EVALUATION OF IN-HOUSE WINDROW COMPOSTING AS A POULTRY LITTER TREATMENT PRIOR TO LAND APPLICATION

A Thesis

by

SCOTT ALAN WINKLER

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

Chair of Committee,	Craig D. Coufal
Committee Members,	Daren Harmel
	Jason T. Lee
Head of Department,	Jimmy Keeton

August 2013

Major Subject: Poultry Science

ABSTRACT

The land application of poultry litter as a fertilizer is a common practice due to the low cost and high availability of poultry litter in some regions. However, land application can create concerns related to runoff water quality and odor. An experiment was conducted to determine the effectiveness of in-house windrow composting (IWC) of poultry litter prior to land application in terms of bacteria, odors and nutrients compared to untreated (fresh) litter. In the second part of the research, the objective was to quantify the number and distribution within poultry houses of selected water quality indicator bacteria in litter.

Comparison of fresh and IWC litter showed that *Escherichia coli (E. coli)* was present in very low concentrations on day 1 in fresh litter (20 cfu/g) and IWC litter (55 cfu/g), but the levels were undetectable in both litter types on day 9 in Trial 1. In Trial 2, *E. coli* levels were undetectable in IWC litter before and after the IWC process. Similarly, fresh litter had undetectable *E. coli* levels on day 1, but 185 cfu/g on day 10. Additionally, nutrient analysis and moisture content results showed no significant differences between fresh and IWC litter.

To evaluate odor differences between fresh and IWC litter, volatile gases were collected onto sorbent tubes and into Tedlar bags from wind tunnel flux chambers placed directly on litter piles prior to land application. The concentrations of 13 compounds commonly associated with animal manure were then determined by GC/MS. Analysis of volatile gas samples resulted in significant changes of various individual odorants,

while olfactometry analysis of Tedlar bag air samples resulted in reduced detection threshold values for IWC litter compared to the fresh litter. These results indicate the possible mitigating effects IWC may have on odors associated with litter.

In the survey of bacterial distribution within poultry houses, litter counts varied greatly within house sections and between farms. Regression analysis revealed that bacterial counts and litter moisture content are significantly related, thus explaining much of the variation in litter bacterial counts within a house.

These results indicate that IWC could be a useful best management practice to reduce *E. coli* levels and odor associated with poultry litter prior to land application, but factors such as moisture content, initial bacteria concentrations, and windrow size all affect the level of bacteria and odor reduction.

DEDICATION

I would like to dedicate this work to my family. Without my family, none of this would have been possible. To my parents, Daniel and Nancy, I would like to thank you for the endless love and constant support you have always provided me with. For as long as I can remember you have instilled in me good work ethic and morals that made me who I am today. To my sister Dawna, you have always been there with constant support and answers to all of my questions. You have been the best big sister anyone could ever want. I thank you for your constant guidance, love and support and continue to look up to you. To my grandparents, A.J. and Bernice Havel, I would like to thank you for the constant support and concern you have always shown me. Our weekly conversations about what the future holds always kept me motivated and focused. I love all of you very much and want to say thank you for everything you have done for me.

ACKNOWLEDGEMENTS

First and foremost I would like to thank God for his grace and guidance though this amazing journey that is my life. You constantly provide me with motivation and strength to continue when times get tough. Through Christ anything is possible.

I would like to thank my committee chair, Dr. Craig Coufal for his constant support and leadership. You have always been there when I had questions and taught me many things useful in graduate school and life. Thank you for giving me the opportunity to work alongside you and accomplish my goal of earning a master's degree.

Additionally, I would like to extend a thank you to Dr. Jason Lee, Dr. Daren Harmel and Eddie Caraway. Jason, you have been influential both in undergraduate and graduate school with your immense knowledge of the poultry industry. Thank you for being available and willing to help with any statistics and any questions about poultry. Daren, you have been extremely helpful throughout the whole project. Although we could not control Mother Nature and make it rain, you made sure everything else necessary for the project to succeed was taken care of. Eddie, thank you for being there to answer any and all of my questions about odors. Your expertise with odors is unmatched, and this project could not have been possible without you.

Thanks also go to my friends and colleagues that have made my time here at Texas A&M an amazing experience. Katlyn Klabunde, Nathan Fuchs, Joseph Klein, Derek Mund, Stephanie Iselt, Mallori Williams, Carolee Hinojosa-Garza, Kendre Stringfellow, T.J. Klein, Trevor Kalich, Sterling Koopmann, Martia Ross, Jacob Prukop,

James Boothe III and Rocky Latham, you have all made my time here interesting and enjoyable. Thank you for your advice, support, many laughs and memories that I will never forget. There was never a dull moment in my time here thanks to all of you. You have become like a second family to me, thank you.

I would also like to thank the Texas State Soil and Water Conservation Board and the USEPA for making this project possible with funding through a Clean Water Act §319(h) grant.

Finally, thanks to all of my family and friends that have been with me my whole life. You never let me forget who I am and how I was able to make it where I am today. Without my family and friends I would not be the person I am today.

NOMENCLATURE

BMP	Best Management Practice
cfu	Colony Forming Unit
DT	Detection Threshold
D/T	Dilution to Threshold Ratio
EPA	United States Environmental Protection Agency
GC/MS	Gas Chromatography-Mass Spectrometry
IWC	In-house Windrow Composting
K	Potassium
N	Nitrogen
NH_3	Ammonia
P	Phosphorus
SE	Standard Error
TCEQ	Texas Commission on Environmental Quality
TMDL	Total Maximum Daily Load
TSSWCB	Texas State Soil & Water Conservation Board
VFA	Volatile Fatty Acid
VOC	Volatile Organic Compound

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Poultry production is an important agricultural industry in Texas. According to the USDA (2011), more than 630,500,000 broilers were produced in Texas in 2011. With a litter production rate estimated at approximately 1 ton per 1,000 broilers (Coufal et al., 2006), roughly 630,000 tons of broiler litter is produced in Texas per year. On a national scale, more than 90% of poultry litter produced is land applied for agricultural use (Moore et al., 1995) because it is a valuable fertilizer source that contains high levels of nitrogen (N), phosphorous (P), potassium (K), and trace minerals (Kelleher et al., 2002). Although land application of poultry litter is known to have positive attributes for plant growth, it can create water quality and odor concerns.

One possible best management practice (BMP) to reduce water quality concerns associated with bacteria/pathogen runoff and odors concerns is heat treatment of the litter through in-house windrow composting (IWC) prior to removal from the house and land application. IWC is a relatively simple technique that utilizes natural bacterial metabolism to generate heat within piles formed lengthwise down a broiler house. It can be successfully completed within the broiler house, requires a shorter time span than traditional composting (about 10 days compared to several months), and can be one of the most effective methods of composting (Bautista et al., 2008). IWC has also been referred to as a "pasteurization" process instead of composting because it uses heat from bacterial metabolism within the litter mass to destroy pathogens but does not completely

convert the litter to a humic-like material as does traditional composting (Timmons, 2009). According to the time-temperature criteria for composting set forth by the United States Environmental Protection Agency (EPA), a compost pile must maintain a temperature greater than 55°C for a minimum of 3 days for pathogen inactivation to occur (Wichuk and McCartney, 2007).

In addition to decreasing pathogen content during composting, Ullman et al. (2004) noted that traditional composting methods of poultry litter can assist in reducing odor releases over time while converting litter to a humic soil amendment product. Nearly 50% of nuisance odor complaints in agriculture are associated with the land application of manure or poultry litter (Ullman et al., 2004). Odors associated with poultry litter are produced from the microbial degradation of the organic matter in the litter. Odor volatilization is attributed to absorption and metabolism of non-absorbable byproducts by microorganisms in the gastrointestinal tract or litter (Jenkins et al., 2008). The perception of odors by a person is a response to odorant compounds and is different for almost everyone (Millner, 2009), but Kreis (1978) recognized 13 different volatile organic compounds (VOC) commonly associated with animal waste odors. Nuisance odor complaints were higher than normal in certain poultry areas in Texas in 2009; therefore, the 81st Texas Legislature passed Senate Bill 1693 to address nuisance odors created by poultry farms and the land application of poultry litter. This bill has 5 basic components: complaint investigations, odor control plans, record keeping, training for odor prevention, and rules for siting new construction (TSSWCB, 2009). Senate Bill 1693 also set a requirement that if the Texas Commission on Environmental Quality

(TCEQ) issues 3 odor violations to the same facility within 1 year, the facility must create an odor control plan approved by the TCEQ. Additionally, the bill requires that owners or operators of a new poultry facility must complete a facility training course on the prevention of odor nuisances no later than the 90th day after birds are placed on the facility (TSSWCB, 2009). Finally, the bill requires poultry facilities selling or transferring poultry litter, along with the purchaser of the poultry litter, to maintain records of sale or transfer of litter for 2 years, and all records can be inspected by the TCEQ upon request (TSSWCB, 2009).

To date, some researchers, including Macklin et al. (2006) and Lavergne et al. (2006), have evaluated the effectiveness of IWC on reducing bacteria in poultry litter, but research combining odor and bacteria is limited. Therefore, research determining the possibility of inactivating bacteria and mitigating odors is important to not only the poultry industry, but also to end-users of poultry litter and the general public. The principal objective of this research was to evaluate the use of IWC to treat broiler litter prior to land application as a BMP to decrease the *E. coli* content in litter and to mitigate the potential for nuisance odors. This research was conducted with funding from the Texas State Soil and Water Conservation Board and the EPA through a Clean Water Act §319(h) grant. Additional research related to soil and water quality impacts of in-house windrow composting of poultry litter prior to land application was conducted within this grant project, but results are not reported in this thesis.

CHAPTER II

LITERATURE REVIEW

Poultry Litter

The recent growth of the poultry industry, driven by the demand for meat and egg products, has subsequently resulted in an increase in poultry litter production throughout the United States. In 2011, Texas produced more than 630 million broilers (USDA, 2011). With an annual litter production rate estimated at nearly 1 ton per 1,000 broilers (Coufal et al., 2006), this equals about 630,000 tons of litter produced per year in Texas. The increase in bedding material cost and the addition of litter amendments between flocks has led many producers to recycle litter for multiple flocks (Macklin et al., 2008).

Animal wastes have been utilized as a nutrient source for crop production for many years (Simpson, 1991). Poultry litter consists of many different organic materials including manure, spilled feed, bedding material and feathers (Kelleher et al., 2002). Since the inception of large scale, commercial broiler facilities, it has become a common practice to apply poultry litter as a fertilizer to croplands and pasture in place of commercial fertilizers (Bosch and Napit, 1992). Litter contains macro plant nutrients, such as N, P, and K, along with secondary plant nutrients, calcium, magnesium and sulfur, and also multiple trace elements, including copper, zinc and molybdenum (Bolan et al., 2010). It has been found that more than 90% of poultry litter produced is land applied for agricultural use (Moore et al., 1995). Studies have shown that the land

application of poultry litter has become the most desirable and most commonly used technique of using manure due to the content of nutrients and organic matter (USDA, 1999). Poultry litter can also be used as a fuel source to produce heat energy, or as an animal feed additive due to the uric acid in the manure (Bolan et al., 2010).

There are multiple advantages to using poultry litter rather than commercial fertilizers. One of the main advantages is that poultry litter can be less expensive than commercial fertilizers (Evers, 1998). Nitrogen, P, and K are the 3 main nutrients used to estimate the value of poultry litter as a fertilizer (Bosch and Napit, 1992). Additionally, Dunkley et al. (2011) estimates poultry litter to be equal to a 3-3-2-grade fertilizer on average. This equates to 60, 78 and 56 lb/ton of N, P_2O_5 and K_2O respectively. The value of poultry litter as an organic fertilizer averages around $80/ton using recent fertilizer prices (Ritz and Merka, 2009). Research conducted by Dunkley et al. (2011) has found that when applying broiler litter to crops, an increase of $103.74/0.4 ha (1 acre) per year can be achieved compared to those crops fertilized with commercial fertilizers. Similarly, Harmel et al. (2008) observed that the greatest annual profit ($56/0.4 ha) can be achieved by incorporating a 2 ton/0.4 ha litter application rate.

Conversely, nuisance odor complaints, variability of nutrient content between litter sources and the cost of transportation of litter have been found as some of the challenges of using litter as a fertilizer (Evers, 1998). With urban development expanding into previously agricultural areas, nuisance odor complaints have become more common. Additionally, broiler litter is bulky to transport, averaging 1.9 m^3/ton (67 ft^3/ton) when removed from the houses (Ritz and Merka, 2009). Ritz and Merka (2009)

calculated an average cost to transport poultry litter at about $20/ton according to current market rates.

In addition, pathogens in litter can adversely affect bird health and water quality. Pope and Cherry (2000) and Terzich et al. (2000) found *Staphylococcus*, *Escherichia coli*, *Salmonella*, and *Campylobacter* as just a few of the pathogens commonly found in poultry litter. Terzich et al. (2000) found that *E. coli* counts in poultry litter differ across states. *E. coli* numbers ranged from 1.22×10^5 (5.08 log) cfu/g in the Carolinas to 8.8×10^{10} (10.9 log) cfu/g in Texas. Conversely, other researchers have found no *E. coli* in litter samples acquired from the outer portions of litter compost piles or in inner portions of the same piles (Martin et al., 1998).

Odor Concerns

Although poultry litter can be used as an alternative to commercial fertilizer, it has the possibility to create nuisance odor complaints. In certain areas, almost half of the total number of agricultural odor complaints originated from the spreading of manure (Ullman et al., 2004). Odorant compounds are byproducts of the incomplete anaerobic digestion process in poultry and other animals (Parker et al., 2010). Odor is a person's olfactory perception, either good or bad, of odorant compounds in the environment (Millner, 2009; Ullman et al., 2004). Odor volatilization is attributed to absorption and metabolism of non-absorbable byproducts by microorganisms in the gastrointestinal tract or litter (Jenkins et al., 2008). The majority of odors result from the microbial degradation of feces and uric acid found in broiler litter (Ullman et al., 2004). Kreis

(1978) recognized 13 different volatile organic compounds commonly associated with animal manure (Table 1).

Table 1. List of 13 odorant compounds and corresponding odor descriptions commonly associated with animal manure (Kreis, 1978).

Odorant	**Description**
Ammonia	Pungent; Irritating
Propionic Acid	Body Odor; Vomitus
Butyric Acid	Body Odor; Vomitus
Isobutyric Acid	Rancid; Butter; Cheese
Valeric Acid	Foul
Isovaleric Acid	Fatty Acid; Sweat; Buttery
Indole	Piggy; Mothball; Burnt; Musty
Skatole	Outhouse; Fecal
Dimethyl Sulfide	Decayed Cabbage
Dimethyl Disulfide	Repulsive
Hydrogen Sulfide	Rotten Egg
Methanethiol	Rotten Cabbage
Ethanethiol	Garlic Odor

Nuisance odors are the source of most complaints from individuals living near animal feeding operations (Dalton et al., 2011). While there are no federal standards regarding odors, odors are regulated as nuisances by state agencies (Lacey et al., 2004). Many different factors affect the frequency of odor complaints. One main factor is location (Carey et al., 2004). Selecting the proper site for land application of poultry

litter can be very important in avoiding nuisance odor complaints. Certain criteria, such as prevailing winds, humidity, litter moisture and tree lines should be taken into consideration prior to land application (Carey et al., 2004; Miner, 1997).

Due to increasing odor complaints near new poultry farms in Texas in 2009, the 81st Texas Legislature passed Senate Bill 1693 to address odors from poultry farms and litter application sites (TSSWCB, 2009). This law has 5 basic segments: complaint investigations, odor control plans, record keeping, training for odor prevention and rules for siting new construction (TSSWCB, 2009). This bill made it mandatory for the TCEQ to investigate nuisance odor complaints within 18 h of the time of complaint (TSSWCB, 2009). Previously, there were no set time requirements to investigate odor complaints.

There are many different ways to measure odors to determine whether or not a nuisance odor condition exists. Field olfactometry, the use of a human nose as a detection instrument, is a common but extremely subjective process (Dalton et al., 2011). One commonly used system incorporates the FIDO principle. This technique takes into consideration the 4 basic attributes of odor: frequency, intensity, duration and offensiveness; where the frequency is based on how often the odor is identified over a certain amount of time, intensity refers to the potency of an odor, duration is how long the odor is detectable, and offensiveness is the character of the odor (Lacey et al., 2004). A more direct method for determining the concentration of odors is to use a field olfactometer such as the St. Croix Nasal Ranger Field Olfactometer, which can be used

to dilute the odor to certain dilution to threshold (D/T) ratios (Dalton et al., 2011; Lacey et al., 2004).

Odorants are comprised of volatile organic compounds (VOC) which are composed of volatile fatty acids (VFA) (Lacey et al., 2004; Parker et al., 2010). Recent research by (Parker et al., 2010) used wind tunnels and flux chambers to collect air samples on stainless steel sorbent tubes to analyze VOCs and VFAs emitting from animal feeding operations. Once the samples were collected on sorbent tubes, gas chromatography/mass spectrometry (GC/MS) analysis was conducted to determine the concentration of 11 specific VOCs associated with animal feeding operations.

Composting of Litter

For many years, composting has been an effective method for treatment of organic wastes such as animal mortalities and food products (Macklin et al., 2008). The goal of composting is to stimulate the growth of natural aerobic microorganisms present in the organic material to alter its physical and chemical features (Walker, 2004). Composting can be conducted in multiple ways. Two relatively simple, yet effective methods include static pile composting, which consists of simply placing the litter into piles and waiting, and windrow composting in which the compost piles are physically "turned" or agitated (Walker, 2004). More complex methods of composting include passively aerated piles where pipes are used to encourage air flow within the pile and forced aeration piles that force air through the piles using a pipe system (Brodie et al., 2000; Walker, 2004). No matter which method is used, the goal is to create an aerobic

environment to utilize heat produced by microorganisms metabolizing organic material (Barker et al., 2011).

Moisture is a major factor in composting. Miles et al. (2011) found that litter moisture can vary from 19% to 37% throughout commercial broiler houses with an average of approximately 30% moisture. This average moisture content has been found to work well for composting (Lavergne et al., 2006; Timmons, 2009).

In-house Windrow Composting

In more recent years, the treatment of broiler litter by IWC between flocks has become a common practice for various reasons (Macklin et al., 2006). Many poultry producers have delayed full house clean-outs to extend the useful life of poultry litter due to the increased cost of replacement bedding material (Bautista et al., 2008). IWC has the potential to help reduce pathogens, mitigate odors, reduce litter volume and increase broiler performance, all while maintaining its usefulness as a soil amendment when conducted correctly (Penn et al., 2010). IWC is less labor intensive than traditional composting, can be successfully completed within the broiler house, requires a shorter time span of about 10 days compared to months for normal composting, and is one of the most effective methods of composting when done correctly (Bautista et al., 2008; Timmons, 2009). Some people consider IWC a "pasteurization" process instead of a composting process since it technically uses heat to destroy disease causing bacteria but does not actually complete a true conversion of litter to humic matter (Timmons, 2009).

In-house windrow composting consists of piling litter into piles (windrows) lengthwise down the house and turning the windrows approximately 4 days after formation (Barker et al., 2011; Malone, 2010). Ideally the windrow will reach a minimum of 55°C (131°F) and maintain that temperature for a minimum of 3 days to completely inactivate pathogenic bacteria (Malone, 2010). Hartel et al. (2000) found that windrowed litter contained fewer bacteria than non-composted litter. Additionally, significant reductions in *Salmonella* were achieved with IWC according to Macklin et al. (2008). A similar study discovered that *E. coli* and *C. perfringens* were completely eradicated after windrow composting was performed (Bautista et al., 2008). Not only did IWC reduce bacterial counts, it also improved flock performance by creating a more advantageous environment for placing day old chicks (Barker et al., 2011; Bautista et al., 2008).

Effects of Composting on Bacteria and Odor

According to EPA time-temperature criteria for composting, a compost pile must maintain a temperature greater than 55°C for a minimum of 3 days in order for pathogen inactivation to occur (Wichuk and McCartney, 2007). Numerous researchers have tested this standard to confirm the validity. Erickson et al. (2010) proved that after inoculating compost piles with *E. coli* and *Salmonella* and composting at 55°C for 3 days, all samples were verified as pathogen-free. Additional research performed by Wilkinson et al. (2011) on poultry litter found that after 8 h, a reduction of greater than 99% of *E. coli* was achieved. Macklin et al. (2008) found a significant 10 log cfu/g of litter reduction

of *E. coli* 7 days after windrow composting. Bautista et al. (2008) and Hartel et al. (2000) found that in multiple scenarios, all fecal coliforms, including *E. coli* and *C. perfringens* were completely eliminated post composting. In addition to eliminating pathogens during composting, Ullman et al. (2004) observed that composting poultry litter can assist in reducing odor releases by stabilizing the animal wastes in poultry litter. However, specific data on the effects of composting on odors are lacking.

Effects of Composting on Litter Value

Composted poultry litter has a fertilizer value of between $70 and $90/ton based on its concentrations of N, P and K (Ritz and Merka, 2009). Nitrogen and P within the litter are primarily found in organic form, but K can be found in an inorganic state (Bosch and Napit, 1992; Ritz and Merka, 2009). Mineral levels in litter have the tendency to increase with successive grow outs until an equilibrium is met (Lavergne et al., 2006). Research has shown that on average litter contains 2.94, 3.22, and 2.03% N, P_2O_5, and K_2O, respectively (Coufal et al., 2006). Tiquia and Tam (2002) and Kelleher et al. (2002) found that during the composting process, there is a loss of N that is mainly attributed to NH_3 volatilization. Conversely, Coufal et al. (2006) discussed that P and K are minerals that do not become volatilized from litter during composting. Some research has shown that in spite of the volatilization of N, there is an increase in total P, K and even trace elements such as copper and zinc because ammonia, carbon dioxide and water are volatilizing, so while the total mass of minerals present does not change, the percentages of minerals increase (Tiquia and Tam, 2002).

Conclusion

The increasing demand for healthy and satisfying poultry products creates corresponding increases in poultry litter production. More people and businesses expanding into traditionally agricultural areas raises concern as to whether the land application of poultry litter will continue to be appropriate and acceptable. Limited research has been conducted into methods that can be used to treat litter prior to land application to reduce bacteria and control odors. Due to limited data specific to Texas, there is also a need to determine bacterial concentration (specifically *E. coli*) in poultry litter produced from modern broiler farms. Some assumptions about the effect that litter composting would have on odors can be made, but data on actual odor concentrations is minimal. Therefore, an experiment that investigates the effects of IWC of poultry litter to reduce bacterial and odor content prior to land application would provide useful data to supplement the deficiencies in current knowledge.

CHAPTER III

EVALUATION OF IN-HOUSE WINDROW COMPOSTING AS A POULTRY LITTER TREATMENT PRIOR TO LAND APPLICATION

Introduction

Broiler litter production rates have been determined to be about 1 U.S. ton per 1,000 broilers harvested (Coufal et al., 2006). According to USDA (2011), there were over 630,500,000 broilers produced in the state of Texas in 2011. Thus, it can be estimated that in 2011 about 630,500 tons of broiler litter was produced in Texas. Since the uses for litter in Texas are limited to just a few, the majority of poultry litter is land applied as a fertilizer. On a national basis, it has been found that more than 90% of poultry litter produced is land applied for agricultural use (Moore et al., 1995). Litter contains plant macro nutrients, N, P and K, along with secondary plant nutrients, calcium, magnesium and sulfur, and also many trace elements, including copper, zinc, and molybdenum. Although the land application of poultry litter is known to have positive attributes for plant growth, negative impacts to environmental quality are a concern under some circumstances.

Counts of *E. coli* bacteria in poultry litter have been reported as high as 8.8×10^{10} cfu/g from litter samples collected in Texas (Terzich et al., 2000). In addition to *E. coli,* poultry litter is known to contain *Salmonella, Campylobacter, Staphylococcus* and *Clostridium perfringens* along with many other bacteria (Pope and Cherry, 2000). Therefore, runoff water from lands receiving poultry litter could potentially be

contaminated with such bacteria and are a cause of concern for possible contamination of surface water.

Odor release during and following the land application of poultry litter is another point of concern, particularly if people living and/or working near the application site are offended by the smell of animal manure. It has been found that nearly 50% of agricultural nuisance odor complaints result from the land application of animal manure (Ullman et al., 2004). It is an inherent fact that odors emanate from manure, and in many situations little can be done to prevent this occurrence. As the demand for meat products continues to grow along with the population, animal feeding operations continue to grow in size and number, thus producing more manure. Producers and end-users of animal manures typically cannot afford to spend excessive amounts of time and money to control odors, so the need for processes that are rapid, economical and easy to implement could be extremely beneficial. Research has been conducted using feed additives and litter amendments to mitigate odors associated with poultry operations, but not much success has been achieved. It has been suggested by Ullman et al. (2004) that proper litter composting can assist in reducing odor releases.

Bautista et al. (2008) and Hartel et al. (2000) found that in multiple scenarios, all fecal coliforms, including *E. coli* and *C. perfringens* were completely eliminated post-composting. Additionally, composting has been known to reduce odors while maintaining a favorable nutrient composition. While traditional composting is a procedure that can achieve desirable changes in poultry litter such as reducing odors and bacteria, it requires an extended amount of time and properly designed facilities. One

possible alternative is the process of in-house windrow composting (IWC), which requires less time than traditional composting and has been found to achieve pathogenic bacterial reductions. The goal of the current study was to evaluate the effects of IWC on poultry litter prior to land application with regards to *E. coli* counts, nutrient analysis, and odor.

Materials and Methods

In-house Windrow Composting

This project was conducted using 2 trials at 2 different commercial broiler farms. The first trial was conducted in October 2011, and the second trial was conducted during May 2012. The age of the litter varied from only 4 flocks reared on the litter in Trial 1 to 16 flocks reared on the litter used in Trial 2. Both trials were conducted using the same methods for all criteria except odor collection, which was expanded in Trial 2. In both trials, a single commercial broiler house was divided in half lengthwise. The litter on one side of the house was formed into a windrow (IWC litter) and the other half of the house was not disturbed (fresh litter). Both trials used a custom made poultry litter windrowing implement designed by students in the Texas A&M Department of Biological and Agriculture Engineering. The windrower consisted of a 24-inch auger powered by the power take off (PTO) of a tractor and connected to the three-point hookup. The windrow machine was approximately 2 m (7 ft) in length, and included modified attachments to assist in windrow formation. As the auger turned, litter was

aerated and pushed into a windrow pile approximately 0.6 m (2 ft) tall and 1.5 m (5 ft) wide.

Each portion of the house contained a minimum of 20 tons of litter to be land applied. Litter samples were collected prior to windrow formation, on either the fourth or fifth day when the windrows were turned, and finally on the ninth or tenth day when litter was transported to the land application sites. Litter moisture analysis was determined by drying at 100°C for 24 h.

Windrow Temperatures

Litter temperature within the windrows was recorded using iButton temperature data loggers throughout the entire IWC period. Data loggers were programmed to record temperatures once per hour. Data loggers were fastened on wooden stakes and inserted into the windrow horizontally. In Trial 1, stakes were inserted in the triangular pattern (Figure 1) at 12 m (40 ft) and 30.5 m (100 ft) from the cool pad end of the house to examine temperature variation throughout the windrow. This pattern is similar to that of Schmidt (2010) to determine temperature variation throughout a windrow pile.

At 3 m (10 ft) and 21 m (70 ft) from the cool pad end of the house, stakes were placed in the windrow so that a single data logger measured temperatures in the core of the pile (Figure 2). In Trial 2, temperature data loggers were placed in the core of the windrow at 12 m (40 ft) and 30.5 m (100 ft) from the cool pad end of the house only.

Figure 1. Temperature data logger positioning at 12 m (40 ft) and 30.5 m (100 ft) from the cool pad end of the house in Trial 1.

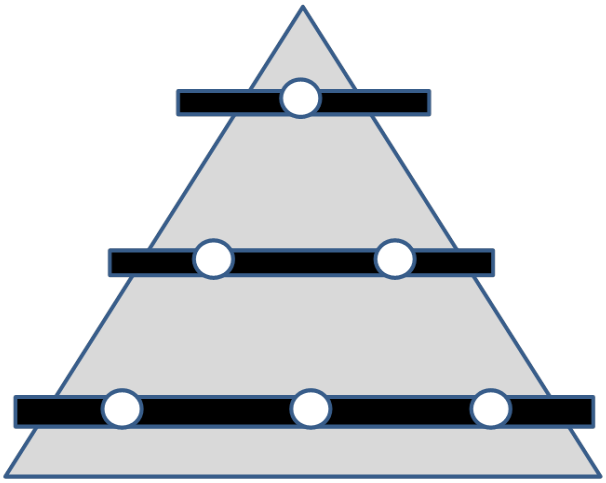
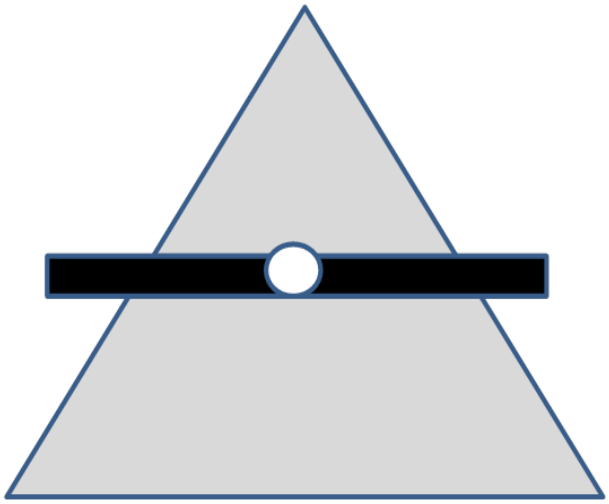


Figure 2. Temperature data logger positioning at 3 m (10ft) and 21 m (70 ft) from the cool pad end of the house in Trial 1, and at 12 m (40 ft) and 30.5 m (100 ft) from the cool pad end of the house in Trial 2.



Description of Land Application Sites

Eight pasture watersheds, located at the USDA-ARS (Agricultural Research Service) Grassland, Soil and Water Research Laboratory's Riesel Watersheds near Riesel, TX (Figure 3), received either fresh or IWC litter. The Riesel Watersheds are dominated by Houston Black clay soil (fine, smectitic, thermic, udic Haplustert), which is recognized throughout the world as the classic Vertisol. These highly expansive clays, which shrink and swell with changes in moisture content, have a typical particle size distribution of 17% sand, 28% silt, and 55% clay. These soils are very slowly permeable when wet (saturated hydraulic conductivity ≈ 1.5 mm/h.); however, preferential flow associated with soil cracks contributes to high infiltration rates when the soil is dry (Allen et al., 2005; Arnold et al., 2005; Harmel et al., 2006). Land management, size and litter application data for each of the 8 watersheds is presented in Table 2.

Figure 3. Watershed sites at the USDA-ARS Riesel Watersheds.

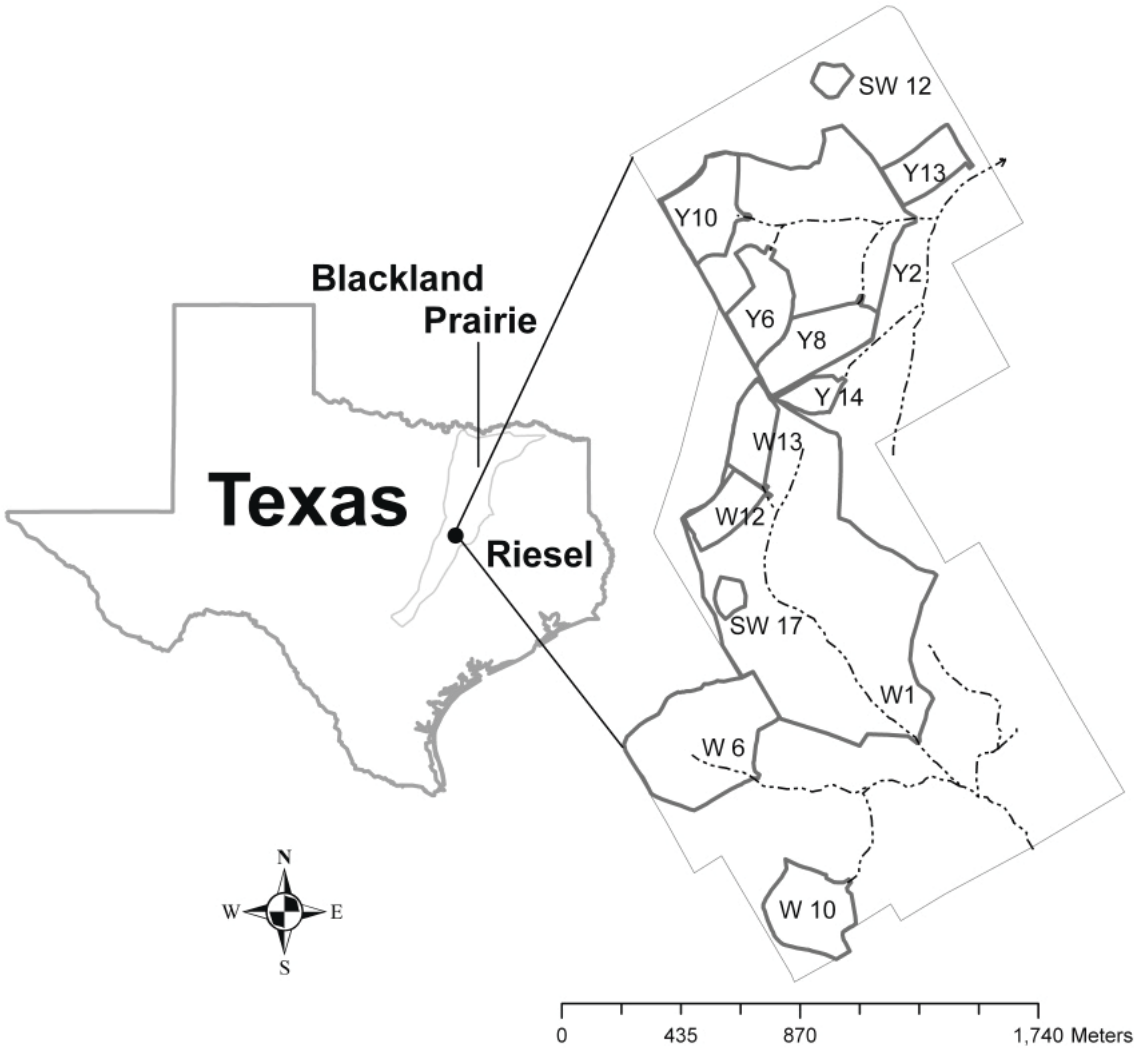


Table 2. Land management, watershed characteristics and litter application data for USDA-ARS Riesel Watersheds pasture sites.

	Watershed Characteristics							
	P1	**P2**	**P3**	**P4**	**SW12**	**SW17**	**W10**	**Y14**
Area, ha	0.1	0.1	0.1	0.1	1.2	1.2	8.0	2.3
Slope, %	2.8	3.0	3.0	2.8	3.8	1.8	2.6	1.6
	Land Management							
2010 -11	renovated	renovated	renovated	renovated	hayed	renovated	grazed	renovated
Litter rate, Mg $ha^{-1}yr^{-1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2011 -12	shredded	shredded	shredded	shredded	shredded	shredded	grazed	shredded
Litter rate, Mg $ha^{-1}yr^{-1}$	6.7	6.7	6.7	6.7	0.0	6.7	0.0	6.7
Litter Type	Fresh	IWC	Fresh	IWC		IWC		Fresh
2012 -13	shredded	shredded	shredded	shredded	shredded	shredded	grazed	shredded
Litter rate, Mg $ha^{-1}yr^{-1}$	6.7	6.7	6.7	6.7	0.0	6.7	0.0	6.7
Litter Type	Fresh	IWC	Fresh	IWC		IWC		Fresh

Pastures P1, P2, P3 and P4 are each 0.1 ha (0.25 ac) watershed plots while the remaining sites were larger and varied in size. Pasture management generally consisted of litter application (surface applied), shredding or grazing, and herbicide application. One of the pasture watersheds, SW12, a native (remnant) prairie that has never received litter or inorganic fertilizer, served as a reference and control site. Another watershed, W10, received litter application from 2001-07 and has been rotationally grazed since then; thus, this watershed served as an additional control. The 2012-2013 application was moved earlier in the year in an attempt to obtain wetter litter and thus observe a greater impact of the IWC process. Litter was applied on a dry weight basis to ensure the IWC and fresh litter solids were applied at the same rate. Litter was applied by a contract applicator with a commercial poultry litter applicator at a rate of 6.7 Mg/ha (3 ton/ac).

Litter Collection and Analysis

For each trial, litter samples were collected immediately prior to land application and analyzed for moisture, nutrient and *E. coli* content. Moisture content was determined by drying at 116°C for 24 h. Organic C was determined using a total C analyzer with the primary sample ignition furnace temperature reduced to 650°C (McGeehan and Naylor, 1988; Schulte and Hopkins, 1996). *E. coli* levels were enumerated following EPA Method 1603 (USEPA, 2006). Total N and C were determined using a combustion process and total P were determined by ICP analysis of a nitric acid digest (Lindsay and Norvell, 1978). Water extractable nitrate plus nitrite N

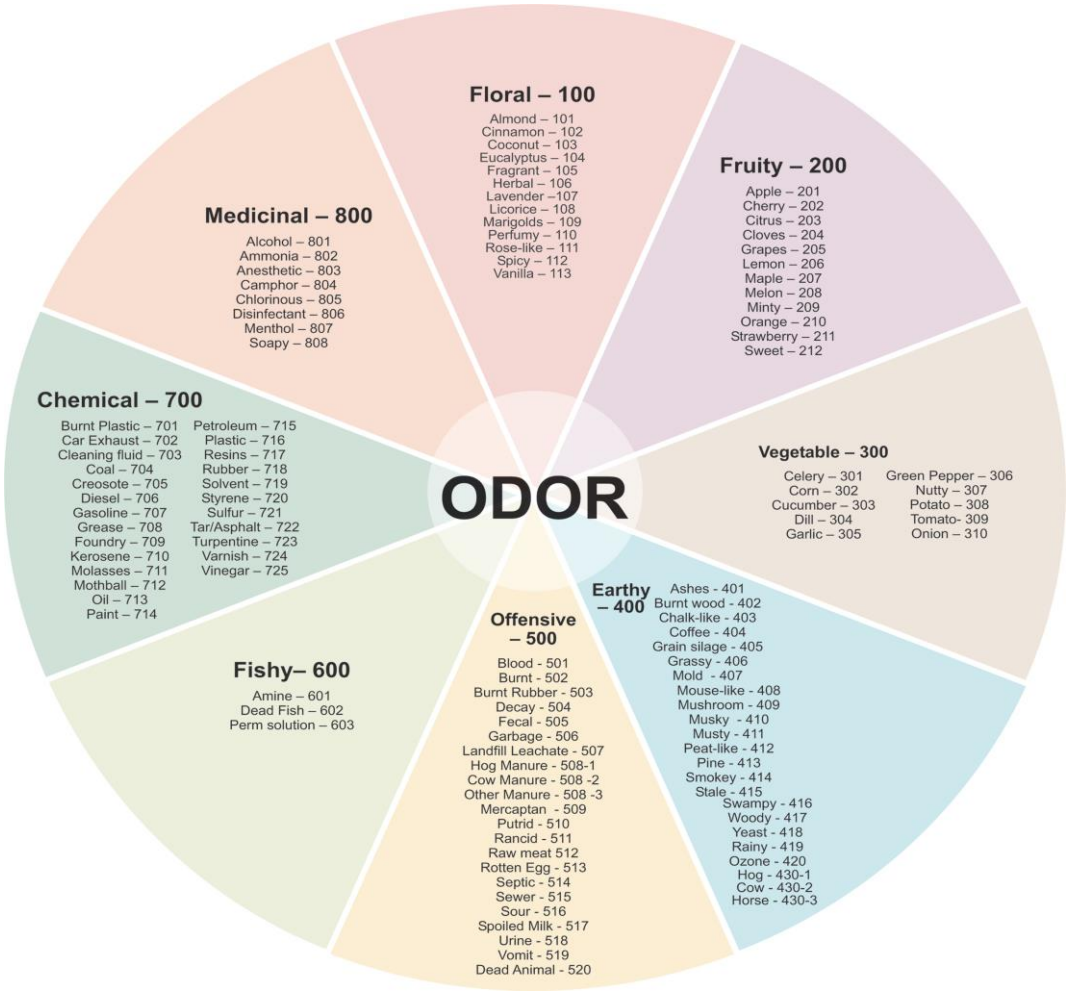
(NO_3+NO_2-N), ammonium N (NH_4-N), and orthophosphate P (PO_4-P) concentrations were determined with extraction methodology described by Self-Davis and Moore (2000) and subsequent colorimetric analysis.

Odor Data Collection and Analysis

Two methods (Nasal Rangers and sorbent tubes) were used to collect odor related data in Trial 1, and olfactometry analysis by trained human panelists was also used in Trial 2. To assess ambient air odor concentrations, 18 human volunteers (referred to as monitors) were recruited from the local community. Monitors were screened for his or her olfactory sensitivity to *n*-butanol "Sniffin" Sticks (St. Croix Sensory). In addition to sensitivity testing, monitors participated in a training session involving odor observation techniques, data recording procedures and proper technique for using the Nasal Ranger. It was also necessary to familiarize the monitors with the perceptual quality of certain odors associated with agricultural processes so that they could characterize the descriptors on the odor wheel (Figure 4). Monitors were divided into groups containing an average of 4 volunteers, and the group remained together for all sampling days in a trial. The majority of monitors were males between the ages of 30 and 40. Odor data were collected on 3 mornings over a 5-day period per trial. Monitors recorded dilution to threshold ratio (D/T) data using a Nasal Ranger every 5 min for 2.5 h. Dilution to threshold ratios were determined by taking the volume of carbon filtered air divided by the volume of odorous air. It is one of the most commonly used ways to objectively determine the presence of odors. On days of data collection, monitors were

instructed to refrain from the use of perfume, aftershave, and cologne, as well as refraining from alcohol and tobacco use so as to not interfere with odor readings.

Figure 4. **Odor wheel used by monitors to identify the quality or source of the odors detected.**



All Nasal Rangers used were calibrated by the manufacturer prior to use and routine maintenance of the equipment, including changing O-rings and air filters, was conducted by the project managers. Data recorded by the monitors included (1) date and

time of the reading; (2) odor intensity (D/T) using the Nasal Ranger; (3) odor descriptors according to the odor wheel (Figure 4); and (4) weather conditions. Monitors were stationed upwind of the litter application sites to assess ambient air and downwind at the edge of the application field to determine the "worst case scenario" of odor perception following the land application of poultry litter.

Dilution to threshold ratios were obtained by placing the Nasal Ranger over the nose, with the dial in the blank position, and breathing normally through the instrument. As the ambient air was drawn through the charcoal filter with the dial in the blank position, it allowed the monitors to "zero" their nose. They then turned the dial to the highest dilution ratio, 60 D/T, and inhaled at the target inhalation rate (16 to 20 L/min as indicated by green LED lights). After inhalation, the dial was rotated to the next blank position, resumed normal breathing, and determined whether they had smelled an odor at that dilution or not. If they did experience an odor, the monitor recorded it on the data sheet along with the D/T and a descriptor (if applicable) for the odor. If the monitor did not smell an odor at that dilution, they turned the dial to the next lower dilution ratio and repeated the process until they either did or did not experience an odor at the lowest dilution ratio.

In addition to human sensory monitors, volatile odorants were collected onto stainless steel sorbent tubes using pocket pumps from wind tunnel flux chambers placed directly onto litter piles in both trials. The wind tunnel flux chambers had a sampling port for the collection of air samples. The top of the lateral flow wind tunnel is a 0.6 cm thick piece of plexiglass with four 0.9 cm holes for air outlet where samples were

collected. The flush gas inlet is a 5.08 x 5.08 cm steel tube with ten 0.3 cm holes spaced 2.5 cm apart. Compressed breathing air was used as the flush gas at a flow rate of 8 L/min. Following the flushing of the chamber, pocket pumps pulled air at a rate of 200 mL/min for 20 min through the stainless steel tubes, and VOCs were absorbed onto the packing material. A total of 4 L of air was sampled over the 20 min time period. Three or 4 sorbent tube samples per litter type were collected from different locations on each litter pile. The sorbent tubes were analyzed using gas chromatography-mass spectrometry (GC/MS) to determine the concentrations of 13 selected odorants (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid hexanoic acid, phenol, p-cresol, 4-ethylphenol, 2'-aminoacetophenone, indole and skatole). Odor activity values (OAV) for each compound were determined by dividing the concentration of a compound by the detection threshold for that compound.

In Trial 2, an additional odor sampling and analytical procedure was used in addition to the human monitors and GC/MS analysis. Air samples were collected directly from the litter piles and in the middle of the application field for fresh and IWC litter on the day following application. Pocket pumps were used to collect samples of approximately 10 L of air to be transported to the West Texas A&M University Commercial Core Laboratory for olfactometry analysis by trained human panelists. Duplicate samples for both the pile and field samples for each type of litter were collected (8 total samples). Only 8 samples were collected due to the high cost of analyzing these samples. The air samples were evaluated by trained odor panelists within 24 h of collection using a commercial olfactometer that was operated in

accordance with international standards (Australia Standards, 2001). The panelists were qualified through training, sensory screening, followed the code of conduct set forth by the lab, and continuous monitoring of their performance. The olfactometer presented the panelist with 3 air samples which consisted of 2 non-odorous samples and one diluted sample from the Tedlar bag to determine if the panelist could differentiate between the samples. Results were reported as detection threshold (DT) values for each air sample.

Statistical Analysis

Litter properties and OAV were compared by one-way ANOVA using the General Linear Model (GLM) procedure in SPSS with means deemed significantly different at $P \leq 0.05$. Means were separated by Duncan's post-hoc test.

Results and Discussion

Litter Properties

Few differences between fresh and IWC litter properties were observed in Trial 1, and none in Trial 2. The average moisture content ranged from 18.5% to 21.3% (Table 3), which is lower than the range of 22% to 29% reported by Edwards and Daniel (1992) and lower than the typical moisture contents of turkey litter from central Texas that had been applied at the Riesel watershed sites from 2001 to 2010 (avg. = 25.1%) (Harmel et al., 2013). Lower than expected litter moisture is most likely attributed to the

extremely dry weather conditions experienced in the drought of 2011 prior to Trial 1. During hot and dry weather conditions, moisture is readily removed from the litter in poultry houses due to the high ventilation rates used to keep the birds cool. Therefore, the litter was drier than expected. No significant differences were noticed between fresh and IWC litter in terms of C, N and P concentrations. These results indicate that litter treated with IWC prior to removal from poultry houses retains almost the same nutrient composition when compared to fresh litter. This result is expected since the IWC process only requires 9 to 10 days, so there is not ample time for dramatic changes in litter composition to occur. In addition, since no additional moisture or C material is added during the IWC process, the microbial metabolism within the piles is limited after only a few days, thus complete breakdown of litter material and loss of volatile nutrients (N and C) is limited.

Table 3. Litter properties presented on an "as-is" basis as means ± SE.

Applied	Samples	Moisture	Organic C	Total N	Total P	Water extractable nutrients		
						NO_3-N	NH_4-N	PO_4-P
	(n)	(%)	(%)	(%)	(%)	---------- (mg/kg) ----------		
Trial 1								
Fresh	8	19.7 ± 0.4	29.5 ± 0.6	2.82 ± 0.03	1.55 ± 0.6	1,071 ± 103	2,148 ± 310	442 ± 9
IWC	8	21.3 ± 0.3	28.9 ± 0.9	2.79 ± 0.04	1.37 ± 0.5	818 ± 110	3,781 ± 153	464 ±79
Trial 2								
Fresh	6	19.6 ± 0.4	30.6 ± 0.3	2.77 ± 0.07	1.43 ± 0.6	324 ± 10	3,831 ± 435	542 ± 7
IWC	6	18.5 ± 0.4	30.3 ± 0.4	2.91 ± 0.04	1.49 ± 0.6	282 ± 25	3,507 ± 69	566 ± 13

Windrow Temperatures

Windrow temperatures recorded on an hourly basis for Trial 1, conducted in October 2011, are presented in Figures 5, 6 and 7. An internal windrow temperature of 58°C (137°F) at the 12 m (40 ft) location and 56°C (134°F) at the 30.5 m (100 ft) location was achieved within the first 24 h. The peak temperatures reached were 65°C (150°F) and 62°C (144°F) at 12 m and 30.5 m, respectively, within 60 h of windrow formation. Although the core temperature exceeded the 55°C standard set by the EPA to deactivate pathogens, the outside of the piles cannot reach the target temperature. For this reason, turning of the windrows is an important part of the process to try to get as much of the litter mass mixed into the core area of the pile where the required temperatures are generated. After turning of the windrows on day 4, the piles reheated to recorded maximums of 58°C at 12 m and 55°C at 30.5 m. It was expected that the windrow would not reheat to temperatures quite as high after the turning process due to the release of moisture and heat. Variation in temperatures recorded by the data loggers may be attributed to litter moisture content at the location of the data logger and its proximity to the core of the pile.

Figure 5. Hourly windrow temperatures at 12 m (40 ft) from the cool pad end of the house in Trial 1**.**

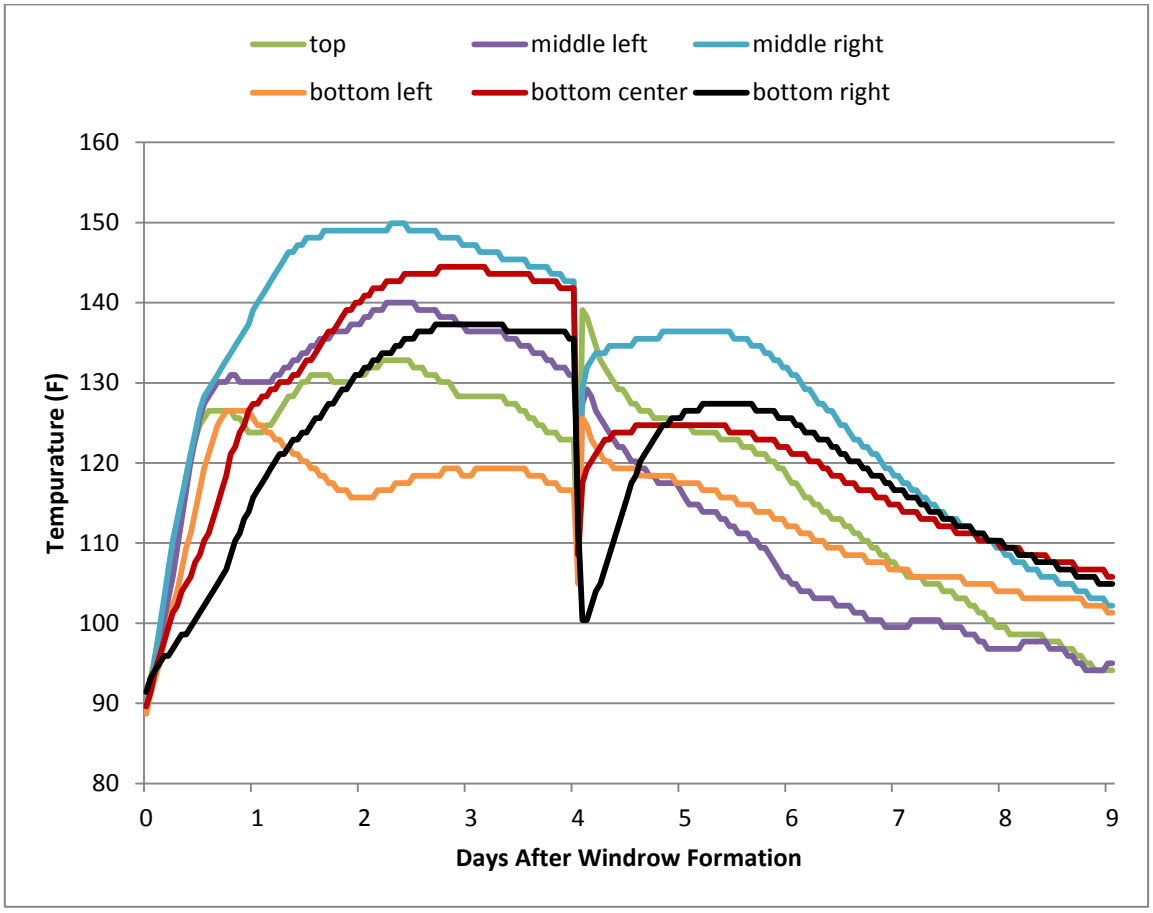
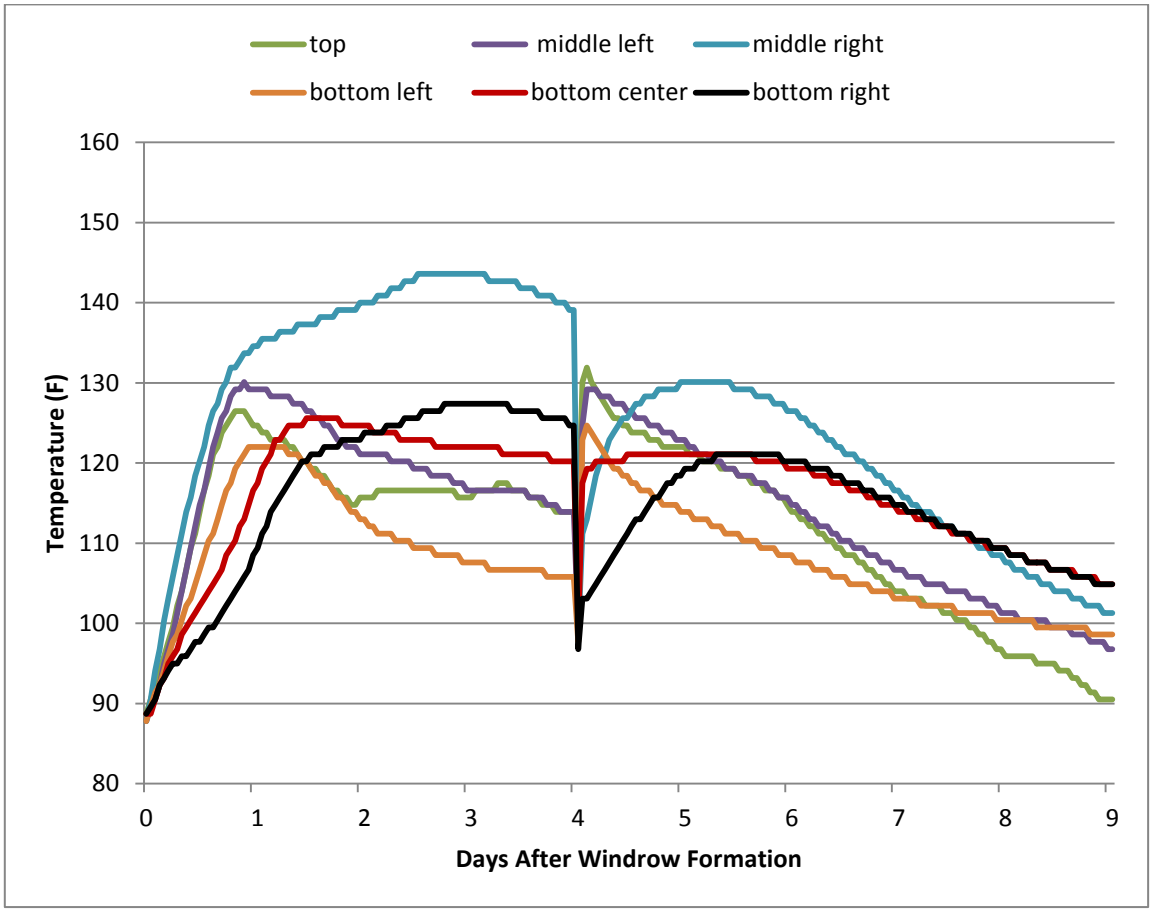


Figure 6. Hourly windrow temperatures at 30.5 m (100 ft) from the cool pad end of the house in Trial 1.

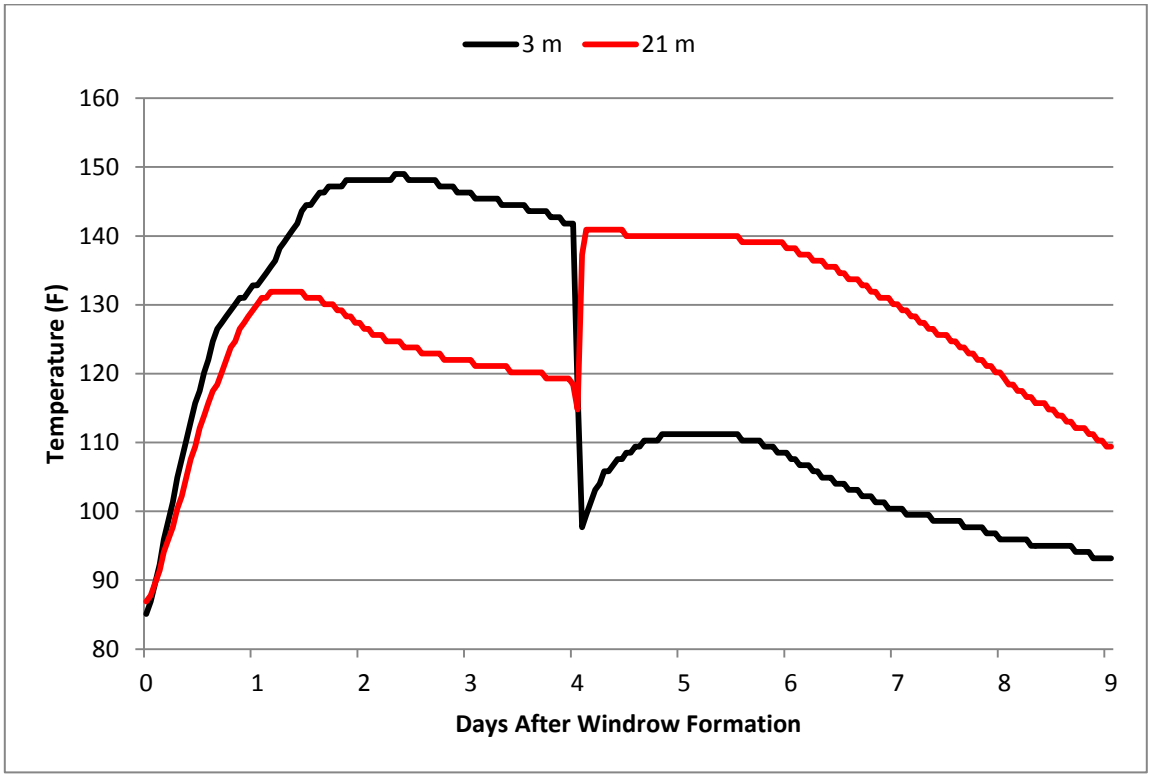


Temperatures recorded by the data loggers placed in the core of the windrow at 3 m (10 ft) and 21 m (70 ft) from the cool pad end of the house are presented in Figure 7. These results indicate opposite trends. The core temperature at the 3 m location rapidly heated to nearly 66°C (150°F) prior to turning on day 4, but after the windrow was turned it only reheated to 44°C (111°F). Conversely, the core temperature at the 21 m location only reached 56°C prior to turning but reheated to 60°C (140°F) after the turning. These results are difficult to explain, as many factors such as litter moisture,

litter aeration, pile size and dimensions and data logger placement could influence the recorded temperatures. However, the data do indicate that temperatures sufficient enough to inactivate *E. coli* bacteria were achieved during the process. The average litter moisture content was 25% at the start of the windrowing process, and decreased to 22% at the completion of the process.

Trial 1 temperature data reiterates the necessity of turning the windrows to expose the maximum amount of litter to the required treatment temperature found in the core of the windrows. The triangular-pattern data logger positioning demonstrated that temperature can vary widely within the cross-section of the windrows at a given location. Additionally, in some cases the temperatures after the turning of windrows will actually be greater than after initial windrow formation.

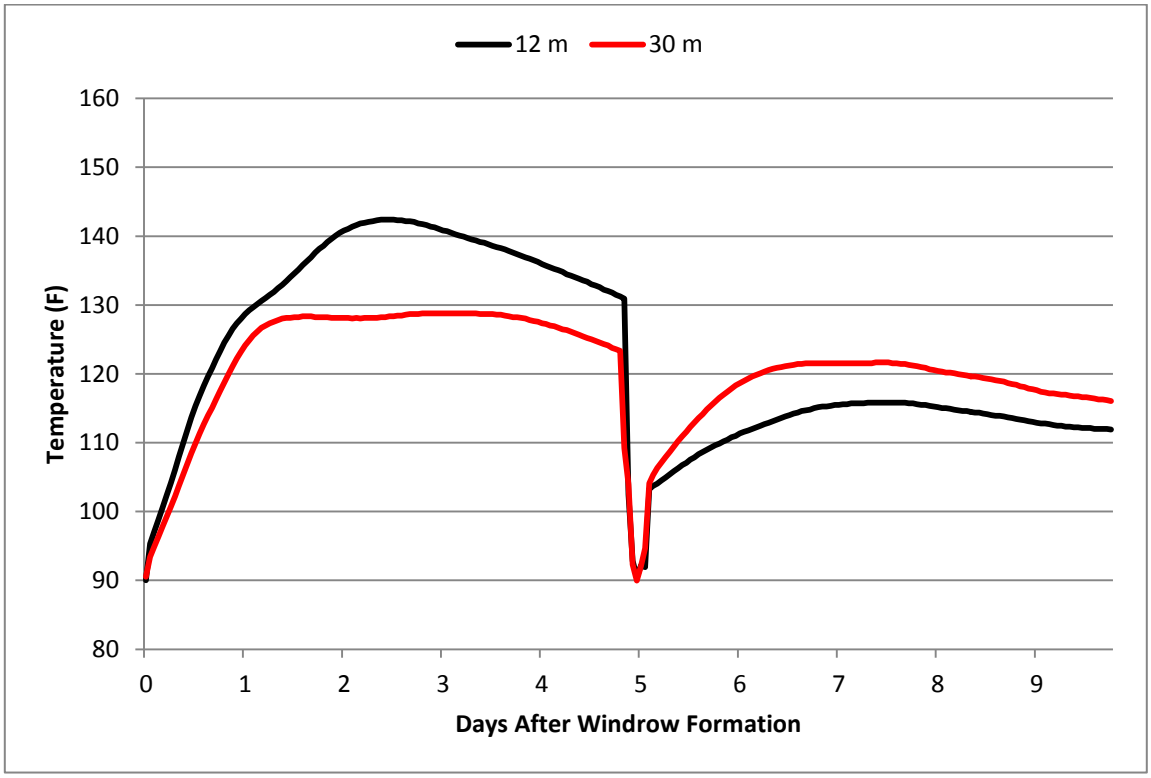
Figure 7. Hourly core windrow temperatures recorded at 3 m (10 ft) and 21 m (70 ft) from the cool pad end of the house in Trial 1.



In Trial 2, conducted in May 2012, similar windrow temperatures to Trial 1 were observed. The peak temperatures achieved were 61°C (142°F) and 53°C (128°F) at 12 m and 30 m, respectively, from the cool pad end of the house. Both of these peak temperatures were reached at 55 h after windrow formation. Although slightly different from Trial 1, due to scheduling, the windrows were turned during the fifth day and litter was removed from the house on day 10. Hourly temperatures for the core of the windrows are presented in Figure 8. The windrow temperature peaked at 61°C at the 12 m location and met the 55°C target temperature to deactivate pathogens, but it did not meet the 55°C standard at the 30 m site with a maximum temperature of 53°C. After

turning of the windrows, litter temperatures did not reheat as high as the first 5 days. This lack of reheating is likely due to the decrease in average litter moisture content during the windrowing process. The average moisture content at the beginning of the process was 25%, but had decreased to 21.9% by day 5 (Table 4). Therefore, the minimum litter moisture content required for the IWC process to achieve proper heat generation is likely near 25% as previously suggested by Malone (2010).

Figure 8. Hourly windrow temperatures recorded at 12 m (40 ft) and 30 m (100 ft) from the cool pad end of the house in Trial 2.



Litter E. coli

Litter *E. coli* counts were typically below the detection limit (10 cfu/g of wet litter) in both fresh and IWC litter (Table 4). As reported in Harmel et al. (2013), warm dry conditions in central Texas often produce conditions unfavorable for *E. coli* survival at the time that litter is removed from the poultry houses and land applied. In Trial 1, *E. coli* counts immediately after flock removal were 20 cfu/g and 55 cfu/g in the fresh and IWC litter, respectively. However, no *E. coli* were found in either type of litter at the time of land application. In Trial 2, no *E. coli* were found in either the fresh or IWC litter at the time of windrow formation. Fresh litter did have low *E. coli* levels of 185 cfu/g at the time of land application, whereas *E. coli* was not detected in the IWC litter. These results were surprisingly lower than those recorded by Terzich et al. (2000). It is unlikely that the amount of *E. coli* in the fresh litter increased while sitting undisturbed in the house during the IWC process, so the lack of *E. coli* in the litter samples prior to windrowing may be attributed to sampling variation. Although the initial litter *E. coli* counts were very low prior to windrow formation, and thus it was not conclusively shown that IWC can reduce litter *E. coli* counts, windrow temperature data indicates that sufficient heating was achieved to inactivate *E. coli* within many areas of the windrows. Thus, if *E. coli* had been present in high numbers in the litter prior to windrow formation, it is likely that differences in *E. coli* counts could have been seen since *E. coli* was found in the fresh litter at the time of litter application in Trial 2. This hypothesis is supported by the results of Macklin et al. (2008) who observed a significant reduction in

both enteric and anaerobic bacteria when the IWC process is completed under ideal moisture conditions.

Table 4. Litter *E. coli* counts and moisture content prior to windrow formation, at the time of windrow turning on day 4 or 5, and post windrowing.

Year	Treatment	Sample	*E. coli*[1] (cfu/g)	Moisture (%)
Trial 1	Fresh	Prior	20	18.51
		Turn	-	16.90
		Post	<10	15.58
	IWC	Prior	55	25.35
		Turn	-	24.83
		Post	<10	22.63
Trial 2	Fresh	Prior	<10	27.25
		Turn	-	18.20
		Post	185	19.24
	IWC	Prior	<10	25.35
		Turn	-	21.89
		Post	<10	22.35

[1] *E. coli* enumeration was not performed on litter samples at turning

Odors

The 267 odor readings recorded by monitors over the 3 sampling days in Trial 1 are presented in Table 5. Ninety-four percent of the upwind readings were non-detectable. This was expected since the field where litter was applied was in a very rural area with no other odor sources nearby. The upwind location was chosen to verify that

the odors being detected by the downwind monitors originated from the application site. In contrast, only 38% of the downwind readings were non-detectable, with the vast majority of those attributed to the fresh litter field and only a few recorded at the IWC field. Therefore, it was determined that the monitors perceived more odor associated with the IWC litter than the fresh litter. Anecdotal observations included an "earthy" odor emanating from the IWC litter application field versus a more offensive "manure" odor originating from the fresh litter application field. Although monitors perceived more odor with the IWC compared to the fresh, no determination was made pertaining to the offensiveness of the odor.

Table 5. Trial 1 frequency of dilution to threshold ratio (D/T) values determined by odor monitors upwind and downwind of litter application fields**.**

Days after application	Site	Dilution to Threshold Ratio (D/T)						
		ND	**2**	**4**	**7**	**15**	**30**	**60**
Day 1	Upwind	29	3	0	1	0	0	0
	Fresh	25	2	4	1	0	0	1
	IWC	2	1	13	9	2	1	0
Day 3	Upwind	31	0	0	0	0	0	0
	Fresh	2	13	12	1	0	0	0
	IWC	0	3	13	15	3	0	0
Day 6	Upwind	29	0	2	0	0	0	0
	Fresh	27	1	0	0	0	0	0
	IWC	7	11	3	0	0	0	0

During this project, the fresh litter remained in the broiler house for 9 days alongside the IWC litter. This amount of time may have allowed the fresh litter to dry out and allow the odors to volatilize. As noted in Table 4, moisture content decreased from 18% to 15% in the fresh litter during this time period. Under normal conditions, the fresh litter would likely have been removed and land applied much sooner instead of on day 10. This difference in time may have reduced the litter odor. In this field project, it was not possible to compare actual "fresh" litter to IWC since this would require applying litter on different days (with possibly different weather conditions) or getting litter from different houses on the same day. Both of these options would likely produce different results compared to litter from the same house on the same day.

The concentration of odorants collected on the litter piles using wind tunnel flux chambers and sorbent tubes were analyzed using GC/MS. While there are potentially hundreds of VOCs that could be produced from animal manures, 13 compounds that have previously been associated with manure and agricultural operations were selected and quantified. GC/MS results indicate that in the first trial, only 1 compound, isobutyric acid, was significantly different in the IWC litter compared to fresh litter, and was 1,163% greater in the IWC litter (Table 6). Indole, with an odor description of "piggy/musty", was nearly nonexistent (0.03 ng/L) in the IWC litter compared to the fresh litter and approached statistical significance ($P = 0.114$). Additionally, 4-ethylphenol was determined to be present in concentrations below the detection threshold, thus not an important odorant of poultry litter.

Table 6. Trial 1 GC/MS odorant concentrations and calculated odor activity values (OAV) ± SE.

Compound	Description	Detection Threshold (mg/m^3)	Treatment[1]	Concentration (ng/L)	OAV[2]	P-Value
2'-aminoacetophenone	Bat cave; taco shell	0.514	Fresh	1.75	3.41 ± 1.40	0.171
			IWC	0.37	0.73 ± 0.03	
4-ethylphenol	Spice; horse manure	13.000	Fresh	4.83	0.37 ± 0.20	0.296
			IWC	1.27	0.09 ± 0.03	
Acetic Acid	Sour; vinegar	2.030	Fresh	2.14	1.05 ± 0.7	0.649
			IWC	3.04	1.50 ± 0.5	
Butyric Acid	Body odor; vomitus	0.034	Fresh	2.47	72.77 ± 41.0	0.134
			IWC	10.48	308.99 ± 146.9	
Hexanoic acid	Foul	0.180	Fresh	7.14	39.57 ± 3.6	0.300
			IWC	12.96	71.82 ± 33.0	
Indole	Piggy; musty	0.004	Fresh	1.18	307.43 ± 132.4	0.114
			IWC	0.03	8.05 ± 2.5	
Isobutyric Acid	Rancid; butter	0.123	Fresh	5.55	45.32 [b] ± 19.1	<0.001
			IWC	70.16	572.77 [a] ± 62.3	
Isovaleric Acid	Foul/sweat; buttery	0.007	Fresh	3.61	555.36 ± 159.0	0.689
			IWC	5.70	876.88 ± 876.9	

Table 6. Continued.

Compound	Description	Detection Threshold (mg/m^3)	Treatment[1]	Concentration (ng/L)	OAV[2]	P-Value
P-cresol	Barnyard	0.010	Fresh	15.26	1,573.44 ± 797.8	0.420
			IWC	7.04	725.89 ± 245.6	
Phenol	Medicinal; floral	0.734	Fresh	41.73	56.85 ± 28.3	0.378
			IWC	18.05	24.58 ± 0.5	
Propionic Acid	Body odor; vomitus	0.350	Fresh	5.86	16.76 ± 5.7	0.566
			IWC	4.31	12.32 ± 3.2	
Skatole	Outhouse; fecal	0.002	Fresh	0.33	146.66 ± 61.7	0.761
			IWC	0.39	174.27 ± 54.4	
Valeric Acid	Foul	0.036	Fresh	1.93	53.19 ± 21.4	0.857
			IWC	2.16	59.49 ± 25.5	

[1]n = 3 for IWC, n = 4 for Fresh
[2] OAV = concentration/detection threshold
[a,b] Means within an individual compound with different superscripts differ significantly (P≤0.05).

Trial 2 consisted of 242 Nasal Ranger odor readings over the 3 sampling days and are presented in Table 7. Ninety-five percent of the upwind readings were non-detectable. In comparison, 89% of the downwind readings were non-detectable, with the vast majority (82.5%) of those attributed to the fresh litter field and only a few (17.5%) recorded at the IWC field. Due to inclement weather on the originally scheduled first sampling day, field data collection was postponed for 2 days. Instead of sampling on days 1, 3 and 6, Nasal Ranger readings by monitors were performed on days 3, 5, and 8. This delay in sampling time following litter application may explain why very few detectable odor readings were recorded by the monitors. No detectable odor readings were obtained from the fresh litter site on any day, while 16 detectable readings were recorded on day 3 at the IWC litter site. As in Trial 1, Trial 2 monitor data indicates that more odor was present from IWC litter compared to the fresh litter.

Table 7. Trial 2 frequency dilution to threshold values (D/T) determined by odor of application field.

Days after application	Site	Dilution to Threshold Ratio (D/T)						
		ND	**2**	**4**	**7**	**15**	**30**	**60**
Day 3	Upwind	24	0	3	1	0	0	0
	Fresh	32	0	0	0	0	0	0
	IWC	12	15	1	0	0	0	0
Day 5	Upwind	27	0	0	0	0	0	0
	Fresh	27	0	0	0	0	0	0
	IWC	26	1	0	0	0	0	0
Day 8	Upwind	24	0	0	0	0	0	0
	Fresh	21	0	0	0	0	0	0
	IWC	20	0	0	0	0	0	0

Following the same procedure as Trial 1, sorbent tubes were used to collect volatilized odorant compounds and analyzed using GC/MS to determine odor concentrations (Table 8). In contrast to Trial 1, 3 compounds were higher in IWC compared to fresh litter, and 4 were lower in IWC compared to fresh litter. Hexanoic acid, phenol and skatole were all higher by 3,533, 46 and 980%, respectively, in IWC compared to fresh. Conversely, acetic acid, butyric acid, valeric acid and isovaleric acid were all lower in IWC compared to fresh litter by 77, 97, 85 and 59%, respectively. P-cresol, propionic acid and isobutyric acid approached being significantly different between the litter types ($P = 0.069$, 0.065 and 0.065, respectively) in the IWC litter. Similar to Trial 1, 4-ethylphenol was determined to be present at concentrations below

the detection threshold, and thus not necessary to evaluate using GC/MS in future projects related to poultry odors.

In addition to the GC/MS and Nasal Ranger data collection conducted in Trial 2, air samples were also collected into Tedlar bags for analysis by a trained human panel. The air samples were analyzed using a commercial olfactometer and trained odor panelists to determine the human perception of the odor. One of the samples for the fresh treatment was damaged in transport and was unable to be processed. Results are reported in Table 9, and indicate consistently lower detection threshold (DT) values, as perceived by panelists, in the samples collected from IWC litter. These data indicate that the process of IWC of poultry litter prior to land application reduces the amount of odors perceived by the trained panelists. Although GC/MS results indicated that odors were higher for many compounds in IWC compared to fresh litter, and the volunteer monitors perceived more odor from the IWC litter at the edge of the field, the olfactometry panelists perceived lower odor concentrations. These results are contrary and difficult to explain as many different factors are involved in each type of measurement.

Table 8. Trial 2 GC/MS odorant concentrations and calculated odor activity values (OAV) ± SE.

Compound	Description	Detection Threshold (mg/m^3)	Treatment	Concentration (ng/L)	OAV	P-Value
2'-aminoacetophenone	Bat cave; taco shell	0.514	Fresh	3.60	7.01 ± 2.5	0.144
			IWC	6.19	12.04 ± 1.2	
4-ethylphenol	Spice; horse manure	13.000	Fresh	3.25	0.25 ± 0.07	0.934
			IWC	3.12	0.24 ± 0.03	
Acetic acid	Sour; vinegar	2.030	Fresh	7.63	3.76^a ± 0.6	0.013
			IWC	1.75	0.86^b ± 0.2	
Butyric acid	Body odor; vomitus	0.034	Fresh	1.11	7.90^a ± 0.4	0.001
			IWC	0.03	1.36^b ± 0.6	
Hexanoic acid	Foul	0.180	Fresh	0.59	3.26^b ± 3.2	0.021
			IWC	21.29	118.30^a ± 31.1	
Indole	Piggy; musty	0.004	Fresh	12.07	3,017.29 ±325.0	0.276
			IWC	10.38	2,595.14 ± 79.8	
Isobutyric acid	Rancid; butter	0.123	Fresh	0.97	35.6 ± 12.5	0.065
			IWC	0.17	1.02 ± 0.5	
Isovaleric acid	Foul/sweat; buttery	0.007	Fresh	1.56	$1,974.87^a$ ± 16.1	<0.001
			IWC	0.64	294.03^b ± 40.5	

Table 8. Continued.

Compound	Description	Detection Threshold (mg/m^3)	Treatment[1]	Concentration (ng/L)	OAV[2]	P-Value
P-cresol	Barnyard	0.010	Fresh	0.14	13.63 ± 4.8	0.069
			IWC	3.89	388.95 ± 152.2	
Phenol	Medicinal; floral	0.734	Fresh	6.49	8.84^{b} ± 0.5	0.006
			IWC	9.45	12.88^{a} ± 0.6	
Propionic acid	Body odor; vomitus	0.350	Fresh	33.63	96.09 ± 13.9	0.065
			IWC	20.57	58.78 ± 5.2	
Skatole	Outhouse; fecal	0.002	Fresh	0.31	153.94^{b} ± 14.3	<0.001
			IWC	3.33	$1{,}663.18^{a}$ ± 55.3	
Valeric acid	Foul	0.036	Fresh	71.09	222.57^{a} ± 10.2	0.018
			IWC	10.59	91.06^{b} ± 32.4	

[1] n = 3 samples per treatment
[2] OAV = concentration/detection threshold
[a,b] Means within an individual compound with different superscripts differ significantly ($P \leq 0.05$).

While the GC/MS data are more quantitative in nature, only 13 of possibly hundreds of potential compounds were measured, so some compounds that might have greatly influenced the character of the actual odor were not quantified. In addition, variation between volunteer monitors, weather conditions and field size and shape could have impacted the Nasal Ranger D/T results. Since the Tedlar bag samples were analyzed under controlled laboratory settings with the use of trained human panelists, that data may be the most reliable of the 3 types collected.

Table 9. Detection threshold (DT) values determined by olfactometry with human panelists for air samples collected in Tedlar bags from litter application sites and litter piles.

Location	Treatment	DT	Average
		----------*(OU/m^3)*----------	
Field	Fresh	1,011	1,220
	Fresh	1,429	
	IWC	602	428
	IWC	254	
Litter Pile	Fresh	4,082	4,082
	Fresh	-[1]	
	IWC	2,030	1,731
	IWC	1,432	

[1]Technical error with sample, therefore no data obtained

Conclusion

Data collected during the 2 trials indicated that temperatures of at least 55°C within the windrow piles at various locations down the length of the houses could be achieved in a commercial broiler house. Very low *E. coli* counts (a maximum of 185 cfu/g of litter) in the litter prior to windrowing did not allow for an adequate determination of *E. coli* reducing potential of the IWC process. The finding of very low *E. coli* counts in broiler litter is contrary to previously reported values in the literature, and indicates that the concern of *E. coli* contamination of runoff water from lands receiving poultry litter application may not be a potential problem as previously thought. In addition, nutrient analysis revealed that IWC litter nutrient values remained virtually unchanged when compared to fresh litter.

Olfactometry data and GC/MS results indicated a possibility for odor mitigation when comparing IWC to fresh litter. Significantly lower concentrations of certain odorants and higher concentrations of others were observed when comparing the IWC litter to the fresh litter in Trial 2. Therefore, it can be concluded that the odor profile of the IWC litter was substantially altered compared to the fresh litter. Due to a low number of samples that were able to be collected in Tedlar bags for olfactometry analysis, a statistical comparison between the litter types was not possible. However, the IWC litter DT values for both the pile and field samples were less than half of the fresh litter DT values. This finding was contrary to the Nasal Ranger data collected by the monitors at the edge of the application fields. It is difficult to determine why this occurred, but perhaps the size of the application fields was a factor. The potential for

odor accumulation in the air is likely greater with an increase in the amount of manure or litter applied. Thus, a field consisting of 40 ha (about 100 ac) would require the application of about 300 tons to successfully fertilize compared to just the 9 tons applied to the 1.2 ha (3 ac) watershed fields. Perhaps different Nasal Ranger results may be obtained if the land application of the different litter types was conducted on a larger scale. If the IWC litter did indeed result in less release of the highly offensive-type odorants with very low DT values compared to the fresh litter, and a larger mass of the fresh litter releasing those highly offensive odorants led to greater accumulation in the air than was possible with the small litter mass applied in this trial, then perhaps the monitors at the edge of the fields would have perceived more odor with the fresh litter and less of a difference between the 2 types of litter.

The practice of IWC as a litter treatment prior to land application has been shown to have the potential to be a successful BMP. In-house windrow composting is a practice that is already used by many broiler producers to manage litter between flocks. One of the main points to consider when implementing IWC is the amount of down time between flocks. A minimum of 13 days between flocks is necessary to properly execute IWC and prepare the house for a new flock. It was noted in Trial 2 that a partial house cleanout was being performed in the other houses of the farm where the litter was obtained. If ample time is allowed and the procedure is implemented correctly, IWC could feasibly be incorporated as part of the partial house cleanout procedure between flocks for broilers.

CHAPTER IV

MICROBIAL SURVEY OF POULTRY LITTER IN TEXAS

Introduction

Poultry litter contains many different types of microorganisms, including some pathogens that have negative effects on bird health and possibly the environment. *E. coli*, *Enterococcus*, *Clostridium perfringens* and total coliforms are important bacteria with regard to poultry litter. *E. coli* and *Enterococcus* are used by the EPA to determine the presence of fecal contamination in water, and both can be found in poultry litter. Outside of the U.S., *C. perfringens* is commonly used as an indicator organism for fecal contamination. *C. perfringens* is also known to be the causative agent for some poultry diseases such as necrotic enteritis and gangrenous dermatitis and can cause food borne illness in humans (Immerseel et al., 2004).

In a study to assess litter microbial populations, Terzich et al. (2000) sampled multiple broiler houses from 12 states in different regions of the U.S. Litter samples were analyzed for total bacteria, Gram-negative, Gram-positive, *Staphylococcus*, *E. coli*, and total coliforms and enumerated. Results concluded that *E. coli* counts in Texas were higher than any other state and averaged at 8.80×10^{10} cfu/g of litter (Terzich et al., 2000). Conversely, Martin et al. (1998) surveyed 86 poultry farms in Georgia to determine the amounts of *E. coli* O157:H7, *Staphylococcus,* and S*almonella* present, but found no pathogenic bacteria in any samples. In a nutritional pen study in which litter *E. coli* was enumerated, Diarra et al. (2007) found average *E. coli* counts of 8.47 log cfu/g

of litter. In Trials 1 and 2 discussed in the previous chapter, 5 out of 8 litter samples collected yielded counts below the limit of detection (<10 cfu/g). The 3 samples that were positive for *E. coli* had very low counts (maximum of 185 cfu/g). These vast differences in bacterial counts between studies could be attributed to many factors, including litter sampling technique, facility management, bacterial enumeration procedures and regional differences.

Another factor to consider that might lead to wide variation in litter *E. coli* counts is that *E.* coli may not be uniformly distributed within broiler houses. Research was conducted by Carr et al. (2000) in the Delmarva area to determine if the presence of *Salmonella* spp. within broiler houses was uniformly distributed. After sampling 86 poultry houses, it was concluded that when *Salmonella* is present, it is unequally distributed throughout the house. It was determined that the bacteria were localized in areas with high litter moisture content and high water activity.

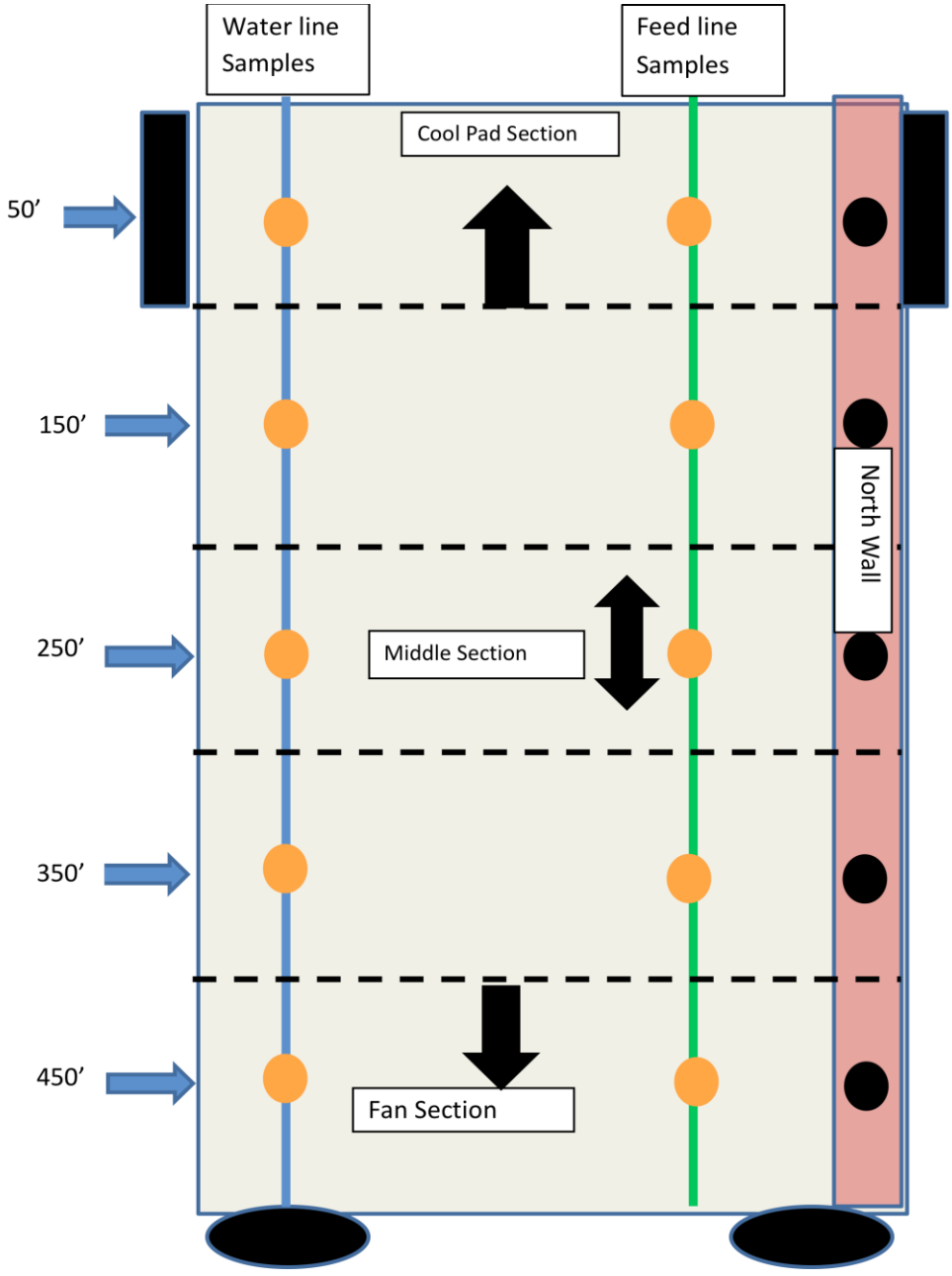
Based on the results of Trials 1 and 2, and the lack of data regarding litter bacterial counts in Texas, a litter sampling survey was initiated to determine accurate numbers of bacteria within the litter of commercial broiler houses in Texas. In addition to determining average counts, spatial variation throughout the house was also investigated.

Materials and Methods

Litter Sample Collection

A total of 19 broiler houses from 5 different farms were sampled between February 2013 and April 2013. Of the 5 farms sampled, litter was collected from 4 houses on the first 4 farms and 3 houses on the last farm. Litter samples were collected from commercial broiler houses using a stainless steel core-sampling tool similar to a soil sampling tool. This tool was used to collect samples up to 15.24 cm (6 in) in depth. Litter samples were collected within 24 h of flock removal and were transported to the laboratory on ice. Samples were collected at 15 predetermined sites within the house and pooled based on 6 sections within the house (Figure 9). Three sections were selected to determine spatial variation across the width of the broiler house and included: 1) water line, 2) feed line and 3) north wall. Three additional sections were used to determine spatial variation down the length of the house and included: 4) cool pad end, 5) middle of house, and 6) fan end. The grid pattern was chosen to determine the variation of litter bacterial counts throughout all areas of the broiler house. The water line, feed line, and north wall sections represented distinctly different areas across the width of the house and included 5 samples per section, whereas the cool pad end, middle and fan end sections represented variation down the length of the house and included 3 samples per section. The samples from each section were collected and composited in 5-gallon buckets. The litter for each section was thoroughly mixed, and duplicate samples were placed sterile Whirl Pak™ plastic bags (NASCO, Fort Atkinson). All samples were stored on ice and immediately transported to the laboratory following collection.

Figure 9. Predetermined litter sampling locations for microbial enumeration across 6 sections of each broiler house.



Moisture Content

Litter moisture content was determined by drying triplicate subsamples at 100°C in a drying oven for 24 h and measuring the difference in the weight of the litter sample before and after drying. The triplicate subsamples were then averaged and recorded.

Litter Bacterial Enumeration

Bacterial enumeration of *E. coli, Enterococcus, C. perfringens,* and total coliforms was performed for each sample within 18 h of collection. One gram of litter from each composite sample was mixed with 99 mL of sterile PBS (phosphate buffered saline) and homogenized using a shaker for 10 min on the low speed. After shaking, a dilution series was prepared for each type of media used. Next, all 10 mL in each dilution tube were filtered through sterile 0.45 µm membrane filters using a vacuum filtration system. Filters were removed using sterile forceps and placed on the corresponding media

E. coli was enumerated using EPA method 1603 (USEPA, 2006). The filters were placed on modified mTEC agar and incubated at 35°C ± 0.5°C for 2 ± 0.5 h to resuscitate injured or stressed bacteria and then incubated at 44.5°C ± 0.2°C for 22 ± 2 h. The red or magenta colonies, which are considered the "typical" *E. coli,* were counted and recorded. For quality control, 100 mL of sterile PBS was filtered and processed as a "blank." Additionally, a positive control BioBall (Biomérieux Industry) consisting of a precise number of bacteria, was dissolved in 100 mL of PBS and filtered following previous methods. In addition to method 1603, 3M Petrifilm™ *E. coli*/coliform plates

(petrifilm) were used to enumerate *E. coli* and total coliform counts following AOAC (Association of Analytical Communities) method 998.08. Six dilutions for each sample were made, and 1mL of each dilution was pipetted onto the center of the petrifilm, spread using the spreading tool provided with the petrifilm, and incubated for 24 h ± 2 h at 35°C ±1°C. Blue colonies with a gas bubble were recorded as *E. coli*, and all colonies present were considered total coliforms.

Enterococcus in the litter were enumerated using EPA method 1600 (USEPA, 2002). Filters were placed onto mEI agar and incubated for 24 h at 41°C. The mEI agar was made using the alternative method that incorporates using nalidixic acid and triphenyltetrazolium chloride (TTC). For quality control, 100 mL of sterile PBS was filtered and used as a "blank", or negative control, and a BioBall dissolved in 100 mL of PBS served as the positive control. All colonies, regardless of color, with a blue halo were recorded as *Enterococcus* colonies.

Clostridium perfringens in the litter was enumerated using CP *ChromoSelect* agar (Sigma Aldrich), which is used for the enumeration and differentiation following the manufacturer's instructions. CP *ChromoSelect* agar has the advantage over many other *Clostridium* media because no confirmation is needed since the green colonies that grow on the plates are highly specific to *C. perfringens*. Since *C. perfringens* requires an oxygen-depleted atmosphere to grow, plates were incubated in an anaerobic environment created using the Anoxomat AN2CTS machine. The Anoxomat system uses a Macintosh and Fildes jar system to create anaerobic conditions by first evacuating a portion of the jar's contents and refilling it with an anaerobic gas mixture (anaerobic,

0% O_2). This procedure is repeated 3 times after which the oxygen concentration is reduced to 0.16%, and small reusable catalysts remove this minute percentage. Once in an anaerobic atmosphere, the petri dishes were incubated for 24 ± 2 h at 44°C to promote ideal colony growth. Once incubation was complete, green colonies were counted and recorded as *C. perfringens*.

Statistical Analysis

One-way ANOVA using the General Linear Model (GLM) procedure in SPSS was used to determine statistical differences in moisture content and bacterial counts between sampling locations. Each farm served as the unit of replicate for each section, and means were deemed significantly different at $P \leq 0.05$. Means were separated using Duncan's multiple range test. Samples resulting in a count of zero were given a value of 50 since the limit of detection was 100 cfu/g of litter. All bacterial counts were converted to log values for statistical analyses. Linear regression analysis using the GLM procedure in SPSS was used to determine the relationship between litter moisture content and bacterial counts of each organism or method for each sample analyzed. Regressions were considered significant at $P \leq 0.05$.

Results and Discussion

Litter Moisture

Litter moisture for all 5 farms sampled averaged 29% (Table 10). Litter moisture content varied from as low as 18% in the feed line section to 42% under the

water line. The amount of water spilled by the birds while drinking along with the possibility of water leaks contributed to the high moisture content found under the water lines. These data indicate that litter moisture is highly variable throughout a broiler house.

E. coli

Throughout the 5 farms sampled, the average litter *E. coli* count was 3.30 log cfu/g of wet litter when enumerated using method 1603. House section average counts ranged from 2.64 log cfu/g of wet litter in the feed line samples to 4.02 log cfu/g of wet litter in the water line samples. This finding can be attributed to the higher moisture content found in the water line section compared to the other sections. The only section resulting with any non-detectable counts (<100 cfu/g) for individual samples using method 1603 was the feed line section, which also had the lowest average moisture content. The north wall and feed line consistently had the lowest counts and consistently had the lowest moisture contents.

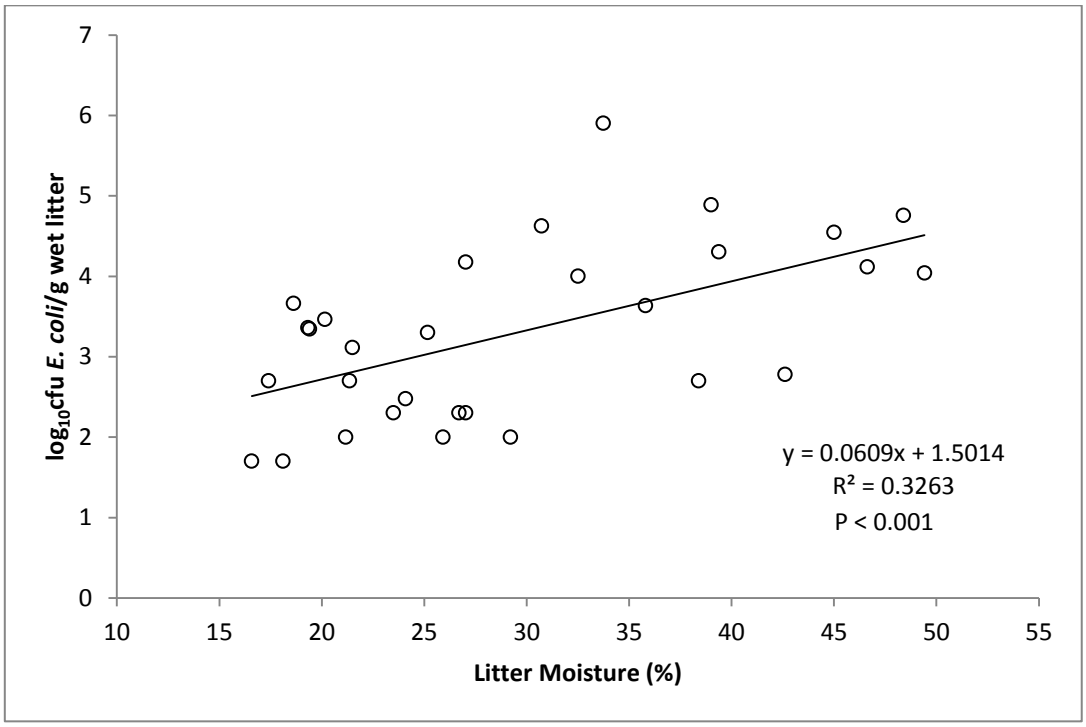
Table 10. Mean litter moisture and bacterial counts by sampling location ± SE (n = 5 samples per location).

Location	Moisture	*E. coli* (1603)	*E. coli* (petrifilm)	Total coliforms	*Enterococcus* 1600	*Clostridium perfringens*
	(%)	---------- (log_{10} cfu/g of wet litter) ----------				
Water line	42.03^{a} ± 7.6	4.02 ± 1.0	3.89 ± 0.7	5.46^{a} ± 1.5	6.63 ± 0.5	4.76 ± 0.8
Feed line	18.18^{d} ± 1.3	2.64 ± 0.9	2.66 ± 1.1	2.69^{bc} ± 1.1	6.65 ± 0.4	3.41 ± 0.8
North wall	22.09^{cd} ± 4.1	2.68 ± 0.7	2.14 ± 0.7	2.22^{c} ± 0.7	6.30 ± 0.6	3.28 ± 0.8
Cool pad	29.18^{bc} ± 7.6	3.01 ± 0.8	2.30 ± 1.0	2.84^{bc} ± 1.3	6.47 ± 0.6	3.59 ± 0.7
Middle of house	32.38^{b} ± 10.1	3.65 ± 0.9	3.38 ± 1.1	4.04^{ab} ± 1.8	6.28 ± 1.1	3.73 ± 0.9
Fan	32.92^{b} ± 4.8	3.77 ± 1.5	3.57 ± 1.9	4.70^{a} ± 1.1	6.57 ± 0.5	3.58 ± 0.9
Average	29.46 ± 10.1	3.30 ± 1.1	2.99 ± 1.2	3.66 ± 1.7	6.48 ± 0.6	3.73 ± 0.9

$^{a-d}$ Means within columns with different superscripts differ significantly ($P \leq 0.05$).

Regression analysis yielded a significant relationship ($P < 0.001$) between *E. coli* and moisture content, with an $r^2 = 0.326$ (Figure 10). Average *E. coli* counts determined in this study were lower than those determined by Terzich et al. (2000) who found 10.9 log cfu/g and Diarra et al. (2007) who found an average of 8.47 log cfu/g of litter.

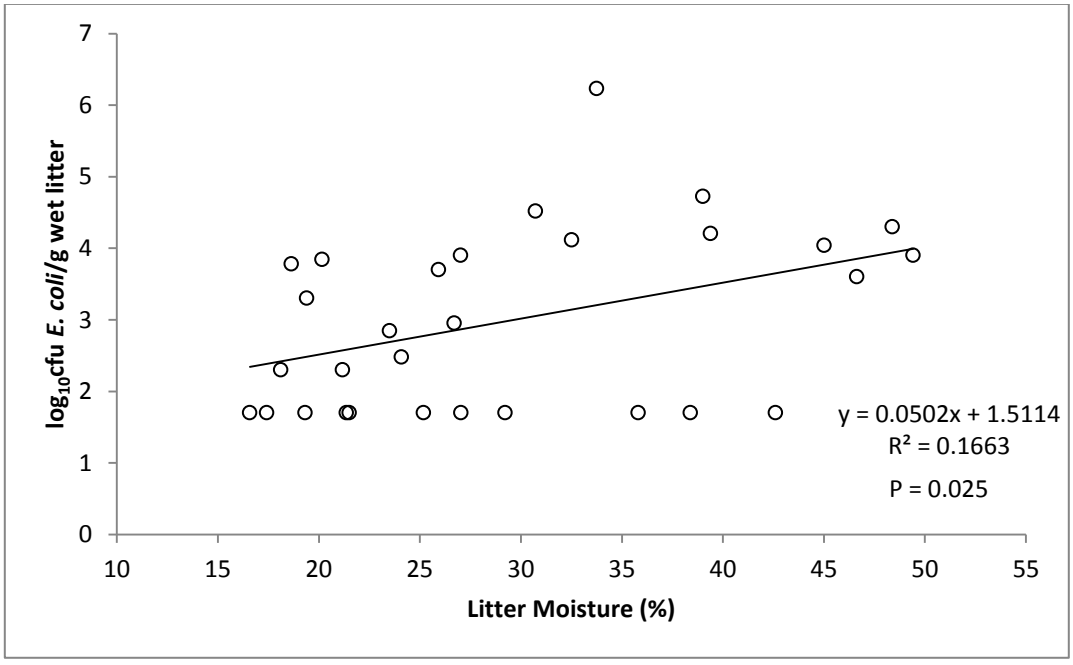
Figure 10. Regression analysis of *E. coli* counts using EPA method 1603 and litter moisture for all sampling locations in broiler houses (n = 30).



Average *E. coli* counts when using the petrifilm were 2.99 log cfu/g of wet litter with house section averages ranging from 2.14 log cfu/g of wet litter along the north wall to 3.89 log cfu/g of wet litter from samples under the drinkers. In general, *E. coli* results were similar between the petrifilm method and method1603 for each section

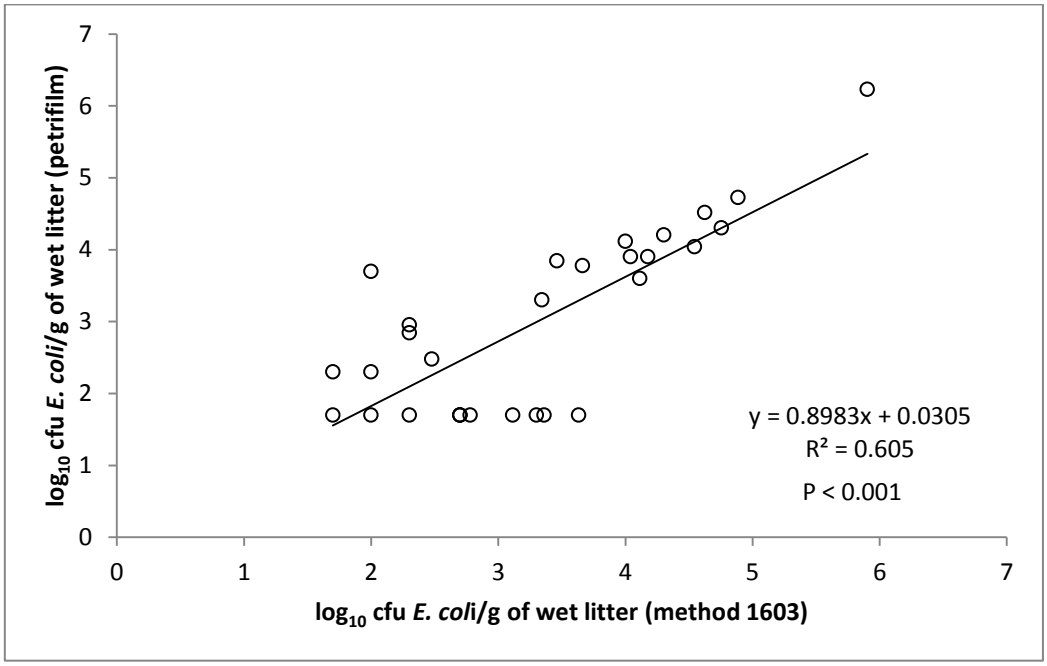
sampled, with the greatest difference being only 0.71 log cfu/g for samples from the cool pad section. There were 11 individual non-detectable samples using the petrifilm method, but none of them were from the water line section. *E. coli* counts using petrifilm were lower in comparison to previous results by Terzich et al. (2000) which found that the average *E. coli* in litter samples from Texas was 8.8×10^{10} (log 10.9) cfu/g when using the petrifilm method. The relationship between *E. coli* counts with petrifilms and litter moisture was found to be statistically significant with an $r^2 = 0.166$ (Figure 11). These results indicate that the petrifilm method is more variable than method 1603, but counts averaged across all sections were similar between methods (log 3.30 vs. log 2.99 for method 1603 and the petrifilm method, respectively).

Figure 11. Regression analysis of *E. coli* counts using petrifilm and litter moisture for all sampling locations in broiler houses (n = 30).



It was originally hypothesized that the petrifilm method would result in higher counts due to the results found by Terzich et al. (2000), but in actuality petrifilm yielded lower counts than method 1603 in 5 of the 6 sections. Figure 12 reports regression analysis comparing method 1603 and petrifilm. Regression analysis resulted in an r^2 = 0.605 for all samples. Higher variation between the two methods was observed among samples with counts below 4 log cfu/g, whereas samples with counts above 4 log cfu/g were more consistent. One major advantage of the petrifilm method is that samples can be processed in a fraction of the time compared to method 1603. In addition to saving time, the petrifilm method costs considerably less due to the minimal supplies required compared to method 1603. The verification of the petrifilm method to yield similar *E. coli* results to method 1603 in this experiment shows promise for future trials in which petrifilm can be used instead of method 1603, although the increased possibility of non-detects should be considered.

Figure 12. Regression analysis comparing *E. coli* counts obtained with method 1603 and petrifilm for the same litter samples (n = 30).

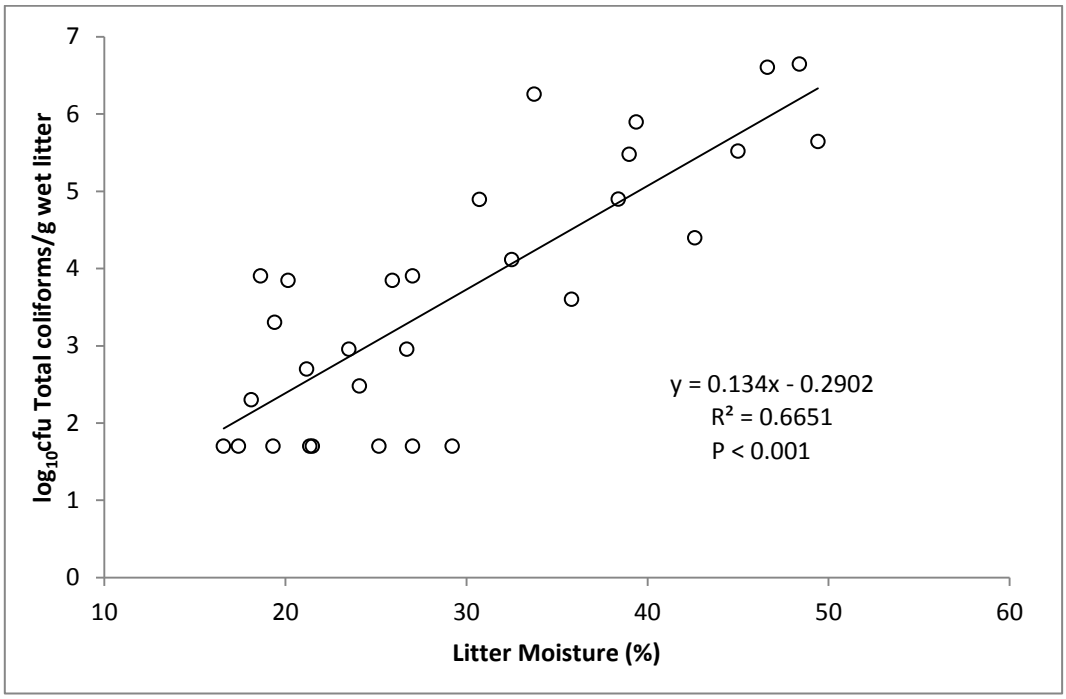


Total Coliforms

Total coliforms were also enumerated using the same petrifilm used for *E. coli*. Results were similar to those of the *E. coli* in that the water line and fan sections had the highest counts, and the feed line and north wall had the lowest counts. Counts ranged from 2.22 to 5.46 log cfu/g of wet litter. Eight samples resulted in counts below the level of detection (<100 cfu/g), but the water line section had countable results at every farm. Additionally, the relationship between total coliforms and moisture was statistically significant with an $r^2 = 0.665$ (Figure 13). Terzich et al. (2000) reported total coliform counts in Texas as 2.67×10^6 cfu/g of litter when using the same petrifilm method. Results were lower in this survey likely due to differences in litter source

and/or litter sampling methodology. Although counts were relatively high in Texas litter samples, Martin et al. (1998) found undetectable amounts of total coliforms in 94% of samples of Georgia poultry litter.

Figure 13. Regression analysis of total coliform counts using petrifilm and litter moisture for all sampling locations in broiler houses (n = 30).

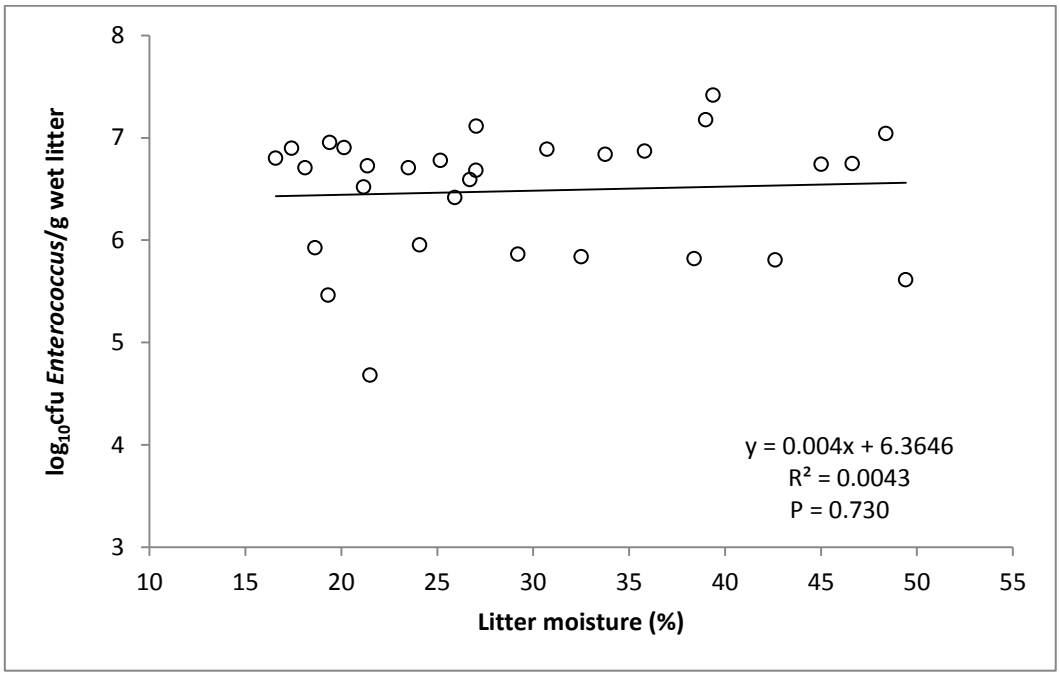


Enterococcus

Enterococcus counts for all sampling sections averaged 6.48 log cfu/g of wet litter and had the highest overall counts of all the bacteria enumerated. The overall mean counts in this research (6.48 log cfu/g of wet litter) were lower than the 7.86 log cfu/g of litter found by Diarra et al. (2007). Regression analysis displayed in Figure 14 depicts that no correlation between moisture content and *Enterococcus* counts exists.

Enterococcus was the only bacteria where a significant relationship with moisture content was not seen. *Enterococcus* results indicate that these bacteria can be found virtually everywhere within the litter in a broiler house in high numbers. This may be due to the fact that *Enterococcus* is Gram-positive and more resistant to desiccation and harsh conditions than *E. coli*. *Enterococcus* counts in bovine manure have been found to average around 4.44 log cfu/g of manure (Sinton et al., 2007). Some states use *Enterococcus* as a fecal contamination indicator for water because of the prevalence of *Enterococcus* in manure from many animals.

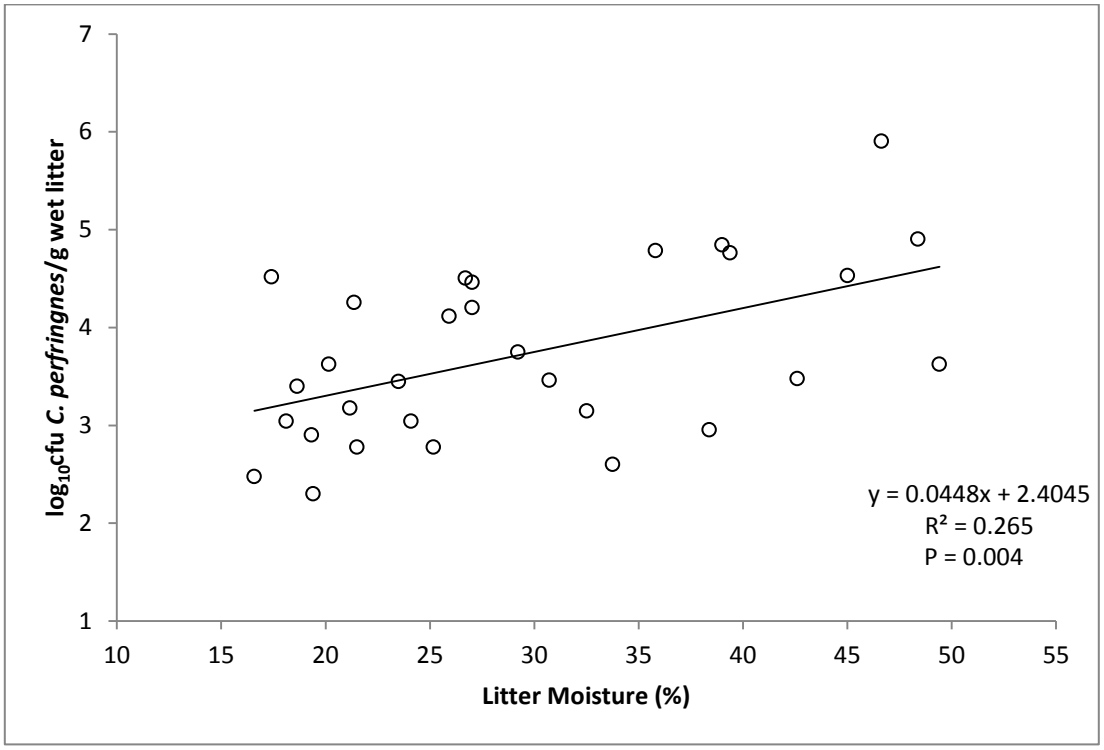
Figure 14. Regression analysis of *Enterococcus* counts using EPA method 1600 and litter moisture for all sampling locations in broiler houses (n = 30).



Clostridium perfringens

Clostridium perfringens results followed the trend of all bacteria surveyed except *Enterococcus*. Similar to *E. coli* and total coliforms, moisture content had a significant effect on *C. perfringens* litter counts (Figure 15). Regression analysis of *C. perfringens* compared to moisture resulted with an $r^2 = 0.265$. The average litter counts across all sections was 3.73 log cfu/g of wet litter, with the highest concentrations being found under the water line (4.76 log cfu/g of wet litter). Results were higher than those recorded by Diarra et al. (2007) who found an average of 2.65 log cfu/g of litter when evaluating samples from 35 day-old broiler chickens, but lower than results determined by Brooks et al. (2010) who found an average of 4 log cfu/g of litter. These data indicate that if producers want to control *C. perfringens*, which is known to cause necrotic enteritis and gangrenous dermatitis in poultry and foodborne illness in humans, the management of litter moisture is an important factor to consider.

Figure 15. Regression analysis of *C. perfringens* counts and litter moisture for all sampling locations in broiler houses (n = 30).



Conclusion

The data collected during the survey of litter bacteria produced many important findings. *E. coli* was present in countable numbers in litter from at least 3 sections sampled from all 5 farms, and all water line litter samples were found to contain *E. coli*. A linear relationship between litter moisture and bacterial counts was noted for *E. coli*, total coliforms and *C. perfringens,* but not *Enterococcus*. Additionally, the petrifilm method was found to produce similar overall average counts to method1603, but was more variable and produced more non-detects. The petrifilm method resulted in 37% of all the samples yielding "zero" counts, whereas method 1603 only resulted in 7% of all

the samples having “zero” counts. Using the petrifilm method is cheaper and less time intensive than the filtration technique used in method 1603. These data indicate that if *E. coli*, coliforms or *C. perfringens* are a concern for growers, actions to reduce litter bacterial counts should focus on the areas with higher litter moisture content.

CHAPTER V

CONCLUSION

Poultry litter will continue to be land applied as an alternative to commercial fertilizers due to its regional availability, low cost and nutrient value. Potential problems associated with land application of animal wastes, including poultry litter, are water quality issues and nuisance odor complaints. Researchers in many disciplines continue to develop best management practices (BMP) that producers can implement to assist in mitigating these issues. A promising BMP could be the use of in-house windrow composting (IWC) of litter prior to land application. Data from the research described herein has shown that proper IWC has the potential to mitigate bacteria found in litter, alter odors associated with litter, and has little effect on beneficial litter nutrients.

The first part of the research consisted of 2 trials that were used to evaluate the effectiveness of IWC on bacteria reduction, odor mitigation, and nutrient content in litter. Bacterial results were not definitive due to the extremely low counts prior to treating the litter by means of IWC. These low counts may be attributed to the dry litter conditions present at the time of sampling. Few differences were found in litter nutrients when comparing IWC to the fresh litter, which suggests that IWC does not decrease the value of litter as a fertilizer.

Additionally, odor monitors using Nasal Rangers noticed a higher concentration of odors when sampling at the edge of the field of IWC litter compared to fresh litter in both trials. However, it was observed anecdotally that the odor from the fresh litter

field, while low in concentration, had a more offensive "manure" smell compared to the IWC field which had an "earthy" smell. While several of the compounds were substantially increased in the IWC litter, these data did not evaluate the offensiveness, and thus how those odors would be perceived by people and how that would correlate with the potential for nuisance odor complaints was not determined. Trial 2 incorporated the use of a commercial olfactometer and trained panelists to determine the detection threshold (DT) values for air samples collected from IWC and fresh piles and plots. This analysis found that fresh litter has nearly double the DT value compared to the IWC litter when evaluated by the panelists. These data are promising that IWC could be an effective BMP used to alter the odor profile of poultry litter, but more research is needed.

The second phase of the research was conducted to estimate the prevalence of multiple bacteria in poultry litter collected from broiler farms in Texas. Litter moisture for each sample was determined along with bacterial counts for *E. coli* using EPA method 1603 and petrifilm, *Clostridium perfringens* using CP *ChromoSelect*, total *Enterococcus* using EPA method 1600, and total coliforms using petrifilm. Data included samples from 19 houses from 5 different broiler farms within a 3-month period. Fifteen spot samples were taken from each house and categorized into one of 6 sections: water line, feed line, north wall, cool pad end, middle of the house, and fan end. All data, with the exception of *Enterococcus*, indicated that as litter moisture increases, bacterial counts also increase. Differences in litter moisture were found throughout the broiler houses. The average moisture throughout the 5 farms was 29.5%, and the water

line section of the house consistently had the highest moisture content. Average litter *E. coli* counts was 3.30 log cfu/g of wet litter using method 1603 and 2.99 log cfu/g of wet litter using the petrifilm. These counts were lower than the 8.8×10^{10} (log 10.9) cfu/g previously found in Texas and reported by Terzich et al. (2000) using the petrifilm method. Total *Enterococcus* counts averaged 6.48 log cfu/g of wet litter and were not related to moisture content. These data have relevance to the commercial industry when a partial or full house litter cleanout without any treatment of the litter prior to land application is undertaken. The data suggests that the wetter litter should be given consideration as to how it is handled and where it is to be applied to prevent possible runoff problems. Relationships have been found between moisture content and bacteria such as *E. coli, C. perfringens*, and total coliforms. These results can also provide insight for the control of certain bacteria. Data indicate that locations within the broiler house that contain elevated moisture content are the most common place for bacteria to be found. The importance of these findings is that if growers have bacterial problems or if the land application of litter is an option, areas in the house with high litter moisture content should be targeted for treatment to reduce bacterial loads. Treatment for *Enterococcus* should include treatment of the whole house since counts were prevalent throughout the house regardless of moisture.

Based on the findings that litter bacteria counts have a positive correlation to moisture content with regards to *E. coli,* total coliforms and *C. perfringens*, a recommendation for future use of IWC is to focus windrowing efforts on the wet areas of the litter to achieve the best results. Combining the wet litter with dry litter from just

a few feet away (for example, under the water and feed lines) during windrowing may have a negative effect on the composting process. If the moisture content becomes too low, the windrow will not achieve temperatures high enough to deactivate bacteria and can possibly produce undesirable effects. Many of the bacteria thrive in warm conditions and can replicate rapidly if the target temperatures are not achieved. If down time between flocks spans a period of at least 13 days, IWC can be used as a BMP to assist in controlling bacteria and promote better flock health by decreasing the load of certain bacteria.

Future research pertaining to IWC effectiveness should focus on areas of the broiler house with high moisture content. Also, bacterial sampling for *E. coli, C. perfringens* and *Enterococcus* prior to and after the windrowing process would provide more detailed results concerning deactivation of these bacteria during the windrowing process. Odor research is both difficult and expensive, but sampling more locations for odor using Tedlar bags for human panelist evaluation would assist in developing a more accurate analysis to determine the effects of IWC on odor.

REFERENCES

Allen, P., R. Harmel, J. Arnold, B. Plant, J. Yelderman, and K. King. 2005. Field data and flow system response in clay (vertisol) shale terrain, north central Texas, USA. Hydrol. Process. 19:2719-2736.

Arnold, J., K. Potter, K. King, and P. Allen. 2005. Estimation of soil cracking and the effect on surface runoff in a Texas Blackland Prairie watershed. Hydrol. Process. 19:589-603.

Australia Standards, (AS). 2001. Stationary Source Emissions – Determination of Odour Concentration by Dynamic Olfactometry, Sydney, NSW, Australia.

Barker, K., C. Coufal, J. Purswell, J. Davis, H. Parker, M. Kidd, C. McDaniel, and A. Kiess. 2011. In-house windrowing of a commercial broiler farm during the summer months and its effect on litter composition. J. Appl. Poult. Res. 20:168-180.

Bautista, D., J. Ruano, G. Malone, K. Phillips, and S. Fitz-Coy. 2008. In-house composting shows promise. Poultry Times 11: 1-2.

Bolan, N. S., A. Szogi, T. Chuasavathi, B. Seshadri, M. Rothrock, and P. Panneerselvam. 2010. Uses and management of poultry litter. World's Poult. Sci. J. 66:673-698.

Bosch, D. J., and K. B. Napit. 1992. Economics of transporting poultry litter to achieve more effective use as fertilizer. J. Soil Water Conserv. 47:342-346.

Brodie, H. L., L. E. Carr, and P. Condon. 2000. A comparison of static pile and turned windrow methods for poultry litter compost production. Compost Sci. Util. 8:178-189.

Brooks, J., M. McLaughlin, B. Scheffler, and D. Miles. 2010. Microbial and antibiotic resistant constituents associated with biological aerosols and poultry litter within a commercial poultry house. Sci. Total Environ. 408:4770-4777.

Carey, J., R. Lacey, and S. Mukhtar. 2004. A review of literature concerning odors, ammonia, and dust from broiler production facilities: 2. Flock and house management factors. J. Appl. Poult. Res. 13:509-513.

Carr, L., E. Mallinson, L. Douglass, and S. Joseph. 2000. Characterization of the contribution of water activity and moisture content to the population distribution of *Salmonella spp*. in commercial poultry houses. Poult. Sci. 79:1557-1561.

Coufal, C. D., C. Chavez, P. R. Niemeyer, and J. B. Carey. 2006. Measurement of broiler litter production rates and nutrient content using recycled litter. Poult. Sci. 85:398-403.

Dalton, P., E. A. Caraway, H. Gibb, and K. Fulcher. 2011. A multi-year field olfactometry study near a concentrated animal feeding operation. J. Air Waste Manag. Assoc. 61:1398-1408.

Diarra, M. S., F. G. Silversides, F. Diarrassouba, J. Pritchard, L. Masson, R. Brousseau, C. Bonnet, P. Delaquis, S. Bach, and B. J. Skura. 2007. Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and *Enterococcus* counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. Appl. Environ. Microbiol. 73:6566-6576.

Dunkley, C., D. Cunningham, and G. Harris. 2011. The value of poultry litter in south georgia. University of Georgia.

Edwards, D. R., and T. C. Daniel. 1992. Environmental impacts of on-farm poultry waste disposal—A review. Bioresour. Technol. 41:9-33.

Erickson, M. C., J. Liao, G. Boyhan, C. Smith, L. Ma, X. Jiang, and M. P. Doyle. 2010. Fate of manure-borne pathogen surrogates in static composting piles of chicken litter and peanut hulls. Bioresour. Technol. 101:1014-1020.

Evers, G. 1998. Comparison of broiler poultry litter and commercial fertilizer for coastal bermudagrass production in the southeastern US. J. Sustainable Agric. 12:55-77.

Harmel, R., C. Richardson, K. King, and P. Allen. 2006. Runoff and soil loss relationships for the Texas Blackland Prairies ecoregion. J. Hydrol. 331:471-483.

Harmel, R. D., B. Harmel, and M. Patterson. 2008. On-farm agro-economic effects of fertilizing cropland with poultry litter. J. Appl. Poult. Res. 17:545-555.

Harmel, R. D., K. L. Wagner, E. Martin, T. J. Gentry, R. Karthikeyen, M. Dozier, and C. Coufal. 2013. Impact of poultry litter application and land use on *E. coli* runoff from small agriculture watersheds. Biol. Eng. Trans. 6:3-16.

Hartel, P., W. Segars, J. Summer, J. Collins, A. Phillips, and E. Whittle. 2000. Survival of fecal coliforms in fresh and stacked broiler litter. J. Appl. Poult. Res. 9:505-512.

Immerseel, F. V., J. D. Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. 2004. *Clostridium perfringens* in poultry: An emerging threat for animal and public health. Avian Pathol. 33:537-549.

Jenkins, M. B., C. C. Truman, G. Siragusa, E. Line, J. S. Bailey, J. Frye, D. M. Endale, D. H. Franklin, H. H. Schomberg, D. S. Fisher, and R. R. Sharpe. 2008. Rainfall and tillage effects on transport of fecal bacteria and sex hormones 17beta-estradiol and testosterone from broiler litter applications to a Georgia Piedmont Ultisol. Sci. Total Environ. 403:154-163.

Kelleher, B. P., J. J. Leahy, A. M. Henihan, T. F. O'Dwyer, D. Sutton, and M. J. Leahy. 2002. Advances in poultry litter disposal technology--a review. Bioresour. Technol. 83:27-36.

Kreis, R. D. 1978. Control of animal production odors: the state-of-the art. US Environmental Protection Agency, Office of Research and Development, Robert S. Kerr Environmetal Research Laboratory.

Lacey, R., S. Mukhtar, J. Carey, and J. Ullman. 2004. A review of literature concerning odors, ammonia, and dust from broiler production facilities: 1. Odor concentrations and emissions. J. Appl. Poult. Res. 13:500-508.

Lavergne, T., M. Stephens, D. Schellinger, and W. Carnet Jr. 2006. In-house pasteurization of broiler litter. Louisiana State University Cooperative Extension, Service Publication.

Lindsay, W., and W. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. J. Soil Sci. Society of America. 42:421-428.

Macklin, K., J. Hess, and S. Bilgili. 2008. In-house windrow composting and its effects on foodborne pathogens. J. Appl. Poult. Res. 17:121-127.

Macklin, K., J. Hess, S. Bilgili, and R. Norton. 2006. Effects of in-house composting of litter on bacterial levels. J. Appl. Poult. Res. 15:531-537.

Malone, B. 2010. Management guidelines for In-house composting of litter. Proc. Virginia Poultry Health and Management Seminar, Harrisonburg, Va.

Martin, S. A., M. A. McCann, and W. D. Waltman. 1998. Microbiological survey of Georgia poultry litter. J. Appl. Poult. Res. 7:90-98.

McGeehan, S., and D. Naylor. 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. Commun. Soil Sci. Plant Anal. 19:493-505.

Miles, D. M., D. E. Rowe, and T. C. Cathcart. 2011. Litter ammonia generation: moisture content and organic versus inorganic bedding materials. Poult. Sci. 90:1162-1169.

Millner, P. D. 2009. Bioaerosols associated with animal production operations. Bioresour. Technol. 100:5379-5385.

Miner, R. J. 1997. Nuisance concerns and odor control. J. Dairy Sci. 80:2667-2672.

Moore, P., T. Daniel, A. Sharpley, and C. Wood. 1995. Poultry manure management: Environmentally sound options. J. Soil Water Conserv. 50:321-327.

Parker, D., E. Caraway, M. Rhoades, N. Cole, R. Todd, and K. Casey. 2010. Effect of wind tunnel air velocity on VOC flux from standard solutions and CAFO manure/wastewater. Biol. Eng. Trans. 53:831-845.

Penn, C., K. VanDevender, and S. Watkins. 2010. Systematic evaluation of in-house broiler litter windrowing effects on production benefits and environmental impact. University of Arkansas.

Pope, M., and T. Cherry. 2000. An evaluation of the presence of pathogens on broilers raised on poultry litter treatment-treated litter. Poult. Sci. 79:1351-1355.

Ritz, C. W., and W. C. Merka. 2009. Maximizing poultry manure use through nutrient management planning. University of Georgia.

Schmidt, A. M. 2010. Design and analysis of static windrow piles for in-house broiler litter composting. University of Arkansas.

Schulte, E., and B. Hopkins. 1996. Estimation of soil organic matter by weight loss-on-ignition. SSSA, Madison, WI.

Self-Davis, M.L., and P. A. Moore. 2000. Determining water-soluble phosphorus in animal manure. In: Methods of phosphorus analysis for soils, sediments, residuals and waters, G.M. Pierzynski (Editor). Southern Cooperative Series Bulletin No. 396, North Carolina State University, Raleigh, NC.

Simpson, T. W. 1991. Agronomic use of poultry industry waste. Poult. Sci. 70:1126-1131.

Sinton, L. W., R. R. Braithwaite, C. H. Hall, and M. L. Mackenzie. 2007. Survival of indicator and pathogenic bacteria in bovine feces on pasture. Appl. Environ. Microbiol. 73:7917-7925.

Terzich, M., M. J. Pope, T. E. Cherry, and J. Hollinger. 2000. Survey of pathogens in poultry litter in the United States. J. Appl. Poult. Res. 9:287-291.

Timmons, J. 2009. In-house composting of litter between flocks. University of Maryland.

Tiquia, S. M., and N. F. Y. Tam. 2002. Characterization and composting of poultry litter in forced-aeration piles. Process. Biochem. 37:869-880.

TSSWCB. 2009. Texas Senate Bill 1693. TSSWCB, Austin, TX.

Ullman, J., S. Mukhtar, R. Lacey, and J. Carey. 2004. A review of literature concerning odors, ammonia, and dust from broiler production facilities: 4. Remedial management practices. J. Appl. Poult. Res. 13:521-531.

USDA. 1999. Unified national strategy for animal feeding operations, United States Department of Agriculture, Washington, DC.

USDA. 2011. Broilers: Inventory by state, United States Department of Agriculture NASS.

USEPA. 2002. Method 1600: *Enterococci* in water by membrane filtration using Membrane-*Enterococcus* Indoxyl-B-D-Glucoside agar (mEI). USEPA, Washington, DC.

USEPA. 2006. Method 1603: *Escherichia coli* (*E. coli*) in water by membrane filtration using Modified Membrane-Thermotolerant *Escherichia coli* agar (Modified mTEC). EPA, Washington, DC.

Walker, F. 2004. On-farm composting of poultry litter. The Agricultural Extension Service, University of Tennessee, Institute of Agriculture.

Wichuk, K. M. W. K. M., and D. M. C. D. McCartney. 2007. A review of the effectiveness of current time-temperature regulations on pathogen inactivation during composting. J. Environ. Sci. Eng. 6:573-586.

Wilkinson, K., E. Tee, R. Tomkins, G. Hepworth, and R. Premier. 2011. Effect of heating and aging of poultry litter on the persistence of enteric bacteria. Poult. Sci. 90:10-18.