EFFECTS OF MICROBIAL LITTER AMENDMENTS ON BROILER PERFORMACE, LITTER QUALITY, AND AMMONIA PRODUCTION

A Thesis

by

MATTHEW JAMES HINKLE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	Craig D. Coufal
Committee Members,	David J. Caldwell
	Michael A. Davis
Head of Department,	John B. Carey

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ABSTRACT

Effects of Microbial Litter Amendments on Broiler Performance, Litter Quality and Ammonia Production. (December 2010)

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Chair of Advisory Committee: Dr. Craig Coufal

The reuse of litter in broiler production can lead to litter pathogen buildup and high levels of ammonia in broiler housing, thus resulting in poor broiler performance. This study evaluated the effects of two microbial litter amendments on litter characteristics, ammonia production and broiler performance. Experiment one, consisting of three trials, utilized eight pens approximately 3 x 3.2 m (10 x 10.5 ft) to rear broilers to 49 d of age. Experiment two, consisting of one trial, utilized twelve 1.8 x 3.7 m (6 x 12 ft) pens to rear broilers to 42 d of age. Used litter was obtained from separate commercial broiler farms for each experiment and placed into the pens at an average depth of 11 cm (4.3 in). Feed consumption and mortality were recorded for each pen for each trial. Ammonia production was measured by placing an enclosed chamber over the litter and measuring the headspace ammonia concentration after 20 minutes for both experiments. Experiment one also utilized a two minute ammonia flux technique. Ammonia measurements were taken at the time of litter treatment, at chick placement, and once per week for the remainder of the grow-out. Litter samples were collected at the same time and location as ammonia measurements. At the end of all trials, caked litter was

removed from each pen, weighed and sampled. Litter and cake samples were analyzed for total aerobic and anaerobic microbial counts in experiment 1. Experiment 2 analyzed aerobic litter samples only. Paw scores were also recorded at the end of each trial for all birds using a 3-point scale. Data was subjected to ANOVA using the GLM procedure with means deemed significantly different at $P < 0.05$. Statistical differences were seen sparingly in different parameters in both experiments; however these differences were random in their distribution and showed no trend. Final results indicated that the microbial litter amendments had no effect on broiler performance, litter characteristics or ammonia production.

DEDICATION

To my mother and father, for without your love, support, and dedication I could never hope to be where I am today; and, of course, to my wife Sara, you are the force that drives me to succeed in all that I do.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Poultry litter, by definition, is a combination of used bedding material, poultry manure, and poultry feathers (Terzich et al., 2008). Optimally, at the end of every flock the used bedding material would be removed and replaced in order to promote an environment with less potential for bacterial growth and ammonia (NH_3) production. Cake is described as crusted litter found around areas of greater moisture, such as drinker lines and evaporative cool pads. The Poultry Waste Management Handbook (NRAES, 1999) suggests that up to 30-35% of the total litter may be made up of cake.

Poultry litter serves multiple purposes. It acts as a cushioning material for broilers to help prevent carcass downgrades that would happen if the broilers were raised on a hard or wire surface, provides a layer of insulation above the ground for chicks during brooding, acts as an absorbing material for moisture, and it provides a non-slick surface for the birds to walk on.

The beginning bedding material comes in many different forms. The most popular bedding materials are generally by-products from agriculture and forestry. Typical bedding materials include pine wood shavings, saw dust from lumber mills, rice hulls, peanut hulls, straw chaff from wheat production and sand. Recycled paper, which can be formed into pellets, has also been utilized as bedding material; however, research has shown an increased likelihood of caking when using paper products (Worley et al.,

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This thesis follows the style of Poultry Science.

1999). Worley et al., 1999 also suggested that some of the most popular bedding materials are becoming more costly due to demand. Instead of selling the by-products to poultry operations, companies are using them in an attempt to save money in their own operations. This has resulted in an increased cost for poultry producers to procure bedding materials if the materials are available at all.

Poultry litter is a major factor in the economic considerations of today's poultry industry. A key component in current industry practices to maintain economic feasibility is the reuse of litter for multiple flocks in an attempt to postpone the complete cleanout of litter and the subsequent replacement with new and more costly bedding material. However, care must be taken during litter reuse to ensure proper management techniques are applied. While the main reason for litter reuse is cost savings, the well-being and successful rearing of the birds must also be given appropriate consideration. If handled correctly, litter can be an excellent source of revenue for poultry growers through its sale after cleanout. Due to its plant nutrient content, poultry litter is widely utilized as a fertilizer for crops. Additionally, poultry litter can be further processed and utilized as a feed additive for cattle as previous research has indicated that it makes a good protein source (Martin et al., 1998). However, if management of poultry litter is neglected, it can result in overall loss due to poor bird performance and health. Poorly managed, reused litter can result in elevated volatilization of NH_3 in broiler housing, increased moisture levels which may result in increased incidences of breast blisters and burns on the paws

of broilers, the potential to harbor increased numbers of total bacteria within the litter, and an increase in insects.

Ammonia is produced as the result of the microbiological breakdown of nitrogenous organic compounds in litter, namely uric acid, urea, and proteins. Farm managers must take into account prevention, control, and mitigation strategies for NH_3. Improper litter management can lead to elevated levels of NH_3 within broiler housing (Reece et al., 1979). Broilers exposed to elevated levels of NH_3 were shown in research by Beker et al. (2004) to have a lower overall performance and an increased susceptibility to disease.

The overall goal of litter management is to manipulate the litter to remove the caked litter, agitate the litter to promote drying, and release of the stored NH_3 before the placement of the next flock (Topper et al., 2008). These practices require time, labor and expense. If sufficient time is not given to these practices, NH_3 and possible pathogenic build-up may occur. Litter amendments have been introduced that impact the NH_3 volatilization in a number of ways. Those that utilize alkaline materials (pH above 7.0) will cause an increased volatilization of NH_3. Some that utilize acidic materials (pH below 7.0) will cause an inhibitory affect on NH_3 volatilization from litter, and some litter amendments will also have an inhibitory effect on microbiological load (Pope and Cherry, 2000; Terzich et al., 1998a; McWard and Taylor, 2000).

The purpose of this study is to determine the effectiveness of two microbiological poultry litter amendment sprays on reduction of NH_3, their effects on litter characteristics, as well as overall broiler performance. Experiment one consisted

of 3 trial flocks and examined the efficacy of the proprietary product marketed as Litter Guard™ (LG). Experiment two consisted of one trial flock and examined the efficacy of a second proprietary product marketed as Poultry Waste Degrader™ (PWD).

Objectives for each experiment are to determine the effects of microbial litter amendments on broiler performance, litter characteristics, and litter ammonia flux. Broiler performance will be determined by observations of final body weights, feed consumption and conversion, total mortalities, and paw scores. Poultry litter characteristics will be determined by analysis of litter pH, water activity (a_w), total nitrogen (N) and carbon (C) content, total caked litter produced and total aerobic and anaerobic bacteria. Poultry litter ammonia volatilization will be determined utilizing a flux chamber placed over the broiler litter.

Literature Review

Litter Characteristics

In order to correctly manage poultry litter, it is first necessary to understand its characteristics. Poultry litter is a highly variable substance, meaning that pH, moisture, and nitrogen can all vary throughout any given house. Tasistro et al. (2004) suggests that this variability is attributed to the state of decomposition in which any portion of litter may be undergoing. Samples observed showed that litter under drinkers showed the most decay, litter under feeders showed the least decay, and mid-house showed a median between the two. The purpose of understanding litter characteristics is to ultimately understand the factors that contribute to litter producing NH_3. In previous research it was found that pH, temperature, and moisture content all contribute to NH_3 release, and that

pH has more effect than temperature which has more effect than moisture content (Elliott and Collins, 1982).

Turnbull and Snoeyenbos (1973) showed in their research that the pH of poultry litter would depend on the age of the litter. New bedding material was found to have a pH between 5.0 and 6.5, whereas thoroughly used litter would have a pH of 8.5 (Elliott et al., 1982). Terzich et al. (2000) found that the average pH of litter across several regions was 8.0. This increasing level of pH over poultry litter life plays a key role in the overall formation of NH_3. When poultry litter increases in age, and therefore, in manure content, the pH will rise. As the pH rises to a more alkaline state, NH_3 will shift to an un-ionized state and become available for volatilization (Ferguson et al., 1998).

This finding is further corroborated by Reece et al (1979) in which it is was suggested that NH_3 release is low to unnoticeable at a pH less than 7.0, and will begin to reach its highest volatilization at pH greater than 8.0. It has been suggested that pH levels below 5.0 are required to completely fix nitrogen within poultry litter (Derikx et al., 1994). Interestingly, lower pH levels have benefits in addition to decreased volatilization levels. When levels fall below pH 6.0, an inhibition of ammonifying and putrefying bacteria are seen, and when levels below pH 5.0 are reached hostile conditions are found for salmonellae (Byrd, 1999).

Elliott et al. (1982) found that directly following pH and temperature, litter moisture has the greatest effect on NH_3 volatilization rates. Because of this, a large portion of litter management is devoted to controlling litter moisture content in poultry broiler operations. Although significant attention is paid to the overall control of

moisture, poultry litter is highly variable in its moisture content. Sampling sites, age and health of the bird as well as diet can all affect total moisture content (Patterson et al., 1998). Malone et al. (1992) found that moisture content for poultry litter averages 27%, with cake moisture averaging 40%. Patterson et al. (1998) found moisture content averages at 31%. Martin et al. (1998) observed that the average moisture content of 21.9% for poultry litter. NRAES (1999) found litter moisture to be 21% with cake moisture being 40%. Carr et al. (1990) suggest that moisture content be kept below 30% to reduce NH_3 levels, stating that levels above 30% result in increased NH_3 volatilization. Terzich et al. (2000) found that the average moisture content across a 12-region area was 25.1%. NRAES (1999) suggest that litter moisture try to be maintained around 20-25%. Carr et al. (1990) concluded that higher NH_3 concentrations at higher moisture levels were a result of increased capillary action as a result of the greater moisture, which in turn resulted in an increased diffusion rate of NH_3.

Research conducted by Hayes et al. (2000) found that within poultry litter there was a positively correlated relationship between a_w and moisture content of the litter. Their findings that showed 74.4% of samples had an a_w above 0.90 with 72.1% of moisture content greater than 30%, and that when moisture content fell below 26% a_w also fell greatly. Furthermore, it has been shown that a_w is a deciding factor in the control of microbial populations. When a_w is held at a level below 0.70, bacterial growth will be reduced (Scott, 1957).

The two main components of N in poultry litter are uric acid, constituting 70% of the total N, and undigested proteins, which constitute 30% of the total N (Groot

Koerkamp, 1994). Nitrogen is a key component in the formation of NH_3 the more N that is present, the more possibility there is for the formation of NH_3. Conversely, if levels of N can be maintained the levels of NH_3 that is volatilized could be potentially reduced. When NH_3 volatilization is reduced, total N increases in poultry litter (Moore et al., 1995). Carr et al. (1990) describes poultry litter as a "nitrogen storehouse", stating that if conditions are appropriate a large quantity of the stored N will be converted to NH_3. The appropriate conditions, in this case referring to findings of Elliott et al. (1982), are elevated pH, temperature and moisture. Conversely Derikx et al. (1994) suggest that complete N fixation can be achieved when pH is maintained at 5.0. Poultry litter, as stated before, is variable in its moisture content as well as pH. Nitrogen is similar in that it is also variable throughout poultry litter. Patterson et al. (1998) found averages of 3.31% and 3.94% total N. Malone et al. (1992) found 2.7% total N for litter and 3.25% total N for cake. NRAES found 3.5% total N for litter and 2.3% total N for cake. There is also a seasonal variation, during summer months volatilization of N was as high as 82%, yet in winter months it can fall as low as 23% (Coufal et al., 2006).

Taking into account the multiple characteristics of poultry litter, which all combine together to bring about the volatilization of N to NH_3, it is necessary to grasp a detailed understanding of these characteristics to be able to effectively manage poultry litter.

Litter Management

In order to effectively rear broilers, it is necessary to control the environment in which they are raised; litter management exerts an essential role in this aspect. While the

production of NH_3 is often difficult to control under commercial conditions, the circumstances that affect the production are not. Such factors include litter management, litter amendments, ventilation, diet formulations, and drinker management. Litter management is an approach to the maintenance of good quality litter. This approach consists of litter age, as well as after flock management, such as cake-out and top dressing.

One of the most important factors for litter management is to control litter moisture, and the most efficient way to control litter moisture is through ventilation. However, this is not always economically feasible. During the first two weeks of growth, temperatures must be kept elevated to promote bird growth. Ventilation is the key to promote moisture control, yet temperature must be kept relatively high. Money must either be spent on fuel for heating or the possibility for higher amounts of NH_3 may become a factor. Carr et al. (1990) states that ventilation can also lead to negative issues such as excessively dry litter, which can lead to dust issues. Research has shown that the combination of *Escherichia coli* and dust particulates could induce a pathogenic response in 4-week-old broilers (Oyetunde et al., 1978).

Understanding that litter moisture is a direct contributor to NH_3 volatilization, it is necessary to control areas in which moisture can be added to poultry litter, mainly drinker lines, and evaporative cool pads. To begin, all equipment should be maintained in a working condition in which no leaks are present and cool pads should only be operated during days of low humidity. According to Carey et al. (2004) during periods of cooler temperatures birds will exhibit a reduced water intake; because of this, the

pressure in water systems lines will need to be reduced in order to prevent excess leakage. Observation of litter conditions are extremely important for moisture management; if wet litter is observed, solutions such as raising drinker heights or decreasing pressure needs to be considered (Carey et al., 2004).

Finally, management between flocks plays an important role in litter management. After removal of a flock, cake should be either scraped and removed or broken and tilled back into the litter. If the cake is removed a "top dress", or addition of additional bedding material, may be necessary. Litter depth has been shown to affect NH_3 volatilization rates, where higher rates were seen with less bedding material (Al Homidan et al., 1997). Furthermore, practices aimed at NH_3 volatilization can be utilized prior to chick placement. Topper et al. (2008) describes a practiced deemed as "cook off" in which litter is brought to a high temperature prior to chick placement; the intended effect of this is to promote an increase in the production of NH_3 in an attempt to reduce NH_3 volatilization during the remainder of rearing.

Ammonia Production in Poultry Litter

Ammonia has been and continues to be a major issue within the poultry industry. Not only does NH_3 have effects on farm management, it also plays a role in poultry health, which will be discussed in following sections, as well as a large role in overall poultry production performance. In order to control NH_3, it is first necessary to understand how NH_3 is produced.

Ammonia is a colorless, water-soluble gas with no ionic charge. Ammonia, or more specifically volatilized NH_3, is described as the result of microbial breakdown of

nitrogenous compounds; in regards to poultry production these are found in uric acid, urea, and other organic nitrogenous compounds. As was mentioned earlier, any litter containing a pH of greater than 7.0 allows for the production of NH_3 (Reece et al., 1979). The reason for this is that NH_3 has no ionic charge, which allows for its volatilization; adding a hydrogen ion transforms NH_3 into ammonium (NH_4^+), which is a nonvolatile form. In order to complete this transformation, however, the pH must be acidic.

Ammonia has a variety of factors that will affect how quickly and the rate at which it will be formed. As was stated earlier, Elliott et al. (1982) found that pH, temperature and moisture content all contribute to NH_3 release, and that pH has more effect than temperature, and that temperature has more effect than moisture content. In turn, these factors can be affected themselves by outside environmental conditions such as seasonal climate changes and weather conditions (Carey et al., 2004).

Studies have shown that litter with pH less than 7.0 showed very little NH_3 volatilization; however, as pH approached 7.0 volatilization of NH_3 began to increase, and at a pH of 8.0 volatilization of NH_3 reached its maximum level (Reece et al., 1979). It can be concluded that poultry manures' natural pH range, shown by Elliott et al. (1982) of 8.5 in well used litter, as well as Terzich et al. (2000) who found an average of 8.0 across several regions, makes it an ideal starting point for the production of NH_3.

As was previously mentioned Coufal et al. (2006) found that N loss in manure due to volatilization in summer reached 81.5% while in winter months it can fall as low as 18.1%. Warm summer conditions will require the use of cooling pads, which, in turn, increase moisture. Cold winter air can promote condensation on external walls and

coupled with a reduction in ventilation such conditions will promote moisture. Carr et al. (1990) states that high moisture content in poultry litter will result in high NH_3 diffusion rates, which results in elevated levels of NH_3 in broiler houses.

Seasonal variations will promote differing effects on temperatures within poultry houses. A study conducted by Visek (1968) showed that an increase of as little as 1-2 degrees Celsius would result in an increase in NH_3 volatilization. The previously mentioned "cook off" procedure (Topper et al., 2008) is an example of the effects of increased temperature on poultry litter NH_3 volatilization. Because of the nature of the factors affecting NH_3 production both internal and external, the control of NH_3 production through the management of pH, temperature, and moisture alone is very difficult.

Litter Microbiology

Poultry litter results in an environment which, given the right conditions, can be excellent for microbial proliferation for both pathogenic and non-pathogenic bacteria. Pathogenic bacteria are of a key concern due to the potential economic impact that could result from their presence. However, non-pathogenic bacteria affect bird performance through their contribution to NH_3 production. Schefferle (1965) suggests that promotion of anaerobic conditions will prevent the proliferation of uric acid decomposing bacteria, which was found to be mainly aerobic. Conversely, Ernst and Massey (1960) suggest that decreasing moisture will best prevent microbial activity. Total bacterial loads for poultry litter are highly variable. Research conducted by Terzich et al. (2000) over several regions saw wide variation between sampling points, however the average total

bacterial load for these samples was 2.54 x 10^{11}. Cook et al. (2008) stated that research findings suggested that poultry litter had a microbial load of 2.8 +/- .08 x 10^{8}. Season has also been shown to have an effect on overall total bacterial loads in poultry litter. For 41 samples taken during spring and summer, the highest count was 8.4 x 10^{7} CFU/g, while the lowest was 1.2 x 10^{3} CFU/g. Conversely, during winter the highest count was 1.0 x 10^{8} CFU/g, with the lowest at 4.0 x 10^{2} CFU/g. This variation was attributed to the higher moisture levels found in winter as opposed to spring/summer (Martin et al., 1998). The majority of research reviewed for total bacterial load counts focused on aerobic bacteria. However, Fries et al. (2005) found that anaerobic bacteria showed an increase after placement followed by a small decrease after removal of broilers. Pope et al. (2000) found that as total bacterial load decreased pH showed decreases as well. Byrd (1990) further demonstrates this where it was found that when pH levels fall below 6.0 ammonifying and putrefying bacteria were found to be inhibited, and Salmonella was found to be inhibited when levels below pH 5.0 were achieved. Terzich et al. (2000) also showed that total bacterial counts increase along with increases in pH. Further research has found that the even addition of birds to poultry litter will increase the total bacterial load of poultry litter. In one instance the addition of birds increased the total bacterial load 9 log per gram of litter from a previous sample with fresh poultry litter (Fries et al., 2005).

Poultry Litter Amendments

Litter amendments can be utilized in litter management to overcome the problem of low ventilation and ammonia accumulation during the initial stages of rearing. The

production of NH_3 can drastically affect the overall economics of the poultry industry. The goal of amendments is to lower levels of NH_3 by decreasing litter pH. This can be accomplished through the use of acidifiers such as alum (aluminum sulfate), Poultry Litter Treatment (PLT), Poultry Guard^{TM}, ferric sulfate, and phosphoric acid can (North Carolina Cooperative Extension Service, 2006). The goal of amendment application is to achieve litter pH levels less than 7.0. As was discussed previously, previous research conducted by Reece et al. (1979) concluded that when poultry litter exhibits pH less than 7.0, NH_3 volatilization was minimal; however, as pH level increased NH_3 increased as well, and volatilization of NH_3 reached its maximum level at pH of 8.0. Alkaline materials such as agricultural lime ($CaCO_3$), slaked lime ($Ca(OH)_2$) and burnt lime (CaO), can also be used as litter amendments (North Carolina Cooperative Extension Service, 2006). Unlike acidifiers, alkaline materials attempt to elevate the pH to such a level that NH_3 volatilization takes place more rapidly in an attempt to deplete the quantities of NH_3 before chick placement. Volatilized NH_3 is then vented from the rearing facility utilizing ventilation equipment. Another form of amendment that is currently available to the poultry industry is microbial litter amendments. According to the North Carolina Cooperative Extension Service (2006), microbial amendments work by introducing beneficial microbes into the poultry litter prior to chick placement that expedites uric acid and urea conversion into NH_3, which is then, vented utilizing rearing facility ventilation. Unfortunately, at this time there is no scientific data to validate the claims of proposed effectiveness of microbial litter amendments.

Alum ($Al_2(SO_4)_3 \cdot 18H_2O$), an acidifying poultry litter amendment, has been evaluated in multiple studies for its effectiveness in reduction of NH_3 volatilization in poultry litter, as well as additional benefits. Alum is also currently marketed under the trade name $Al^{+}Clear^{TM}$. Cook et al. (2008) suggest through research findings that alum reduces NH_3 volatilization through both chemical means, in other words the reduction of pH, and biological means, or the reduction of ureolytic bacteria. Moore et al. (2000) found that when compared to control pens with no poultry litter amendment treatment, alum-treated pens exhibited lower NH_3 levels during the first three weeks of rearing. Worley et al. (1999) found that alum application as a poultry litter amendment held additional benefits such as fuel savings as a result of decreased ventilation demands, darkling beetle suppression, as well as an increase in available litter N. In earlier research conducted by Moore et al. (1996) it is stated that in testing the effects of multiple poultry litter amendments alum, ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and phosphoric acid (H_3PO_4) showed the lowest pH and highest inorganic N along, but that alum would be preferred due to its lack of toxicity issues (ferrous sulfate), and its ability to reduce phosphorous runoff after land application of the treated poultry litter. McWard et al. (2000) found in research comparing different litter amendments that alum; sodium bisulfate ($NaHSO_4$) and Poultry Guard^{TM} (acidified clay) would provide control over NH_3 volatilization for up to 4 weeks after application, as well as offer significantly reduced darkling beetle counts. Finally, research conducted on alum found that it reduced total bacterial populations by up to 50% in the first 4 weeks after application (Cook et al., 2008). Another acidifying agent utilized as a poultry litter amendment is

manufactured under the name Poultry Litter Treatment (PLT). Research conducted by Terzich et al. (1998a) utilizing PLT found that pH levels were lowered and that NH_3 was significantly lower than non-treated pens. These conclusions are affirmed in further research conducted by Pope et al. (2000) in which similar findings of significantly lowered pH and NH_3 volatilization were seen in PLT treatments as compared to control pens; there was also a reduction seen in total bacterial loads for week one with PLT treatments. Terzich et al. (1998a) theorizes that the PLT effectiveness in reduction of NH_3 stems from the ability to have a direct chemical effect on uric acid, an ability to reduce pH, and the ability to reduce populations of ammonifying bacteria.

Effects of NH_3 and Poultry Litter Amendments on Poultry Health

The effects of NH_3 on poultry often have dual consequences. When exposure levels are high enough to produce effects on performance they are also at high enough levels to produce effects on poultry health. Furthermore, oftentimes performance and health are linked in regards to NH_3. Ammonia has been linked by research to lowering performance as well as increasing incidence of disease (Becker et al., 2004).

However, Anderson et al. (1964) states that even at a lower amount of 20 ppm NH_3 there would be noticeable effects to both health and performance if poultry were subjected to such an amount for the length of the rearing period. Research in which Leghorn cockerels were subjected to 0, 25, and 50 ppm of NH_3 for four weeks, with an addition of infectious bronchitis vaccination, found that the cockerels subjected to NH_3 exhibited reduced feed efficiency and a reduction in overall body weight (Kling and Quarles, 1974). A parallel study, which utilized similar parameters, stated that body

weights were significantly lower in test units containing 50 ppm of NH_3 (Quarles and Kling, 1974). For bird performance it was seen that subjection of birds to levels of NH_3 of 25 ppm and greater resulted in a lower overall body weight and in correlation to such that both feed consumption and efficiency fell (Miles et al., 2002; Charles and Payne, 1966; Johnson et al., 1991). The response to increasing NH_3 is exponential in poultry health. Exposure levels of 20 ppm NH_3 for extended periods have shown to cause respiratory tract complications (Anderson et al., 1964). Further research conducted by Reece et al. (1980) in which broilers were subjected to NH_3 levels of 50, 100, and 200 ppm, for four weeks, found that groups exposed to 100 and 200 ppm NH_3 exhibited reduced body weight and lowered feed conversion when compared with controls, and that these trends increased along with increases in NH_3 so much so that those birds exposed to 200 ppm of NH_3 showed only half the body weight of control birds. These trends continued even after exposure to NH_3 had ceased. In a separate trial conducted at the same time, it was seen that the exposure to NH_3 had an exponential effect on the amount of time recovery took. For 50 ppm it took birds an additional three days to overcome the effects of NH_3 and reach control weights; for 100 ppm, it required 4 days; and after 8 days, the 200 ppm group had still not meet control group levels. Mortality for both of the trials conducted in the experiment show that 50-ppm of NH_3 had little effect on mortality, but that 100 and 200-ppm treatments showed a larger amount of mortalities than did the control group.

In addition to effects on mortality, body weight, feed conversion and general bird performance that elevated levels of NH_3 have been shown to exhibit on commercial

poultry, there are also other physiological effects. Levels at 75 to 125 ppm NH_3 for extended periods have shown to cause not only respiratory tract complications but also the loss of respiratory tract features, such as increases in mucous producing cells as well as losses in cilia numbers (Al-Mashhadani, and Beck, 1985; Oyetunde et al., 1978). Quarles et al. (1974) suggests that elevated levels of NH_3 can also promote keratoconjunctivitis. Kristensen and Wathes (2000) suggest that keratoconjunctivitis can both cause physical pain for birds as well as affect their overall growth rate due to a decreased ability to find food and water.

Poultry litter amendments, as stated before, are designed to reduce the overall production of NH_3 in commercial poultry operations, and therefore combat the effects of NH_3 on commercial poultry. With a reduction in NH_3 come positive effects. As was discussed above, NH_3 can have a multitude of effects on poultry performance. Terzich et al. (1998b) stated in research findings that the application of PLT to poultry litter significantly reduced the number of mortalities attributed to ascites when compared to control group birds. Research showed that PLT treated litter produced broilers with a mean body weight that was significantly higher than control groups (Terzich et al., 1998a). McWard et al. (2000) showed in research findings that Poultry Guard™ and sodium bisulfate significantly improved average weight when compared to control groups and that when mortalities and body weight were adjusted that feed conversion was also significantly improved for birds raised over Poultry Guard™.

CHAPTER II

EFFECTS OF LITTER GUARD ON BROILER PERFORMANCE, LITTER QUALITY, AND AMMONIA PRODUCTION

Introduction

Reuse of litter for multiple flocks is a widely accepted practice in current commercial poultry operations. This is done to get the longest practical life out of litter before complete clean out and reduce the cost of buying new increasingly expensive and harder to obtain bedding material. By definition poultry litter is a combination of bedding material, poultry feathers, and poultry manure (Terzich et al., 2008). As a rearing period progresses, cake, which is crusted litter caused by excess moisture will begin to form around areas of higher moisture, such as drinker lines, and evaporative cool pads. When proper management practices are applied to reused litter, it can result in the savings of costs that otherwise would be spent on new litter. However, if improperly managed, reused litter may result in poor bird performance, an increased possibility of disease, housing, and increased moisture levels. Neglected management also has the possibility of increasing volatilization of NH_3 in broiler houses (Reece et al., 1979).

Ammonia is a colorless, water-soluble gas with no ionic charge, and is formed as a result of the microbial breakdown of uric acid, urea, and other nitrogenous compounds. Elliot et al. (1982) stated that pH, temperature, and moisture content contribute to the volatilization of NH_3, stating that pH has more effect than temperature, which in turn has more affect than moisture content on volatilization rates.

Elevated volatilization rates of NH_3 in a broiler house can promote negative effects in both performance as well as physiology on broilers that are subjected to those conditions during rearing. When $NH_{3,}$ levels are allowed to reach elevated levels, Becker et al. (2004) showed that broilers will experience lowered performance and increased incidence of disease. Levels of 20ppm NH_3 were shown to negatively affect broiler performance and promote respiratory tract complications (Anderson et al., 1964). Reece et al. (1980) found that when groups of broilers were exposed to varying levels of NH_3, 50ppm 100ppm, and 200ppm that the higher exposure groups 100-200 ppm exhibited reduced body weight gain lowered feed conversion, and increased mortality. When levels of NH_3, reach 75-125ppm, research has shown a respiratory tract complications, in conjunction with increases of mucous producing cells, and a loss of cilia (Al-Mashhadani, and Beck, 1985; Oyetunde et al., 1978). Research has also suggested that elevated NH_3 can promote karetoconjunctivitis in broiler chickens (Quarles et al., 1974).

LG is a microbial litter amendment, which utilizes a mixture of bacteria and humic acids in an attempt to promote improved broiler performance through better litter quality. LG attempts to improve litter quality by introducing beneficial bacteria, to competitively exclude, pathogenic bacteria to reused litter while simultaneously utilizing humic acid to promote an increase in NH_3 volatilization prior to broiler chick placement (Jim Antwine, Pittsburgh, Texas, personal communication).

At this point there is a lack of scientific literature on the effects of microbial litter amendments on broilers, poultry litter, or microbial amendments effects on NH_3

volatilization. Objectives for this experiment were to determine the effects of LG a microbial amendment on broiler performance, poultry litter characteristics, and poultry litter NH_3 flux. Broiler performance was determined by observations of final body weights, total feed consumption, total mortalities, and paw scores. Poultry litter characteristics was determined by analysis of litter pH, water activity, carbon/nitrogen content, total caked produced and total aerobic and anaerobic bacteria. Poultry liter ammonia flux was determined by observations collected utilizing an NH_3 flux chamber, placed over the broiler litter.

Materials and Methods

Experiment one utilized a microbial amendment that was sprayed onto the poultry litter. This made it necessary to isolate control and treated litter in the research pens during the rearing period to prevent cross contamination of groups. This would have been a difficult objective to meet in a commercial setting without the use of multiple houses. In order for results of experiment one to maintain significance to the commercial industry, it was designed to replicate commercial broiler production settings as closely as possible in a research unit that allowed for segregation of control and treatment groups in a single building. Pens were equipped with commercial-style feeder[1] and drinkers[2]. Lighting and ventilation programs were used to parallel commercial industry standards.

[1] Chore Time Brock, Inc., Milford, IN
[2] Ziggity Systems, Inc., Middleburg, IN

Housing, Equipment, and Broiler Husbandry

The house utilized in experiment one was of solid wall construction, with concrete floors divided into 8 (3 x 3 m) pens. Environmental conditions were controlled through the use of mechanical ventilation. Evaporative cool pads provided cooling during warmer periods and heat was supplemented with natural gas furnaces during colder periods. At chick placement and until one week of age, pens were also equipped with infrared heat lamp brooders to provide additional warmth. These brood lamps were equipped with thermostats so that, if the temperature exceeded the maximum set point this the brood lamps would power off until the temperature fell, at which point the lamps would resume function. Cycle timers were installed on the ventilation fans to provide minimum ventilation at all times and thermostats were installed that would turn the fans on continuously if temperatures exceeded the set point and would run until temperature again fell within the acceptable range. Temperature overrides for the house were set at approximately 32C at chick placement and were reduced 2C each week until the end of each trial. Full lighting was provided for 24 h/d for the first week of rearing, which lighting intensity was reduced, and 1 hr. of darkness was provided per day (until the end of the trial). The Experiment utilized a standard industry-feeding program in which each pen was equipped with a nipple drinker line, and four industry style feeders. This allowed ad-libitum feed and water intake for broilers. Feed and water lines were observed daily and adjusted as needed to prevent unnecessary spillage. A commercial strain of broilers was reared at a density of 0.8 square feet per bird to 49 days of age. Of the 8 pens utilized, 4 served as controls, with no application of litter amendments, the

other four pens were treated with LG by the manufacture one week prior to broiler chick placement. Broiler litter (used for approximately 14 flocks) was obtained from a local commercial broiler farm. This litter was spread at an average depth of 11cm in all pens. All broilers were reared in accordance with Texas A&M University Animal Use Protocols.

Broiler Performance

Broiler performance was determined by measurements of final body weights, total feed consumption, paw scores, and total mortalities. Body weights were taken at the time of broiler chick placement and at the conclusion of each trial to obtain total beginning and ending body weights per pen. Beginning body weights were taken by placing chicks in trays and recording their weights, before placement into pens. Final body weights were recorded on day 49, (after a 12 h feed withdraw) as broilers were removed from the each pen and placed 10 at a time into a coop As broilers were placed into the coops an individual would visually determine a paw score for each bird based on a system used by Bilgili et al. (2006) in which 0 indicated no presence of lesions on the broiler paw, 1 indicated lesions < 7.5 mm and 2 indicated lesions >7.5 mm. Feed consumption was recorded throughout each trial for each pen and each diet, which allowed for a final calculation of total feed consumed for the trial. Mortalities were recorded daily for each pen. The weight of each mortality was recorded after removal. Due to an unfortunate ventilation failure on an unusually hot and humid day at the end of trial one, mortality was approximately 15% in a 24 h period, thus, performance data for trial one was lost.

Litter Analysis

Litter samples were collected at three separate points in each pen. The samples were collected prior to product application, at broiler chick placement, and each week thereafter until the conclusion of each trial. At the conclusion of each trial caked litter was removed from each pen, weighed and samples collected. All litter and cake samples were frozen until analysis. Litter and cake samples were removed from the freezer and allowed to reach room temperature, then analyzed for pH, a_w, percent moisture, and percent N, percent C, and C:N ratio. pH of litter and cake samples was analyzed by weighing 12 g of litter and adding de-ionized water until the final volume was 60 mL. The sample was then mixed and allowed to rest before the pH with a pH meter[3]. Water activity of litter and cake samples was analyzed by weighing 6 g of cake or litter into plastic dished that were then placed into the measurement chamber of the a_w analyzer[4] for a final calculation. Litter moisture was calculated for litter and cake samples by weighing 10 g samples of material into dry pans and drying in an oven at 100 C for 24 h. The weighing again to obtain a dry weight, the difference between the wet and dry weights, results in a total moisture calculation for the sample. After moisture analysis was completed the litter or cake sample was finely ground, For N and C analysis, 100 mg of the finely ground sample was packaged in an aluminum foil wrapper and formed

[3] Model UP-25, Denver Instruments, Aruada, CO
[4] Model Hygro Palm A_w 1, Rotronic Instrument Corporation, Huntington, NY

into a sample pellet. The sample was then analyzed by combustion method using an Elementar Vario CNS analyzer[5].

Litter Microbiological Analysis

Prior to litter treatment, at the time of chick placement, and at the end of each trial, litter samples were analyzed for total aerobic and anaerobic bacteria. At the end of each trial cake samples were also analyzed for total aerobic and anaerobic bacteria. Litter microbial enumeration was accomplished by placing 10 g of litter or cake sample into a Whirl-pak bag with a filter, then mixed with 90 mL of phosphate buffered solution (PBS). The mixture was then stomached for 30 seconds. 1.0 mL of the mixture was then pippeted into 9.0 mL of sterile PBS, and appropriate serial dilutions performed. Each dilution was plated on Tryptic Soy Agar (TSA) all plates were incubated at 37 C for 24 h. Anaerobic samples were incubated in an anaerobic chamber with gas concentration of 5.0% CO_2 and 5.0% H balanced with N. After incubation, aerobic and anaerobic plates were manually counted and total colony forming units (CFU) were calculated.

Litter Ammonia Flux Analysis

Ammonia flux measurements were collected prior to litter treatment, at chick placement, and each week thereafter until the conclusion of the trial. Ammonia flux measurements were taken using two methods. Method one utilized a Drager XAM 7000[6] unit to measure NH_3 concentrations at 60, 90, and 120 seconds, after a dome-shaped

[5] Model Vario N, CN, CNS, Elementar Analysensyteme, GmbH, D-63452, Hanau, Germany.
[6] Draeger Safety Inc., Pittsburgh, PA.

chamber was placed on the litter and method two utilized a Drager CMS unit[7] to measure NH_3 concentrations in the chamber after a 20 minute equilibrium period. Ammonia measurements were taken at three locations in each pen for method one and two locations in each pen for method two. Both methods utilized a static flux chamber with a small fan installed inside the top to prevent gas stratification within the chamber. The chamber design was adopted from Ferguson et al., (1998), and was placed over the litter allowing an accumulation of volatilized ammonia to be sampled. In combination with the static flux chamber, method one also utilized compressed air from outside the rearing area to force clean air into the chamber prior to sampling. The chamber was placed on a plexiglas sheet, allowed to purge with fresh air until the NH_3 reading was stable at or near 0 ppm, and then placed onto the litter. The moment the chamber made contact with litter, was deemed 0 seconds, and a timer was started. For method two, the chamber was placed on the litter and a timer was set for 20 minutes. At 20 minutes, a plug was removed and the sampling hose attached to the CMS unit was lowered into the chamber for analysis to begin. Due to equipment failure, NH_3 data for trial one was not collected.

Statistical Analysis

All data was analyzed by one-way ANOVA using the GLM procedure of SPSS[8], with the treatment serving as the fixed variable, and observation serving as the dependent variable. Means were determined significantly different at $P<0.05$.

[7] Draeger Safety Inc., Pittsburgh, PA
[8] SPSS for Windows, Version 11, IBM, Armonk, NY

Results and Discussion

Data collected during this experiment are summarized in the following tables. Tables 2.1 and 2.2 summarize broiler performance for Trials 1, 2 and 3. Tables 2.3 through 2.11 contain data pertaining to litter parameters for Trials 1, 2, and 3.

Broiler Performance

Broiler performance parameters of beginning chick weights, ending body weights, average gain, and feed consumed, feed conversion ratio, and adjusted feed conversion ratio are presented in Table 2.1. As mentioned previously data for Trial 1 is absent due to a ventilation failure during an unusually hot weather period which resulted in above normal mortality and a loss of production data. No statistical differences were seen between treatments for the parameters of in Table 2.1.

Table 2.1. Experiment 1 broiler body weights, gain, feed consumption and feed conversion.

	Treatment	Trial 2	Trial 3
Day 1 (g)	Control	44.47 ± 0.13	39.99 ± 0.21
	Spray	44.58 ± 0.13	40.11 ± 0.25
	P-value	0.545	0.725
Ending Body Weight (kg)			
	Control	407.98 ± 4.92	363.29 ± 6.91
	Spray	411.33 ± 7.93	348.16 ± 9.58
	P-value	0.732	0.247
Average Gain (kg)			
	Control	3.20 ± 0.06	2.97 ± 0.04
	Spray	3.15 ± 0.06	2.87 ± 0.06
	P-value	0.557	0.219
Feed Consumed (kg)			
	Control	699.95 ± 17.09	668.68 ± 3.95
	Spray	689.84 ± 18	649.77 ± 17.04

Table 2.1 continued.

Treatment	Trial 2	Trial 3
Feed Consumed (kg)		
P-value	0.706	0.321
Feed Conversion		
Control	1.74 ± 0.02	1.72 ± 0.02
Spray	1.70 ± 0.02	1.87 ± 0.01
P-value	0.151	0.522
Adjusted Feed Conversion		
Control	1.72 ± 0.02	1.83 ± 0.04
Spray	1.69 ± 0.02	1.86 ± 0.02
P-value	0.243	0.507

Broiler performance parameters of percent mortality, average paw score, percent paw scores of 0, 1, and 2 and total ending cake weight are presented in Table 2.2. Again, broiler performance is absent for Trial 1. Since mortality on the ventilation failure day was approximately equal across treatments, statistical differences were observed between treatments for ending caked litter weight the treated groups had less cake than controls in Trial 1, and for percent mortality with the treated group having were less than control in Trial 2. While the percent mortality in Trial 2 is significantly different between treatments in favor of treated groups, the trend did not continue in Trial 3, and due to the loss of performance data for Trial 1, the difference in Trial 2 mortality can not be fully explained. The remainder of the observations between treatments in Table 2.2 showed no statistical differences.

Table 2.2 Experiment 1 broiler mortality, paw scores and ending caked litter weight.

Treatment	Trial 1	Trial 2	Trial 3
Mortality (%)			
Control	---[1]	3.64 ± 0.21[a]	6.84 ± 1.57
Spray	---	2.09 ± 0.37[b]	6.90 ± 1.86
P-value	---	0.011	0.982
Average Paw Score			
Control	---	0.75 ± 0.13	0.71 ± 0.18
Spray	---	0.69 ± 0.18	0.96 ± 0.22
P-value	---	0.786	0.427
Percent Paw Score 0 (%)			
Control	---	45.72 ± 8.22	57.65 ± 10.29
Spray	---	49.59 ± 11.78	44.88 ± 11.30
P-value	---	0.797	0.435
Percent Paw Score 1 (%)			
Control	---	33.52 ± 3.97	13.38 ± 3.17
Spray	---	31.96 ± 6.19	14.73 ± 2.57
P-value	---	0.840	0.754
Percent Paw Score 2 (%)			
Control	---	20.76 ± 5.29	28.97 ± 7.14
Spray	---	18.45 ± 5.98	40.39 ± 10.94
P-value	---	0.782	0.415
Ending Caked Litter Weight (kg)			
Control	100.40 ± 4.76[a]	128.20 ± 27.83	89.85 ± 8.57
Spray	56.42 ± 5.52[b]	103.53 ± 22.56	86.63 ± 11.77
P-value	0.001	0.517	0.832

[1] Information missing due to ventilation failure

Litter/Cake Parameters

Tables 2.3 through 2.5 show the observations of litter moisture, a_w, and pH for Trials 1, 2, and 3, for litter and caked litter. Trial 1 is missing data for the first five weeks for a_w, and the first three weeks for pH due to a loss of samples following a freezer

failure. Statistical differences were observed on 0 d of Trial 1 for moisture, and 28 d of Trial 3 for a_w. The difference in moisture on 0 d of Trial 1 moisture (Table 2.3) showed the treatment group to contained more moisture than the control. This followed the first addition of treatment to the litter which had been agitated and desiccated through transport process. The addition of LG required mixture with water, the addition of the water to litter explains the added moisture in the treated groups and also why the statistical difference is not continued in latter sampling periods. In latter measurements moisture was available to all pens through environmental conditions as well as drinker lines. The statistical difference at 28 d of Trial 3, a_w (Table 2.5) could be attributed to variation amongst pens. This is taking into account that the difference does not continue into later observations. The treated group displays a slightly lower a_w than the control group. No other statistical differences were noted in Trials 1, 2, or 3 for litter moisture, a_w, or pH. Litter moisture averages were similar to those found by Terzich et al. (2000). They observed an average of 25.1% across a 12-region area. Caked litter moisture averages were also found to be similar to those observed by Malone et al. (1992) who measured caked litter moisture averages at 40%. pH also showed results similar to previous research where thoroughly used litter had a pH averaging around 8.5 (Elliott et al., 1982).

Table 2.3 Experiment 1 litter moisture, a_w, pH, Trial 1.

Day	Treatment	Moisture (%)	a_w	pH
-6 d	Control	20.60 ± 0.55	---[1]	---
	Spray	20.98 ± 0.62	---	---
	P-value	0.665	---	---
0 d	Control	19.67 ± 0.07[a]	---	---

Table 2.3 continued.

Day	Treatment	Moisture (%)	a_w	pH
0 d	Spray	20.55 ± 0.27[b]	---	---
	P-value	0.020	---	---
7 d	Control	201.11 ± 0.66	---	---
	Spray	20.31 ± 0.77	---	---
	P-value	0.856	---	---
13 d	Control	24.87 ± 0.68	---	8.24 ± 0.09
	Spray	24.60 ± 0.52	---	8.24 ± 0.06
	P-value	0.760	---	0.984
18 d	Control	33.04 ± 0.66	---	8.61 ± 0.05
	Spray	31.95 ± 0.59	---	8.67 ± 0.04
	P-value	0.267	---	0.417
30 d	Control	26.44 ± 0.38	0.91 ± 0.01	8.75 ± 0.05
	Spray	25.55 ± 0.48	0.92 ± 0.01	8.72 ± 0.05
	P-value	0.201	0.453	0.674
40 d	Control	28.74 ± 0.41	0.92 ± 0.01	8.80 ± 0.04
	Spray	27.81 ± 0.59	0.92 ± 0.01	8.74 ± 0.03
	P-value	0.244	0.981	0.388
48 d	Control	26.81 ± 1.19	0.90 ± 0.01	8.74 ± 0.08
	Spray	25.80 ± 0.97	0.89 ± 0.01	8.70 ± 0.07
	P-value	0.537	0.396	0.709
Cake	Control	38.29 ± 1.01	0.94 ± 0.01	8.92 ± 0.05
	Spray	39.68 ± 0.80	0.96 ± 0.01	8.92 ± 0.05
	P-value	0.323	0.129	0.946

[1]Information missing due to loss of samples following freezer failure

Table 2.4. Experiment 1 litter moisture, a_w, pH, Trial 2.

Day	Treatment	Moisture (%)	a_w	pH
-7 d	Control	26.47 ± 0.31 0.91 ± 0.01	8.79 ± 0.07	
	Spray	26.29 ± 0.46	0.90 ± 0.01	8.82 ± 0.06
	P-value	0.755	0.835	0.806
0 d	Control	22.56 ± 0.83	0.85 ± 0.02	8.78 ± 0.02
	Spray	23.86 ± 0.50	0.87 ± 0.01	8.83 ± 0.02

Table 2.4 continued.

Day	Treatment	Moisture (%)	a_w	pH
0 d	P-value	0.229	0.455	0.231
7 d	Control	22.35 ± 0.42	0.85 ± 0.01	8.62 ± 0.01
	Spray	21.80 ± 0.41	0.84 ± 0.01	8.64 ± 0.01
	P-value	0.382	0.712	0.248
14 d	Control	24.28 ± 0.59	0.87 ± 0.01	8.63 ± 0.05
	Spray	24.12 ± 0.50	0.87 ± 0.01	8.62 ± 0.03
	P-value	0.848	0.753	0.846
21 d	Control	26.98 ± 1.01	0.89 ± 0.01	8.82 ± 0.03
	Spray	26.46 ± 0.47	0.91 ± 0.01	8.82 ± 0.02
	P-value	0.657	0.056	0.995
28 d	Control	28.42 ± 0.80	0.92 ± 0.01	8.96 ± 0.03
	Spray	27.25 ± 1.40	0.93 ± 0.01	8.90 ± 0.03
	P-value	0.495	0.765	0.185
35 d	Control	26.01 ± 0.94	0.89 ± 0.004	9.03 ± 0.06
	Spray	27.02 ± 1.23	0.89 ± 0.02	9.05 ± 0.04
	P-value	0.540	0.925	0.851
42 d	Control	26.77 ± 1.08	0.88 ± 0.02	8.95 ± 0.06
	Spray	26.45 ± 1.43	0.88 ± 0.02	8.98 ± 0.03
	P-value	0.862	0.932	0.707
49 d	Control	24.22 ± 0.81	0.88 ± 0.01	8.97 ± 0.04
	Spray	22.70 ± 0.78	0.86 ± 0.02	8.80 ± 0.06
	P-value	0.225	0.365	0.057
Cake	Control	44.96 ± 3.23	0.99 ± 0.01	8.42 ± 0.22
	Spray	41.47 ± 2.65	0.99 ± 0.01	8.42 ± 0.18
	P-value	0.471	0.955	0.416

Table 2.5 Experiment 1 litter moisture, a_w, pH, Trial 3

Day	Treatment	Moisture (%)	a_w	pH
-9 d	Control	21.94 ± 0.81	0.81 ± 0.01	8.86 ± 0.04
	Spray	20.27 ± 0.60	0.80 ± 0.01	8.85 ± 0.04
	P-value	0.149	0.524	0.855

Table 2.5 continued.

Day	Treatment	Moisture (%)	a_w	pH
-3 d	Control	20.71 ± 0.60	0.82 ± 0.02	8.84 ± 0.05
	Spray	19.01 ± 0.38	0.79 ± 0.02	8.77 ± 0.01
	P-value	0.055	0.309	0.178
7 d	Control	19.13 ± 0.69	0.78 ± 0.03	8.67 ± 0.07
	Spray	18.21 ± 1.45	0.77 ± 0.04	8.65 ± 0.04
	P-value	0.590	0.853	0.808
14 d	Control	20.65 ± 0.44	0.83 ± 0.01	8.58 ± 0.04
	Spray	19.52 ± 0.34	0.82 ± 0.02	8.49 ± 0.05
	P-value	0.088	0.768	0.272
21 d	Control	23.49 ± 0.14	0.94 ± 0.01	8.67 ± 0.03
	Spray	22.80 ± 0.39	0.93 ± 0.01	8.62 ± 0.07
	P-value	0.144	0.389	0.514
28 d	Control	26.93 ± 1.41	0.93 ± 0.01[a]	8.86 ± 0.08
	Spray	23.51 ± 0.36	0.90 ± 0.01[b]	8.71 ± 0.08
	P-value	0.073	0.029	0.273
35 d	Control	26.26 ± 1.24	0.94 ± 0.01	8.88 ± 0.02
	Spray	26.57 ± 0.43	0.94 ± 0.005	8.89 ± 0.07
	P-value	0.836	0.988	0.967
42 d	Control	22.06 ± 0.57	0.94 ± 0.01	8.60 ± 0.12
	Spray	21.98 ± 0.84	0.92 ± 0.02	8.59 ± 0.09
	P-value	0.938	0.683	0.955
49 d	Control	24.61 ± 0.64	0.95 ± 0.005	8.90 ± 0.04
	Spray	24.71 ± 0.95	0.94 ± 0.01	8.90 ± 0.06
	P-value	0.934	0.947	0.919
Cake	Control	35.73 ± 2.78	0.98 ± 0.01	8.99 ± 0.04
	Spray	37.63 ± 3.55	0.99 ± 0.02	8.96 ± 0.01
	P-value	0.731	0.885	0.508

Tables 2.6-2.8 show the recorded observations for litter N, C, and C: N ratio, for Trials 1, 2, and 3, for litter and caked litter. A statistical difference was noted for litter C

on -6 d of Trial 1(Table 2.6), and litter N on 35 d of Trial 2 (Table 2.7). In both instances treated groups show to have slightly higher percentages than control groups. However, these two circumstances are again isolated incidences in the trials, the trend does not continue, and the explanation should be attributed to the variation found within in litter and sampling rather than an affect by the treatment. Analysis of all samples was not completed for Trial 3; rather the first two and last sampling periods were analyzed to test for statistical differences. It was inferred that if a statistical difference was observed in any of these analyses, further testing would be warranted. However, no statistical differences were observed. Litter N results were similar to those found by Patterson et al. (1998) in which observations of averages of 3.31% and 3.94% were found for N.

Table 2.6 Experiment 1 litter N, C, C: N ratio Trial 1

Day	Treatment	Litter N	Litter C	C: N ratio
-6 d	Control	3.53 ± 0.06	33.37 ± 0.11[a]	9.46 ± 0.15
	Spray	3.57 ± 0.02	34.46 ± 0.32[b]	9.66 ± 0.12
	P-value	0.524	0.018	0.356
0 d	Control	3.54 ± 0.06	33.73 ± 0.75	9.55 ± 0.16
	Spray	3.54 ± 0.05	34.47 ± 0.26	9.74 ± 0.15
	P-value	0.964	0.384	0.413
7 d	Control	3.64 ± 0.06	34.67 ± 0.54	9.83 ± 0.12
	Spray	3.73 ± 0.05	34.84 ± 0.69	9.84 ± 0.15
	P-value	0.964	0.846	0.938
13 d	Control	3.67 ± 0.06	34.29 ± 0.86	9.35 ± 0.25
	Spray	3.73 ± 0.07	34.54 ± 0.40	9.26 ± 0.15
	P-value	0.520	0.802	0.775
18 d	Control	4.04 ± 0.46	34.66 ± 0.29	8.87 ± 0.88
	Spray	3.56 ± 0.07	35.07 ± 0.38	9.87 ± 0.10
	P-value	0.520	0.802	0.775
30 d	Control	3.44 ± 0.05	33.97 ± 0.43	9.90 ± 0.21

Table 2.6 continued.

Day	Treatment	Litter N	Litter C	C: N ratio
30	Spray	3.43 ± 0.05	34.94 ± 0.45	10.19 ± 0.06
	P-value	0.955	0.169	0.232
40 d	Control	3.57 ± 0.07	33.11 ± 0.53	9.29 ± 0.16
	Spray	3.41 ± 0.11	32.93 ± 0.98	9.66 ± 0.15
	P-value	0.273	0.873	0.145
48 d	Control	3.61 ± 0.11	33.54 ± 0.63	9.33 ± 0.36
	Spray	3.73 ± 0.17	35.14 ± 0.32	9.50 ± 0.48
	P-value	0.561	0.063	0.796
Cake	Control	3.90 ± 0.10	34.80 ± 0.12	8.96 ± 0.20
	Spray	3.99 ± 0.06	34.74 ± 0.30	8.72 ± 0.08
	P-value	0.463	0.864	0.320

Table 2.7 Experiment 1 litter N, C, C: N ratio Trial 2

Day	Treatment	Litter N	Litter C	C: N
-7 d	Control	3.44 ± 0.01	33.15 ± 0.33	9.63 ± 0.09
	Spray	3.53 ± 0.08	33.64 ± 0.56	9.54 ± 0.15
	P-value	0.305	0.476	0.622
0 d	Control	3.35 ± 0.05	34.12 ± 0.54	10.21 ± 0.08
	Spray	3.33 ± 0.04	33.61 ± 0.35	10.12 ± 0.19
	P-value	0.798	0.461	0.700
7 d	Control	3.49 ± 0.05	33.27 ± 0.53	9.53 ± 0.09
	Spray	3.53 ± 0.05	33.06 ± 0.34	9.38 ± 0.14
	P-value	0.613	0.746	0.403
14 d	Control	3.56 ± 0.07	33.23 ± 0.56	9.33 ± 0.10
	Spray	3.53 ± 0.02	33.38 ± 0.50	9.44 ± 0.11
	P-value	0.690	0.850	0.487
21 d	Control	3.54 ± 0.09	34.77 ± 0.49	9.86 ± 0.37
	Spray	3.61 ± 0.12	34.89 ± 0.53	9.73 ± 0.44
	P-value	0.677	0.877	0.822
28 d	Control	3.53 ± 0.08	35.14 ± 0.90	9.96 ± 0.34
	Spray	3.58 ± 0.12	35.30 ± 0.38	9.91 ± 0.30
	P-value	0.780	0.870	0.903

Table 2.7 continued.

Day	Treatment	Litter N	Litter C	C: N
35 d	Control	3.66 ± 0.03[a]	34.80 ± 0.60	9.57 ± 0.13
	Spray	4.01 ± 0.10[b]	36.24 ± 0.18	9.08 ± 0.19
	P-value	0.014	0.060	0.080
42 d	Control	3.84 ± 0.07	37.12 ± .13	9.70 ± .13
	Spray	3.93 ± 0.08	36.25 ± .37	9.26 ± .18
	P-value	0.385	0.070	0.100
49 d	Control	3.98 ± 0.08	36.83 ± 0.14	9.28 ± 0.21
	Spray	3.91 ± 0.07	37.33 ± 0.23	9.58 ± 0.16
	P-value	0.531	0.113	0.302
Cake	Control	4.30 ± 0.16	37.17 ± 0.49	8.71 ± 0.22
	Spray	4.12 ± 0.12	36.72 ± 0.12	8.97 ± 0.23
	P-value	0.400	0.408	0.447

Table 2.8 Experiment 1 litter N, C, C: N ratio Trial 3

Day	Treatment	Litter N	Litter C	C: N
-9 d	Control	3.63 ± 0.04	35.47 ± 0.30	9.78 ± 0.10
	Spray	3.74 ± 0.10	36.02 ± 0.47	9.63 ± 0.16
	P-value	0.333	0.363	0.481
-3 d	Control	3.44 ± 0.06	34.87 ± 0.58	10.15 ± 0.07
	Spray	3.48 ± 0.07	35.42 ± 0.36	10.18 ± 0.26
	P-value	0.629	0.453	0.905
49 d	Control	3.93 ± 0.15	37.41 ± 0.05	9.55 ± 0.33
	Spray	3.87 ± 0.07	37.23 ± 0.29	9.63 ± 0.14
	P-value	0.703	0.573	0.818

Table 2.9 presents data for total colony forming units of aerobic and anaerobic bacteria observed for litter and caked litter for Trials 1, 2, and 3. No statistical differences were observed for any of the testing periods in any of the trials for either litter or caked litter.

Table 2.9 Experiment 1 total plate counts (log10CFU/g)

Trial	Day	Treatment	Aerobic	Anaerobic
1	-6 d	Control	6.76 ± 0.16	5.91 ± 0.19
		Spray	6.55 ± 0.06	5.86 ± 0.22
		P-value	0.247	0.865
	0 d	Control	6.43 ± 0.19	5.78 ± 0.21
		Spray	6.15 ± 0.33	5.70 ± 0.15
		P-value	0.507	0.763
	48 d	Control	7.41 ± 0.11	6.97 ± 0.18
		Spray	7.50 ± 0.09	7.28 ± 0.09
		P-value	0.553	0.169
	Cake	Control	7.40 ± 0.07	7.07 ± 0.11
		Spray	7.35 ± 0.14	7.10 ± 0.11
		P-value	0.551	0.903
2	-7 d	Control	7.22 ± 0.14	6.93 ± 0.17
		Spray	7.16 ± 0.13	6.87 ± 0.09
		P-value	0.747	0.761
	0 d	Control	7.38 ± 0.16	7.02 ± 0.20
		Spray	7.32 ± 0.19	7.13 ± 0.15
		P-value	0.847	0.655
	49 d	Control	7.76 ± 0.25	7.30 ± 0.17
		Spray	7.78 ± 0.21	7.14 ± 0.10
		P-value	0.942	0.458
	Cake	Control	8.16 ± 0.22	7.66 ± 0.09
		Spray	8.33 ± 0.17	7.68 ± 0.22
		P-value	0.556	0.936
3	-9 d	Control	7.15 ± 0.21	---[1]
		Spray	7.60 ± 0.13	---
		P-value	0.118	---
	-3 d	Control	7.31 ± 0.07	6.93 ±0 .13
		Spray	7.65 ± 0.23	7.31 ± 0.14
		P-value	0.200	0.097
	49 d	Control	8.18 ± 0.02	8.81 ± 0.11
		Spray	7.87 ± 0.12	8.93 ± 0.14

Table 2.9 continued.

Trial	Day	Treatment	Aerobic	Anaerobic
3	49 d	P-value	0.054	0.414
	Cake	Control	7.88 ± 0.13	8.04 ± 0.27
		Spray	7.73 ±0 .09	8.01 ± 0.42
		P-value	0.472	0.943

[1]Anaerobic Chamber unavailable for use

Tables 2.10 and 2.11 60, 90, 120 second calculations NH_3 flux and 20 minute NH_3 chamber equilibrium NH_3 for Trials 2 and 3. Statistical differences were seen for 14 d of Trial 2, (NH_3 60 sec) (Table 2.10) where the control group exhibited a lower volatilization rate than that of the treated group. On -3 d of Trial 3, NH_3 60, 90 and 120 sec (Table 2.11) where the spray group exhibited a lower volatilization rate than that of the control group. However, several different factors play important roles in the volatilization of NH_3, and the consideration that these observations do not show a pattern of continuance throughout the trial must be taken into account. The likely explanation for the differences is that one of the many variables that affect volatilization played a role and the statistical difference is just a variation in observations.

Table 2.10 Experiment 1 60, 90, 120 second, 20 minute NH_3 volatilization Trial 2

Day	Treatments`	60sec	90sec	120sec	20min
0 d	Control	---[1]	---	---	191.58 ± 12.76
	Spray	---	---	---	195.17 ± 20.07
	P-value	---	---	---	0.890
7 d	Control	200.84 ± 40.22	194.61 ± 33.12	170.74 ± 27.58	87.38 ± 15.91
	Spray	195.95 ± 9.35	190.79 ± 5.96	164.79 ± 3.50	97.63 ± 8.96
	P-value	0.910	0.913	0.838	0.595
14 d	Control	80.34 ± 6.38[a]	102.60 ± 11.37	100.18 ± 12.71	75.00 ± 9.18
	Spray	111.89 ± 2.47[b]	122.88 ± 1.39	115.19 ± 1.86	68.75 ± 3.92
	P-value	0.004	0.127	0.287	0.554

Table 2.10 continued.

Day	Treatments`	60sec	90sec	120sec	20min
21 d	Control	72.44 ± 12.56	94.67 ± 17.72	93.22 ± 16.87	64.38 ± 7.59
	Spray	108.43 ± 29.31	135.68 ± 23.54	130.81 ± 19.49	76.50 ± 5.91
	P-value	0.302	0.213	0.195	0.254
28 d	Control	109.09 ± 24.23	138.40 ± 20.0	134.08 ± 17.95	81.38 ± 12.71
	Spray	93.01 ± 33.02	124.28 ± 30.69	133.86 ± 24.03	97.63 ± 11.90
	P-value	0.708	0.713	0.994	0.387
35 d	Control	64.32 ± 12.79	97.96 ± 22.51	102.49 ± 20.78	64.13 ± 13.07
	Spray	82.21 ± 3.77	129.20 ± 15.47	153.62 ± 8.83	79.50 ± 11.41
	P-value	0.228	0.292	0.064	0.410
42 d	Control	---	---	---	132.25 ± 10.91
	Spray	---	---	---	125.50 ± 15.20
	P-value	---	---	---	0.731
49 d	Control	---	---	---	135.13 ± 35.85
	Spray	---	---	---	126.00 ± 20.63
	P-value	---	---	---	0.833

[1]Measurements unavailable due to equipment malfunction

Table 2.11 Experiment 1 60, 90, 120 second, 20 minute NH_3 volatilization Trial 3

Day	Treatments`	60sec	90sec	120sec	20min
-9 d	Control	269.53 ± 22.4	229.72 ± 43.29	192.93 ± 17.19	100.13 ± 5.53
	Spray	214.05 ± 43.3	189.21 ± 18.8	161.40 ± 25.5	85.38 ± 11.24
	P-value	0.298	0.327	0.345	0.284
-3 d	Control	228.0 ± 21.34[a]	195.76 ± 16.62[a]	161.0 ± 13.50[a]	99.38 ± 5.93
	Spray	164.74 ± 4.81[b]	146.82 ± 4.64[b]	125.14 ± 3.37[b]	84.00 ± 4.42
	P-value	0.028	0.030	0.038	0.083
7 d	Control	150.12 ± 24.79	135.19 ± 22.02	118.05 ± 18.11	63.25 ± 6.22
	Spray	140.98 ± 11.27	127.90 ± 10.44	108.36 ± 8.43	52.13 ± 4.04
	P-value	0.749	0.775	0.645	0.184
14 d	Control	129.78 ± 31.28	103.37 ± 23.06	85.62 ± 18.76	63.63 ± 7.07
	Spray	175.47 ± 18.33	139.59 ± 14.77	114.98 ± 12.03	65.88 ± 4.76
	P-value	0.254	0.234	0.236	0.801

Table 2.11 continued.

Day	Treatments`	60sec	90sec	120sec	20min
21 d	Control	137.19 ± 46.20	118.11 ± 37.36	101.33 ± 31.36	89.75 ± 8.33
	Spray	144.49 ± 7.13	116.19 ± 6.05	96.55 ± 5.03	74.11 ± 11.86
	P-value	0.881	0.961	0.885	0.319
28 d	Control	117.83 ± 44.25	103.15 ± 38.32	90.60 ± 32.56	78.48 ± 18.02
	Spray	69.03 ± 16.90	69.95 ± 17.16	60.42 ± 15.53	84.25 ± 7.60
	P-value	0.409	0.516	0.491	0.785
35 d	Control	366.52 ± 65.82	330.80 ± 60.81	276.39 ± 47.70	101.38 ± 39.71
	Spray	380.95 ± 70.97	323.10 ± 55.18	267.39 ± 45.84	122.25 ± 30.24
	P-value	0.886	0.928	0.896	0.690
42 d	Control	142.50 ± 13.95	138.82 ± 12.83	125.55 ± 13.08	65.88 ± 10.93
	Spray	224.12 ± 50.06	188.36 ± 34.92	158.01 ± 27.44	62.63 ± 5.4
	P-value	0.167	0.231	0.327	0.799
49 d	Control	267.90 ± 25.60	220.43 ± 16.39	189.90 ± 13.49	102.75 ± 6.34
	Spray	195.57 ± 62.10	203.39 ± 44.55	193.95 ± 47.23	94.88 ± 17.10
	P-value	0.323	0.732	0.928	0.681

CHAPTER III

EFFECTS OF POULTRY WASTE DEGRADER ON BROILER PERFORMANCE, LITTER QUALITY, AND AMMONIA PRODUCTION

Introduction

The overall characteristics of poultry litter and its reuse have a significant impact on broiler growth and performance in the current commercial poultry industry. Poultry litter as defined by Terzich et al. (2008) is the combination of previously used poultry bedding with the addition of poultry feathers and manure. In addition, areas of high moisture near drinker lines and evaporative cool pads can have crusted litter known as cake. Currently, managers in commercial poultry operations utilize litter management and poultry litter amendments in an attempt to prevent excessive amounts of volatilized NH_3 from affecting commercial poultry broiler performance. Litter management, while important, can only extend the life of reused poultry litter so far, and current acidified poultry litter amendments only offer effective NH_3 mitigation for the first 3-4 weeks of a broiler flocks grow out. If proper management techniques such as moisture control and ventilation are not followed, an increased level of NH_3 volatilization is likely (Reece et al., 1979).

Ammonia is a colorless, water-soluble gas with no ionic charge, volatilized as a result of the microbiological breakdown of urea, uric acid, proteins and other nitrogenous compounds. The volatilization rate of NH_3 is affected most by pH of the litter, followed by temperature, and finally by moisture content of the litter (Elliot et al., 1982). When broilers are subjected to elevated levels of NH_3, studies have shown a

marked decrease in overall performance and increased likelihood of disease susceptibility (Becker et al., 2004). Quarles et al. (1974) suggest elevated levels of NH_3, can promote karetoconjunctivitis in broilers. When broilers are subjected to levels of NH_3 up to 20 ppm for extended periods of time, findings show that not only is broiler performance affected but respiratory tract complications are seen (Anderson et al,. 1964). At exposure levels of 75-125 ppm for extended periods of time respiratory tract complications are again seen but are compounded with increases in mucous producing cells and a loss of cilia (Al-Mashhadani, and Beck, 1985; Oyetunde et al., 1978). When extended exposure to NH_3, levels of 100-200 ppm findings suggest that broilers suffer reduced body weights, lower feed conversions, and increased mortalities (Reece et al., 1980).

Prior research in the field of microbiological poultry litter amendments is lacking. It was the intent of this experiment to determine the effectiveness of a microbiological poultry litter amendment on the reduction of NH_3 volatilization when applied to reused poultry litter, the effects of this amendment on the characteristics of the litter, as well as its effects on the overall performance of commercial broilers. The objectives of this experiment were to conduct a trial to determine the effects of a microbial litter amendment, PWD on broiler performance, litter characteristics, and poultry litter NH_3 flux. Broiler performance was determined by observations of final body weights, total feed consumption, total mortalities, and paw scores. Poultry litter characteristics were determined by analysis of litter pH, water activity, total caked litter produced and total aerobic and anaerobic bacteria. Poultry litter NH_3 flux was

determined by equilibrium chamber utilizing a measurement flux chamber placed over the litter.

Materials and Methods

Experiment two utilized a microbial amendment that was sprayed onto the poultry litter, thus it was necessary to segregate control and treated litter samples during the rearing period to prevent cross contamination of groups. This would have been a difficult objective to meet in a commercial setting without the use of multiple houses. In order for results of experiment two to maintain significance to the commercial industry, it was designed to replicate commercial broiler production settings as closely as possible in a research unit that allowed for segregation of control and treatment groups in a single building. Experiment two also utilized commercial style feeder, drinkers, lighting and ventilation schedules, and previously used litter along with commercial strain broiler chicks to parallel commercial industry standards as closely as possible while still maintaining research integrity.

Housing, Equipment, and Broiler Husbandry

The house utilized in experiment two was of solid wall construction, with concrete floors divided into 12 (2 x 4 m) pens. Environmental conditions were controlled through the use of mechanical ventilation. Evaporative cool pads provided cooling during warmer periods and heat was supplemented with natural gas furnaces during colder periods. At chick placement and until one week of age, pens were also equipped with infrared heat lamp brooders to provide additional warmth. These brood lamps were equipped with thermostats so that, if the temperature exceeded the maximum set point

this the brood lamps would power off until the temperature fell, at which point the lamps would resume function. Cycle timers were installed on the ventilation fans to provide minimum ventilation at all times and thermostats were installed that would turn the fans on continuously if temperatures exceeded the set point and would run until temperature again fell within the acceptable range. Temperature overrides for the house were set at approximately 32^{o}C at chick placement and were reduced 2^{o}C each week until the end of each trial. Full lighting was provided for 24 h/d for the first week of rearing, which lighting intensity was reduced, and 1 hr. of darkness was provided per day (until the end of the trial). The Experiment utilized a standard industry-feeding program in which each pen was equipped with a nipple drinker line, and four industry style feeders. This allowed ad-libitum feed and water intake for broilers. Feed and water lines were observed daily and adjusted as needed to prevent unnecessary spillage. Experiment two utilized commercial strain broilers which were reared at a density of 0.75 square feet per bird. Of the 12 pens utilized, 6 served as controls, with no application of litter amendments, the other 6 pens were treated with PWD. 15 oz of PWD was mixed with de-ionized water to bring the final volume to 1 gallon. This mixture was then applied to the surface of the litter and agitated with a rake to thoroughly promote mixing in the top 1 inch of the litter mass. The mixture was applied one week prior to broiler chick placement. At this time control pens received 2 gallons of de-ionized water and treatment pens received an additional 1 gallon of ee-ionized water, to provide additional moisture to the litter of the control pens to aid in bacterial placement, the control pens received additional water so that moisture levels would remain constant across

treatments. Experiment two utilized used broiler litter obtained from a local commercial broiler producer. This litter was spread at an average of 4 to 6 inches in depth in all pens. Broilers were reared to an age of 42 days. All broilers were reared in accordance with approved Texas A&M University Animal Use Protocols.

Broiler Performance

Broiler performance was determined by observations of final body weights, total feed consumption, paw scores, and total mortalities. Body weights were recorded at the time of broiler chick placement and at the conclusion of each trial to obtain total beginning and ending body weights. After a 24 hr feed withdraw period final body weights were taken on 42 d, broilers were removed from the each pen and placed 10 at a time into a coop for weighing. While broilers were set into the coops a individual would visually determine a paw score for each bird, this scoring system is based on a system used by Bilgili et al. (2006), in which 0 indicated no presence of lesions on the broiler paw, 1 indicates lesions < 7.5 mm and 2 indicates lesions >7.5 mm. Feed consumption was calculated throughout the trial for each pen and each diet, which allowed for a final calculation of total feed for the trial. Mortalities were recorded daily for each pen; each mortalities weight was recorded after its removal.

Litter Analysis

Litter samples were collected at three separate points in each pen. The samples were collected prior to product application, at broiler chick placement, and each week thereafter until the conclusion of the trial. At the conclusion of the trial caked litter was removed from each pen, weighed and samples collected. All litter and cake samples

were frozen until analysis. Litter and cake samples were removed from the freezer and allowed to reach room temperature, then analyzed for pH, a_w, percent moisture. pH of litter and cake samples was analyzed by weighing 12 g of litter and adding de-ionized water until the final volume was 60 mL. The sample was then mixed and allowed to rest before the pH with a pH meter. a_w of litter and cake samples was analyzed by weighing 6 g of cake or litter into plastic dished that were then placed into the measurement chamber of the a_w analyzer for a final calculation. Litter moisture was calculated for litter and cake samples by weighing 10 g samples of material into dry pans and drying in an oven at 100^{o}C for 24h. The weighing again to obtain a dry weight, the difference between the wet and dry weights, results in a total moisture calculation for the sample.

Litter Microbiological Analysis

Prior to litter treatment, at the time of chick placement, at days 21, 28, 35, and at the end of the trial, litter samples were analyzed for total aerobic bacteria. Litter microbial enumeration was completed by using 10g of litter or cake sample. Samples were weighed, and then mixed with 90 mL of PBS; the mixture was then stomached for 30 seconds. 1.0 mL of the mixture was then pippeted into 9.0 mL of PBS, appropriate serial dilutions were performed. Each dilution was plated on TSA all plates were incubated at 37 C for 24 h. After incubation, plates were manually counted and total CFU were calculated.

Litter Ammonia Flux Analysis

Ammonia flux measurements were collected prior to litter treatment, at chick placement, and each week thereafter until the conclusion of the trial. Ammonia flux

measurements were taken using a Drager CMS unit to measure NH_3 concentrations in the chamber after 20 minute equilibrium. Ammonia measurements were taken at two locations in each pen for. Measurements utilized a static flux chamber incorporated with a small fan to prevent gas stratification within the chamber. The chamber design was adopted from Ferguson et al., (1998), and was placed over the litter allowing an accumulation of volatilized ammonia to be sampled. The chamber was placed on the litter and a timer was set for 20 minutes. At 20 minutes, a plug was removed and the sampling hose attached to the CMS unit was lowered into the chamber for analysis to begin.

Statistical Analysis

All data was analyzed by one-way ANOVA using the GLM procedure of SPSS, with the treatment serving as the fixed variable, and observation serving as the dependent variable. Means were determined significantly different at $P<0.05$.

Results and Discussion

Data collected during this experiment are presented in Tables 3.1 and 3.2. Table 3.1 contains data pertaining to broiler performance where as Table 3.2 contains data concerning litter parameters.

Broiler Performance

Broiler performance parameters of beginning chick weight, ending body weight, average gain, feed consumed, feed conversion, adjusted feed conversion, percent mortality, average paw score, percent paws scored 0, 1, and 2, and ending caked litter

weight are presented in Table 3.1. No statistical differences between treatments were observed for any of the measured parameters.

Table 3.1 Experiment 2 broiler performance and caked litter

	Control	Spray	P-value
Day 1 Chick Weight (g)	40.43 ± 0.37	40.08 ± 0.29	0.47
Ending Body Weight (kg)	229.83 ± 0.03	226.22 ± 0.06	0.46
Average Gain (kg)	2.41 ± 0.03	2.36 ± 0.06	0.49
Feed Consume (kg)	393.20 ± 5.63	382.08 ± 10.85	0.38
Feed Conversion Ratio	1.74 ± 0.02	1.72 ± 0.02	0.41
Adjusted Feed Conversion Ratio	1.72 ± 0.01	1.71 ± 0.02	0.57
Percent Mortality (%)	2 26 ± 0.50	1.74 ± 0.22	0.57
Average Paw Score	0.96	0.95	0.95
Percent Paw Score 0 (%)	48.38 ± 3.77	48.26 ± 9.15	0.89
Percent Paw Score 1 (%)	6.95 ± 1.65	8.22 ± 1.86	0.62
Percent Paw Score 2 (%)	44.66 ± 3.50	43.48 ± 7.82	0.95
End Caked Litter Weight (kg)	42.37 ± 5.28	40.37 ± 7.84	0.84

Litter/Cake Parameters

Litter parameters of 20 minute equilibrium chamber NH_3 measurements, litter moisture, a_w, pH and total colony forming units of aerobic bacteria are presented in Table 3.2. With the exception of a_w prior to treatment -7 d (a) and on 35 d, no statistical difference between the control group and the treatment group were found. NH_3 volatilization shows its highest mark at -7 d (Table 3.2); this is likely due to the

movement during collection and transportation of the litter which increased volatilization rates. At 7 d volatilization rates begin to decrease and continued to remain low for the rest of the experiment. Litter moisture content for the experiment (Table 3.2) could play a role in the ineffectiveness of the treated groups to show significant differences when compared to control groups. The experiment was conducted at a time of year, which requires substantial use of brood lamps, heaters, and minimal use of ventilation to produce favorable environmental conditions. This equated to extremely dry conditions, which could have affected PWD effectiveness. Litter moisture samples were found to be similar to results seen in Experiment one discussed in Chapter II.

Table 3.2 Experiment 2 litter characteristics

Day	Treatment	NH_3	Moisture	a_w	pH	Aerobic
-7 d (a)	Control	217.75 ± 16.57	20.82 ± 0.44	0.88 ± .01[a]	8.65 ± .04	7.14 ± 0.12
	Spray	232.33 ± 8.81	20.17 ± 0.27	0.86 ± .01[b]	8.6 ± 0.03	7.40 ±0.11
	P-value	0.46	0.24	0.04	0.30	0.57
-7 d (b)	Control	---[1]	24.52	0.93 ± .01	8.57	---[2]
	Spray	---	24.87	0.94 ± .01	8.61	---
	P-value	---	0.28	0.29	0.58	---
0 d	Control	120.50 ± 7.42	19.01 ± 0.26	0.83 ± 0.02	8.41 ± 0.03	6.87 ± 0.16
	Spray	131.92 ± 9.50	19.06 ± 0.40	0.84 ± 0.01	8.39 ± 0.02	6.88 ± 0.15
	P-value	0.37	0.92	0.97	0.51	0.56
7 d	Control	41.10 ± 3.15	16.23 ± 0.47	0.79 ± 0.01	8.28 ± 0.05	---
	Spray	42.83 ± 1.44	16.49 ± 0.40	0.77 ± 0.01	8.34 ± 0.01	---
	P-value	0.63	0.68	0.27	0.19	---
14 d	Control	35.20 ± 6.09	17.69 ± 0.27	0.82 ± 0.01	8.27 ± 0.03	---
	Spray	29.34 ± 3.26	17.86 ± 0.25	0.79 ± 0.01	8.31 ± 0.03	---
	P-value	0.42	0.66	0.56	0.41	---
21 d	Control	24.98 ± 2.99	22.26 ± 0.42	0.90 ± 0.01	8.15 ± 0.06	8.63 ± 0.15
	Spray	35.90 ± 4.99	23.06 ± 1.12	0.90 ± 0.01	8.24 ± 0.09	7.97 ± 0.38
	P-value	0.09	0.52	0.53	0.38	0.17

Table 3.2 continued.

Day	Treatment	NH_3	Moisture	a_w	pH	Aerobic
28 d	Control	30.40 ± 2.01	24.86 ± 1.14	0.91 ± .03	8.21 ± 0.10	9.03 ± 0.08
	Spray	27.88 ± 3.64	25.89 ± 1.19	0.90 ± .01	8.46 ± 0.07	9.02 ± 0.07
	P-value	0.56	0.54	0.86	0.07	.079
35 d	Control	33.97 ± 3.81	25.77 ± 1.01	0.94 ± 0.01	8.66 ± 0.07	8.83 ± 0.13
	Spray	38.16 ± 3.40	26.34 ± 0.48	0.92 ± 0.01	8.67 ± 0.09	8.93 ± 0.05
	P-value	0.43	0.62	0.05	0.93	0.69
42 d	Control	37.80 ± 3.09	25.78 ± 0.91	0.93 ± .01	8.56 ± 0.08	8.95 ± 0.06
	Spray	39.59 ± 2.66	26.01 ± 0.59	0.93 ± .01	8.68 ± 0.10	8.95 ± 0.04
	P-value	0.68	0.84	0.61	0.36	0.89
Cake	Control	---[3]	41.30 ± 1.79	0.99 ± 0.01	8.76 ± 0.06	---
	Spray	---	41.75 ± 2.41	1.0± 0.001	8.76 ± 0.06	---
	P-value	---	0.89	0.26	0.94	---

[1]Addition of water only, NH_3 flux, and total CFU were not calculated twice

[2] Total CFU were not calculated on these dates

[3] NH_3 flux was not measured for cake

CHAPTER IV

CONCLUSION

The overall characteristics of poultry litter and its reuse can play a significant role in the commercial poultry industry. Currently, managers in commercial poultry operations utilize litter management and poultry litter amendments in an attempt to prevent excessive amounts of NH_3 from affecting poultry performance. Litter management, while important, can only extend the life of reused poultry litter so far, and current acidified poultry litter amendments only offer effective NH_3 control for the first 3 to 4 weeks of a broiler growout. If proper management techniques such as moisture control and proper ventilation are not followed, an increased level of NH_3 volatilization is assured (Reece et al., 1979). When poultry are subjected to elevated levels of NH_3, studies have shown a marked decrease in overall performance and an increased likelihood of disease susceptibility (Becker et al., 2004).

Prior research regarding the use of microbiological poultry litter amendments is lacking. At the point this study was conducted, no scientific literature had been published concerning the effects of microbiological litter amendments on poultry performance, litter characteristics, or NH_3 volatilization. Currently, there are products being sold and utilized as microbial litter amendments in the poultry industry with the intention of improving performance and reducing NH_3 volatilization. It was the intent of this study to determine the effectiveness of two such microbial litter amendments on the

reduction of NH_3 when applied to reused poultry litter, the effects of those amendments on the characteristics of the reused poultry litter, and their effects on the overall performance of commercial broilers.

Litter Guard

Experiment one tested the efficacy of Litter Guard™ (LG) over three trial flocks. Final results from experiment one indicate that the product had little effect on broiler performance, litter characteristics or NH_3 volatilization. As demonstrated in Tables 2.1 and 2.2, broiler performance between control and treated groups showed no statistical differences between treatments with the exception of ending caked litter weight in Trial 1. However, this difference is not seen again in the next two trials, and cannot be fully explained. Some statistical differences are seen in the data showing litter moisture, a_w, and pH (Tables 2.3 through 2.5); however, trends again are not seen throughout the experiment and are most likely due to variation in samples. The same conclusion can be rawn for litter N, C, C:N and litter microbiological results. Ammonia volatilization recorded at 60, 90, and 120 seconds, showed one point on day 14 at 60 seconds observation in trial two where a statistical difference was recorded, and at -3d 60, 90, 120 second observations in trial three where a statistical difference was recorded. However, this pattern did not continue in Trial 2 or 3 into latter observations, and must be considered circumspect as a result of variation in samples.

Poultry Waste Degrader

Experiment two tested the efficacy of PWD to improve overall broiler performance, litter characteristics and NH_3 volatilization in litter that had been treated

with the product. Final analyzed results from experiment two indicate that PWD had very little to no effect any parameters measured. No data with the exception of day -7a and day 35 a_w showed any statistical differences in the experiment. The previously mentioned data points, while they do demonstrate statistical difference, must be attributed to variation as no trend was observed to continue in the experiment. However, data from a preliminary trial testing a formulation of PWD with the addition of surfactants and application that took place twice daily indicated that PWD may have the opportunity to be developed further. In the preliminary trial the addition of the product daily kept additional moisture in the litter, while in experiment two moisture levels were low, a factor which may have affected the efficacy of PWD. A final factor to take into account is that control group broilers, as well as test group broilers, performed very well and very similarly in this experiment. This in and of itself left a small amount of room for PWD to have any effect on broiler performance.

Summary

Findings in both experiments of this study indicate that both LG and PWD had little to no effect on broiler performance, litter characteristics, or NH_3 production under the test conditions. There are, however, several possible explanations as to why the microbial litter amendments were ineffective under the conditions of these tests. Perhaps one method to re-examine the effectiveness of the amendments would be to begin an application regime concurrent with the placement of new bedding material. Terzich et al. (2000) showed that total bacterial counts increase along with increases in pH. Previous research has shown that the addition of manure from the birds increased

the total bacterial load 9 log/g of litter from a previous sample of fresh poultry litter (Fries et al., 2005). Therefore, as litter microbial populations become established with litter use over multiple flocks, the addition of smaller amounts of bacteria in the microbial amendments to the well-used litter may be out competed by the larger microbial loads already present in the litter.

Another factor that could be responsible for the ineffectiveness are the low moisture and a_w levels found in the poultry litter used for both experiments. The a_w impacts microbial growth and was found in this experiment to be relatively low at the time of amendment application. Chen and Alexander (1972) showed that a strain of drought resistant gram-positive rods required minimum a_w levels of 0.880, and that a strain of drought resistant as well as drought susceptible gram-positive rods required minimum a_w of 0.980 for growth. In both experiments $a_{w,}$ levels rarely reached the previously mentioned levels. Some findings have suggested lower thresholds, and that when a_w is held at a level below 0.70, bacterial growth will be reduced (Scott, 1957).

A final point of consideration may be that the C: N ratio may have been too low. Nahm (2003) suggest that the addition of high C materials such as saw dust and rice hulls could play a large roll in the immobilization of N in poultry litter. The addition of C to poultry manure increases the C: N ratio in turn allowing microbial populations to develop and allow for N immobilization in the poultry litter (Alexander, 1977; Serna and Fomares, 1991).

An important finding in both experiments was that litter a_w was positively correlated to litter moisture, meaning that as litter moisture increased so did a_w. This

finding is substantiated by research conducted by Hayes et al. (2000) in which similar findings found that within poultry litter there was a positive correlation between a_w and moisture content of the litter. Figures 4.1, 4.2, and 4.3 show regression analysis of moisture and a_w. They are divided as LG treatments (4.1), PWD treatments (4.2), and a combination of only control pens (4.3). Figure 4.4 graphically depicts the regression analysis of a_w compared to litter moisture for all treatments combined in Experiment 2. Figure 4.5 graphically depicts the regression analysis of a_w when compared to litter moisture for control pens only for Experiments 1 and 2.

The equation y=46.706x -17.701 is derived from the regression analysis for the combination of control pens from both experiments in Figure 4.3. The x in the equation represents a_w, where the y represents percentage of moisture. By substituting 0.90, just above the minimum a_w required for vegetative growth as measured by Chen et al. (1972), for x, the equation yields 24.33 for y. This would be the minimum moisture required to achieve a a_w of 0.90 and microbial growth, and this would need to be maintained. Referring to Tables 2.3, 2.4, 2.5, and 3.2 it is seen that moisture levels seldom reach this level and do not maintain it if they did manage to obtain it. Additional calculations based on this regression equation ranging from 0.800 to 0.999 are provided in Table 4.1.

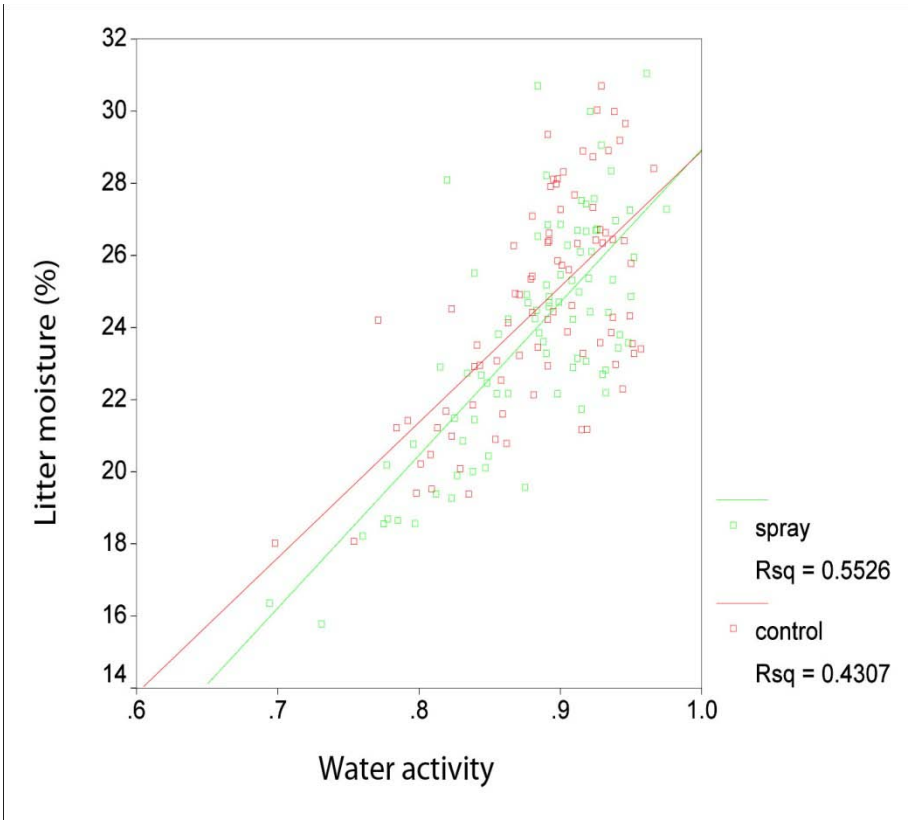

Figure 4.1 Regression analysis of a_w vs. litter moisture for Experiment 1.

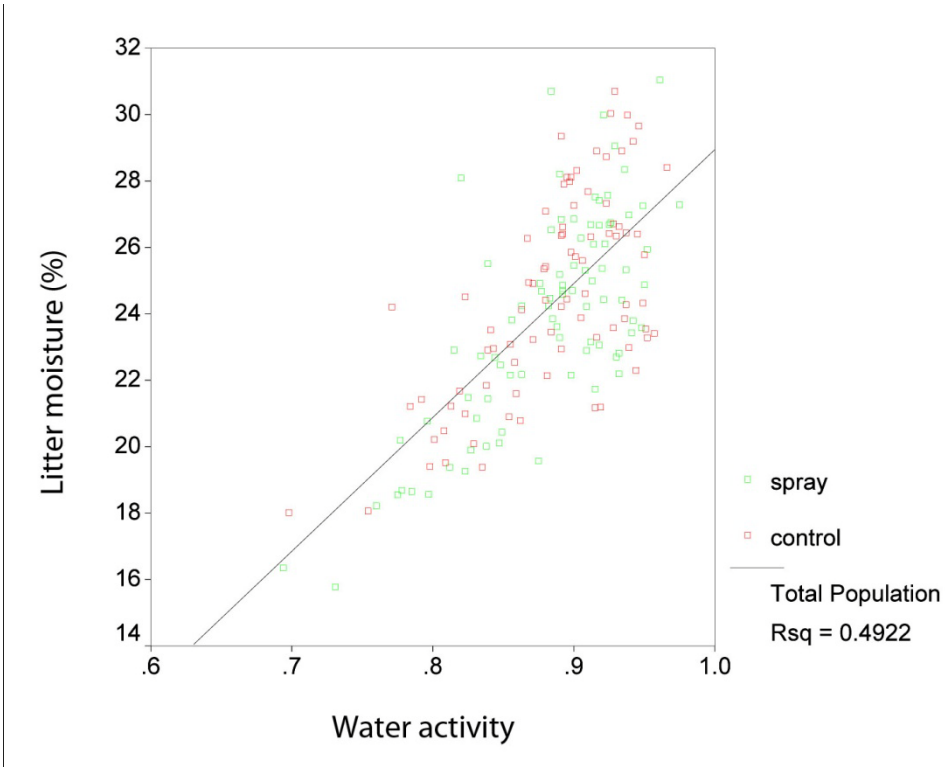

Figure 4.2 Regression analysis of a_w vs. litter moisture for Experiment 1 treatments combined.

Table 4.1 Linear equations relating aw to moisture for all Experiment 1 and 2 pens and all control pens.

	beta	constant	x (a_w)	y (moisture)
All Exp. 1 pens	40.321	-11.373	0.800	20.88
			0.850	22.90
			0.875	23.91
			0.900	24.92
			0.925	25.92
			0.950	26.93
			0.975	27.94
			0.999	28.91
All Exp. 2 pens	54.357	-25.578	0.800	17.91
			0.850	20.63
			0.875	21.98
			0.900	23.34
			0.925	24.70
			0.950	26.06
			0.975	27.42
			0.999	28.72
All Control pens	46.706	-17.701	0.800	19.66
			0.850	22.00
			0.875	23.17
			0.900	24.33
			0.925	25.50
			0.950	26.67
			0.975	27.84
			0.999	28.96

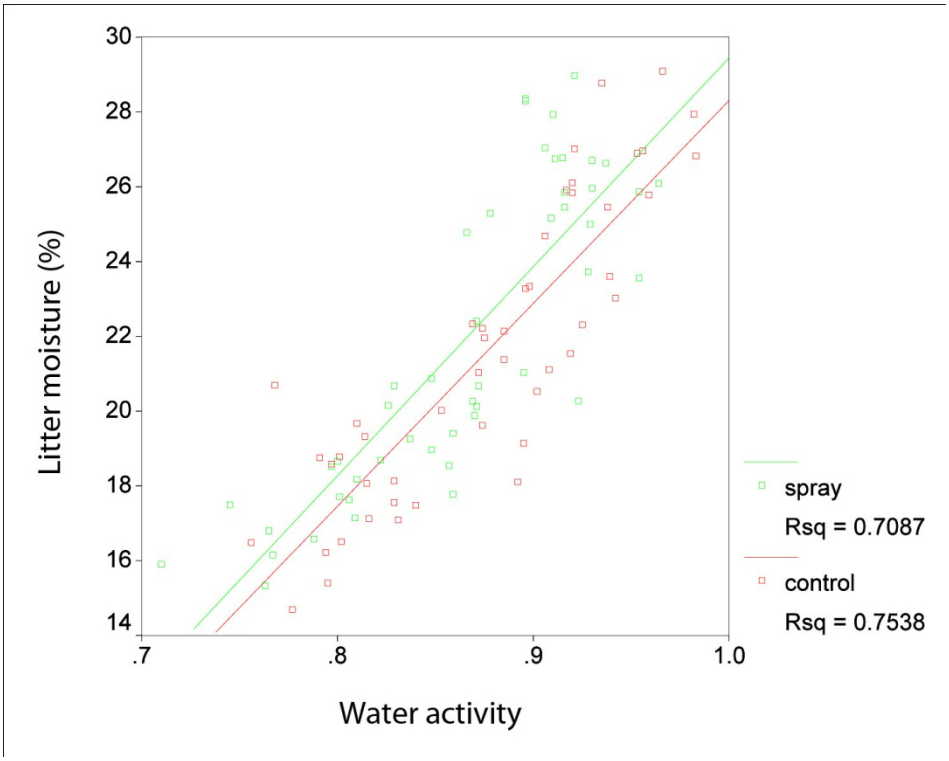

Figure 4.3 Regression analysis of a_w vs. litter moisture for Experiment 2.

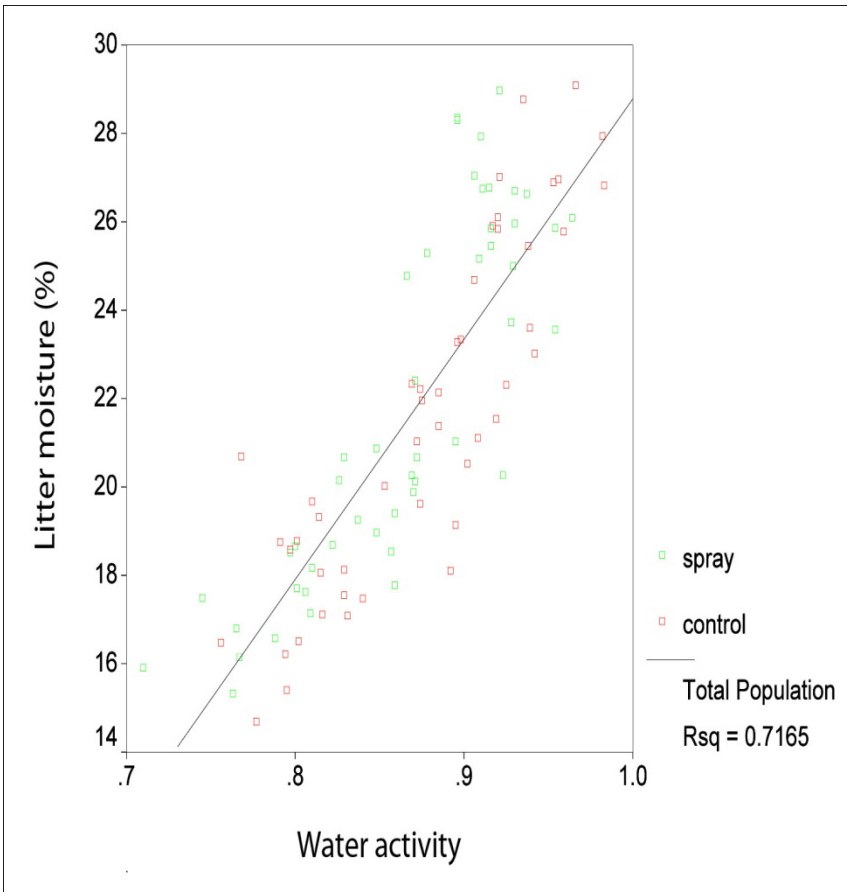

Figure 4.4 Regression analysis of a_w vs. litter moisture for Experiment 2 treatments combined.

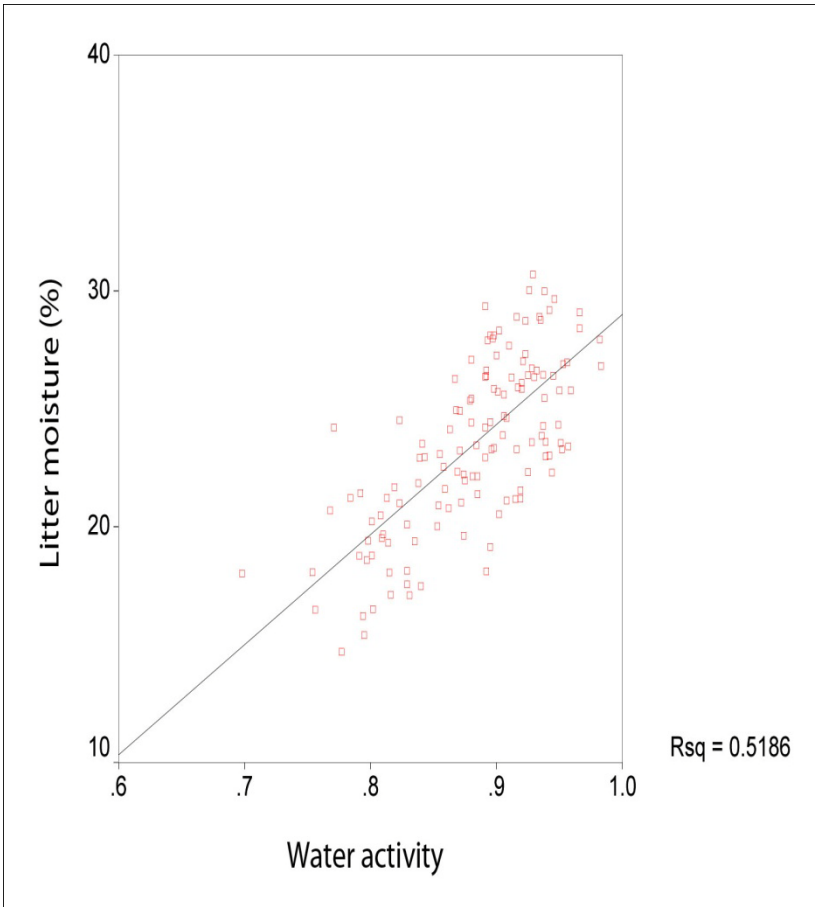

Figure 4.5 Experiment 1 and 2 control pen a_w vs. litter moisture.

Figure 4.6, 4.7, and 4.8 show the relationship through regression analysis between 20 minute NH_3 equilibrium chamber measurements as compared to 60, 90, and 120 second flux measurements. The results indicate the two methods are positively related. The 120 second measurement resulted in the best r^2 when compared with the 60 and 90 second measurements.

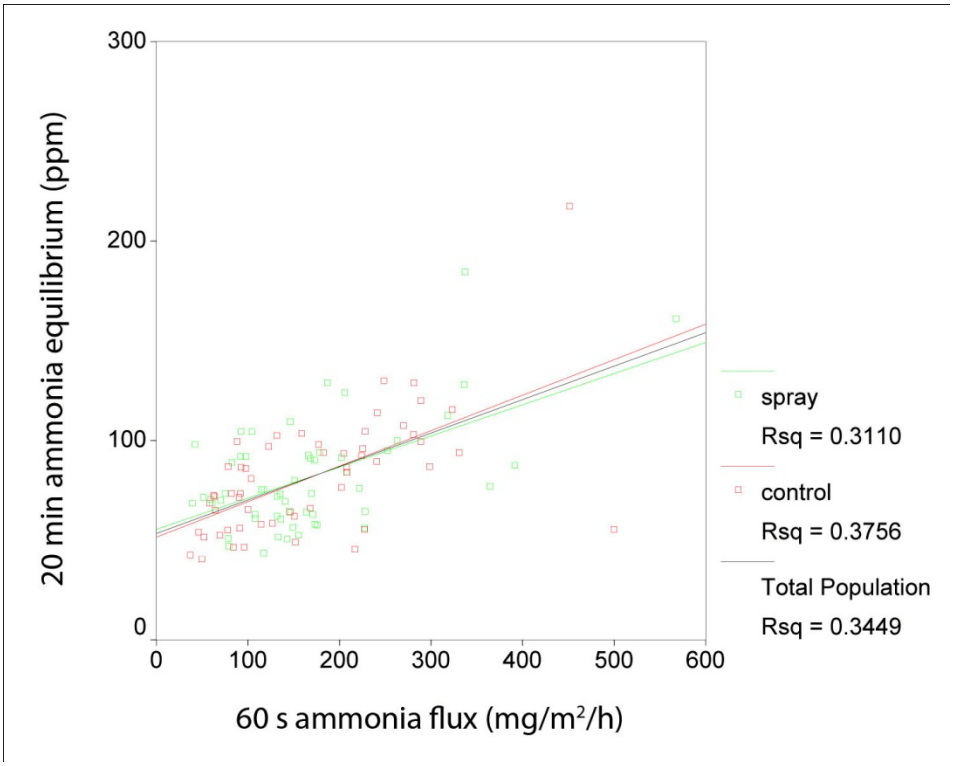

Figure 4.6 Experiment 1 20 minute NH_3 equilibrium chamber compared to 60 second NH_3 flux.

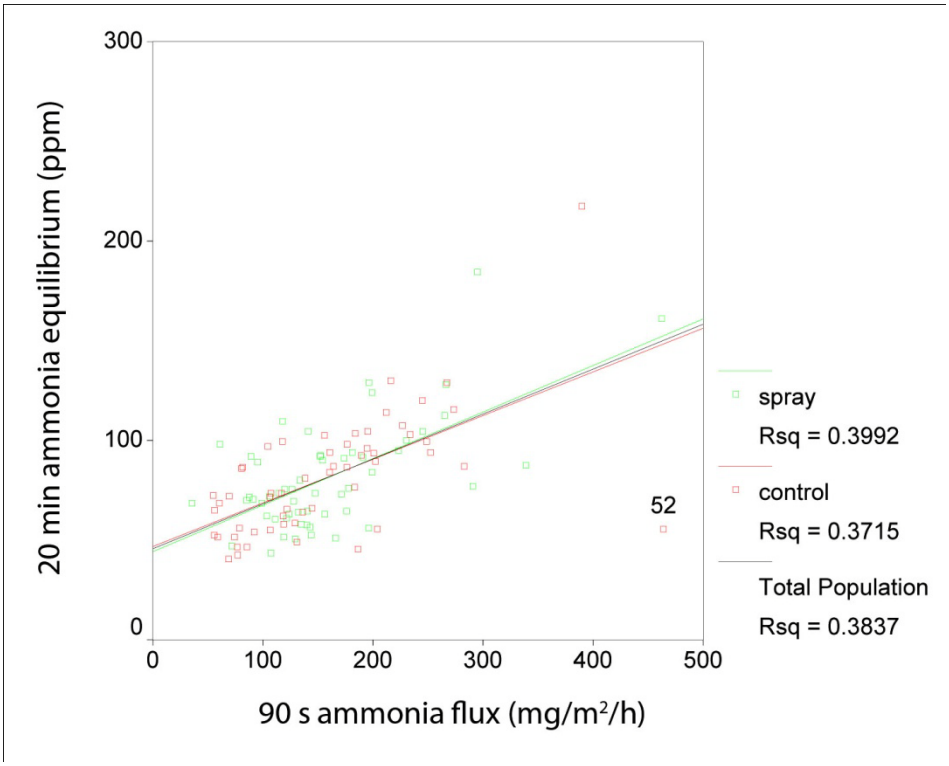

Figure 4.7 Experiment 1 20 minute NH_3 equilibrium chamber compared to 90 second NH_3 flux.

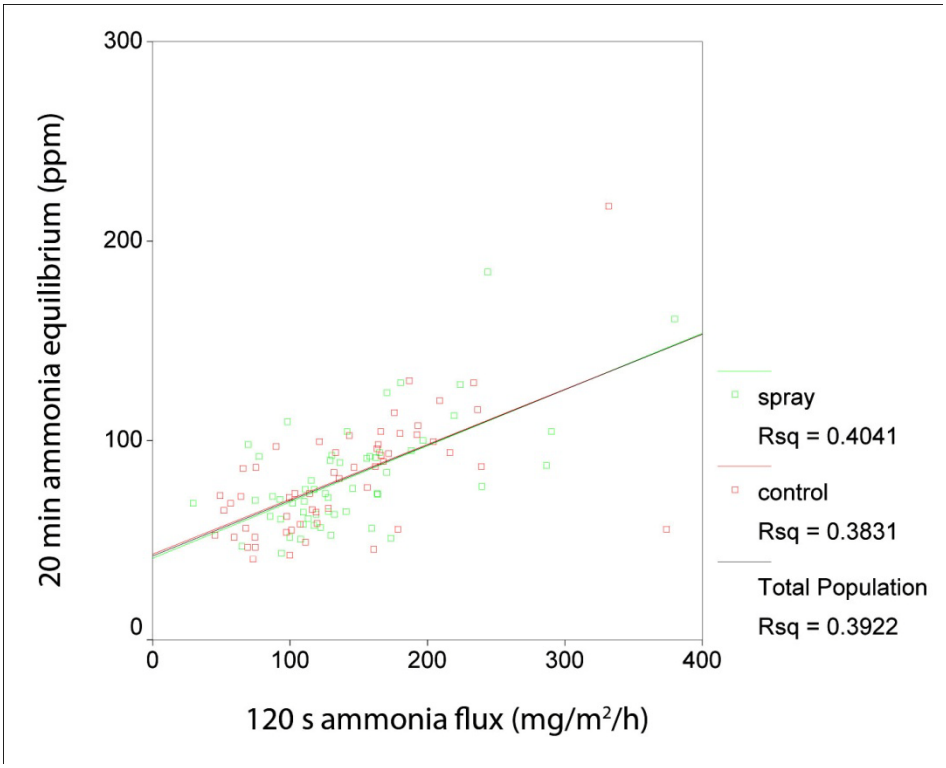

Figure 4.8 Experiment 1 20 minute NH_3 equilibrium chamber compared to 120 second NH_3 flux.

Figures 4.9 and 4.10 show regression analysis both linear and curve linear for the relationship of moisture and NH_3 20 minute equilibrium concentration measurements. From the earlier examination of the relationship of a_w to moisture, it was concluded to provide appropriate amounts of a_w for bacterial proliferation it would be required to have a minimum litter moisture percentage of 25%. However, a concern with this rationale is that elevated moisture levels will result in elevated levels of NH_3 volatilization. Carr et al. (1990) concluded that higher NH_3 concentrations at higher moisture levels were a result of increased capillary action as a result of the greater moisture, which in turn

resulted in an increased diffusion rate of NH_3. The addition of increased moisture percentages to promote increased a_w must be maintained throughout the life of the flock. If they are, and the beneficial microbes are allowed to proliferate and microbial proliferation is successful, NH_3 volatilization should decrease as a result. Figures 4.5 to 4.8 indicate at the recommended moisture percentages, there is no increase in NH_3 volatilization. In order to confirm this hypothesis, however, more research is needed. Additional consideration must be given to the effects of increased moisture on microbial elements that have not been introduced, namely pathogenic bacteria already present in the litter microbial flora that have the potential to create damage if proliferation is allowed to go uncontrolled. If increased litter moisture, which leads to increased a_w, can affect beneficial bacteria, it has equal ability to affect harmful bacteria.

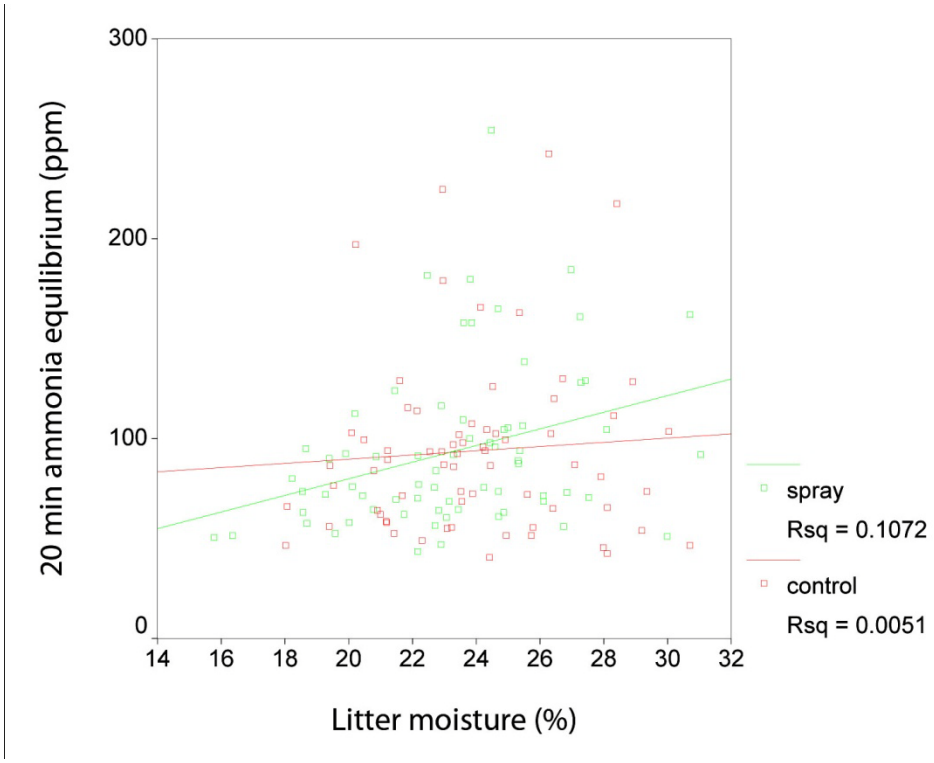

Figure 4.9 Experiment 1 moisture vs. NH_3 equilibrium chamber.

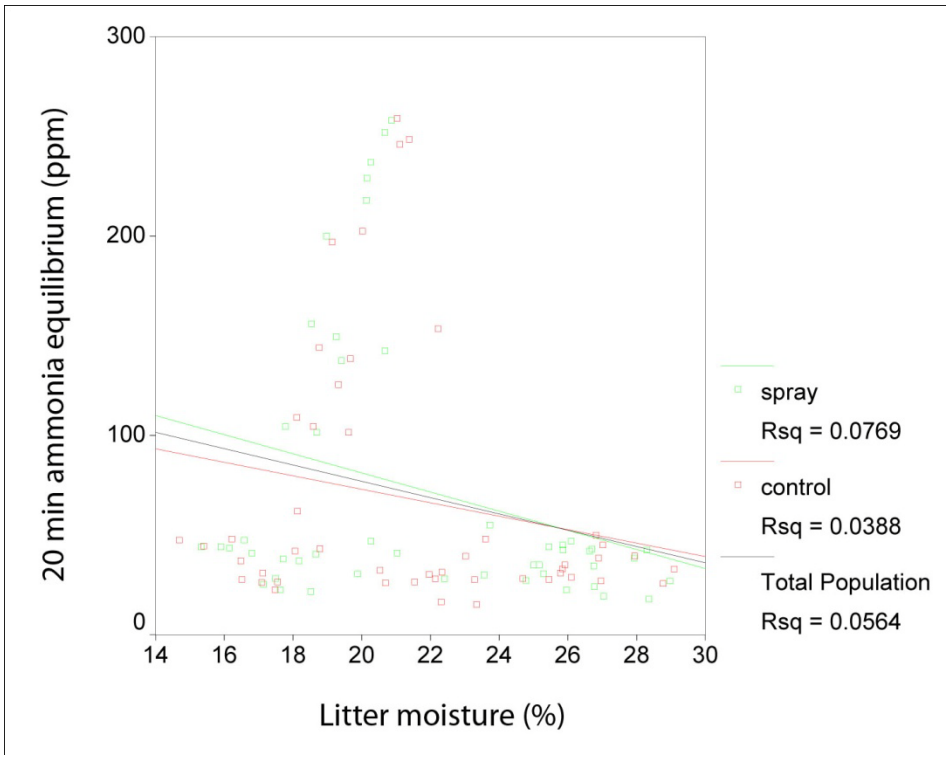

Figure 4.10 Experiment 2 moisture vs. NH_3 equilibrium chamber.

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