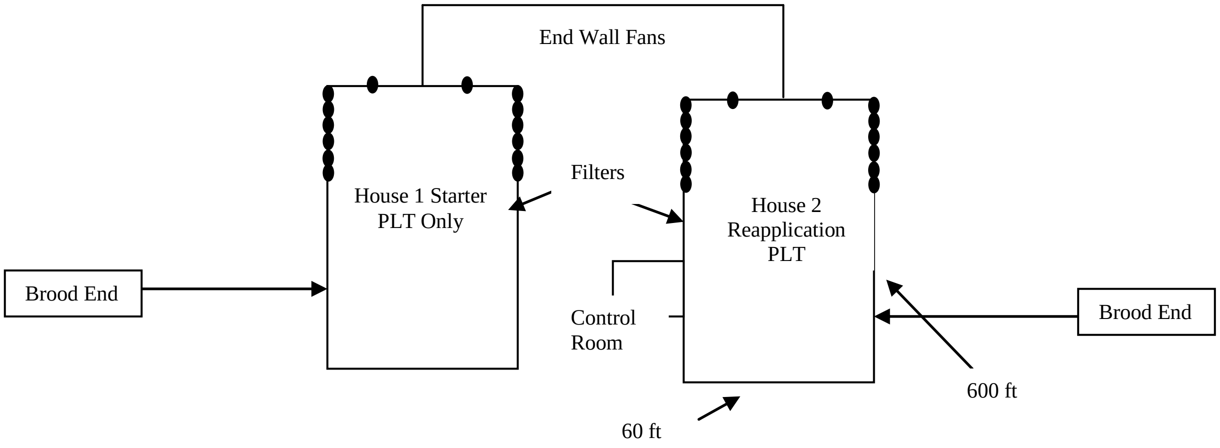

Figure 3.2. Layout of Broiler Houses and Air Sampling Mechanism

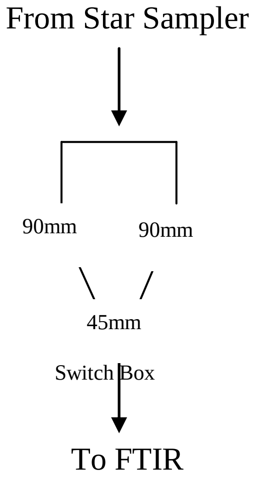

Figure 3.3. Filter setup

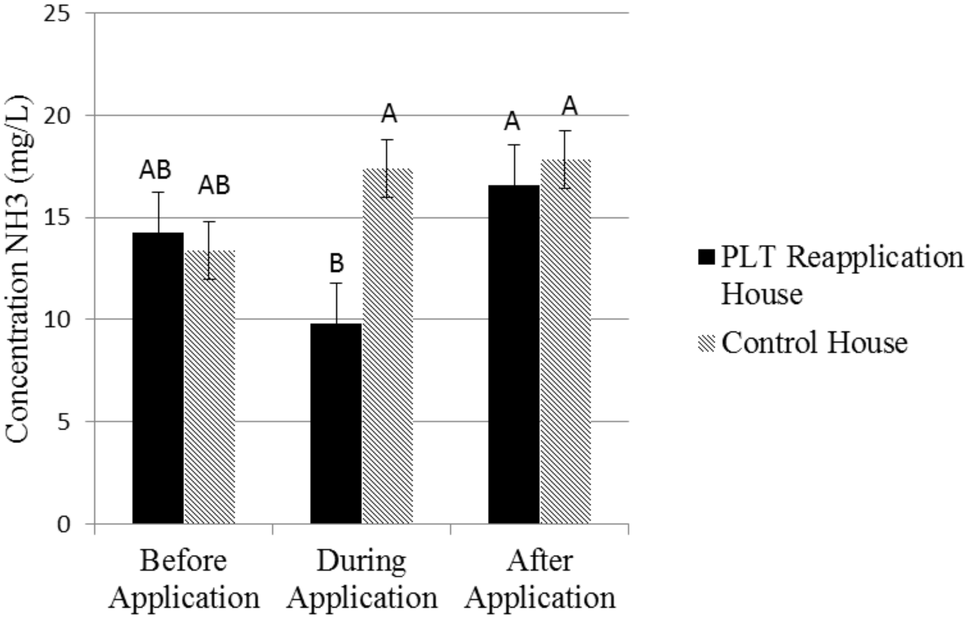

Figure 3.4. Ammonia concentration (mg/L) at each stage of PLT reapplication. Flocks 2 and 4 (4 reapplications) were combined. “Before Application” represents the four hours previous to PLT reapplications. “During Application” represents the time from initiation to end of the PLT reapplications. “After Application” represents the time from two hours post PLT application until 6 hours post application.

† Letters indicate significance at $p \leq .0.05$

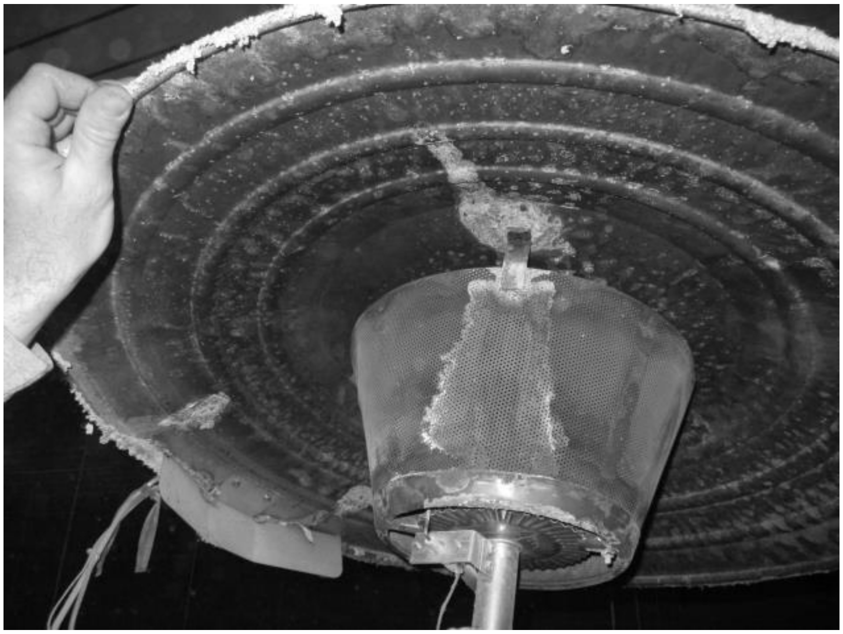

Figure 3.5. Damage to heater from PLT in reapplication house.

Figure 3.6. Plastic covering over heater in reapplication house.

Figure 3.7. Plastic gutter over feed lines in reapplication house.

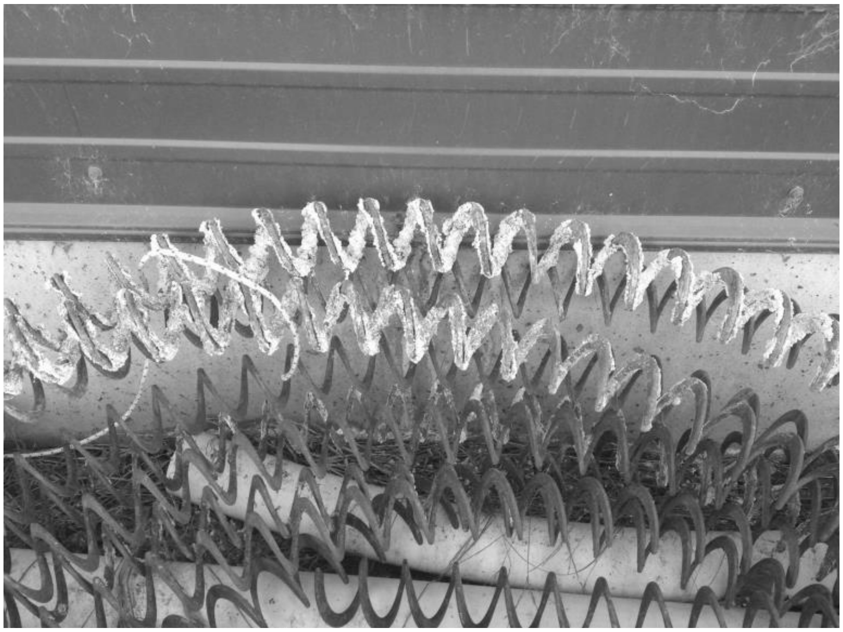

Figure 3.8. Buildup on auger removed from reapplication house.

Figure 3.9. PLT deposit below spinner after fuse blowout in reapplication house.

Figure 3.10. Spinner tilt causing distorted spread in reapplication house.

Figure 3.11. Distorted spreading of PLT in reapplication house.

Figure 3.12. Holes in the ceiling above a spinner in reapplication house.

Figure 3.13. Melting of ceiling in reapplication house.

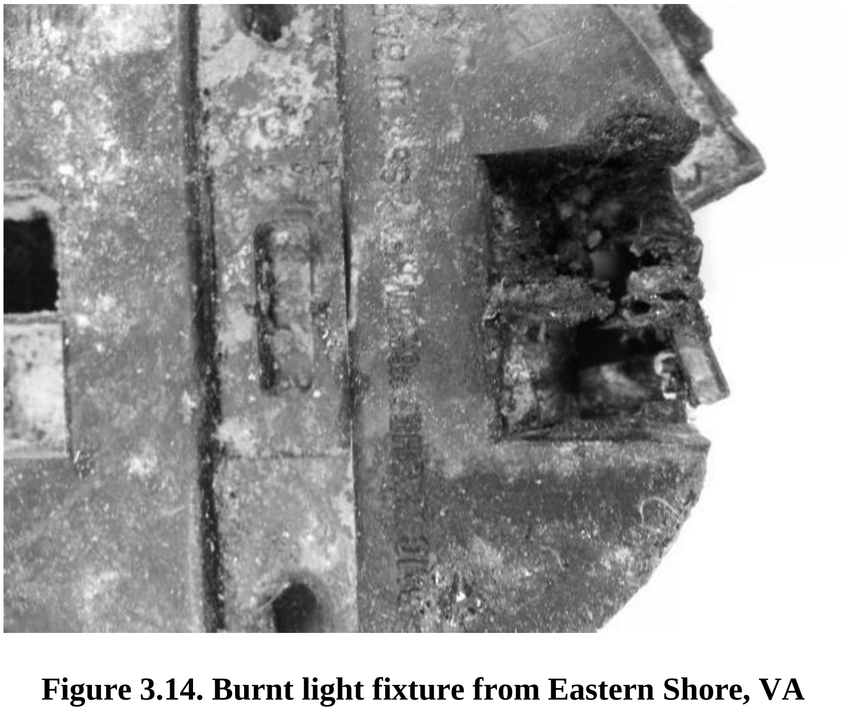

Figure 3.14. Burnt light fixture from Eastern Shore, VA

CHAPTER 4: EFFECT OF SODIUM BISULFATE (PLT) REAPPLICATION SYSTEM ON *E. COLI* PRESENCE IN A COMMERCIAL BROILER HOUSE

Abstract: Poultry litter has a ubiquitous microbial bacterial presence. *Escherichia coli (E. coli)* is of particular concern in litter due to its negative health effects in both birds and humans. Chemical amendments such as poultry litter treatment (PLT, sodium bisulfate) can be used to control microbial populations in poultry litter; however, they are currently applied only once before flock arrival and are not effective indefinitely. This paper analyzes the effect of a new auger and spinner system that applies several additions of PLT to poultry litter throughout the growth of three flocks on *E. coli* coliforms, pH, moisture, and nitrogen (N) in litter. Litter properties for a total of six reapplications over three flocks were compared between a reapplication house and control house which received no PLT reapplications. Reapplication of PLT temporarily reduced litter pH and *E.coli*; however, by the end of each flock, neither pH nor *E. coli* was significantly affected by PLT reapplication. Litter moisture was greater in the reapplication house when compared to the control after three flocks, because of PLT's hygroscopic properties. The N content of litter increased after three flocks with PLT reapplication and would therefore increase the litter fertilizer value. Although short-term benefits were seen in this study, there is no indication that PLT reapplication with this system and application timing provided long-term reduction of *E.coli* in litter. However, short term reductions in *E. coli* indicate a PLT application immediately before bird removal may reduce *E.coli* export.

Keywords: *Broilers, E. coli, PLT, Sodium Bisulfate*

Introduction

Escherichia coli (*E. coli*) is a gram-negative, rod-shaped, facultative anaerobe that is found naturally in the intestinal tracts of organisms (Williams, 2012). Many strains can be beneficial or harmless to hosts; however, several are pathogenic and are capable of causing disease (CDC, 2014). Due to its ubiquitous presence, *E. coli* is used as a sign of fecal contamination in surface waters (Harmel et al., 2013). Human or animal fecal matter can contribute bacterial pathogens to waterways by both point and non-point sources. Some major point sources include sewers and waste water treatment plants that are easier to monitor and control. Nonpoint sources, on the other hand, are predominantly from agriculture and are unpredictable. Agricultural sources can include both runoff from land application of manures or directly from livestock farms (Chen and Jiang 2014; Erickson et al., 2004; Wilkinson et al., 2011).

E. coli colonies in poultry houses comprise approximately 12.5% of the bacteria in litter; however, due to variations in broilers, excretion rates, feed, and day-to-day house management changes, colonies are hard to pinpoint and are not uniform throughout a house (Lu et al., 2003; Williams, 2012). Even so, only a portion of *E. coli* in litter is pathogenic, and these strains can be difficult to find. The possibility of contracting infections from pathogenic *E.coli* increases with decreased ventilation and increased pH, moisture, and dust counts in the house (Homidan et al., 2003; Pope and Cherry, 2000). As the in-house infection potential increases, so does the possibility of waterway or processing plant contamination. Commercially raised birds are in constant contact with litter and can hold bacteria on their feathers, feet, and skin, allowing the easy transfer of infectious bacteria (Pope and Cherry, 2000).

In Virginia, many large-scale poultry production facilities operate in a concentrated area because of economies of scale and close proximity to feed mills and processing plants. Though this helps in transportation and production costs, the large quantities of litter from intensive flock growth can be problematic when considering storage and disposal. Fortunately, poultry litter from these farms can be recycled and used as fertilizer on agricultural lands (Campbell-Nelson and Herbert, 2009; Sims and Wolf, 1994; Wilkinson et al., 2011). Litter is made up of bedding material, poultry excreta, feathers, and spilled feed (Chen and Jiang, 2014; Espinoza et al., 2007). Application of litter onto soils for crop growth is effective as a fertilizer (Funderburg, 2009; Soupir et al., 2006). Although there are benefits of litter application to agricultural fields, fecal contamination of food and aquatic environments is a concern (Campbell-Nelson and Herbert, 2009; Edwards and Daniel, 1993; Harmel et al., 2013; Sims and Wolf, 1994; Soupir et al., 2006).

Some treatments exist for the reduction of pathogens in litter; however none are 100% effective. Chemical amendments to litter have been effective in the past for microbial reduction and may provide a way to decrease *E. coli* in litter, thereby lowering the possibility of bird, water, or food contamination (Pope and Cherry, 2000). Several chemical additives exist, such as acid amendments that lower litter pH below 8 are the most common and are able to decrease microbial enzymatic activity, ammonia (NH_3) emissions, and dust particles (Aneja et al., 2008; Blake and Hess, 2001; Delaune et al., 2004; Madrid et al., 2012; Parsons, 2006; Rothrock et al., 2010). In fact, acid amendments can lower NH_3 concentrations in the air and be beneficial for decreasing heating and ventilation costs in houses due to less fan run time in addition to increasing bird productivity (Madrid et al., 2012).

Poultry litter treatment (PLT, $NaHSO_4$) is a common acidifier used to decrease pH of litter. It is currently applied to litter before flock arrival on only the brood end of a house at a rate of 24.4 kg/100 m^2 (50 lb/1000 sq ft) (Blake and Hess, 1997; Jones-Hamilton, 2010; Tasistro et al., 2008). When PLT is applied to litter, it forms ammonium sulfate [$(NH_4)_2SO_4$] and prevents the release of NH_3 (Charles and Payne, 1966; Blake and Hess, 2001). Reducing NH_3 emissions improves air quality in and around broiler houses. In the past, bird performance has improved and microbial activity has decreased with the addition of PLT (Charles and Payne, 1966; Blake and Hess, 2001).

As birds grow and defecate, the initial application of PLT is no longer beneficial for keeping microbes or NH_3 under control as acidic properites are reduced. The PLT is safe to apply while broilers are present; however, the current method of application with a tractor does not allow spreading after bird arrival. If PLT can be applied several times throughout a flock cycle, it may be possible to render litter less hospitable to pathogens, improve bird health, and ensure food safety (Blake and Hess, 1997). For this reason, a system was developed to apply PLT several times during a flock. This system spreads the chemical overhead to the whole house rather than just the brood end, which should allow for a greater decrease in NH_3 volatility from litter. A small-scale study has been done using this equipment; however, the effects on *E. coli* in a large-scale broiler house needs to be evaluated (Li et al., 2013).

Based on the potential risks for broilers and land application of litter, solutions for decreasing *E. coli* in poultry litter need further study and may lead to better poultry and environmental health. The objectives of this project were to assess the ability of a PLT reapplication system in whole-house trials to reduce litter *E. coli*, and to study the impact on the litter properties pH, moisture, and N on a large-scale broiler operation.

Materials and Methods

House Management

Two broiler houses measuring 19 × 192 m (60 × 600 ft) were used in this study. The houses were in the Shenandoah Valley of Virginia and were identical in size and management. Houses had six 1.37m (54”) Chore-Time galvanized HYFLO® fans on each sidewall, and two end wall 1.37m (54”) Chore-Time galvanized HYFLO® tunnel exhaust fans opposite to the brood end. Temperature, humidity, and NH_3 levels determined fan run time, which was monitored by the farmer. The fan run time, and thus ventilation rate, was kept identical in both houses regardless of treatment effects. Cool cells measuring 30.5 m were on each side wall on the brood end of both houses.

Houses were cleaned out previous to study initiation, which involved bedding and litter removal, to eliminate variability between houses. Fresh bedding composed of 50% peanut hulls and 50% wood chips was applied to a depth of 7.62 cm (3 in) after cleanout. The first flock after complete cleanout had the recommended pre-flock PLT treatment at 24.4 kg/100 m^2 (50 lb/1000 sq ft) in both houses; however, there were no additional applications to ensure that the houses were under the same management. The intention of this first flock was to prove both houses were statistically identical and any effects on litter properties were due to PLT treatments and not affected by selection of the house. The PLT reapplication treatment post-cleanout began with flock 2.

For each of the flocks observed, chicks were placed in the brood end of the house for 2 weeks before the whole house was open to them. Birds were removed after about 56 days when they reached an approximate weight of 3.4 kg (7.5 pounds). Between each flock, houses were decaked by removing the top hardened moist layer of litter wherever the farmer deemed necessary. Treatments were assigned to houses randomly and reapplications of PLT was the only management variation between them regardless of in-house variations in NH_3 levels caused by the PLT.

Treatments

The control house received the recommended industry standard treatment of 24.4 kg/100m^2 (50 lbs/1000 sq ft) on the brood end 24 hours prior to flock arrival. The PLT reapplication house had the same initial application of 24.4 kg/100 m^2 on the brood end 24 hours before chick arrival and two additional whole-house reapplications of 24.4 kg/100 m^2 at weeks 3 and 5 of the birds' growth cycle using an overhead auger and spinner reapplication system (Fig. 4.1).

More specifically, for each application, 25 bags of PLT weighing 22.68 kg (50 lb) were placed one at a time in hoppers outside each end of the house. Fig. 4.1 illustrates where each auger carried PLT into the house and split into "T"s. As the PLT reached each rotating spinner position, it dropped onto the surface through a hole in the auger pipe, which spread the chemical in a circular fashion. Spinners were simple attached below the auger. Cleaning of the auger was done after each application by running two 18.93-liter (5-gallon) buckets of feed through the system.

Litter Sampling and Analysis

Litter was collected and analyzed for three flocks post cleanout from September 2013 to May 2014. Litter samples were collected at 2.54 cm (1 in.) in depth. Three subsamples were collected and mixed to make one sample. Samples were collected around waterers, feeders, and sidewalls from both the brood and opposite ends of houses. Six samples per house (from 18 subsamples) were placed in plastic bags. Samples were collected from the PLT reapplication house before flock arrival, after flock removal, as well as immediately before and after PLT reapplication. The same sampling protocol was used for the control house, but as there was no PLT reapplication only one sample was collected on that day, rather than the immediately before and after PLT reapplication samples collected in the reapplication house. Samples were immediately placed in a cooler with ice and analyzed within 24-hrs of collection for *E.coli*. Labeled plastic bags containing samples were stored in the freezer at -20°C prior to all other analysis.

Moisture content was determined by percent change in weight after drying litter at 105 ^{o}C for 24 hours (Peters et al., 2003). Litter pH was analyzed with a 2:1 deionized water to litter ratio and pH meter (Senyondo, 2013). *E. coli* colony-forming units (cfus) of litter samples were determined using the most probable number (MPN) method with Colilert® enzyme-substrate liquid-broth medium (IDEXX Laboratories, Inc., Westbrook, Maine). This included extraction, incubation at 35°C for 24 hours, and detection under a long-wave ultraviolet light (366 nm). Volume used in extraction and mass of litter differed for each sample due to coliform variations. Litter was analyzed for NH_4-N using 3g subsamples with a 1:100 2*M* KCl extraction ratio and shaken for 1hour (Bremner and Keeney, 1966). The extracts were then allowed to settle and filtered through Whatman #42 filters. Filtered samples were analyzed using the salicylate method by Lachat flow injection analysis for NH_4-N (Lachat Instraments, 1992). Finally, total N was determined by combustion using a Vario Max C/N-Analyzer (Elementar Americas, Mt. Laurel, NJ). Analysis was done on an “As Is Basis”.

Statistical Analysis

For statistical analysis samples were separated by house and not identified based on positioning within house. A completely randomized design was used for this study with each house as an experimental unit. Paired t tests were done using JMP Pro 10 statistical package (SAS Institute, 2012). Statements of statistical significance were accepted at $p \leq 0.05$. The statistical model was $y_{ij} = \square + \square_i + e_{j(i)}$ where:

- y_{ij} is the dependent variable (pH, Moisture, nitrogen, NH_3, *E.coli*) on the j^{th} house with treatment *i*
- $\square\square$is the overall average of the dependent variable.
- $\square_i$ is the average effect of treatment *i* on dependent variable
- $e_{j(i)}$ is the residual effect of the j^{th} house receiving treatment *i* on a variable

Results and Discussion

Litter Analysis

After the first flock post-cleanout, the litter pH, litter moisture content, *E. coli*, NH_4-N, and litter total N did not significantly differ between houses before treatment (data not shown). This demonstrated that both houses were identical at study initiation and any impacts on litter properties were due to treatment effects and not inherent to the houses.

Litter Moisture

There were no differences in litter moisture following each PLT reapplication in the reapplication house in every instance (Table 4.1). During and at the end of Flock 2, the reapplication house had a significantly greater litter moisture than the control house both before and after PLT reapplication ($p<0.0001$). By the first reapplication in Flock 3, there were no differences between houses regardless of PLT reapplication. After reapplying PLT to three flocks (6 PLT reapplications total), the control house had a significantly lower litter moisture content at 21.4% than did the reapplication house at 34.2% (*p*<0.0001) (Table 4.1). This result can be explained by PLT's hygroscopic properties, which causes water to be attracted to the litter where PLT is reapplied (Sun et al., 2008). Additionally, there was also a decrease in bird performance and weight in the reapplication house compared to the control house, as reported to the farmer by the poultry integrator (personal communication). The farmer thought this was linked to the higher moisture and NH_3 in the PLT reapplication house.

Reduction in litter moisture is important for decreasing NH_3 in houses and can be achieved by increasing air circulation or temperature, or through complete litter cleanout (Esmay, 1978; Kentucky, 2010). Solely increasing broiler house heat is not cost-effective for NH_3 reduction due to ventilation needs to supplement higher temperatures. Similarly, complete cleanout is expensive, labor-intensive, and generates more waste. To reduce moisture levels and remove NH_3 in-house, continuous air flow is suggested as the most beneficial option and can be used in combination with chemical amendments (Esmay, 1978). In fact, Esmay (1978) showed that increased air circulation is correlated with increased bird weight and survival as well as decreased litter moisture and caking. The benefits are presumably caused by an improvement in the ability of the litter to absorb or trap uric acid and NH_3 as solids before NH_3 volatilization occurs (Weaver and Meijerhof, 1991). Although our study showed an increase in litter moisture with PLT reapplication, a laboratory study involving PLT reapplication to litter in Delaware showed no significant differences in litter moisture (Li et al., 2013). Therefore, studies of repeated PLT or alternate acidifier applications and their contributions to the moisture content in litter need further study.

Litter pH

After house cleanout and prior to initiation of the reapplications, the litter pH was not significantly different between houses. During the first reapplication, PLT resulted in an immediate and significant drop in litter pH from 8.69 to 7.03 ($p<0.001$; Table 5.1) which was also significantly less than that in the control house (pH 8.69; $p<0.001$). However, the differences in litter pH between houses were temporary and did not last until the next measurement 3 weeks later, showing that PLT is not effective indefinitely (Table 4.1). In fact, litter from both houses had a pH of 8.57 after the first flock receiving reapplications. This trend was the similar for all 3 flocks, with both houses having the same litter pH prior to each PLT reapplication and at the end of each flock. At the conclusion of the study, after 3 flocks with 2 whole-house PLT reapplications each, litter pH was not different between houses (Table 4.1).

Decreases in pH seen directly following PLT application are consistent with PLT having a pH of close to 2 (Jones-Hamilton, 2010). In addition to simply lowering pH, when extreme changes in pH occur in the litter, enzymes denature and die which is important for NH_3 generation from uric acid (Kentucky, 2010). At higher pH and moisture levels, enzymatic and microbial activity is increased and the environment becomes ideal for pathogens (Blake and Hess, 1997). Previous small-scale studies have shown that 6 reapplications at a 244 g/2wk-m^2 rate of PLT to litter decreased the pH from 8.05 at the beginning of the flock to 7.50 at the end of the flock (Li et al., 2013). However, it should be noted that in the study, the control; which received no PLT reapplication treatment, also showed a decrease in litter pH from 8.12 to 7.65 at the end of the flock, indicating that the reduction may be due to other aspects of house management such as ventilation. Moreover, Li et al. (2013) conducted their study on a small-scale farm with 1/3 the number of birds and in a different location than that in the current investigation. Drier, more manageable litter may be able to extend PLT reaction time.

Litter Nitrogen

The two houses were not significantly different in terms of total N in the litter at the beginning of the study (31.2 g/kg in both houses). After Flocks 2 and 3, the total N was statistically the same in both houses, with no effect of the PLT reapplications seen. However, at the end of Flock 4; which included a total of 6 additional PLT reapplications to the assigned house over 3 flocks, the total N content of litter was significantly lower in the control house (38.6 g/kg) compared to in the reapplication house (42.5 g/kg; *p*=0.0031) (Table 4.2).

After the cleanout Flock 1 where the two houses were treated identically, the NH_4-N content in the PLT reapplication and control house litters was not significantly different. After three flocks and six PLT reapplications, the reapplication house had the same litter NH_4-N content (15.28 g/kg) in the PLT reapplication house as in the control (13.24 g/kg) at study completion (*p*=0.11) (Table 4.2). Decreasing litter pH with PLT application can result in N retention in litter as solid NH_4^+ rather than being lost as gaseous NH_3. As NH_4-N did not increase over the 3 flocks where PLT was reapplied, the increase in litter total N seen after Flock 4 must have been an organic form of N such as the uric acid excreted by the birds. Therefore, PLT reapplications decreased microbial degradation of uric acid. This can be beneficial when litter is used as a fertilizer for its N value, as well as enhanced poultry house and surrounding air quality.

In a previous study, the organic and total N contents of litter increased by at least 1% with three additional applications of PLT at a rate of 244 g/m^2 (Li et al., 2013). Using poultry litter as a crop fertilizer is a cost-effective way of recycling the nutrients it contains. Having manure fertilizers rich in organics and nutrients is beneficial for both crop growth and soil health (Choi and Moore, 2008). If the N content of poultry litter can be increased, the need for synthetic N fertilizer in crop production can be decreased (Miles et al., 2011).

Litter E. coli

Post house cleanout and after Flock 1 where the houses were treated the same, the *E. coli* counts were the same in both houses (Table 4.3). At the ends of Flocks 2, 3 and 4 where PLT reapplications were made to one house, the *E.coli* counts were also the same in both houses. The only significant differences seen in *E.coli* were immediately before and after PLT applications in the PLT reapplication house, and when comparing the control house to the reapplication house post PLT reapplication. For example, the first PLT reapplication in Flock 2 dropped *E.coli* in litter significantly from 2.63×10^7 cfus to 5.00×10^2 cfus in the reapplication house ($p<0.0001$), which was also less than the 2.46×10^7 in the control house ($p<0.0001$) (Table 4.3). By the time the next samples were taken 3 weeks later, there was again no difference in *E.coli* in litter between houses. This trend was repeated for every PLT reapplication in every flock throughout the study. Although there was a consistent drop in litter *E.coli* by at least 99% post reapplication, which shows that PLT was effective in killing *E.coli* , the rebound in *E. coli* between PLT reapplications indicates that effects of PLT on *E.coli* in litter are temporary. This rebound can occur from the continued defecation of birds, as birds and all animals have *E. coli* naturally in their intestines. Reapplication of PLT was shown in the study to temporarily decrease *E. coli* in litter in a large-scale broiler farm; however, the benefit of several applications in terms of lowering bacteria present on birds is unknown and should be studied.

The *E. coli* detected in poultry litter can vary greatly due to excretion by individual birds, number of birds in a house, sample collection and transport, and bedding material type (Durairaj and Clark, 2007). Though the exact numbers may not be recreated, it is obvious that with the application of an acidifier, coliforms are less likely to survive. The addition of an acidifier creates a less than ideal environment for microbes, so a resulting decrease in bacteria from treatments was expected. Previous studies with single applications of PLT also showed a decrease in *E.coli* presence in litter as well as on birds grown on the treated litter; however, studies of multiple application effects in a field or lab setting are lacking (Blake and Hess, 2001; Pope and Cherry, 2000).

Conclusions

A single application of PLT to the brood end of poultry houses before chick arrival repeatedly showed to improvement in bird health, while decreasing NH_3 emissions and heating

costs. However, the reapplication of PLT in whole-house trials using a new overhead system provided no long-term benefits to decreasing poultry litter pH or *E. coli*. However, due to the acidic nature of PLT rendering the litter an inhospitable environment for bacteria, *E. coli* coliform levels temporarily decreased by 99% directly after application; which may have benefits for bird health and food security if done immediately before flock removal. Alternatively, the hygroscopic properties of PLT caused the moisture content of litter to significantly increase with reapplications, which can have negative effects on bird health and increase NH_3 volatilization. The increase seen in the total N content of litter receiving multiple reapplications of PLT after three flocks and 6 reapplications would be a benefit when litter is used as a fertilizer.

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Table 4.1. Comparisons of reapplication effects on litter pH and moisture content before study, immediately before and after PLT reapplications, between flocks, and after four flocks at the study completion.

		pH			Moisture content		
		PLT Reapplication House		Control	PLT Reapplication House		Control
	Date	Before	After		Before	After	
					---------------------------%--------------------		
After Flock 1	10/18/13	8.58a		8.06a	24.99a		28.27a
				Flock 2			
Reapplication 1	11/14/2013	8.69a	7.03b	8.69a	28.82a	28.41a	22.50b
Reapplication 2	12/3/2013	8.65a	7.56b	8.71a	26.04a	25.32a	13.38b
Between Flock 2 & 3	12/21/2013	8.57a		8.57a	25.7a		23.60b
				Flock 3			
Reapplication 1	1/23/2014	8.49a	6.46b	8.63a	29.00a	29.36a	30.35a
Reapplication 2	2/20/2014	8.64a	6.56b	8.60a	29.61a	26.87a	22.78a
Between Flock 3 & 4	3/7/2014	8.32a		8.65a	28.50a		26.30a
				Flock 4			
Reapplication 1	4/9/2014	8.24a	6.95b	8.24a	31.22a	29.99a	27.77a
Reapplication 2	4/25/2014	8.57a	7.75b	8.62a	28.77a	30.55a	28.20a
After Flock 4	5/15/2014	8.55a		8.51a	34.21a		21.40b

†Different letters indicate significance across rows for either pH or moisture at $p \leq 0.05$

±Flock 2 begins reapplications post house cleanout

Table 4.2. Comparison of Total-N and NH_4-N in litter for 4 flocks determined on an “As Is Basis”.

	Date	Total-N		NH_4-N	
		PLT Reapplication	Control	PLT Reapplication	Control
		----------g/kg----------			
After Flock 1	10/18/13	31.2a	31.2a	10.12a	9.09a
After Flock 2	1/10/14	30.3a	30.2a	10.52a	10.25a
After Flock 3	3/17/14	34.5a	34.1a	5.08a	4.33a
After Flock 4	5/15/14	42.5a	38.6b	15.28a	13.24a

†Different letters indicate significance across rows, for either Total-N or NH_4-N, at $p \leq 0.05$

±Flock 2 begins receiving multiple PLT applications

Table 4.3. Comparisons of reapplication effects on litter *E.coli* content before study, before and after PLT reapplications, between flocks, and after four flocks at the study completion determined on an "As Is Basis".

		E.coli		
		PLT Reapplication House		Control
	Date	Before	After	
		-----------------------------cfu-----------------------------		
		Flock 4		
After Flock 1	10/18/2013	2.48x10^{7}a		1.83x10^{7}a
Reapplication 1	11/14/2013	2.63x10^{7}a	5.00x10^{2}b	2.46x10^{7}a
Reapplication 2	12/3/2013	2.61x10^{7}a	1.00x10^{3}b	2.19x10^{7}a
Between Flock 2 & 3	12/21/2013	1.36x10^{8}a		1.60x10^{8}a
		Flock 5		
Reapplication 1	1/23/2014	9.79x10^{7}a	4.63x10^{3}b	9.97x10^{7}a
Reapplication 2	2/20/2014	9.53x10^{7}a	1.60x10^{3}b	8.69x10^{7}a
Between Flock 3 & 4	3/7/2014	1.00x10^{8}a		8.54x10^{7}a
		Flock 6		
Reapplication 1	4/9/2014	8.49x10^{7}a	5.28x10^{3}b	1.47x10^{8}a
Reapplication 2	4/25/2014	2.00x10^{8}a	5.08x10^{3}b	1.67x10^{8}a
After Flock 4	5/15/2014	9.00x10^{7}a		1.01x10^{8}a

†Different letters indicate significance across rows at $p \leq 0.05$

±Flock 2 begins reapplications post house cleanout

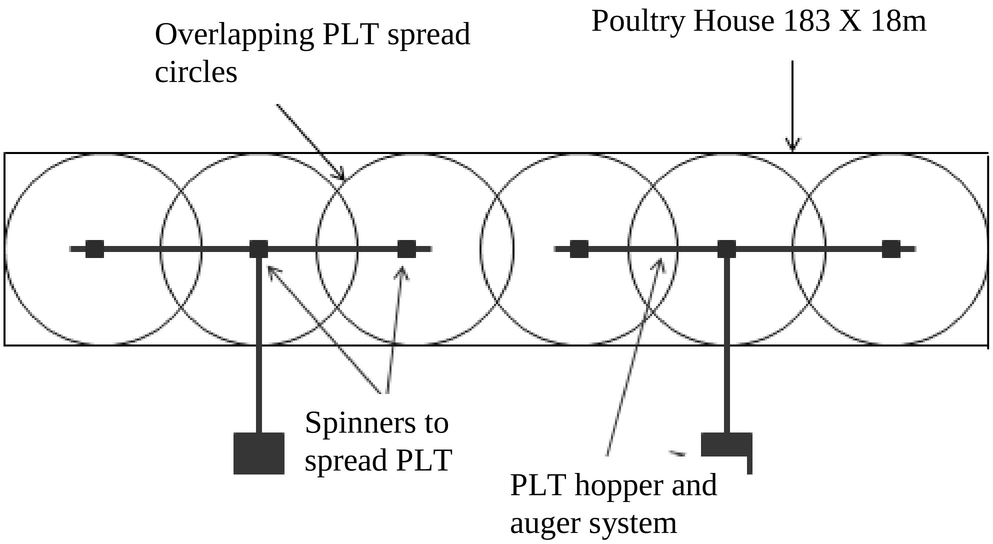

Figure 4.1. The PLT reapplication schematic of the auger and spinner system installed in House 2, not drawn to scale. There were 8 spinners on each auger system for a total of 16.

CHAPTER 5: EFFECTS OF SODIUM BISULFATE (PLT) REAPPLICATION ON AMMONIA VOLATILIZATION FROM POULTRY LITTER USING LABORATORY CHAMBERS

Abstract: Ammonia (NH_3) volatility from poultry manure can be harmful to surrounding ecosystems. The acid sodium bisulfate (Poultry Litter Treatment, PLT) is currently used to decrease NH_3 volatilization from litter in poultry houses, but is only applied pre-flock. This study analyzed the effect on NH_3 emissions of several additions of PLT to poultry litter. Six treatments were compared, 1) single initial PLT application, 2) initial PLT application plus reapplication every five days, 3) untreated poultry litter, 4) initial PLT and poultry manure added every 5 days 5) initial PLT plus reapplication of PLT and poultry manure every five days 6) untreated poultry litter with poultry manure added every 5 days. Volatized NH_3 was captured using acid traps over a 14 day period. At the end of these 14 days, poultry litter was sampled and analyzed for moisture, pH, *E. coli*, and NH_4^+-N. The PLT applications caused a wet layer and a greater moisture content in the top 1.5 cm of litter. The pH of litter samples was decreased from above 7 to below 7 with both the single and reapplied PLT. *E.coli* in litter was decreased significantly with both single and reapplied PLT. After 14 days, NH_3 released from litter treated with reapplied PLT was significantly less than litter with both single and no applications. Reapplication of PLT was most beneficial in reducing NH_3 volatilization from litter in this study. Furthermore, NH_4^+-N content of litter was greatest in litter treated with reapplied PLT increasing its fertilizer value.

Keywords: *Broilers, Volatility Chambers, PLT, Sodium Bisulfate*

Introduction

Environmental health can be negatively affected by confined poultry facilities that contribute to excess ammonia (NH_3) in the atmosphere (Pinder et al., 2007; Walker et al., 2000). Where poultry production facilities are densely populated, this becomes an even bigger issue particularly for the surrounding air and water quality (Moore et al., 1999; Rothrock et al., 2010; Zaman and Blennerhassett, 2010). The NH_3 emissions from poultry facilities can travel long distances and react with urban emissions such as NO_x and SO_x leading to fine particulate matter <2.5 µm (PM 2.5) production. This PM 2.5 can cause visibility reduction, acid rain, climate change, and respiratory ailments (Delaune et al., 2004; Krupa, 2003; Mutlu et al., 2004; Pathak et al., 2009; Speizer, 1989; Subramaniam et al., 2003; Van Breemen et al., 1982). Though air quality degradation from NH_3 production is important, contamination of waterways stemming from poultry facilities is also a concern. Excessive nitrogen (N) stemming from agriculture that enters aquatic systems can alter habitat, increase bacteria, and cause eutrophication (Aneja et al., 2008; Campbell-Nelson and Herbert, 2009; Chesapeake Bay Foundation, 2014; Paerl and Fogel, 1994; Williams et al., 1999).

Application of manure to agricultural lands can both supply nutrients to crops and provide an effective way to dispose of the by-product, however, this disposal has the potential of increasing fecal contamination to food and waterways (Soupir et al., 2006). In particular, *Escherichia coli* (*E. coli*) can be transferred from manure to crops or waterways by runoff after rainfall events leading to sickness. *E. coli* is a common fecal gram negative facultative anaerobe rod shaped bacterium that causes several diseases in poultry and is harmful to human health (Williams, 2012). Colonies make up about 12.5% of the total bacteria population in poultry litter, but are variable due to house management and individual chicken variances (Lu et al., 2003;

Williams, 2012). Lower ventilation, pH increase, increased moisture, and dust counts can all raise infection potential of *E. coli* (Pope and Cherry, 2000).

Litter and ventilation management are the best ways to decrease NH_3 volatilization and pathogenic microbes by creating an environment where enzymes in manure are less likely to mineralize and decompose urea ($CO(NH_2)_2$) to ammonium (NH_4^+). The NH_4^+ is a solid and remains in the litter until it is broken down and released as NH_3. This conversion is positively correlated with pH, moisture, and temperature of litter (Delaune et al., 2004; Reddy et al., 1979; Sims and Wolf, 1994).

Ventilation management can improve temperature, dust, and moisture of litter (Bucklin et al., 1998; Gay and Knowlton, 2009). In fact, by moving warm, moist air outside, in-house NH_3 concentrations are generally able to be kept below the EPA recommended 25 mg/L, thereby decreasing potentially harmful health effects to birds and workers (Aneja et al., 2008; Charles and Payne, 1966; Homidan et al., 2003; Miles et al., 2004; Reece et al., 1981; Stokstad, 2014). Nonetheless, ventilation is beneficial for in-house air quality only and contributes to surrounding air and water quality degradation. Chemical litter amendments are also available as a best management practice (BMP) to decrease NH_3 emissions, food borne pathogens, odors, and increase litter fertilizer potential (Blake and Hess, 2001; Choi and Moore, 2008; Miles et al., 2011). Poultry litter pH usually ranges from 8-10, allowing for maximum NH_3 release (Aneja et al., 2008; Blake and Hess, 2001). Acid amendments are able to lower litter pH below 8 thereby decreasing NH_3 volatility, and are the most commonly used amendments. As a result, more N is held by litter as NH_4^+ thereby decreasing volatilization and increasing fertilizer potential (Aneja et al., 2008; Blake and Hess, 2001; O'dell et al., 1960; Parsons, 2006; Rothrock et al., 2010). At lower pHs, enzymes are also less likely to breakdown the uric acid ($C_5H_4N_4O_3$) excreted by poultry. In addition to improving air quality, acidifiers can be cost beneficial by decreasing ventilation or heating needs.

Poultry Litter Treatment (PLT, $NaHSO_4$) is a common litter acidifying pretreatment currently used once before flock arrival at a rate of 24.4 kg/100 m^2 (50 lb/1000 sq ft) on the brood end of the house (Blake and Hess, 1997; Jones-Hamilton, 2010; Tasistro et al., 2008). The PLT reacts with NH_3 to form ammonium sulfate $(NH_4)_2SO_4$ and hold N in the non-volatile form (Blake and Hess, 2001). In the past, PLT has been shown to decrease fuel usage, microbial populations, NH_3 emissions, and improve bird survival (Blake and Hess, 2001; Jones-Hamilton, 2010). Litter amendments including PLT are not effective indefinitely. After birds are released in house and begin to excrete, litter pH will inevitably rise rendering acid treatments ineffective. To overcome this decreased efficacy with time, there has been recent work to develop a reapplication system to reapply PLT while the birds are in the house (Li et al., 2013). It analyzed repeated applications of PLT on flocks with approximately 2,400 birds. When compared to a control, the treatments reduced NH_3 emissions and pH of litter. Further studies with PLT reapplications are needed to confirm efficacy (Li et al., 2013). Solutions for decreasing *E.coli* in poultry houses also needs further study and can lead to better poultry and environmental health as well as our food supply.

If reapplication of acid amendments to poultry litter is going to be developed fully and implemented by farmers, it is necessary to understand how reapplication affects NH_3 loss and poultry litter properties. Therefore, the objective of this research was to examine how reapplication of the acid amendment PLT affected poultry litter pH, moisture, *E.coli*, and NH_3 volatilization.

Materials and Methods

Source of Litter

Poultry litter was collected from the non-brood end of a commercial broiler house in Mauzy, VA for use in this study (November 20, 2014). Collection was done on the non-brood end due to it having received no acid litter amendment as is standard in poultry houses. Initial broiler house bedding material of litter used consisted of 50% peanut hulls and 50% wood chips that was applied at 7.62 cm in depth. Litter was present for three flocks prior to collection, and birds were 10 days old at the time of collection. Litter was collected at 2.5 cm (1 in) in depth from 10 random points and mixed thoroughly in a bucket. Samples were placed in plastic bags and refrigerated prior to use and analysis as described below (Ritz et al., 2009).

Experimental Setup

Three temperature controlled cabinets were utilized for this study, as described by Woodward et al. (2011) and also used for poultry litter experiments by Kulesza et al. (2014). Each cabinet contained the same treatments in six volatility chambers, and were maintained at 100% humidity. Each cabinet was considered a replication for a total of three reps per treatment. Chambers were threaded 100 mm diameter by 150 mm deep beakers fitted with three-hole caps for air inlets, outlets, and thermometers. Each chamber contained 250g of undried poultry litter with assigned treatments surface applied (Fig. 5.1). Air passed at a constant flow rate of 1 L/min over the surface of litter and exited into acid traps allowing the recovery of all NH_3 lost to volatilization. Acid traps were 295 mL plastic bottles containing 200 mL of 0.04 *M* phosphoric acid (H_3PO_4). At each sampling point acid bottles were capped and placed in a refrigeration unit until analysis and new acid bottles immediately replaced the removed samples. Sampling times were at 2, 6, 12, 18, 30, 54, 78, 126, 174, 270, and 336 hours (14 days total).

Application Rates of Treatments

Single and reapplications of the acidifier PLT were compared for the effectiveness on decreasing NH_3 volatilization from poultry litter. Fresh poultry manure was collected by laying a large plastic sheet on the floor of a poultry house for four hours and allowing broilers to walk over it. This manure was kept refrigerated until use and used for application to appropriate chambers for the purpose of simulating poultry excretion in a house environment (Ritz et al., 2009).

The six treatments in this study were 1) single initial PLT application, 2) initial PLT application plus reapplication every five days, 3) untreated poultry litter, 4) initial PLT and poultry manure added every 5 days 5) initial PLT plus reapplication of PLT and poultry manure every five days 6) untreated poultry litter with poultry manure added every 5 days. Initial surface PLT application was in the amount of 3.83 g based on a rate of 48.8 kg/100 m^2. The PLT reapplications were applied to treatments 2 and 5 every five days in the amount of 1.92 g based on a rate of 24.4 kg/100 m^2. Poultry manure was added at 6.35 g every 48 hours based on one bird in each chamber with an excretion rate of 3.17 g-manure/day (Naber and Bermudez, 1990).

Litter and Acid Trap Analysis

Pre-and post-study analysis included litter and manure moisture, pH, *E.coli*, NH_4^+-N, NO_3-N, and Total-N. Two sampling depths were taken in each chamber post study, 0-1.5 cm and 1.5 cm-14 cm to observe surface and subsurface reactions to treatments. Sampling depth was chosen based on the deepest visible wet layer in each chamber. Subsamples were mixed thoroughly before analysis. Acid trap analysis for NH_4^+-N was completed for cumulative NH_3 released.

Moisture was determined by percent change in weight after drying litter at 105 °C for 24 hours (Peters et al., 2003). Litter and manure pH were found with a 2:1 deionized water to litter ratio and pH meter analysis (Senyondo, 2013). *E.coli* colony forming units (cfu) of litter samples were determined using the most probable number (MPN) method with colilert enzyme-substrate liquid-broth medium (IDEXX Laboratories, Inc., Westbrook, Maine) (Olstadt, 2007). This included extraction, incubation at 35°C for 24 hours and detection under a long-wave ultraviolet light (366 nm). For litter NH_4^+-N and NO_3-N, 3-g litter subsamples were extracted with 30 mL of 2 *M* KCl, shaken for 1 hour, and filtered through a 0.45-µm filter connected to a vacuum pump (Bremner and Keeney, 1966). Filtered samples were analyzed on a Lachat flow injected colorimeter to determine NH_4-N concentrations with QuickChem sodium salicylate method 12-107-06-2-A (Hofer, 2001) and NO_3-N concentrations with QuickChem 12-107-04-1-B using Cd reduction (Knepel, 2001). Acid traps were analyzed for NH_4^+-N using the QuickChem phenol method 10-107-06-1-G (Kulesza et al., 2014; Lachat Instraments, 1990). Finally, Total-N was determined by combustion using a Vario Max C/N-Analyzer (Elementar Americas, Mt. Laurel, NJ). Analysis was done on a dry weight basis.

Statistical Analysis

For statistical analysis samples were separated and compared by depths 0-1.5 cm and 1.5-14 cm. A completely randomized design was used for this study with each chamber as an experimental unit and each cabinet as a replication. Student t tests were done for litter properties and cumulative NH_3 release using JMP Pro 10 statistical package (SAS Institute, 2012).

Statements of statistical significance were accepted at $P \leq 0.05$. The statistical model was

$y_{ij} = \square + \square_{i} + e_{j(i)}$ where:

- y_{ij} is the dependent variable (pH, Moisture, nitrogen, NH_{3}, *E.coli*)on the j^{th} chamber receiving treatment i
- $\square$ is the overall average.
- $\square_{i}$ is the average effect of treatment i on dependent variable
- $e_{j(i)}$ is the residual effect of the j^{th} chamber receiving treatment i on dependent variable

Results and Discussion

Litter Analysis

Nutrients in poultry litter vary due to house management and bird differences. Litter collected for this study had a 36.2% moisture content and 2.88 g/kg NH_4^+-N which was similar to the average of 27.8% moisture content and 5.7 g/kg NH_4^+-N found in Virginia poultry litter (Table 5.1) (VADCR, 2005). Initial Total-N and pH of litter were also similar to poultry litter samples in previous studies (Johnson et al., 2011; Steiner et al., 2010; Terzich et al., 2000).

Litter Moisture

At the conclusion of the study, chambers that received applications of PLT had visible wet layers (Fig. 5.2). This can be explained by a combination of PLT's hygroscopic properties (Sun et al., 2008) in addition to the humidity of each chamber. A wet layer is not desirable, because previous studies have shown wet litter can increase NH_3-N volatilization and footpad dermatitis (Liu et al., 2007; Senyondo et al., 2013). Depth of wet layers varied among treatments, however did not exceed 1.5 cm (Table 6.2). The greatest wet depth was 1.49 cm in the chamber receiving repeated PLT applications and manure additions ($p<0.0001$), while chambers receiving no PLT had no visible wet layer. This agreed with the trend seen for moisture content in litter. For example, in the top 1.5 cm, litter receiving PLT had a moisture content above 43% regardless of single or reapplications, or manure additions. This was greater than litter receiving no PLT at less than 40% ($p<0.0001$) (Table 5.2). Conversely, in the 1.5-14 cm of litter, moisture content in litter receiving no PLT was always above 33%, while litter receiving PLT was always below 32% moisture. This suggests PLT drew water out of litter below 1.5cm as well as out of the air. For example, litter with PLT reapplications had a moisture content of 44.8% in the upper

1.5 cm and 29.6% below 1.5 cm, while litter with no PLT had 37.2% and 36.8% respectively. The gap in moisture seen with PLT application, and lack thereof from litter with no PLT, is indicative of the acids hygroscopic properties. Deeper litter consistently had a lower moisture content than wet layer litter (Table 5.2). Additionally, moisture content of litter receiving no PLT was similar to initial litter collected (Table 5.1), while litter receiving PLT treatments was higher (Table 5.2).

This is consistent with the field trial in Chapter 3 which showed reapplication of PLT resulted in greater moisture content of litter. However, a field trial in Delaware found reapplications of PLT resulted in lower litter moisture, when compared to a control, contradicting this data (Li et al., 2013). Geographic location and season of the experiment along with ventilation and other house management protocols in a field setting vary and can alter results as well as the effectiveness of PLT, so additional field studies should be completed.

Litter pH

Initial pH of litter was 7.58 (Table 6.1), but the pHs of 0-1.5 cm litter with PLT applied were all below 7, while litter receiving no PLT had pHs above 7 ($p<0.0001$). Litter pH followed the trend No PLT > PLT applied once > PLT reapplied. For example, the lowest pH was 6.01 in the first 1.5 cm of litter receiving reapplied PLT and no manure additions ($p<0.0001$). Where PLT was applied, manure application significantly increased the pH, which was not surprising as the manure had a pH of 8.05 (Table 5.1 and 5.2). On average a single application of PLT decreased pH by 0.76 units, while repeat application of PLT decreased litter pH by 1.13 units, relative to No PLT. This trend was also observed in two field studies where reapplied PLT resulted in a decrease in litter pH (Chapter 3; Li et al., 2013). Litter pH influences NH_3

concentrations in poultry facilities and as the pH increases above 7, more NH_3 is released as a gas (Burgess et al., 1998). The decrease in pH observed in chambers with the addition of an acidifier is expected due to PLT having a pH of 2, which also holds the potential for a decrease in microbial and enzymatic activity and subsequent NH_3 volatilization (Pope and Cherry, 2000).

Litter E.coli

Where no manure was added, *E. coli* in the 0-1.5 cm litter followed the pattern No PLT > PLT once > PLT reapplied, demonstrating the effectiveness of PLT at killing *E. coli*. This agrees with previous studies that confirmed a decrease in *E.coli* resulting from one application of PLT in a commercial broiler house (Pope and Cherry, 2000).Manure additions resulted in greater *E.coli* content in 0-1.5 cm litter by at least 10 times compared to litter with no manure added in every case except for litter receiving no PLT (Table 5.2). This demonstrates how defecation overcomes the *E. coli* decreases due to PLT, as the manure had 3.69×10^7 cfu. This agrees with the field study in Chapter 4 where PLT reapplication immediately dropped *E. coli*, but by the time of the next sampling coliform presence in litter was once again the same as the control. There was no trend in *E.coli* of litter deeper than 1.5 cm, indicating PLT treatment effects on microbial *E.coli* may be limited by depth. Addition of an acidifier creates a less than ideal environment for microbes, so the resulting decrease in bacteria from PLT additions was predicted. Though it is naturally found in the gut, decreasing *E.coli* of litter can be beneficial for bird performance, food safety, and environmental health and should be pursued.

Ammonia Volatilization

At the conclusion of the 14 day study, cumulative NH_3-N release followed the pattern No PLT > PLT applied once > PLT reapplied (Fig. 5.3). For example, cumulative NH_3-N released from litter receiving no PLT was 3.87 g which was 136% greater than the 1.64 g from litter receiving a single PLT application ($p<0.0001$) (Fig. 5.3). Additionally, litter receiving no PLT released 469 % more NH_3-N than the 0.68 g volatized from litter with reapplied PLT ($p<0.0001$). Manure addition never significantly increased NH_3-N release, so the trend for No PLT > PLT applied once > PLT reapplied remained the same. For example, when manure was added, litter receiving no PLT released 3.91 g of NH_3-N which was 269% more than reapplied PLT (1.06 g) after 14 days ($p<0.0001$) (Fig. 5.3). When manure was added, litter with one application of PLT still released 62% more NH_3-N than litter with reapplied PLT ($p=0.013$)(Fig. 5.3).

These results agree with several studies that observed single application of PLT decreased NH_3 emissions (Blake and Hess, 2001; Pope and Cherry, 2000). Additionally, one study with reapplications showed a decrease in NH_3 emission rate (Li et al., 2013), and another showed a temporary decrease of NH_3 concentration in broiler house air (Chapter 3).

The decrease in NH_3 observed may be attributed to the decrease in pH seen from reapplied PLT (Table 5.2). The reapplied PLT had a greater wet layer depth and moisture is positively correlated with N volatilization, but this was counter acted by the decrease in pH from acidic PLT amendments. Enzymatic activity leading to NH_3 release from litter is ideal above 7, and PLT additions brought the surface layer of litter pH below that point for both single and repeat PLT treatments (Table 5.2, Burgess et al., 1998).

Litter Nitrogen

In the first 1.5 cm with no manure applied, the trend in NH_4^+-N was PLT reapplied > PLT applied once > No PLT. For example, reapplying PLT resulted in the greatest NH_4^+-N at 13.45 g/kg while no PLT had the least at 1.24 g/kg (p=0.0016). The same trend was providing evidence that differences are due to PLT and not manure. Similar results were seen in a previous study where there was an increase in NH_4^+-N content of litter as application rate of PLT increased (Johnson et al., 2011). In litter deeper than 1.5 cm, no trend in NH_4^+-N was seen among treatments. Without PLT applied, litter NH_4^+-N was lower than the initial concentration (Table 5.1 and Table 5.3). This is consistent with conversion to NH_3 gas observed (Fig. 5.3). The amount of NH_4^+-N contained in the litter is an indication of PLT effectiveness due to N retention in the solid form thereby decreasing NH_3-N emissions.

At the 0-1.5 cm depth with no manure applied, reapplying PLT to litter resulted in a Total-N content of 26.55 g/kg; which was greater than litter with no PLT added of 24.58 g/kg ($p \leq 0.045$). This would be a result of less NH_3-N lost through volatilization as discussed previously for Fig. 5.3. However, as organic-N makes up the majority of litter-N, the changes in Total-N in litter were relatively small (Espinoza et al., 2007). In the deeper litter from 1.5-14cm, there was no clear trend in Total-N, indicating PLT effects were focused near the top of the litter (Table 5.3).

Using the NH_4^+-N measured in the litter plus NH_3-N lost to volatilization, Total inorganic N was calculated per chamber (Table 5.3). Total inorganic N was least in litter with PLT reapplied (1.78g, 2.02g with manure; $p \leq 0.0159$) while it was greatest in litter receiving no PLT (4.42, 4.52g with manure; $p < 0.0001$). Single application of PLT was intermediate of the other treatments. These results are consistent with acidifiers tendency to alter microbial behavior (Shah et al., 2006). As seen in a previous study, with PLT additions, there is a decrease in microbial activity which slows the breakdown of organic N and NH_4^+ (Rothrock, 2010). By decreasing microbial activity, less organic N was converted to inorganic N and is therefore not as available to be lost through volatilization (Cook et al., 2011). This decrease in microbial breakdown of organic N is a second pathway for the effectiveness for PLT to decrease NH_4-N volatilization, in addition to dropping the litter pH as discussed above. This may slow the release of N to plants when litter is used as a fertilizer, as inorganic-N is an important source for immediate N availability when litter is used as a fertilizer (Gale et al., 2006).

Conclusions

Currently litter acidifiers are applied once before birds enter a house, but reapplication of acidifying litter amendments is a concept thought to decrease NH_3 emissions from poultry litter. In volatility chambers, reapplication of PLT was more beneficial in reducing NH_3 volatilization from litter than a single application. In addition, reapplication of PLT resulted in significantly less NH_3 released from litter than litter with no PLT applied. This could be explained by litter pH, which was decreased as more PLT was applied. Moisture content increased and wet layers increased in depth as more PLT was applied which agrees with the hygroscopic properties of PLT. The NH_4^+-N and Total inorganic-N content increased in litter as more PLT was applied which can be beneficial for fertilizer use. The result that several applications of PLT decreased NH_3-N volatilization and *E. coli* raises the possibility that the system can be advantageous for decreasing fuel usage, ventilation rates, health and performance of birds, environmental health, and food safety. However, the moist layer where PLT was applied raises some concerns, as wet litter is not desirable. In the future, research should be done over longer periods, with different litter amendments, and on field trials to truly determine reapplication effectiveness.

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Table 5.1. Initial manure and poultry litter chemical properties determined on "As Is" basis.

	Moisture	pH	E.coli	NO_3-N	NH_4^+-N	Total-N
	%		cfu	-------------------g/kg-------------		
Manure	52.1	8.05	3.69×10^7	0.33	2.60	22.20
Litter	36.2	7.58	2.78×10^7	0.31	2.88	27.87

Table 5.2. Litter moisture, pH, and *E.coli* at two sampling depths for the six treatments after the 14 day experiment.

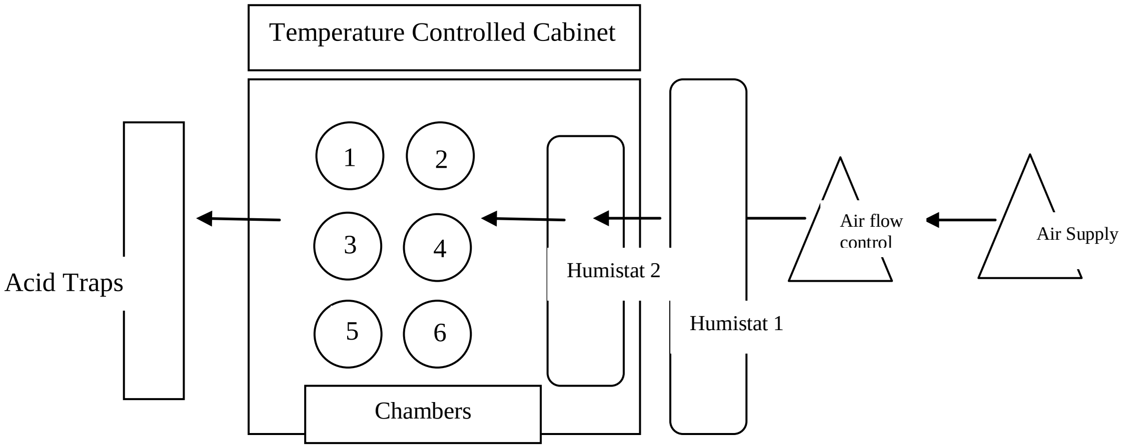
	PLT applied once		PLT reapplied		No PLT	
	no manure applied	manure applied	no manure applied	manure applied	no manure applied	manure applied
Wet layer depth, cm	0.55 c	1.23b	1.18 b	1.49a	0 d	0d
	0-1.5cm					
Moisture %	43.6 a	44.3 a	44.8 a	45.7 a	37.2 c	40.5 b
pH	6.40 c	6.64 b	6.02 e	6.28 d	7.31 a	7.24 a
E.coli, cfu	2.53×10^6 d	2.41×10^7 b	1.34×10^4 e	1.42×10^7 c	2.56×10^7 b	3.38×10^7 a
	1.5-14cm					
Moisture %	28.3 c	31.4 bc	29.6 c	31.0 bc	36.8 a	33.5 b
pH	7.18 ab	7.14 b	7.20 ab	6.97 c	7.26 a	7.15 b
E.coli, cfu	5.00×10^6 d	2.56×10^7 b	2.56×10^5e	2.37×10^7 c	2.78×10^7 a	2.78×10^7 a

†Letters indicate significance across rows at $p \leq 0.05$

Table 5.3. Litter nitrogen content at two sampling depths for the six treatments after the 14 day experiment. Total inorganic N per treatment was calculated as NH4++ NH3 volatized. Results were determined on a dry weight basis.

	PLT applied once		PLT reapplied		No PLT	
	no manure applied	manure applied	no manure applied	manure applied	no manure applied	manure applied
	------------g/kg------------					
	0-1.5cm					
NH_4^+-N	5.32 b	4.49 bc	13.45 a	6.36 b	1.24 c	1.43 c
Total-N	24.37 b	26.04 ab	26.55 a	26.52 ab	24.58 b	24.48 b
	1.5-14cm					
NH_4^+-N	2.97 a	3.15 a	2.29 b	2.95 a	2.05 b	2.25 b
Total-N	24.69 b	27.95 a	29.08 a	30.13 a	25.55 b	24.83 b
	------------g------------					
Total Inorganic N	2.48 b	2.56 b	1.70 c	1.93 c	4.35 a	4.44 a

†Letters indicate significance between treatments across rows at $p \leq 0.05$



Chamber 1: PLT

Chamber 2: Re-application PLT

Chamber 3: Untreated poultry litter

Chamber 4: PLT with re-applied manure

Chamber 5: Re-application PLT with re-applied manure

Chamber 6: Untreated poultry litter with re-applied manure

Figure 5.1. General Schematic of Cabinet

Figure 5.2. **Post study wet layer example resulting from PLT. Arrows indicate bottom of wet layer and top of dry litter.**

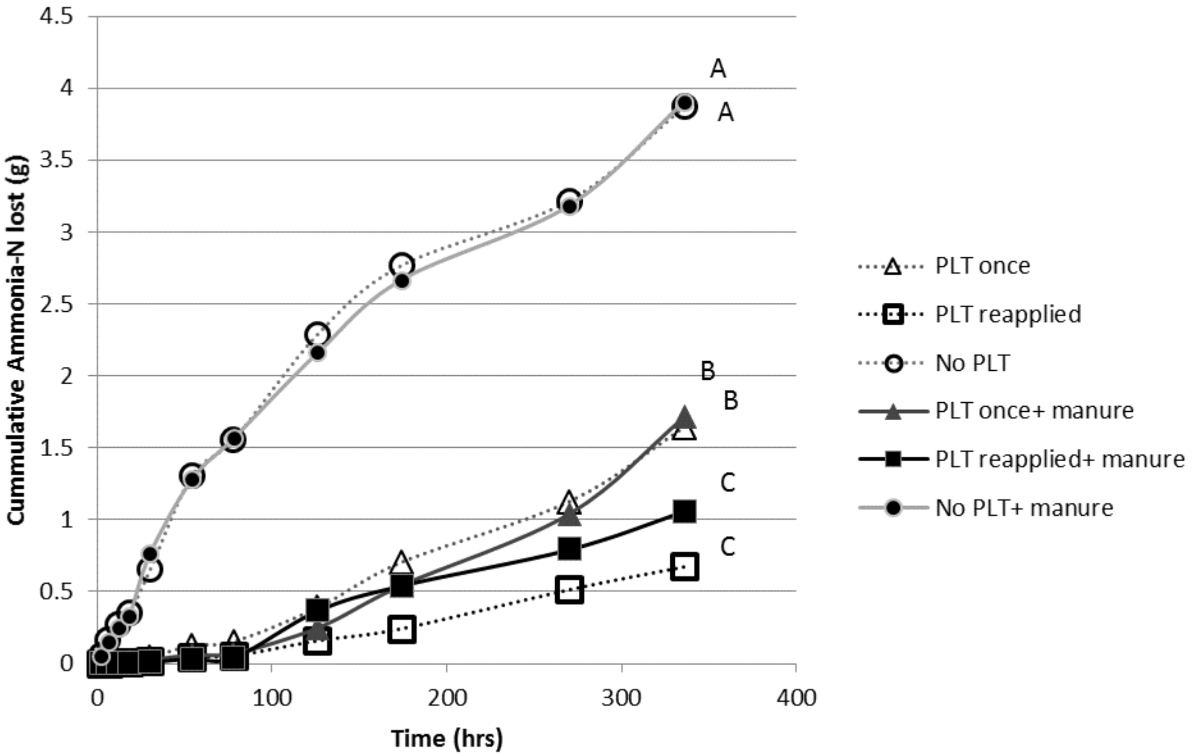


Figure 5.3. Cumulative NH3-N loss from single PLT, reapplied PLT, and control, with and without manure applied, throughout a 15-day volatilization study.

CHAPTER 6: GENERAL CONCLUSIONS AND RECOMMENDATIONS

Ammonia (NH_3) emissions from broiler operations can have negative effects on the surrounding environment. Mitigating NH_3 with acidic pretreatments once before flock arrival has been beneficial at NH_3 reduction in the past. The acidifying litter amendment PLT is safe to apply while birds are in house making multiple applications feasible with the development of reapplication equipment.

An overhead PLT reapplication system was used to apply PLT several times throughout a flock while birds were present. This technology temporarily decreased litter pH and *E.coli* which may have benefits for air quality, bird health and food security. As a direct result of litter pH and microbial activity decrease, NH_3 concentrations in broiler house air was reduced during aerial spread. The continued excretion of birds rendered PLT ineffective within two weeks of applications. Litter moisture increased with reapplications which be problematic for bird health and result in increased NH_3 volatility. Additionally, the overhead PLT reapplication system had several mechanical problems including blown fuses, uneven application, damage to ceiling, and short circuits. Reapplication of PLT during a flock for NH_3 reduction and resulting moisture should be further studied, however this system needs modifications and improvements.

Volatility chambers were used to evaluate and quantify effects on litter and NH_3 volatility from reapplication of PLT. Confirming results of the field study, litter pH and *E.coli* were decreased with PLT reapplications. In addition, several applications of PLT to litter greatly decreased NH_3 volatility from litter while increasing litter NH_4^+-N and Total inorganic-N which can be beneficial for fertilizer use. Similar to the field study performed, reapplication of PLT also increase moisture of litter which can be undesirable.

Reapplication of PLT to poultry litter was proven to be an effective way to control NH_3 in a controlled environment with no broilers present. However, the evaluation of a reapplication system in a commercial broiler house proved to only be temporarily effective and was not mechanically sound. Further development or alteration is necessary of the reapplication system used in this study. Additionally, more data is needed on the effect of reapplications of PLT on litter properties, and bird health. Several applications of an acidifying litter amendment can be advantageous with decreasing fuel usage, ventilation rates, health and performance of birds, environmental health, and food safety, and should be further investigated.