

**EFFECTS OF ACIDIC LITTER  
AMENDMENTS WITH MULTIPLE APPLICATION  
ON AMMONIA, MICROBIAL ENVIRONMENT, PRODUCTION  
PERFORMANCES, AND HEALTH OF BROILERS**

by

Alyson Weiss

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment  
of the requirements of the requirements for the degree of Master of Science in Animal  
Science

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## ABSTRACT

High ammonia (NH<sub>3</sub>) levels in poultry production houses can have damaging effects on the birds inside, such as respiratory disease outbreaks, reduced growth rate, high mortality and low feed efficiency. Acid based litter amendments have been widely used in broiler operations to reduce NH<sub>3</sub> concentration during the brooding period. Laboratory and small-scale field studies showed that frequent litter amendment application during grow-out could significantly reduce NH<sub>3</sub> emission and improve production performances. Two studies were performed to evaluate the effectiveness of frequent PLT application on air emissions and bacterial load and to compare the effectiveness of Klasp and Al+ Clear with PLT on reducing ammonia while not adversely impacting the health of the birds. Using a commercial broiler farm, the first study evaluated the effects of frequent PLT application during a 6-wk grow out by looking at air emissions and bacterial load in the litter. Two identical broiler houses were used, with one house having PLT applied twice with a rate of 244 g per m<sup>2</sup> (50 lb per 1000 ft<sup>2</sup>) on days 21 and 35 and the other serving as control. NH<sub>3</sub> emissions and concentration were monitored throughout. Litter samples were collected three times per flock then diluted and applied to Petrifilm™ plates to be incubated, counted and compared. Immunoassays tested for presence or absence of Salmonella and Campylobacter. Results indicated frequent litter amendment application significantly

reduced NH<sub>3</sub> emissions and concentration and improved performance. There was no significant difference between the houses in the quantities of aerobic, coliforms and yeasts and molds. There was no growth of Salmonella in the treated house, but no effect on Campylobacter. The second study compared the effectiveness of Al<sup>+</sup> Clear and Klasp to PLT in reducing ammonia while not negatively affecting the birds. Three flocks of 30 birds were raised in isolated emission chambers for a standard grow-out period. Beginning on day 21, the three amendments were applied at two different rates (high - 976 g/m<sup>2</sup> and low - 244 g/m<sup>2</sup>) for a total of six treatments weekly until the end of the flock. Amendments were also applied to feeders (1.4 g/bird for high and 0.35 g/bird for low) to simulate spreading in commercial production. Throughout the flock, NH<sub>3</sub> emissions and growth performance were observed. At the end of each flock the birds were euthanized and necropsied to evaluate the health. The results of this study showed no adverse effects on bird performance and health when amendments were applied weekly from 3-wk of age. PLT was the most effective at controlling ammonia while Al<sup>+</sup> Clear was the least.

Keywords: Litter, amendment, ammonia, bacteria, broiler

## Chapter 1

### INTRODUCTION

Broiler can be stressed during growout by many factors, such as thermal environment, microbial environment, and litter management practices (Beker et al., 2004). Litter consists of excreta, feed, feathers and bedding material (Karamanlis et al., 2008). Poultry litter and its management is an important aspect that can keep birds under healthy condition for optimum growth. Ammonia ( $\text{NH}_3$ ) volatilization from litter in production houses is one of the overlooked areas (Moore et al., 1999). Aerial  $\text{NH}_3$  is a pungent, colorless gas and usually the most abundant of the air contaminants in broiler operations while it is presents throughout the birds living areas and can be severely detrimental to the health and welfare of the birds and their caretakers (Kocaman et al., 2006).  $\text{NH}_3$  can build up to high concentrations under poor management and poses an inherent risk to people and animals exposed to it, even for short periods of time (Ritz et al., 2004). It is the leading cause of tracheitis and keratoconjunctivitis in chickens and can increase susceptibility to diseases such as Newcastle disease (Moore et al., 1999).  $\text{NH}_3$  emission is also an issue for both laying hen and broiler growout productions (Kocaman et al., 2006). In the United States, animal agriculture provides an estimated 50% of ammonia emissions, with 5-8% of it coming from poultry operations. The U.S. Environmental Protection Agency (USEPA) estimates these numbers will increase over the coming years, unless immediate action is taken (Lahav et al., 2008).

Birds excrete nitrogen in the form of uric acid and  $\text{NH}_3$  accumulates in the air of poultry houses as bacteria present in the litter break down the uric acid. Uric acid and one of its break-down products, urea, and makes up 70% of the nitrogen content of litter (Rothrock et. al., 2008). Uric acid and urea are degraded to  $\text{CO}_2$  and  $\text{NH}_3$ , which volatilize from the litter into the air. The volatilization of  $\text{NH}_3$  from litter can be affected by several factors: ventilation, moisture and age of litter (Moore et. al. 1995).  $\text{NH}_3$  volatilization happens when the ammonium ( $\text{NH}_4^+$ ) in manure is transformed to  $\text{NH}_3$  gas in this reaction:



As the reaction occurs  $\text{NH}_3$  is produced when pH or temperature and  $\text{NH}_4\text{-N}$  concentration increases. The release rate of  $\text{NH}_3$  is a function of the difference of  $\text{NH}_3$  concentration in manure and air (Meisinger and Jokela, 2000). Environmental and management practices can affect  $\text{NH}_3$  volatilization while good practices will improve air quality in poultry houses.

Birds are often in conditions with 50ppm  $\text{NH}_3$ , but  $\text{NH}_3$  levels can rise to and above 200ppm under poor ventilation (Beker et al., 2004). Over the course of a growout, uric acid builds up in the litter. In the United States, poultry litter is not changed frequently, primarily due to cost and availability of fresh bedding materials. Litter is replaced with fresh bedding materials approximately once a year in Delmarva. Built-up litter accumulates large amounts of manure and  $\text{NH}_3$ -producing and increase aerobic and anaerobic bacterial activity. (Bilgili et al., 2009). Bacteria and enzymes convert the uric acid into  $\text{NH}_4^+$ , which they also raise the pH of the litter so litter

becomes a better site for growth of bacteria and for NH<sub>3</sub> volatilization. Litter has an average pH of 8.0-9.0 (Meisinger and Jokela, 2000) and many types of bacteria at this level thrive and produce urease and uricase breaking down uric acid to produce NH<sub>3</sub>.

NH<sub>3</sub> can be damaging to the health of the birds when they are exposed to high levels for a prolonged period (Kocaman et al., 2006). The mucus membranes in the respiratory tract and the conjunctivae and cornea of the eyes can become irritated by NH<sub>3</sub> due to its high water solubility. Corneal ulcers can develop at levels as low as 60ppm of ammonia (Quarles and Kling, 1974). Tracheal mucus membrane damage and lung arterial wall thickness can increase if birds are exposed to levels as low as 20ppm for extended periods of time (Beker et al., 2004). Damages to mucus membranes leave the birds vulnerable to bacterial infections, especially *E. coli*, due to impaired mucus flow and tracheal ciliary action (Beker et al., 2004). Even decreased vaccination efficiency has been seen in high exposure situations (Quarles and Kling, 1974). Inhalation of airborne irritants, like NH<sub>3</sub>, can cause reduced pulmonary gas exchange and may exacerbate conditions such as ascites. Birds may also have decreased O<sub>2</sub> consumption when compared to healthy individuals (Beker, et al., 2004). Respiratory damage may not always be evident until an infectious agent is introduced (Meisinger and Jokela, 2000). NH<sub>3</sub> poisoning can occur, with symptoms including snickering (sneezing), tracheal irritation, air sac inflammation, conjunctivitis and dyspnea (Ritz et al., 2004). Studies have shown that chickens, turkeys, guinea pigs and mice raised in 20ppm ammonia for six weeks developed histopathological signs of respiratory damage (Quarles and Kling, 1974).

Birds can also show signs of decreased egg production and incidences of airsacculitis. For commercial production this is a severe economic loss as there are fewer quality eggs to sell and the shelf-life of carcasses is shortened (Quarles and Kling, 1974). High NH<sub>3</sub> levels can also affect feed efficiency in vaccine-stressed birds. Liver and kidneys can also be affected at high levels, leading to further damage from disease (Quarles and Kling, 1974).

Increased production costs can occur due to high NH<sub>3</sub> and increasing ventilation and reducing NH<sub>3</sub> volatilization can to alleviate the problem. Reduced body weights and feed efficiency and even bird viability as a consequence of NH<sub>3</sub> exposure (Kocaman, et al., 2006) cause significant monetary losses to farmers. Studies in recent years have shown broilers raised in 50 and 75ppm NH<sub>3</sub> compared to 0 ppm have decreased body weights (Wang et al., 2010). Some studies have shown that birds raised in 25 and 50ppm NH<sub>3</sub> had smaller bursae of Fabricius than birds raised ammonia free, making it difficult for them to fight infections (Wang et al., 2010). If the houses are poorly ventilated, it is possible the birds may be exposed to levels exceeding 200 ppm (Quarles and Kling, 1974). Recommended levels in broiler houses are lower than 25ppm (Moore et al., 1999).

Exposure at levels of 46 to 102 ppm can cause symptoms include huddling of birds, eye rubbing, closing of eyes, sensitivity, and matted and damp feathers. After eye damage, the birds may struggle to find feed and water sources, which can cause poor performance (Ritz et al., 2004). As the birds continue to be exposed, the respiratory system can be severely damaged. Tracheal cilia are paralyzed or decimated

and the epithelium becomes impaired to the point of forming lesions. This damage to the trachea can lead to susceptibility to numerous bacterial pathogens, particularly *E. coli*. Overall livability, weight gain, feed conversion, condemnation rate at processing and the immune system of the birds were compromised when the exposure continues (Ritz et. al., 2004).

The level of 25ppm was set by regulatory agencies such as the National Institute of Occupational Safety and Health Administration as the maximum level for humans' 8-hour exposure limit. Growers often spend 8 to 10 hours a day in poultry houses, especially when birds are young. At a level of 300 ppm, there is an immediate danger and risk to health and life (Ritz et al., 2004). Years of chronic exposure to high NH<sub>3</sub> levels can be disastrous to the health of the people raising poultry (Vučemilo et al., 2007). People working with poultry have exhibited signs of decreased respiratory health. They have higher occurrences of asthma, chronic bronchitis and organic dust toxic syndrome than other workers. Reports have shown cases of hypersensitivity pneumonitis (Rylander and Carvalheiro, 2006). Epidemiological studies exhibited the presence of extensive chest symptoms and changes in respiratory function. Even people living in the vicinity of these areas have been shown to have increased in number and frequency of sore throats, coughing, diarrhea and skin and eye irritation (Lahav et al., 2008). Studies have shown NH<sub>3</sub> to be the most likely cause. NH<sub>3</sub> emissions can lead to the formation of ammonium nitrate and ammonium sulfate in the air and the resulting particulates can cause premature death, chronic bronchitis and asthma attacks (Tasistro et al., 2007).

The poultry litter provides the ideal environment for microbial growth. Population can reach levels as high as  $10^9$  to  $10^{10}$  cells per gram of litter (Rothrock et al., 2008) as temperature, moisture and nutrients are suitable for bacteria to proliferate. Some of these bacteria are beneficial; however, detrimental bacteria are also present. Broilers can be contaminated by pathogens from the litter and introduce them to other birds and into the processing plant. Since the birds raised in commercial broiler flocks are in constant contact with their litter, they are always susceptible to pathogens (Rothrock et al. 2008).

There have been suggestions to lower the  $\text{NH}_3$  concentration maximum in poultry houses to 10 ppm (Ritz et al., 2004). This number is extremely low and will be difficult to achieve under current practices. Litter can be managed in multiple ways to help alleviate the  $\text{NH}_3$  problem in the houses. Dietary manipulation and improvement in ventilation rates have been considered for  $\text{NH}_3$  control (Singh et al., 2009). One method is to use fresh bedding between flocks, but this is not always a viable option. Depending on the location of the farm, it may be very costly to bring in large truckloads of shavings from the sawmill. There is the cost for shipment, labor spent spreading the bedding and for the product itself. There is also the issue of storing or handling the bedding materials after their use as space and proper management are needed to be used for other means (Bilgili et al., 2009).

$\text{NH}_3$  levels in the air can also be limited by simply keeping bedding as dry as possible; however, moisture levels are difficult to control since birds release moisture in several ways including respiration through mouth or respiratory tract surfaces.

Broilers produce fresh manure with approximately 76% moisture, which is partially evaporated into the air and the remainder incorporated into the bedding. The use of nipple drinkers also adds to the moisture as spillage is prevalent and leaking drinkers occur easily (Carey et al., 2004).

The most widely accepted option for  $\text{NH}_3$  control is litter amendments (Vučemilo, et al., 2007). There are several different types including adsorbents, microbes and inhibitors and acidifiers. Adsorbents function by absorbing the  $\text{NH}_3$  and preventing it from entering the air and reaching dangerous levels. Microorganisms can be added to feed to change the bacterial composition of the intestines and thus the bacteria present in manure. Studies have shown benefits with the use of these agents including reduced incident of ascites, occurrence of respiratory lesions, *E. coli* presence and water-soluble phosphorous concentrations in litter (Ritz et. al., 2004). Research has indicated an ability to reduce the bacteria containing urease and uricase by these amendments, which could decrease the amount of  $\text{NH}_3$  produced in the litter (Cook et. al., 2011).

Theoretically, since bacteria that convert uric acid to  $\text{NH}_3$  grow well at a high pH, decreasing the pH of the litter should reduce the number of bacteria and thus  $\text{NH}_3$  levels. Acidifiers have been recognized as one of the top performers in preventing nitrogen loss to the atmosphere (Cook et. al., 2011) and have been an area of interest for a variety of groups in the poultry industry. Since the 1950s, various acid based amendments have been tested for their efficiency in preventing microbial growth and in neutralizing  $\text{NH}_3$  (Moore et. al., 1995). Studies have shown that inhibiting  $\text{NH}_3$

volatilization from poultry litter using chemical amendments can increase productivity. Lowering the pH of poultry litter will not only control the  $\text{NH}_3$  levels, but will also save on fuel usage, reduce brooding stress and improve litter management.

Several commercial acid litter amendments are currently on the market. Among these are sodium bisulfate, aluminum sulfate and ferric sulfate. These compounds lower the pH and are thought to limit bacterial growth and  $\text{NH}_3$  volatilization (Pope and Cherry, 2000).

Poultry Litter Treatment (PLT) is one product marketed for reducing litter pH in broiler houses. It is a dry granular product made up of sodium bisulfate. It can also be used for pest management and pathogen reduction (Pope and Cherry, 2000). PLT has been proven to be an effective acidifier when applied prior to the grow-out phase. A study by Pope and Cherry in 2000 showed broiler houses treated with PLT at a rate of 50 lb/1000ft<sup>2</sup> had a lower pH value than those that were untreated. The pH of litter in untreated houses remained high at a value of 8 while that from treated houses averaged 6.2. PLT produces  $\text{H}^+$  and sulfate ions as it dissolves. The protons ions combine with  $\text{NH}_3$  to form  $\text{NH}_4^+$  ions and the resulting salt is a water-soluble fertilizer. The amount of nitrogen is increased in the litter and pH is decreased. According to a study by Tasistro et al. (2007), sodium bisulfate was highly effective in reducing  $\text{NH}_3$  in the first 3 weeks of the flock. By applying PLT throughout the life span of the flock, it may be possible to maintain the pH and beneficial effects a longer period of time.

Studies have shown aluminum sulfate, alum, to be efficient in reducing NH<sub>3</sub> emissions when added to litter. A study by Worley and Cabrera (1999) examined the efficacy of Al<sup>+</sup> Clear in commercial flocks. Their work provided evidence that the use of Al<sup>+</sup> Clear in commercial flocks can reduce energy usage, beetle populations and NH<sub>3</sub> release. Al<sup>+</sup> Clear functions by producing hydrogen ions as it dissolves. The ions react with the NH<sub>3</sub> in the litter to form NH<sub>4</sub><sup>+</sup> ions, which can form ammonium sulfate remains in the litter and does not become gaseous.

Previous practices have involved placing amendments on the litter prior to bird placement. Typically this is done at a rate of 100 to 200 lb/1000ft<sup>2</sup>; however until recently, there was no information available for applying the amendments during the grow-out period. With PLT applied once every two weeks starting on day 21 at a rate of 50lb/1000ft<sup>2</sup>, a reduction in atmospheric NH<sub>3</sub> was seen. The reduction was up to 50% and health and welfare of the birds was improved (Pope and Cherry, 2000).

There have been no studies to examine other commercially available products applied during grow-out. Most research done on the use of chemical agents only involved the time period before bird placement. Variables such as litter accumulation, litter moisture, bird type and brooding temperature can affect how these products work over time (Ritz et. al., 2004). The hope is that applying amendments during growout will further reduce NH<sub>3</sub> levels in the air and that there will be an improvement in bird health and welfare. By establishing efficacy and safety in other products, growers are able to select the products that best suit their needs.

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## **Chapter 2**

# **EFFECTS OF MULTIPLE LITTER AMENDMENT APPLICATION ON LITTER MICROFLORA AND AERIAL AMMONIA EMISSION OF COMMERCIAL BROILER HOUSES**

A paper to be submitted to Applied Poultry Research

Alyson Weiss, Chen Zhang, Chongyang Lin, Hong Li, Rolf Joerger, Pei Chiu, Eric  
Benson

### **Abstract**

Elevated ammonia (NH<sub>3</sub>) concentrations in poultry operations can cause detrimental effects, such as respiratory system disease outbreaks, slow growth rates, high mortality, and low feed efficiency. Acid-based litter amendments have been widely used in broiler operations to reduce NH<sub>3</sub> concentrations during the brooding period. Moreover, laboratory and small-scale field studies have shown that frequent litter amendment application during grow-out could significantly reduce NH<sub>3</sub> emission and improve production performances. A large-scale field verification study was conducted on a commercial broiler farm aimed at evaluating the effects of frequent PLT application during a 6-wk grow-out on air emissions and bacteria load in the litter of two identical broiler houses. In one house PLT was applied at 244 g per m<sup>2</sup> (50 lb per 1000 ft<sup>2</sup>) on days 21 and 35 while the other remained as the control house. NH<sub>3</sub> concentrations and emission were monitored throughout three flocks. Litter samples

were collected three times per flock and evaluated for total aerobic, coliform and yeasts and mold counts using Petrifilm™. Immunoassays were used to test for the presence or absence of *Salmonella* and *Campylobacter jejuni* in litter enrichment cultures. Frequent litter amendment application significantly reduced NH<sub>3</sub> concentration and emission ( $P < 0.01$ ) and improved the growth performance. No significant difference was observed between treated and untreated poultry litter with respect to microbial counts. *Salmonella* was not detected in the treated house over the three flocks. *Campylobacter* was not influenced by frequent litter amendment application.

Keywords: Litter, amendment, ammonia, bacteria, broiler

### **Introduction**

Since environmental stress is known to increase the susceptibility of broilers to diseases, such as necrotic enteritis, botulism, gangrenous dermatitis, air sacculitis and cellulitis, poultry producers have recognized the need to provide an environment to the birds that minimizes stress (Payne et al., 2002). One important stressor is NH<sub>3</sub> in the air generated by bacterial degradation of nitrogenous compounds in the litter (Beker et al., 2004). Growers may be able to control the release of NH<sub>3</sub> into the air or poultry houses by applying acid based litter amendments. These acidifiers can reduce the pH of the litter to levels less conducive to NH<sub>3</sub> volatilization from litter. In addition to their influence on NH<sub>3</sub>, these acid amendments in litter can also inhibit the growth of

bacteria that break down uric acid and generate  $\text{NH}_3$  and (Vicente et al., 2007) and bacterial foodborne pathogens that can be transmitted to humans through poultry products (Trampel et al., 2000). Sodium bisulfate, Aluminum sulfate, and ferric sulfate are the acid litter amendments commercially available and most commonly used from broilers for the brooding period. Previous studies with sodium bisulfate have shown it to be effective at reducing  $\text{NH}_3$  in broiler houses and improving the health condition of the birds when applied (Li et al., 2013); however, it appears that the effect of a one-time application of the litter amendments prior to introduction of the birds into the house diminishes over time (Li et al., 2013). Therefore, the multiple applications of the amendments during grow-out have been proposed and showed promising reduction on  $\text{NH}_3$  during broiler growout under laboratory conditions (Li et al., 2013). However, more research is needed for broilers under commercial conditions to verify the efficacy of the multiple applications over a prolonged period. The objective of the present study was to determine the effect of multiple applications of sodium bisulfate during six-week growout on  $\text{NH}_3$  emissions, production performances, and the bacteria and pathogen populations of the litter in two commercial broiler houses.

## Materials and Method

### Broiler Housing

Two full-size broiler houses in Delaware were selected. Housing style (tunnel ventilation) and environmental control strategy (radiant tube heaters), bird management (central house brooding), and litter management and handling schemes (de-caking of litter between flocks and applying sodium bisulfate in brooding chamber) at these facilities were typical for current broiler production. The two houses each measured 18.3 m x 152.5 m (60 ft x 500 ft). Each house had insulated drop ceilings, box air inlets along the sidewalls, three 91-cm (36-inch) diameter end wall exhaust fans, and 15 123-cm (48-inch) diameter tunnel fans. Two 91-cm (36-inch) fans (SW1 and SW3) used for minimum ventilation were located in each end of the house (Figure 1). Two sections of evaporative cooling pads were located in the opposite end from the tunnel fans.

### Air Quality Measurements

A continuous monitoring and measurement system was developed to quantify air emissions from the two broiler house. This system has been widely used to measure air emissions from broiler operations and the details of the system can be found in the report (Burns et al., 2007). The brief introduction of the system is as followed. The air pollutant emission is the product of the pollutant concentration difference between outgoing and incoming air streams and volume of air exchanged through the facility.

A photoacoustic multi-gas analyzer (INNOVA 1412, Lumasense, Santa Clara, CA) was used measure gaseous concentrations in conjunction with a custom-made multi-point sampler and data acquisition system. Ventilation rate of the building was based on individual fan curves obtained from two Fan Assessment Numeration Systems (FANS). Exhaust fans' runtime were monitored with current switch sensors attached to the power supply cords of the fans. The signals of the current switch sensors were recorded by compact Fieldpoint modules (cFP-2220 modules, National Instruments, Austin, TX). One static pressure sensor was used to measure house static pressure and determine the ventilation rates with the fan curved. Indoor and outdoor temperature and relative humidity (RH) were measured with HOBO RH/Temp sensors with loggers.

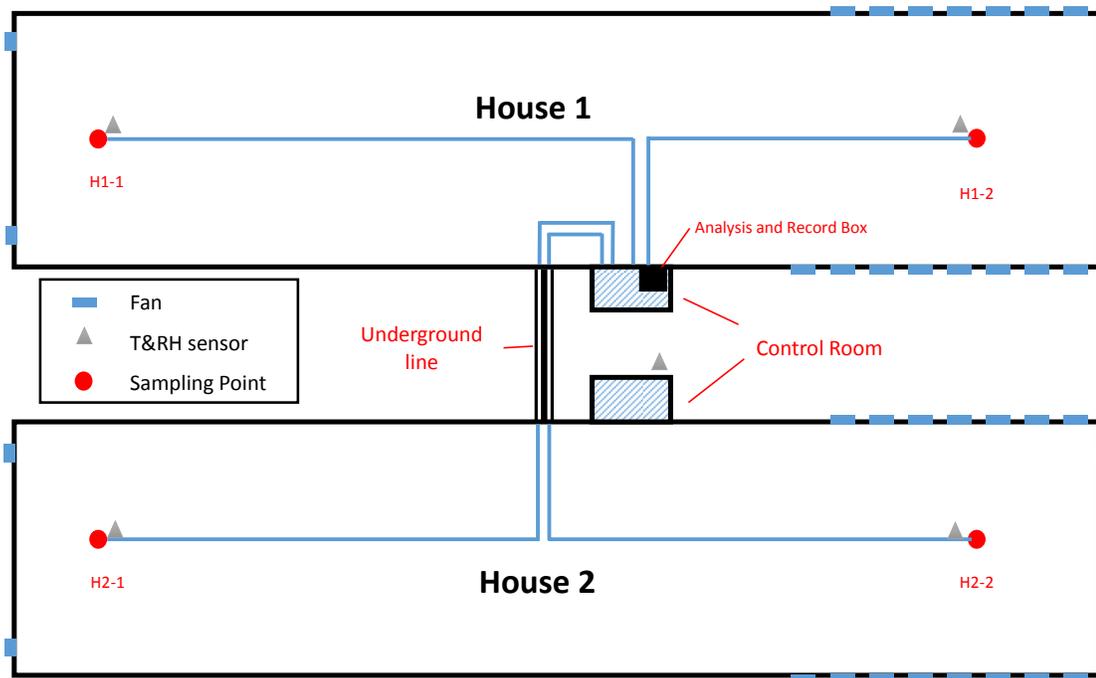


Figure 1 Schematic layout of the two broiler houses at a commercial site.

A temperature controlled equipment chamber was located in one of the control rooms and used to house the gas analyzers, data acquisition system, and gas sampling system. Air sampling lines from the broiler house sampling points (representing the building exhaust air streams) to the equipment chamber were protected against in-line moisture condensation with insulation and heating cable. Gaseous samples were continuously collected and analyzed every 30 seconds for 3 minutes, with every sixth concentration value used as the stabilized reading in the emission calculation. Air samples were drawn from two locations in each house as well as from an outside location to provide the ambient background data. One sampling location was near the primary minimum ventilation (36-in) endwall fan (SW1) used for cold weather and brooding ventilation. The second sampling location was at the tunnel end (TE). The ambient sample location (A) was near the control room of the control house. A gas sampling system was used for measurement of broiler house air emissions. The gas sampling system continuously pumped sample air from all locations using two pumps, one for gas analyzers and the other for bypass. The sample air was bypassed when not analyzed.

#### Litter Amendment Application

The sodium bisulfate application system consisted of three separate delivery systems. Two of the three delivery systems had three and the other one has four applicators (Figure 2). Each delivery system had two augers: one delivers sodium

bisulfate across the house and the other transports sodium bisulfate to each applicator along the house. The applicators are 13.7 m (45 ft) apart and at the center of the house.

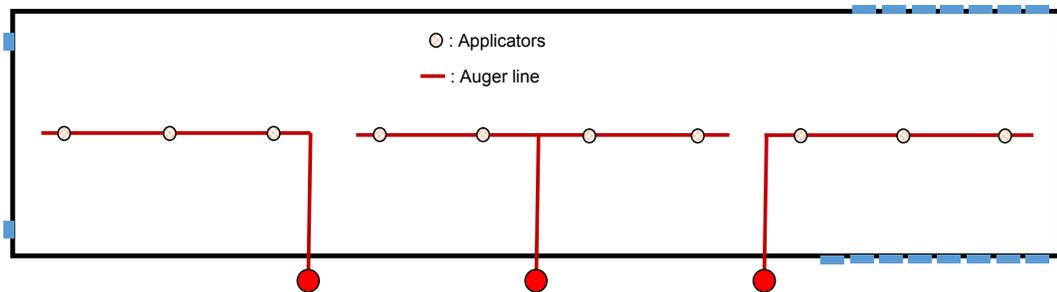


Figure 2 Schematic of sodium bisulfate application system.

Three flocks were studied, with Flock 1 being between Nov 2013 and Jan 2014, Flock 2 being between Jan 2014 and March 2014 and Flock 3 being between March 2014 and May 2014. The farm used central house brooding, so for the first 12-14 days the birds were contained within the central 40% of the house and then released into the whole house. Prior to bird placement, sodium bisulfate was applied manually into the brooding chamber at a rate of  $488 \text{ g/m}^2$  ( $100 \text{ lb}/1000\text{ft}^2$ ) by using pushing applicators. Then sodium bisulfate was applied at 21 and 35-d of age with a rate of  $244 \text{ g/m}^2$  ( $50 \text{ lb}/1000\text{ft}^2$ ) during the grow-out.

### Bird Weight and Health Monitoring

Bird weights were collected and paw quality was examined twice during the three flocks, before 21-d sodium bisulfate application and before bird catching. Three group of birds, 20 birds per group were selected for the weight and paw quality check. Each house had an initial, nominal placement of around 31,400 broilers (Hubbard ×

Cobb). The average grow-out period was 42 days. Both houses had built-up litter at the beginning of monitoring. During the five-month period, there was no total cleanout on litter.

#### Litter Sample Collection and Analyses

Surface litter samples were collected one day before bird placement, one day before sodium bisulfate application, and before bird catching. Two 250-g litter samplers were taken one day before bird placement: one in brooding chamber and the other at the tunnel end. Eight 250-g samples from each house were collected for each sampling event: four in the brooding chamber and four at the tunnel end. The samples were tested for aerobic, coliform, yeasts and mold counts, and the presence or absence of *Salmonella* and *Campylobacter*. To perform the bacterial counts, 10g of each sample was placed in a filtered stomacher bag with 90mL of buffered peptone water. Each sample was stomached for two minutes followed by serial dilution and plating on 3M petrifilms for total aerobic, yeast and mold and coliform counts, respectively. Colony counts were performed after 24 and 48 h of incubation at 37°C. The total number of colony-forming units per gram of litter sample was then calculated.

Immunoassays were used to test for the presence and absence of *Salmonella* and *Campylobacter*. For detection of *Salmonella*, 25 g of litter was placed into 225mL of enrichment medium in sterile plastic bags. Enrichment was carried out at 37°C for 18-22 hours. One-hundred µL of this culture was added to 9.9 mL Rappaport-Vassiliadis (RV) medium incubated at 37°C for 18-24 hours.

To test for *Campylobacter*, 25g of litter sample was added to a stomacher bag with 225mL of *Campylobacter* enrichment medium and incubated at 45°C for 18-22 hours. Antibiotic supplement was added and the cultures were incubated for an additional 24 hours.

Immunoassays were carried out according to the manufacturer's protocol on heat-treated samples. The presence/absence of the bacteria to be detected was determined by comparison of the colors of the positive and negative control samples and the litter cultures with those of the color chart provided by the manufacturer.

### **Statistical Analysis**

Body weight measurements, mortality rates, footpad scores, NH<sub>3</sub> concentrations and emissions, and environmental parameters from the control and treatment houses (in each of three replicate trials) were combined for analysis. A one-way ANOVA and student's t test were performed to determine differences in those categories between treatment means. To test the significant factors that affected bacteria counts and *Salmonella* and *Campylobacter* presence, an ANOVA using standard least squares of JMP 11 (SAS Institute, 2013) was performed. The factors included litter amendment, sample location, bird age, and litter moisture. Significance was accepted at  $P < 0.05$ . The analysis of numbers of aerobic, coliforms and yeasts and molds counts and *Salmonella* and *Campylobacter* presence in the litter was derived from a total of 36 litter samples from each grow-out.

## **Results and Discussions**

The flock information is reported in Table 3.1. The range of daily average ambient temperatures was -10.0 to 30.0 °C (14 to 86 °F) for the two sites. The average inside temperature of the control house over the three flocks was 1 °C lower (21 vs. 22 °C) than for the treatment house, which presumably was due to a slightly higher ventilation rate in the control house. The relative humidity of the treatment house was significantly lower than that of the control house (75.8 % vs 81 %) ( $P < 0.01$ ).

### **Litter Moisture Content and pH**

Litter moisture content and pH values varied with bird age, season, and sodium bisulfate application (Table 1). The litter moisture content increased with bird growth and decreased with higher ventilation in both control and sodium bisulfate treatment houses. The results showed that the sodium bisulfate house had slightly lower moisture content than the control house. Sodium bisulfate is very hygroscopic and can bond moisture from surrounding air and litter that makes water much less available for evaporation. Therefore sodium bisulfate might contribute to the lower humidity in the air and moisture content in the litter. The pH of the litter samples in the control house increased from 6 to 7.9 from day of placement to the end of each flock. The litter pH on the date prior to bird placement was low because sodium bisulfate with 244 g/m<sup>2</sup> rate was used in the brood chamber to suppress NH<sub>3</sub> during the brooding period. With the increasing NH<sub>3</sub> in the litter, the litter pH increased to 6.8 to 7.8 at 21-d of age. The two sodium bisulfate applications at 21 and 35-d of age lowered the litter pH and the pH remained below 7 at the end of the flocks.

Table 1 Daily average temperature, relative humidity (RH), ventilation rate, litter moisture, and pH summary for control (Ctrl) and treatment (Trt) houses.

Flock #		1	2	3	Mean
Start		11/28/2013	1/20/2014	3/20/2014	
End		1/10/2014	3/3/2014	5/1/2014	
Outside Temp, °C		3.11	-0.56	10.5	4.4
Outside RH, %		84.2	75.5	73	77.6
Inside Temp, °C	Ctrl	21.2	19.8	21.9	21.0
	Trt	23.1	21.1	22.6	22.0
Inside RH, %	Ctrl	75.4	82.9	85.1	81 <sup>a</sup>
	Trt	74.7	77.6	75.1	75.8 <sup>b</sup>
Ventilation rate, m <sup>3</sup> /hr	Ctrl	22396	22685	45893	30325 <sup>a</sup>
	Trt	21842	21351	42558	28584 <sup>b</sup>
Litter moisture, % (-1-d)	Ctrl	-	19	21.1	20.1
	Trt	-	19.6	18.6	19.1
Litter moisture, % (21-d)	Ctrl	23	32.1	34.6	29.4 <sup>a</sup>
	Trt	22.9	21.3	31.9	25.4 <sup>b</sup>
Litter moisture, % (40-d)	Ctrl	27.2	30.5	30.4	29.4
	Trt	31.1	24.8	22.8	26.2
pH (-1-d)	Ctrl	-	5.4	6.5	6.0
	Trt	-	5.8	6.3	6.1
pH (21-d)	Ctrl	7.5	6.4	6.4	6.8 <sup>a</sup>
	Trt	7.9	7.6	-	7.8 <sup>b</sup>
pH (40-d)	Ctrl	7.9	7.8	7.9	7.9 <sup>a</sup>
	Trt	6	7.6	5.8	6.5 <sup>b</sup>

### Body Weight and Mortality

The body weight gains of the three flocks of birds in the two houses were compared (Table 2). The birds in the treated house over three flocks were 0.11 kg (0.24 lb) heavier than those in the control house ( $P < 0.01$ ). The body weight gains over the last three weeks for the 2<sup>nd</sup> and 3<sup>rd</sup> flocks were 0.04 kg higher in the treatment house than in the control. The body weight of the 1<sup>st</sup> flock before sodium bisulfate application was not determined. The average daily mortality rates of the two houses over 42 days

for the three flocks varied from 0.65 ‰ to 4.12 ‰. The house with sodium bisulfate treatment had lower overall daily mortality rate (1.58 ‰ vs. 2.02 ‰) than the control although the difference was not significant ( $P = 0.65$ ). Due to the difficulty in feed ticket record keeping, the feed consumption and feed conversion ratio could not be derived.

### **Footpad Score**

Footpad scores were given based on welfare assessments established by Welfare Quality protocols (Welfare Quality, 2009). Scores were between 0 and 4, with 0 being no lesions or signs of lesions and scores of 3 and 4 being evidence of dermatitis. A bird with a score of 1 or 2 had evidence of minimal dermatitis. Based on an average of scores for each house, the treated house had a lower score of 1.02 after treatment, compared to the untreated house, which had an average score of 1.8 ( $P < 0.01$ ). Footpad dermatitis is usually the result of high  $\text{NH}_3$ , wet, sticky, and compact litter when litter quality is not managed properly, such as when a low ventilation rate is applied during the colder season, poor bedding materials are used, and when leaky nipple drinkers are present (Shepherd and Fairchild, 2010). The results of this study indicated that the footpad scores were affected by the sodium bisulfate application and footpad quality was improved likely due to lower litter moisture content and relative humidity in the ambient air.

Table 2 Body weight gain and paw quality score (footpad dermatitis) over the three growouts.

Flock		1	2	3	Mean
Daily mortality rate, ‰ (42-d)	Ctrl	0.65	1.28	4.12	2.02
	Trt	0.50	2.40	1.84	1.58
Daily mortality rate, ‰ (21 to 42-d)	Ctrl	0.65	1.68	7.63	3.32
	Trt	0.41	4.00	3.01	2.47
Body weight, kg (21-d)	Ctrl	-	0.57	0.52	0.55 <sup>a</sup>
	Trt	-	0.59	0.54	0.57 <sup>b</sup>
Body weight, kg (42-d)	Ctrl	2.06	1.99	2.05	2.04 <sup>a</sup>
	Trt	2.30	2.10	2.05	2.15 <sup>b</sup>
Body weight gain, kg (21 to 42-d)	Ctrl	-	1.42	1.53	1.47 <sup>a</sup>
	Trt	-	1.51	1.51	1.51 <sup>b</sup>
Paw quality score (40-d)	Ctrl	-	1.78	1.83	1.80 <sup>a</sup>
	Trt	-	1.1	0.93	1.02 <sup>b</sup>

\* Body weight before sodium bisulfate application and paw quality score were not determined for the 1<sup>st</sup> flock.

### Ammonia Concentrations and Emissions

Sodium bisulfate application can immediately reduce aerial NH<sub>3</sub> concentration by lowering pH and subsequent retention of the NH<sub>4</sub><sup>+</sup> in the litter. Figure 3 shows that NH<sub>3</sub> concentration dropped about 50% in 24-hr after the first and second applications in the treated house. For flock 1, daily average NH<sub>3</sub> concentrations in the control and treated house on day 21 were 43 and 37 ppm, respectively. One day after first application the NH<sub>3</sub> concentrations were 39 and 19 ppm. The daily emission rate (ER) of each house was calculated based on the gaseous concentrations and the building ventilation rates. NH<sub>3</sub> emissions of the two houses were the same before sodium bisulfate application. NH<sub>3</sub> emission from the flocks in the treated house was significantly lower than that in the control house after sodium bisulfate was applied at 21 and 35-d of age (Figure 4). The NH<sub>3</sub> emission rate (ER) from the treated house was

about 53.1% to 23.1% lower during the first 5 to 6 days after each sodium bisulfate application during the grow-out. Daily NH<sub>3</sub> emission reduction varied from 13.1% to 23.5% from 7 to 14 days post application. Overall cumulative NH<sub>3</sub> emission over the last three-wk period from the treated house was 30.2 % lower than that from the control (Fig. 5). In contrast, the reduction rate over the entire 42-d grow-out period was 25.2%. A regression analysis was performed on the NH<sub>3</sub> emission reduction rate by 244 g/m<sup>2</sup> (50lbs per 1000ft<sup>2</sup>) rate. The first application reduced emission by 43% after 24 h while the second application declined by emission by 53%. Then the reduction rate reduced 3% every 24 h. Based on the regression analysis, NH<sub>3</sub> emission can be further reduced if sodium bisulfate is applied more frequently. For example, the emission over the last three weeks can be reduced by 42% if sodium bisulfate was applied on weekly basis at 244 g/m<sup>2</sup> (50 lb per 1000ft<sup>2</sup>) with the current system. In addition, a spatial distribution test on the current system was conducted. Most of the sodium bisulfate was dispersed in an area with a 6- to 7.6-m (20 to 25 ft) diameter under each spreader. Therefore 30% to 40% of the litter may not be treated by sodium bisulfate since the houses were 18 m (60 ft) wide. NH<sub>3</sub> emission can be further reduced by redesigning the spreaders and improving the uniformity of sodium bisulfate distribution.

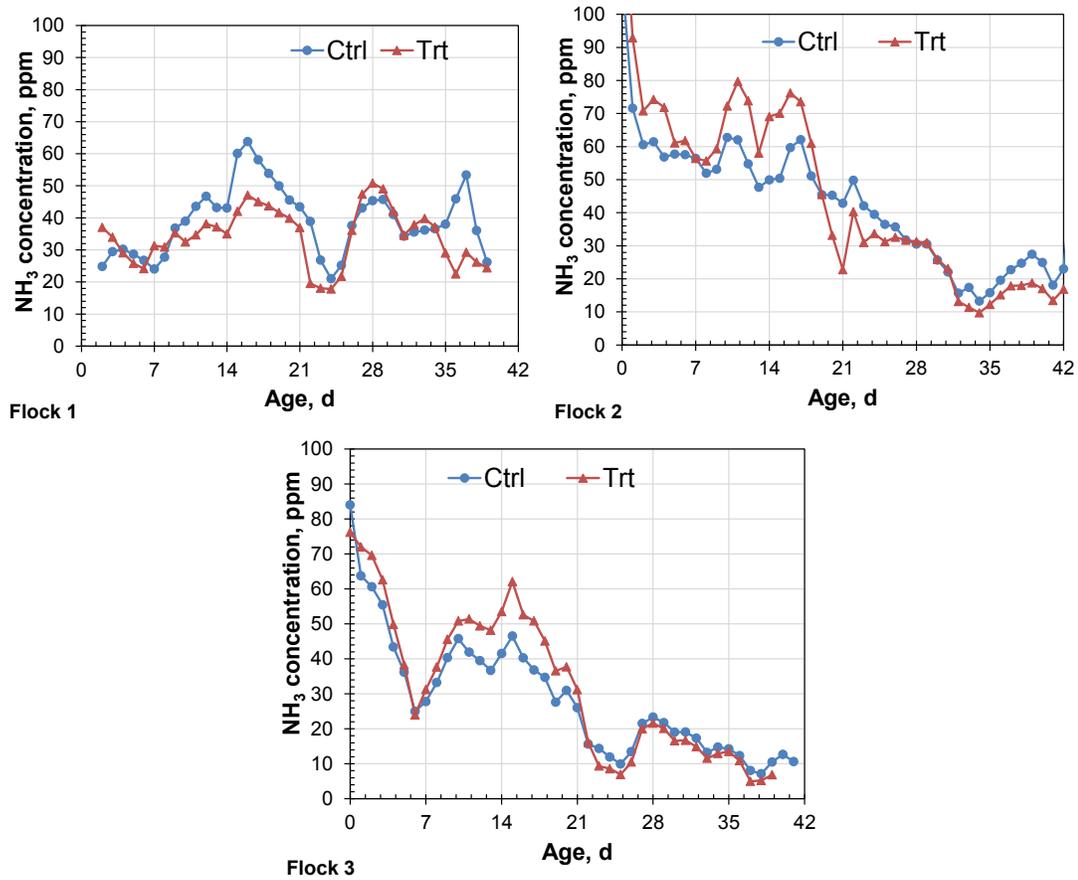


Figure 3 Ammonia concentrations over the three flocks.

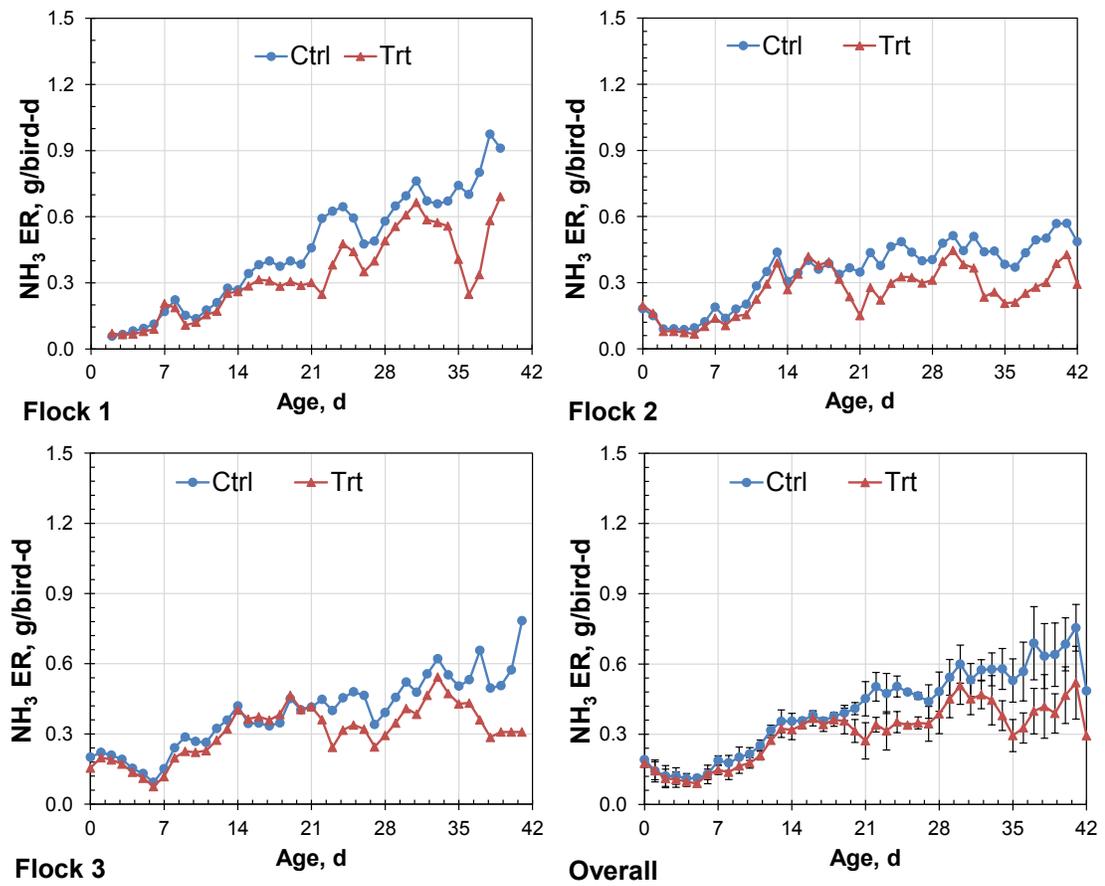


Figure 4 Ammonia emission rates over the three flocks.

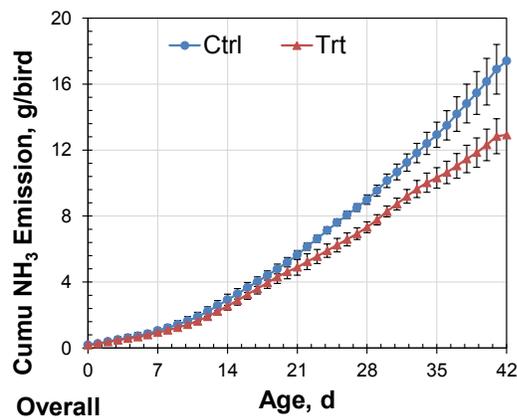


Figure 5 Mean cumulative ammonia emission from the three flocks.

## **Bacterial Counts**

Aerobic bacteria of litter samples from the three flocks are shown in Figure 6. In each flock, the levels of aerobic bacteria were similar at the three different sampling times. There were no differences between the control and sodium bisulfate treatment for all paired comparisons over the three flocks. In Flock 1, the aerobic bacteria counts at 21-d of age were the highest compared to those at 40-d of age and one day prior to bird placement while the aerobic counts gradually increased with bird growth in Flocks 2 and 3. The control house had lower aerobic counts than the treatment house of Flock 1 on 40-d of age; however, the aerobic counts of the control houses for Flocks 2 and 3 were higher on the same day. There was no consistent trend between aerobic counts and sodium bisulfate treatment, pH, and moisture content, and location ( $P > 0.1$ ). The overall average showed that the aerobic counts of all three flocks were not impacted by sodium bisulfate application. Different flocks showed an effect on the counts ( $P < 0.01$ ) due to different characteristic of the build-up litter over time associated with different ventilation modes, moisture contents, temperatures, and humidity levels, etc.

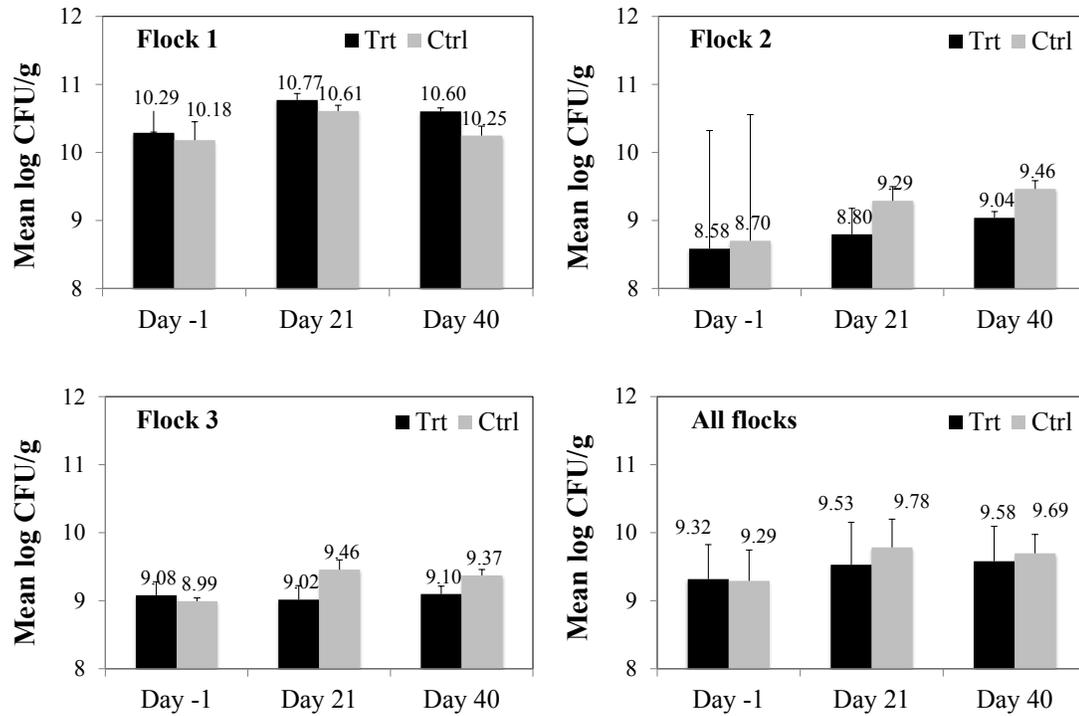


Figure 6 Average aerobic count of three flocks

The results of yeast and molds counts also did not show differences between sodium bisulfate treated and untreated litter (Fig. 7). Flock 1 had a relatively higher yeast and mold level ( $10^5$  CFU/g) at all three sampling events ( $P < 0.01$ ) while Flocks 2 and 3 showed lower levels ( $10^4$  CFU/g). There was no clear trend between yeast and mold level and bird age, litter pH and moisture content besides the flock effect. The average yeast and mold for both control and treatment houses over the three flocks ranged from 5.01 to 5.41 log CFU/g.

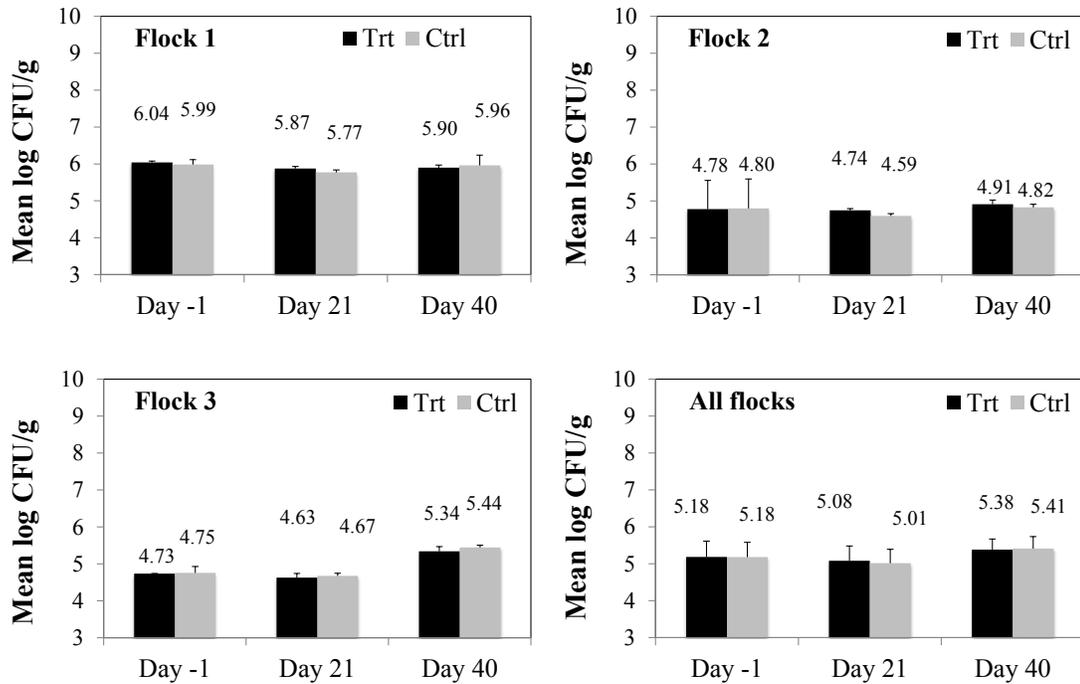


Figure 7 Average yeasts and molds counts of the three flocks

Coliform counts varied over a large range (3 to 7 log CFU/g) over the three flocks (Fig. 8). The litter prior to chick placement of Flock 3 had the lowest counts, 3 and 3.15 log CFU/g for treated and control houses, respectively. Flock 1 had higher coliform bacteria than the other two flocks ( $P < 0.01$ ), which was similar as observed for aerobic bacteria and yeast and molds. The coliform level of sodium bisulfate-treated litter in the flocks was significant lower than that of the control ( $P = 0.012$ ). There was a trend that the coliform level in the treated house was lower when litter was treated with sodium bisulfate, but the trend was not significant. The effects of bird age, litter pH and moisture content, and location were also tested and the results showed that none of these factors had clear relationship with coliform counts.

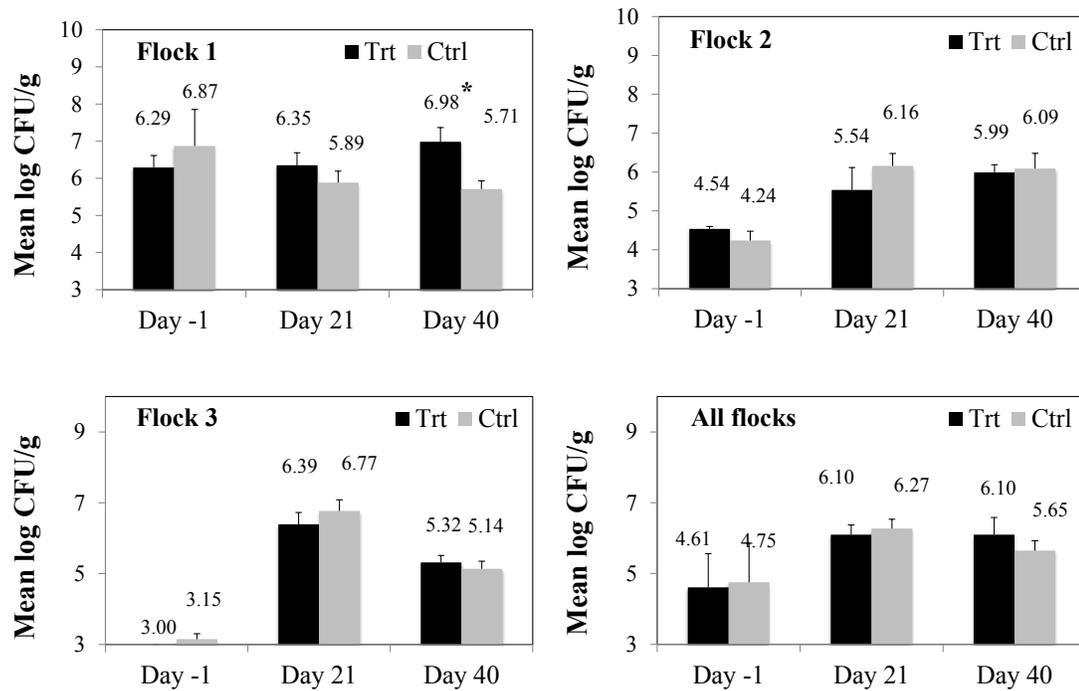


Figure 8 Average coliform counts of the three flocks

Fries et al. (2005) studied the dynamics of microbial populations and found that there was very little difference between bacterial populations after chicks were placed on the bedding material. Macklin et al. (2005) reported aerobic bacteria in broiler litter with fresh pine shaving varied from  $10^8$  to  $10^{10}$  CFU/g. The aerobic bacteria, by the second week, reached a plateau of  $10^8$  CFU/g that lasted until wk 6. Thaxton et al. (2003) evaluated microflora in builtup litter of commercial broiler houses in Mississippi and the results indicated that the broiler litter samples had lower counts than those in the current study:  $10^8$  to  $10^9$  CFU/g for aerobic bacteria,  $10^3$  CFU/g for

yeast and molds, and  $10^2$  CFU/g for coliforms. Another study (Barker et al., 2010) found that the aerobic and coliform levels in broiler litter changed with litter depth. The aerobic level was around  $10^7$  CFU/g while that for coliforms was  $10^6$  to  $10^7$  CFU/g. These studies showed large variation on bacterial populations under commercial production conditions.

Reductions in *Salmonella* and *Campylobacter* from the sodium bisulfate application may have occurred throughout the three flocks. However, the testing done was only qualitative, not quantitative. When the sodium bisulfate was applied to one house and then compared to a control, the results reveals that the control house had more incidences of *Salmonella* than the treated house (zero incidence). In the control house, *Salmonella* was not always detected, but did show up in several samples. Figure 9 shows the percentages of positive samples for each sampling period and indicated that the sodium bisulfate may have suppressed the growth of *Salmonella*.

When the samples were tested for *Campylobacter jejuni*, all control and sodium bisulfate treatment samples were positive. Thus, sodium bisulfate application did not affect the incidence *Campylobacter jejuni* in the litter samples.

Previous studies have examined the bacterial populations in poultry litter after litter amendment application. A study by Rothrock and coworkers (2008) tested Al<sup>+</sup> Clear (aluminum sulfate) effectiveness in reducing pathogens in poultry litter. They examined the application of Al<sup>+</sup> Clear on used litter kept in isolation chambers and its effect on fungi, *C. jejuni*, *Salmonella* and *E. coli*. Using QRT-PCR, they found Al<sup>+</sup> Clear to be significantly efficient at reducing *C. jejuni* and *E. coli*. *Salmonella* was

always below detection; however, their study showed a large increase in diversity of fungal species. The results led to the conclusion that Al<sup>+</sup>Clear could decrease bacterial populations, which would eliminate competition in the litter for the fungal species to grow. Cook and coworkers (2011) had a comparable study to Rothrock and coworkers (2008) that looked at total bacterial populations in litter with several acidifiers including aluminum sulfate and sodium bisulfate. Litter removed from the house was maintained in isolation chambers mixed with amendments. They found Al<sup>+</sup>Clear to cause an average of 1.0 log decrease in total bacterial populations, while sodium bisulfate led to a slight increase.

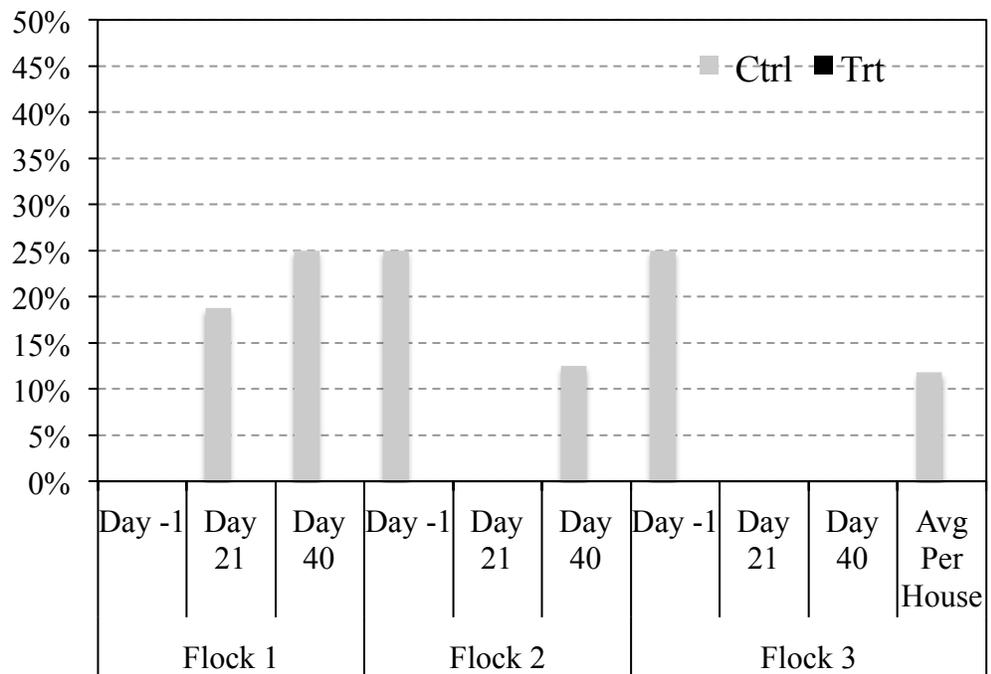


Figure 9 Percentage of litter samples found with positive *Salmonella* over the three flocks

The pH of the litter has been shown to be a major factor in the growth and survival of pathogens. Because many of these litter amendments are acidic compounds, it is reasonable to suspect that proper application could significantly lower pH and water activity of poultry litter, conditions that directly affect the survivability of microorganisms present in the litter (Line, 2002). Payne and colleagues' (2007) data suggest that by reducing litter pH to 4, paratyphoid *Salmonella* populations can be reduced below detectable limits within 20 h or less when litter is previously contaminated with high populations of paratyphoid *Salmonella* ( $\sim 10^7$ ). Once the birds are placed, the pH of the litter over time will start to rise towards a more neutral environment due the organic material and other factors present in the house. This may lead to the reapplication of litter treatments throughout the growout of the birds. Ivanov (2001) agrees through research experiments that lowering the pH (below 5.0) will create unfavorable conditions for the growth of ammonifying bacteria, *E. coli* and *salmonellae*.

The results from our study showed that frequent sodium bisulfate application can significantly control  $\text{NH}_3$ , but the impacts on total aerobic bacteria and coliforms was not significant under commercial conditions. It is possible that the litter treatments impacted certain groups of bacteria and favored survival or growth of others, but such changes would only be observable using selective media or genetic identification methods.

## CONCLUSIONS

Previous research found frequent sodium bisulfate application during broiler growout was effective in mitigating NH<sub>3</sub> in broiler houses and led to healthier and larger birds reaching the processing plants. A full scale field study was carried out to evaluate the effects of two additional sodium bisulfate applications during growout on bacterial populations and NH<sub>3</sub> emissions in two commercial broiler houses. The following conclusions and observations were made based on the results:

- 1) Multiple application of sodium bisulfate led to significant reductions in NH<sub>3</sub> emissions from broilers;
- 2) The reduction rate of multiple sodium bisulfate on NH<sub>3</sub> emission was 30% over the last 3 wks of a 6-wk growout;
- 3) Multiple sodium bisulfate applications showed improved production performance and foot pad quality;
- 4) Effects of sodium bisulfate on aerobic, coliform or yeasts and molds were insignificant;
- 5) Multiple sodium bisulfate applications reduced the incidence of *Salmonella* but not *Campylobacter jejuni*; and
- 6) Further investigation is warranted to assess higher application rate and different litter amendments with more frequent application. This study suggests some pathogens can be affected by amendment, but more quantitative tests are needed. By reducing the pathogen further, the health and welfare of the birds will be improved and results better and safer products.

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## **Chapter 3**

### **ASSESSMENT OF LITTER AMENDMENTS WITH MULTIPLE LITTER AMENDMENT APPLICATION ON AMMONIA EMISSION, GROWTH AND HEALTH OF BROILERS**

A paper to be submitted to Poultry Science

Alyson Weiss, Hong Li, Daniel Bautista

#### **Abstract**

Ammonia from poultry litter causes a myriad of health problems for birds raised in broiler houses and the people who care for them even at low levels. Currently, there are several different products available for commercial use including PLT (sodium bisulfate), Klasp (ferric sulfate) and Al<sup>+</sup> Clear (aluminum sulfate) to reduce ammonia emissions by applying them to the litter of poultry houses. Previous studies have shown PLT to be a food-grade product safe for bird consumption. When applied during grow-out it was noted to be effective at reducing ammonia and to allow for the production of healthier birds. A small-scale study was performed to compare the effectiveness of Klasp and Al<sup>+</sup> Clear with PLT on reducing ammonia during grow out while not adversely affecting the health of the flock. Three flocks of 30 birds were raised in isolated emission chambers for a standard grow-out period. Starting on day 21, Al<sup>+</sup> Clear, Klasp, and PLT were applied to six chambers weekly at two different rates (high - 976 g/m<sup>2</sup> and low - 244 g/m<sup>2</sup>) until the end of the flock. These

amendments were also added to the corresponding feeders following each application at two rates (1.4 g/bird for high and 0.35 g/bird for low) to mimic spillage of amendments into feeders under commercial production conditions. Ammonia emissions and bird growth performance were monitored. The birds were euthanized and necropsied to evaluate the health. The results showed no adverse effects were seen on bird performance and health in regards to the amendment applied to the litter weekly from 3-wk of age. PLT and AI<sup>+</sup> Clear were the most and least effective, respectively at controlling ammonia.

**Keywords:** Litter, amendment, feed, ammonia, broiler, health

### **Introduction**

Broilers are susceptible to a wide variety of diseases. Due to the close contact of the birds with litter, its quality can have a prominent effect on the health and performance (Nagaraj et al., 2007). Frequently, health issues with birds are caused by high ammonia (NH<sub>3</sub>) levels in the broiler house that is due to microbial degradation (Malone and Chaloupka, 1984). Ammonia in the air can adversely affect the body weight, feed conversion and livability of the birds (Seltzer et al., 1969; Line, 2002) and it can even negatively affect the workers involved in the care of the birds (Rylander and Carvalheiro, 2006).

Incidences of airsacculitis, increased susceptibility to diseases such as Newcastle and more frequent cases of keratoconjunctivitis are noted in birds raised in higher than average NH<sub>3</sub> levels (Do et al., 2005). A number of studies have suggested

that NH<sub>3</sub> levels should be not exceed 25 ppm, but even as little as 10 ppm can be damaging to tracheal mucous membranes (Weaver and Meijerhof, 1991). In the winter NH<sub>3</sub> concentrations can reach as high as 50-100 ppm in commercial houses (Kling and Quarles, 1974) and cause irreparable damage to health and welfare of the birds, possibly resulting in carcass condemnations.

Acidifier have been introduced to the market to improve litter quality by reducing pH, preventing growth of pathogens and NH<sub>3</sub> build-up (Weaver and Meijerhof, 1991). Improving litter quality can also alleviate foot and leg abnormalities and reduce the number of carcasses condemned at processing (Weaver and Meijerhof, 1991). A variety of products have been developed to combat this including aluminum sulfate, ferric sulfate, and sodium bisulfate. These products are typically applied prior to bird placement on the used litter between flocks.

The goal of this study was to determine the safety of these products when applied several times throughout the life of the flock.

### **Materials and Methods**

This study was conducted using six environmentally-controlled emission chambers (EC), measuring 74 x 72 x 74 cm (29 x 28 x 29 in) each, to mimic commercial production settings. The six chambers were housed in an environmentally-controlled room where air temperature was maintained at 20 °C (68 °F). Each chamber was equipped with an air blower on top with PVC pipes directing fresh air inside. Airflow rate through each chamber was measured via an air mass flow meter

placed in the supply stream. All chambers received the same amount of NH<sub>3</sub>-free fresh air, which led to 0.5 to 5 cfm/bird based on bird body weight and NH<sub>3</sub> concentration (less than 25 ppm). Samples of exhaust air from each chamber were taken using an air sampling pump at 5-min intervals. The first 4 min were for stabilization and the last 1 min was for measurement. The entire system took 35 minutes for a complete measurement cycle, including 5 min for the sampling of the ambient air. Successive sampling was accomplished through the controlled operation of six solenoid valves. A multi-gas analyzer, Innova 1412, was used for analysis. Analog outputs from the thermocouple used for temperature and mass flow meters and digital outputs from multi-gas analyzer were recorded at 1-s intervals into a computer through a data acquisition module. All measurements were averaged over the 60-s measurement period. Temperatures in each chamber were maintained by air allowed into chamber and wattage of the incandescent lightbulbs located in the middle of the top of each chamber. All chambers had the same type of bulbs at any given time.

Each chamber contained five female Ross 708 broiler chicks totaling thirty for each trial. They were kept at a stocking density of 10 bird/ m<sup>2</sup> (1.1 ft<sup>2</sup>/bird). Standard commercial diets were used. The birds in each chamber were raised on pine shavings. Four inch fresh shavings were applied to each chamber before a new flock. Water and commercial feed were available to the birds *ad libitum*.

Three commercially available products used were Al<sup>+</sup> Clear™ (General Chemical, Parsippany, NJ), PLT™(Jones-Hamilton CO., Walbridge, OH), and Klasp™ (Kemira Chemicals Inc., Atlanta, GA) which are already widely used by

broiler and turkey operations for the brooding period. Two rates were used: 244 g/m<sup>2</sup> (50 lb/1000 ft<sup>2</sup>) and 976 g/m<sup>2</sup> (200 lb/1000 ft<sup>2</sup>). Once the birds were 3 weeks old, the amendments were applied once per week. Amendments were also added to the corresponding feeders following each application at different rates (0.35 g/bird added for 50 lb/1000 ft<sup>2</sup> and 1.4 g/bird added for 200 lb/1000 ft<sup>2</sup>) to mimic spillage of amendment granules into feeders under commercial production at 0.85 g/bird. The amendments were broadcast-applied in a manner meant to simulate the application in a commercial house. Moisture contents of the whole litter profile samples were analyzed at 105°C (221°F) for 24 h at the end of each trial. Litter samples from the top 2.5 cm (1 inch) layer were collected every 2, 5 and 7 days post application for pH analysis. Ten-g litter sample were mixed with 100mL of distilled water for pH measurement.

During the trial, performance data was collected and recorded and included feed consumption, body weight, and feed efficiency. Body weight was measured weekly and feed consumption was recorded daily. Poultry health and welfare indicators associated with exposure to different levels of NH<sub>3</sub> exposure were recorded as a function of different types and application rates of selected litter amendments and included determination of adverse cutaneous reactions (skin and eyes) to direct contact with acid-based litter amendments and intestinal lesions associated with ingestion of the litter amendments. At the end of each trial all living birds were humanely euthanized and necropsied. Health and welfare parameters were measured between treatment groups and included 1) incidence and severity of ocular keratoconjunctivitis,

2) evidence of chemical burns to the skin on any part of the bird's body, 3) breast blisters, 4) incidence and severity of aseptic footpad dermatitis, 5) loss of body condition, 6) dehydration, 7) lung congestion, 8) *E. coli* septicemia lesion incidence and severity, 9) gastrointestinal lesion incidence (proventriculitis, gizzard erosion, intestinal tone, mucosal congestion and other signs of gut irritation). All necropsy results were recorded during the examination. Lung, kidney, trachea, duodenum and intestines samples from at least three birds of each chamber were collected for histopathology analysis. The samples was trimmed, processed, embedded in paraffin, sectioned to 4µm thickness and stained with hematoxylin. Results were broken down by tissue and recorded.

### **Statistical Analysis**

Results of NH<sub>3</sub>, body weight, feed conversion ratio, pH, moisture and necropsy results were compared and analyzed. A student's t test was performed to determine differences in those categories between treatment means. Significant factors were tested using standard least squares of JMP 11 (SAS Institute, 2013). Factors included treatment, rate and flock. Significance was accepted at  $P < 0.05$ . For moisture and pH, 11 samples per chamber per flock were tested.

## Results and Discussion

### Production Performances

The growth curves of each flock were plotted in comparison with the expected values from the Ross 708 manual (Fig. 10). The birds with low PLT and Al<sup>+</sup> Clear had lower body weight than the expected weight, but there was no significant difference ( $P = 0.37$ ) among the results from all treatments and the expected results (Table 3). In addition, the FCRs among the six treatments were not affected by the different amendments and two rates ( $P = 0.26$ ).

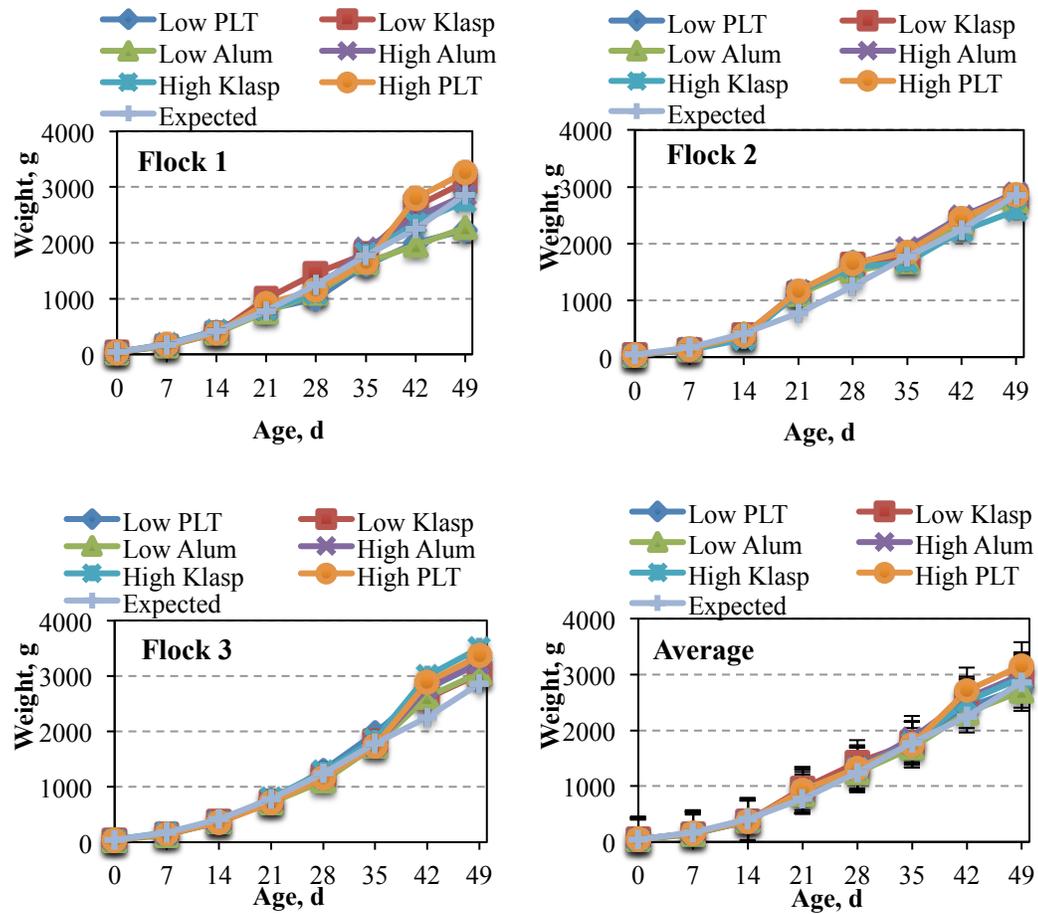


Figure 10 Growth curve of broilers with different litter amendments

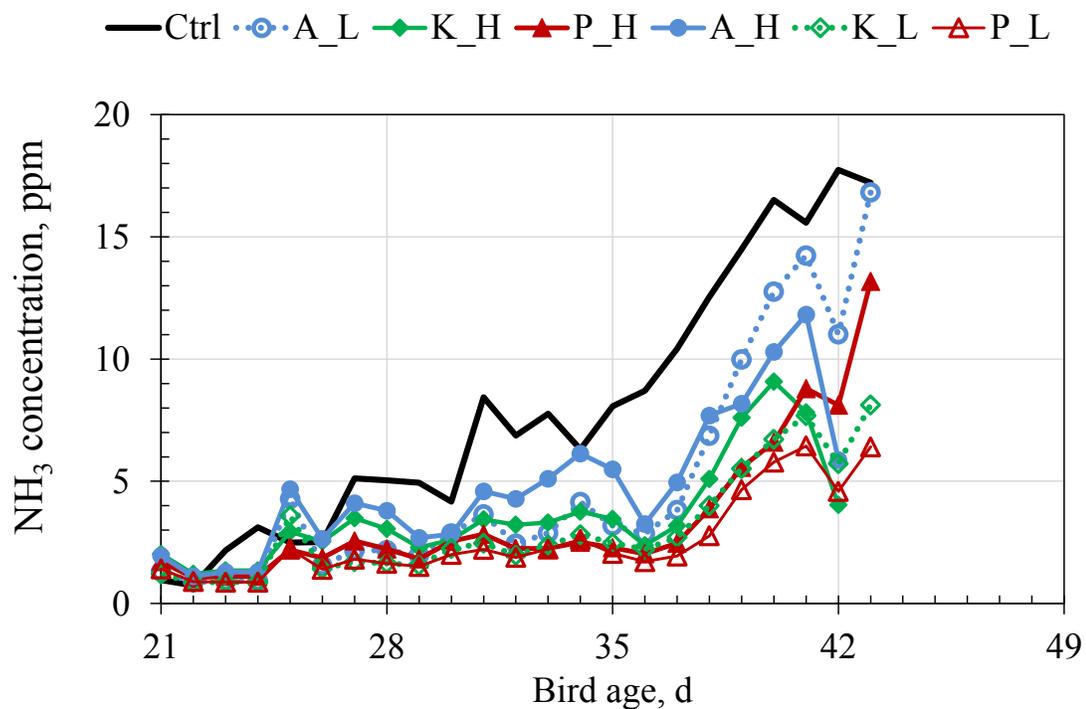
Table 3 Mean ( $\pm$ standard error) body weight and feed conversion ratio (FCR) of 7-wk broilers

<b>Litter Amendment</b>	<b>Rate</b>	<b>FCR</b>	<b>Weight, g</b>
PLT	Low	2.15 $\pm$ 0.37	2767 $\pm$ 506
	High	1.76 $\pm$ 0.21	3162 $\pm$ 267
Klasp	Low	1.98 $\pm$ 0.03	2968 $\pm$ 166
	High	2.00 $\pm$ 0.19	2942 $\pm$ 489
Al+ Clear	Low	2.04 $\pm$ 0.42	2697 $\pm$ 395
	High	1.90 $\pm$ 0.11	2999 $\pm$ 212

### **Ammonia Concentration and Emissions**

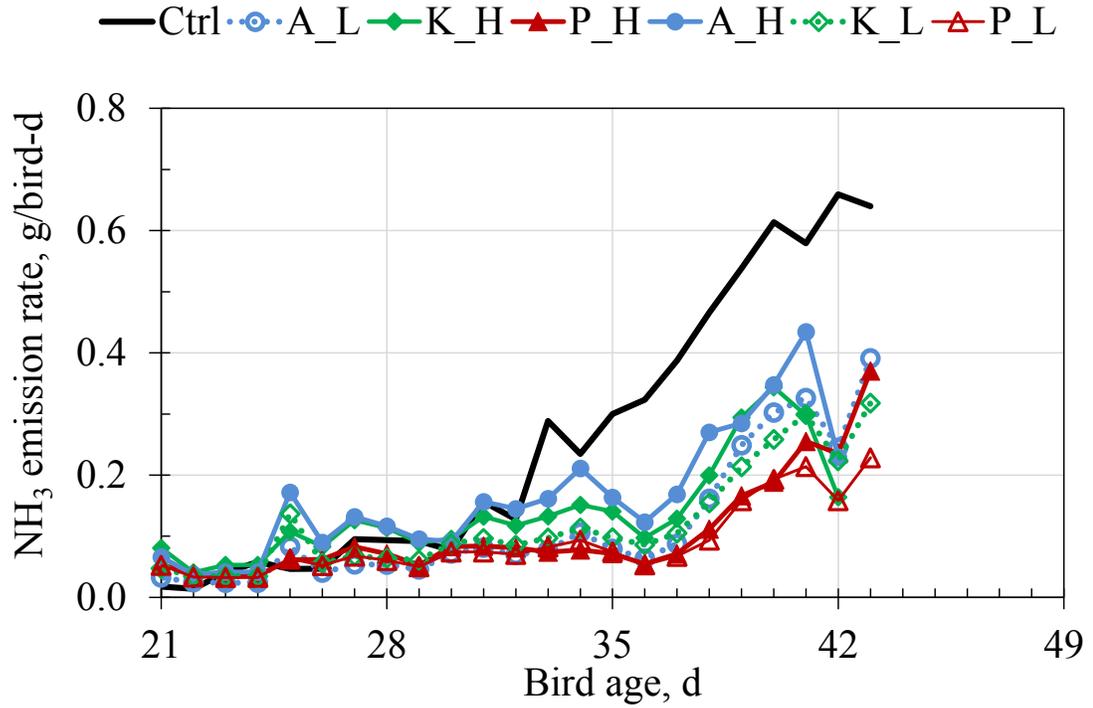
During the study, the airflow rates of each chambers were maintained between 3.4 to 6.8 m<sup>3</sup>/hr (2 to 4 cfm/bird) and NH<sub>3</sub> concentration in each chamber was monitored and kept below 25 ppm. The NH<sub>3</sub> concentration gradually increased with the age of the birds. Figure 11 shows the NH<sub>3</sub> concentrations of each treatment over the 7-wk period compared with a flock of birds that was previously raised and served as a control. The results indicate that all litter amendments lowered NH<sub>3</sub> concentration after litter amendments were applied on days 21, 28, 35, and 42 while NH<sub>3</sub> concentration of the control birds gradually increased from day 21 to day 49 ( $P < 0.01$ ). NH<sub>3</sub> emission rates were also compared among the six treatments and the control (Fig. 12). The reductions

in the NH<sub>3</sub> concentration and emission rate were more significant when birds were older than 28-d of age. The reduction rate of NH<sub>3</sub> emission rates between weekly applications was compared with student's t paired comparison (Fig. 13). The results suggest that PLT provided higher ( $P < 0.01$ ) NH<sub>3</sub> emission rate reductions than Klasp and Al<sup>+</sup> Clear while there were no significant differences in NH<sub>3</sub> emission reductions among the treatments ( $P > 0.1$ ).



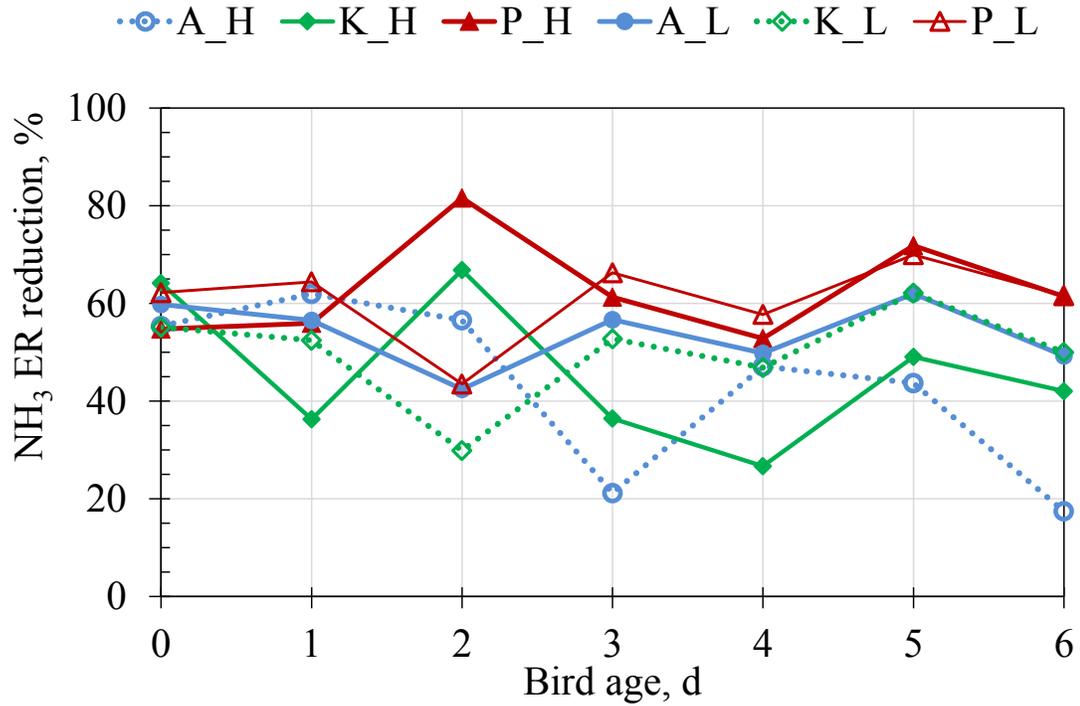
(A\_H: Al<sup>+</sup>Clear high rate, A\_L: Al<sup>+</sup>Clear low rate, K\_H: Klasp high rate, K\_L: Klasp low rate, P\_H: PLT high rate, P\_L: PLT low rate)

Figure 11 Daily mean ammonia concentration in emission chambers with three different litter amendments and rates (data points from 43 to 49 are missing due to instrument and drinker malfunction)



(A\_H: Al<sup>+</sup>Clear high rate, A\_L: Al<sup>+</sup>Clear low rate, K\_H: Klasp high rate, K\_L: Klasp low rate, P\_H: PLT high rate, P\_L: PLT low rate)

Figure 12 Daily mean ammonia emission rates of birds with three different litter amendments and rates (data points from 43 to 49 are missing due to instrument and drinker malfunction)



(A\_H: Al<sup>+</sup>Clear high rate, A\_L: Al<sup>+</sup>Clear low rate, K\_H: Klasp high rate, K\_L: Klasp low rate, P\_H: PLT high rate, P\_L: PLT low rate)

Figure 13 Daily ammonia emission reduction rates of the three litter amendments and two rates between weekly applications

### Litter Moisture and pH

Moisture content and pH values of the litter from the three flocks were pooled and analyzed with multiple pair comparison. Moisture content was not different among all treatments ( $P = 0.75$ ). Litter pHs were below 7.5 for all treatments at the end of the 7-wk growout. Klasp and PLT treatments with high rate further reduced litter pH value to 6.1 and 6.6. There was not a distinct relationship between application

and pH. The discrepancy could be caused by variation of litter samples at different locations and uneven distribution of the litter amendments on the floor.

Table 4 Mean ( $\pm$  standard error) moisture and pH values of litter samples and NH<sub>3</sub> emission reduction at end of the flocks

Amendment	Rate	Moisture, %	pH	NH <sub>3</sub> reduction, %
PLT	Low	39.3 $\pm$ 0.58	7.4 $\pm$ 0.39	60.8 $\pm$ 3.23
	High	37.5 $\pm$ 0.50	6.6 $\pm$ 0.14	62.8 $\pm$ 3.93
Klasp	Low	35.0 $\pm$ 1.41	6.6 $\pm$ 0.35	49.9 $\pm$ 3.78
	High	39.3 $\pm$ 1.15	6.1 $\pm$ 0.16	45.9 $\pm$ 5.67
Al <sup>+</sup> Clear	Low	37.5 $\pm$ 7.78	7.1 $\pm$ 0.23	53.8 $\pm$ 2.60
	High	39.0 $\pm$ 4.24	7.5 $\pm$ 0.12	43.3 $\pm$ 6.63

### Necropsy Score

Across the flocks, most parameters were in the healthy range. No birds in any chamber presented with oral ulcers. All the eyes were bright and clear with no ulcers or signs of irritation. Each flock was also free of breast blisters and had good, strong bones. Upon observation of each bird, air sacs were clear and healthy and organs appeared normal, other than a few fatty livers. During necropsy, the tissues seen with lesions or ulcers were trachea, foot pads and gizzards.

Among the 15 birds exposed to the low PLT treatment, two had slight hock burns and low-grade gizzard ulcers. The high PLT-treated birds had similar results. Only slight hock burns were seen in two out of the 15 birds. Fewer gizzard ulcers were observed, and were low-grade. The birds in the low Klasp trial showed very few health issues. Of all 15 birds, only two presented tracheal lesions. The gizzards of the

low Klasp group had mostly low-grade ulcers; however, some had more severe cases. The high Klasp birds were relatively healthy, but two birds showed tracheal lesions. A few also had agonal hemorrhaging in the trachea. One bird had a very low-grade early ulcer on one foot. About half of the birds presented some low scoring ulcers in their gizzards, with only one bird scoring above 1. In the low Al<sup>+</sup> Clear birds, there were few significant findings. Only one bird had tracheal lesions, but its organs appeared healthy and normal. However, most of the gizzards had low-scoring ulcers. In the high Al<sup>+</sup> Clear-treated group, about one third of the birds had tracheal lesions. Of the fifteen birds, one exhibited the start of footpad dermatitis on one foot. The other health parameters were in the normal range; but two birds presented with pale livers.

Table 5 Presence of tracheal lesions in each flock

Amendment	Rate	Flock 1	Flock 2	Flock 3	Average
PLT	Low	20%	0%	0%	7%
	High	0%	0%	0%	0%
Klasp	Low	40%	0%	0%	13%
	High	20%	20%	0%	13%
Al <sup>+</sup> Clear	Low	0%	0%	0%	0%
	High	40%	20%	40%	33%

The percentage of birds with observable irritation, such as redness or mucosal exudate, in each chamber of each flock was recorded (Table 5). Using the fit model function to look at the effects of flock, amendment rate, and rate, the results showed no significant differences among the other five treatments. Birds with obvious signs of

early ulcers or irritation were recorded (Table 6). Flock 1 only had hock burns while Flocks 2 and 3 had footpad dermatitis. Most of the gizzards were free of ulcers or other lesions. Some birds had been eating bedding despite adequate amounts of food in the feeders, but otherwise had normal gizzards. The percentage of birds in each treatment of each flock with any visible ulcers was recorded (Table 7). The differences in dermatitis and gizzard ulcer presence among the six treatments was not significant ( $P = 0.77$  and  $0.72$ )

Table 6 Presence of footpad dermatitis and hock burn in each flock

Amendment	Rate	Flock 1	Flock 2	Flock 3	Average
PLT	Low	0%	0%	0%	0%
	High	40%	0%	0%	13%
Klasp	Low	0%	0%	0%	0%
	High	0%	0%	20%	7%
Al <sup>+</sup> Clear	Low	40%	0%	0%	13%
	High	0%	20%	0%	7%

Table 7 Gizzard ulcer prevalence in each flock

Amendment	Rate	Flock 1	Flock 2	Flock 3	Average
PLT	Low	100%	100%	20%	73%
	High	40%	0%	40%	27%
Klasp	Low	100%	20%	40%	53%
	High	100%	0%	20%	40%
Al <sup>+</sup> Clear	Low	100%	0%	20%	40%
	High	100%	0%	40%	47%

## **Histopathological Analysis**

The histopathological observations noted no significant enteric damage (mucosal erosion, ulceration or necrosis) in a single intestinal sample (Table 8). Some of the bird groups (low Klasp, low AI<sup>+</sup> Clear, and high PLT) contained liver specimens with mild to marked hepatic lipidosis, which could be symptomatic of a primary metabolic disturbance within the hepatocytes, or it could be secondary to anorexia/inappetence from any cause. Within the groups for low Klasp, low AI<sup>+</sup> Clear, high AI<sup>+</sup> Clear, and high Klasp occasional liver specimens exhibited lymphocytic infiltrates with no evident cause. Some of the experimental groups, low PLT, high AI<sup>+</sup> Clear and high PLT, lymphocytic infiltrates observed in some of their pancreatic samples. The significance of this finding remains uncertain since this can be observed in clinically normal birds, however there was no associated tissue degeneration or necrosis in surrounding tissues. Some of the birds exhibited renal lymphocytic infiltrates, which is suggestive of low grade inflammation or immune response in this tissue. A single sample from the high PLT group revealed inflammation of the renal pelvis (pyelitis) but no parenchymal damage was seen in surrounding tissue of the kidney. Most interestingly, one bird in the low PLT group displayed significant expansion of, and nodular infiltration by, a lymphoid population with intermittent mitotic figures, which is suggestive of lymphoproliferation disease or lymphoma. Fairly common across the experimental groups was the occurrence of moderate bronchial-associated lymphoid tissue diffuse (BALT) or mucosal associated lymphoid

tissue (MALT) and follicular lymphoid hyperplasia in respiratory tissue. These findings are most likely due to the inhalation of environmental antigens. The results from this indicate the birds showed no negative effects based on the treatment applied.

The results found here indicate the use of PLT, Klasp and AI<sup>+</sup> Clear had no adverse health effects on the birds. Based on the gross observations in the necropsies and results from the histology samples, the birds remained healthy and clinically normal. The use of these treatments provided a reduction in NH<sub>3</sub> and caused no detrimental health effects to the birds that makes them available to be studied on a larger scale for their efficacy during grow out of a broiler flock.

Table 8

Histology results of three amendments with two application rates

Amendment	Rate	Histology		
		Intestinal	Renal	Respiratory
PLT	Low	Flock 2: LI in liver	Flock 1: Lymphoma	Flock 2: Pneumonia
	High	Flock 1: Lipidosis in liver Flock 2: LI in liver Flock 3: HP in liver	Flock 1: Pyelitis Flock 2: LI	Flock 2: HP in lungs
	Low	Flock 1: Lipidosis (L), LCI Flock 2: LI and HT in liver	Flock 1: LI Flock 2: LI	Flock 1: LCI in lungs, HP in trachea Flock 2: HP in lungs
Klasp	High	LI in all flocks	Flock 1: LCI Flock 2: LI	Flock 1: HP in lungs, HP in trachea Flock 2: HP in lungs, HP in trachea Flock 3: HP in lungs, HP in trachea
	Low	Flock 1: Lipidosis and LI in liver Flock 2: Lipidosis and LI in liver	Flock 2: LI	Flock 1: HP and HT in lungs, HP in trachea Flock 2: Lipidosis and LI in lungs
Al <sup>+</sup> Clear	High	Flock 1: Lipidosis and LI Flock 2: LI in liver	Flock 2: LI	Flock 1: HP in lungs and trachea Flock 2: HP in lungs and trachea Flock 3: HP in trachea

LI: lymphoid infiltrates; lymphocytic infiltrates: LCI; hyperplasia: HP; hematopoiesis: HT

## CONCLUSIONS

A small scale study was performed to evaluate the effectiveness of multiple application of PLT, Klasp and Al<sup>+</sup> Clear with two different rates during broiler growout. With each application occurring weekly after Day 21 of a seven-week growout the following observations were made based on the results:

- 1) Amendment and rate made no significant difference on the growth or feed conversion ratio of the birds;
- 2) Results indicated PLT provided the most NH<sub>3</sub> reduction, however there was no significant difference among amendments;
- 3) Litter moisture and pH were not affected by amendment or rate; and
- 4) Histological results indicated there was no adverse health effects caused by the amendments.

The results found here suggest the use of PLT, Klasp and Al<sup>+</sup> Clear were effective at reducing NH<sub>3</sub> in broiler houses. Based on the observations in the necropsies and results from the histology samples, the birds remained healthy and clinically normal. The use of these treatments in a small scale study provided a reduction in NH<sub>3</sub> and caused no detrimental health effects to the birds which makes them available to be studied on a larger scale for their efficacy during grow out of a broiler flock.

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Appendix

AACUC PERMISSION

UNIVERSITY OF DELAWARE

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

AGRICULTURAL ANIMAL CARE AND USE COMMITTEE

Application for Use of Agricultural Animals

In Teaching or Research

AACUC Protocol Number: (43) 08-28-13R1

TITLE OF PROJECT: Developing and Evaluating an Innovative Litter Amendment Application System for Poultry Operations

INSTRUCTOR/PRINCIPAL INVESTIGATOR

Hong Li  
Printed Name



Signature

8/27/2013  
Date

.....  
(This section for Committee use only)

Application Approved (date): 09-20-13

Application Rejected (date) \_\_\_\_\_

Reason for Rejection \_\_\_\_\_



Signature, Animal Care and Use Committee

Date

9-20-2013

APPLICATION INFORMATION:

Title: Developing and Evaluating an Innovative Litter Amendment Application System for Poultry Operations

Instructor/Principal Investigator: Hong Li

Address: 237 Townsend Hall

Telephone: 302-831-1652 Email: hli@udel.edu

Co-Investigators:

Address:

Telephone: Email:

People involved in animal care for this protocol:

Name	Email	Office Phone #	Home/Cell Phone #	Received Animal Care Training	
				Yes	No
Hong Li	hli@udel.edu	3028311652	5154411331	X	
Stephen Collier	slc@udel.edu	3022459894		X	

Has everyone listed above read the application and is familiar with the proposed work?

YES  NO

If no, identify those needing to read application.

\_\_\_\_\_  
\_\_\_\_\_

New or Three Year Review (mark one)

NEW  THREE YEAR

If this is a 3 year renewal, what is the assigned existing protocol number?

\_\_\_\_\_

Teaching or Research Application (mark one)

TEACHING  RESEARCH

If TEACHING box was checked, select from the following:

Demonstration  Laboratory  Student Project

**Proposed start date:** 10/1/2013 **End date:** 9/30/2015

Are all proposed animal care management procedures 1) defined as “pre-approved” by the Animal Care and Use Committee, or 2) part of the Standard Operating Procedures developed by the Animal Care and Use Committee for that particular species?

YES  NO  to be determined by AACUC

Has everyone been trained? YES  NO

Who has not been trained?

\_\_\_\_\_  
\_\_\_\_\_

Name the person responsible for conducting the training.

Hong Li

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If after hours participation is required by students, please describe how this is being handled. (e.g. supervisors, assistants, etc.) Please include the times and days that students may be on site.

The students will be trained by Hong Li to properly handle the bird during weekend and after hours. If any emergency happens, students will call and report to Hong Li.

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#### **ANIMAL INFORMATION:**

Common Name of the Animal Requested: Broiler Chicken

Amount Being Requested: 12,090 from Amick and 228,000 from Mountaire

Source of Animals: Amick farm

Where are the animals being held: One small colony houses and one BLK houses with isolators at Poultry research farm in Newark, the environmental house at Carvel research and education center, 12 environmental room at UMES, and two commercial houses at UMES

Briefly Describe the Goals or Objectives of this Application (use additional space as needed).

This research project aims at developing and evaluating an innovative mitigation technology to reduce reactive nitrogen (ammonia) emission from poultry feeding operations, conserve nitrogen in poultry litter and increase its fertilizer value, and improve animal welfare, health and production. The project will focus on accomplishing the following objectives: 1) to develop an automated application system (AAS) in broiler houses for acid-based litter amendments under field conditions; 2) to evaluate and delineate the efficacy of various amendments and application rates/intervals on air emissions and animal health; 3) to conduct field verification tests in two commercial poultry houses and economic analysis on acid-based litter amendments regarding production performance, microbial communities, and air emissions over an extended period; and 4) to investigate and characterize the microbial environment of treated poultry litter with AAS emphasizing specific microbiological agents that impact broiler chicken health and ammonia formation.

The knowledge and technology developed will contribute to effective air emission abatement for the protection of the environment and human health, and the conservation of nitrogen to contribute to the economic competitiveness of the animal industry. The results of the project have the potential for nationwide application. The longer-term outcome is attaining the goal of an environmentally sustainable animal production system.

Please state or attach your animal protocol.

**Experiment 1.**

Initially three commercially available products will be evaluated. They include Al<sup>+</sup> Clear™ (General Chemical, Parsippany, NJ), PLT™ (Jones-Hamilton CO., Walbridge, OH), and Klasp™ (Kemira Chemicals Inc., Atlanta, GA) which are already widely used by broiler and turkey operations for the brooding period. The test will be conducted using six environmentally-controlled emission chambers (EC) measuring 29 x 28 x 29 in (74 x 72 x 74 cm) each. The emission chambers allow better mimicking production settings than can be achieved with small emission vessels. The EC system will be located in an environmentally-controlled

room where the air temperature will be held at 68 °F (20 °C). Fresh air to each chamber will be supplied by a blower through a PVC pipe. The airflow rate through each chamber will be measured with an air mass flow meter placed in the supply air stream. All the ECs will receive the same amount of NH<sub>3</sub>-free fresh air, which will lead to 0.5 to 5 cfm/bird based on bird body weight and NH<sub>3</sub> concentration (less than 25 ppm). The system will be set up and handled in a way that mimics real house conditions.

Each chamber will contain five Ross or Cobb female broiler chicks from 21 to 56 days with a stocking density of 1.1 ft<sup>2</sup>/bird (10 bird/ m<sup>2</sup>). The birds in each EC (Horsfall-Bauer isolation chambers) will be fed standard commercial diets. Used litter (4 in. depth) from a commercial broiler farm with no known significant disease issues will be used for the test. The used litter will have 2.5 to 2.75% TKN and 25% moisture. Three litter amendments will be broadcast-applied to the treated chamber at two different rates (25 lb/1000 ft<sup>2</sup> and 100 lb/1000 ft<sup>2</sup>). Application requirement or interval of the agent will be determined by examining continuous NH<sub>3</sub> emission of ECs and pH profiles of the treated litter. When NH<sub>3</sub> emission begins to increase appreciably, for example when the daily NH<sub>3</sub> emission rate reduction becomes less than 60%, another application of the agent will be carried out. Litter amendments will be added to the corresponding feeders following each application at different rates (1.75 g/bird added for 25 lb/1000 ft<sup>2</sup> and 7 g/bird added for 100 lb/1000 ft<sup>2</sup>) to mimic the spillage of amendment granules into feeders under commercial production at 0.85 ft<sup>2</sup>/bird.

### **Experiment 2. Animal Care protocol is being sought at UMES for this portion of the work**

One or two litter amendments, screened as described in section 3.2.1, that did not compromise production performance and health of broilers will be tested further to determine application requirements. Preliminary evidence indicated that PLT (sodium bisulfate) should work. This test will be conducted at the University of Maryland Eastern Shore (UMES) by using one large house with 12 environmental rooms (ERs), 20 ft x 20 ft each. Each ER will be managed separately, but shares the same bird genetics and production stage. Each room will have an initial placement of 500 straight-run birds (mixed sex, Cobb or Ross) with used litter. The production rooms have insulated ceilings, box air inlets along the central alley, one brooding heater (30,000 BTU), one 12-inch centrifugal fan and one 24-inch diameter fan located on the side wall of the house. Independent environmental controllers will coordinate control of air temperature, ventilation fan and heater operation, and lighting programs. Daily NH<sub>3</sub> emission rates (ER) and cumulative emissions from each room will be calculated and used for data analysis. Production performance data for both control and treatment rooms, including feed consumption, body weight, feed efficiency, and bird mortality, will be collected and necropsied daily. The live weight of the birds in each room will be measured and recorded with a bird scale. Bird mortality will be recorded daily. The feed added into each room will be weighed and recorded. At the end of the trial, the birds will be weighed again and feed conversion ratio (FCR) will be calculated.

Three application rates and four application intervals will be evaluated. There will be five different combination treatments for application rates and intervals plus one control during each trial. The application rates and intervals will be refined based on the results of the screening test. Two rooms will be assigned for each treatment and control. Three trials will be conducted at different seasons to cover seasonal variation.

**Experiment 3. Animal Care protocol is being sought at UMES for this portion of the work**

One litter amendment and application rates and intervals identified as described in Experiment 2 will be tested further to determine air emission reduction, production performance, and litter quality under field conditions at a commercial broiler farm managed by the University of Maryland Eastern Shore and at the University of Delaware research farm. A paired comparison will be conducted for both sites. The two identical commercial houses on the commercial site have dimensions of 500 ft x 60 ft (150 m x 18 m) each and a holding capacity of approximate 35,000 broiler bird. The university research house, measuring 114 ft x 37 ft (34.6 m x 12.1 m), is divided into two partitions, 57 ft x 37 ft (17.3 m x 12.1 m) each. The two partitions (2000 birds/partition) are identical and share the same end wall and control room. On both sites, one house/partition will be equipped with the litter amendment application system developed as described in Objective 1, and the other house/partition will be considered as control. Three flocks of birds will be raised in each house to cover warm and cold weather conditions. All houses will start with used litter with known nutrient and moisture contents and pH. The broilers will be fed for 49 –56 days to reach a market body weight of approximately 7 lb.

How did you determine the number of experimental animals you are requesting? If you have a table showing treatment groups and animal numbers please insert here or include as an attachment.

**Experiment 1**

Each environmental chamber (EC) of the EC system can hold up to 5 birds with 1.1 ft<sup>2</sup>/bird stocking density. 30 birds are required for each batch. Each batch will include 6 treatments (3 amendments X 2 rates). A power analysis was conducted to determine the sample size. Based on the preliminary study (table 1), sample sizes were determined while  $\alpha$  is 0.05 and power is 0.8. The theoretical value of total sample sizes is 16 for 6 treatments. We decided to have 3 replicates for each treatment. Therefore, total 90 birds (30 birds/rep x 3 reps) are requested.

Table 1. Emission rate (g/bird) from litter treated with PLT

Treatment	1	2	3	4	5	6
Mean, g/bird	6.1	7.8	8.8	8.9	9	9.2
SE	0.9	0.9	0.9	0.9	0.9	0.9

**Experiment 2**

Six large environmental rooms will be used to mimic the commercial condition for each litter amendment. Each room will have an initial placement of 500

straight-run birds (mixed sex, Cobb or Ross) with used litter. Each room will represent one treatment. 3000 birds are requested for each batch and each litter amendment. Each batch will include 6 treatments. Three replicates (batches) are required for each treatment based on the power analysis for Experiment 1. Total 9,000 birds are requested for each litter amendment in the large environmental room study. If two litter amendments are identified from the Experiment 1, total 18,000 birds are requested for Experiment 2.

**Experiment 3**

Three flocks are needed at the two sites under commercial production conditions. Three replicates (flocks) are required for each treatment at each site based on the power analysis for Experiment 1. 210,000 birds (70,000 birds/ flock X 3 flocks) are requested at University of Maryland Eastern Shore site. 12,000 birds (4,000 birds/ flock X 3 flocks) are requested at University of Delaware research site.

Please verify that the research involving this protocol is new and is not a duplication of work already performed.  
A literature search looking for duplication of work has been done. There is no such study has been done.

Does this procedure involve surgery? YES  NO

If yes, explain in detail the surgery.

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Will the animals experience pain? YES  NO

If so, what is your pain management protocol? Please insert here or include as an attachment (euthanasia is an acceptable means of pain management):

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Are drugs and/or medications being used? YES  NO

If yes, describe what is being used. Include dosages and sites.

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How often are animals monitored and how are sick or injured animals being handled?

The birds will be monitored daily. The sick or injured animal will be euthanized.

What is the method of euthanasia?

Cervical dislocation

List the veterinarian who is on-call.

Dr. Miguel Ruano

302-831-1539

Name

Telephone

Does this application need approval from OHS? YES  NO

If yes, what form(s) are attached? \_\_\_\_\_

NOTE: OHS approval is required for experiments involving the administration of hazardous or biological materials such as pathogens, carcinogens, highly toxic, or radioactive materials.