BROILER HOUSE PARTICULATE MATTER, AEROBIOME, AND ANTIBIOTIC RESISTANT *E. COLI* UNDER "RAISED WITHOUT ANTIBIOTICS" PRODUCTION

By GREGORY ZOCK

(Under the direction of Lilong Chai)

ABSTRACT

Broiler houses introduce high levels of particulate matter (PM) and airborne bacteria which may lead to health concerns and pathogenic transfer in birds and handlers. The purpose of this study is measure levels of PM and airborne bacteria at different locations in a small-scale research poultry house over the course of a broiler chicken grow-out while monitoring other air quality and house quality factors such as humidity, temperature, and litter moisture. Furthermore, antibiotic susceptibility testing was performed on airborne *E. coli* isolates to develop an antibiotic resistance profile across 14 different drugs. Our goal was to better understand PM and airborne bacteria concentrations and antibiotic resistant profiles to determine human and bird risk associated with PM and airborne bacteria exposure during grow-out. Understanding the factors that most significantly impact increased levels of PM and bacteria will allow future remediation effects to focus efforts to save time, money, and animal life.

INDEX WORDS: Particulate matter, Airborne bacteria, *E. coli*, Antibiotic resistance, Broiler chicken

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DEDICATION

I dedicate my thesis to my family and friends. Thank you for believing and supporting me even when I did not do so myself. I would not be here without your strength and understanding.

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CHAPTER 1

INTRODUCTION

Broiler houses introduce high levels of particulate matter (PM) and airborne bacteria which may lead to health concerns and pathogenic transfer in birds and handlers. Commercial broiler production has conventionally used large amounts of antibiotics for both growth promotion and the therapeutic treatment of bacterial infections. There is an epidemic increase in antibiotic resistant bacteria in health care systems, and food animals have been linked to the increase in resistance (Singer, Randall S., et al., 2020).

Particulate matter from agricultural practices is one of the leading causes of atmospheric PM (Shen et al. 2022). Specifically, from a poultry house, this can include feces, dust, feed, feathers, dander, and more. PM can vary in sizes from 1, 2.5, 4, to 10 microns in diameter. PM_{10} is a respirable particle as this is the size that can enter the respiratory tract. Smaller sizes, such as $PM_{2.5}$ are of particular concern to humans and animals due to their penetrating abilities into air sacs and lungs. The World Health Organization (WHO) has air quality guidelines with particulate matter of both sizes 2.5 and 10 microns being one of the six criteria pollutants. For an eight-hour workday, the Occupational Safety and Health Administration (OSHA) standards set total particulate matter levels to 15 mg/m^3 and respirable particles to 5 mg/m^3 (OSHA, 2006). Despite seemingly low guidelines for exposure to PM, there is evidence to support that adverse

health effects and increased risk of mortality can be observed at levels lower than 10 μg/m^3 (Pinault, Lauren, et al., 2016). Particulate matter, especially the finer particles, can be difficult to control and remediate because of the low levels that must be achieved to see significant reductions in adverse health effects (WHO Global Air Quality Guidelines, 2021). Poultry house air is recommended to have less than 5 milligrams per cubic meter (mg/m^3) total particulate solids at bird level. Up to 8 mg/m^3 can be tolerated with ideal conditions where birds are not being stressed by other air quality factors such as ammonia and high humidity (University of Kentucky Agricultural Extension, 2009). Several studies have shown that particulate matter levels in a broiler house can exceed the recommended levels for the course of an entire grow-out if not properly ventilated (Redwine, et al., 2002; Viegas, S., et al., 2013; Yasmeen, Roheela, et al., 2019).

Poultry workers have been shown to suffer from higher rates of respiratory problems (Viegas, S., et al., 2013) which could have direct ties to elevated particulate matter levels. Short-term PM exposure can result in minor adverse health effects such as irritation with the eyes, nose, and throat. This may include sneezing, coughing, or labored breathing. There has also been positive association shown between short-term exposure to $PM_{2.5}$ and PM_{10} with all-cause mortality rates. Long-term exposure to $PM_{2.5}$ and PM_{10} in humans has been shown to have positive association with all-cause mortality and cardiovascular disease. (Chen, Jie, and Gerard Hoek, 2020). In addition, long-term exposure to high levels of PM can cause respiratory illnesses such as asthma, allergic alveolitis, organic dust toxic syndrome, lung cancer, and chronic

obstructive pulmonary disease (Hartung, J., and J. Schulz, 2011; Viegas, S., et al., 2013). Although birds typically have a short lifespan, the constant exposure to particulate matter can have several adverse health effects on the bird. Broiler chickens were shown to have elevated levels on lung inflammatory factors and injury at high levels of particulate matter exposure. There is also correlation between high PM exposure, inflammatory response, microbial changes in the lungs, and metabolic problems that ultimately cause a significant decrease in average daily weight gain (Shen, Dan, et al., 2021). This high level of exposure to PM can weaken the immune system by damaging cilia which allows for pathogens such as bacteria and viruses to be more likely to colonize within the respiratory system (Hofstetter, Dan, and Gino Lorenzoni, 2020).

Airborne bacteria play a major part in the air quality problems that commercial poultry houses face. Bacteria have the capability to attach to particles in the air causing high airborne bacteria concentrations that transfer throughout the house (Pal, Amrit, et al., 2021). Potential bacterial contaminations can come from poultry litter, feces, water, feed, hatcheries, or handlers with the possibility of becoming airborne. Airborne bacteria must have the ability to survive as well, and certain factors such as relative humidity and temperature may play a part in the viability of these microbes. Higher temperatures can cause rapid desiccation, and higher relative humidity may allow for water to absorb into the bacteria for better survival (Cox, C. S., and Christopher M. Wathes, 1995).

Beneficial bacteria such as *Nocardiopsis, Bifidobacterium*, certain types of *Clostridium, Ruminococcus, Lactobacillus, Bacteroides, Bacillus,* and

Streptococcus are present in a poultry house. The function of these beneficial bacteria includes residing in the gastro-intestinal tract to aid in digestion, producing antimicrobial metabolites to prevent the colonization of pathogenic bacteria, and aiding in the breakdown of feathers, feces, dander, and other detritus (Adil, S., and S. N. Magray, 2012).

Pathogenic bacteria like *Salmonella* sp., *E. coli, Clostridium perfringens, Listeria monocytogenes, Campylobacter* sp., and *Klebsiella pneumoniae* can produce harmful endotoxins that cause diarrhea, fever, vomiting, abdominal cramps, and in some extreme cases can be fatal (Adil, S., and S. N. Magray, 2012; Scallan, E., et al., 2013). Prior research has shown the ability for beneficial and pathogenic bacteria to aerosolize, colonize in chickens throughout the house, and even travel great distances from the poultry house (Sauter, E. A., et al., 1981; Duan, H., et al., 2007; Adell, Elisa, et al., 2014; Plewa, K., and E. Lonc, 2011; Zhang, Jianlong, et al., 2019).

This study focuses on the presence of airborne coliforms and *E. coli* as a sentinel for pathogenic presence. Coliforms and *E. coli* are often considered indicator organisms in certain environments because it shows conditions that may be favorable for enteric pathogens like *Escherichia, Salmonella, Shigella,* and *Campylobacter* (Halkman, H. B. D., and A. K. Halkman, 2014). Coliforms are Gram-negative, facultatively anaerobic, lactose fermenting bacteria of several genera of the family Enterobacteriaceae including *Enterobacter, Escherichia, Klebsiella,* and *Citrobacter* (Carl A. Batt, Pradip Patel, 2014) which can include total and fecal coliforms. Fecal coliforms like *E. coli*, are coliforms that naturally

occur in the intestines of chickens while other coliforms are associated with plant material.

Escherichia coli is a Gram-negative, rod-shaped facultative anaerobe that is in the intestines of birds and spread widely through feces. Birds in a poultry house are continuously exposed to *E. coli* contaminated poultry litter, feces, dust, and water. Damage to bird's disease resistance can allow for more pathogenic strains of *E. coli* to infect the bird (Charlton, B. R., et al., 2006). Most *E. coli* are harmless and an important part of a healthy gastro-intestinal tract, but certain types of *E. coli* carry virulence genes and antibiotic resistance that can be harmful to humans and animals. Avian pathogenic *Escherichia coli* (APEC) are a class of pathogens that cause avian colibacillosis, one of the biggest diseases harming global poultry industries. It is also a public health concern as it is one of the most common avian diseases that is communicable to humans (Lutful Kabir, S. M, 2010).

Antibiotic usage in commercial poultry in America is trending downwards in the USA, and that can partially be attributed to "Raised Without Antibiotics" production, which is estimated to exceed 50% of annual poultry production in the USA (Singer, Randall S., et al., 2020). Antibiotics are not solely selective against pathogens and can also affect and cause selection in commensal bacteria. Treating pathogenic bacteria with antibiotics may solve the immediate concern, but commensal bacteria can acquire antibiotic resistance and act as reservoirs for resistance that may continue to spread these genes via horizontal gene transfer (Juricova, H., et al., 2021). In one study under "Raised Without

Antibiotics" production, whole genome sequencing revealed that a commensal *E. coli* population was the main reservoir for a plasmid carrying antibiotic resistance that was horizontally transferred to *Salmonella* Heidelberg. This implies that simply reducing antibiotics is insufficient in eradicating antibiotic resistance in the poultry house (Oladeinde, Adelumola, et al., 2021).

The air quality problems within a poultry house have been well known for decades, and the health hazards for agricultural workers are continuously studied. Many remediation effects have been suggested to improve air quality such as oil and water sprays, electrostatically charging the air, and using litter additives. Despite an understanding for poor air quality and health hazards associated with the poultry industry, there remains a gap in the research that shows how particulate matter concentrations and airborne bacteria concentrations change over the course of a broiler grow-out and vary spatially within the house. Furthermore, little research is done on phenotypic expression of antibiotic resistance in airborne bacteria over the course of a broiler grow-out and how location in the house may affect these levels. Finally, there is a limitation on understanding the correlations between other factors in the poultry house and how they may affect air quality. Specifically, this research aims to:

1. Determine the particulate matter profile in the poultry house for PM sizes 1, 2.5, 4, and 10 microns at different spatial and temporal measurements over grow-out.
2. Determine airborne *E. coli* and coliform concentrations at different spatial and temporal measurements over grow-out.

3. Measure house and litter parameters such as temperature, relative humidity, litter pH, litter moisture, and litter *E. coli* concentrations to determine correlative effects on the concentrations of airborne *E. coli.*
4. Determine the antibiotic resistant phenotype of recovered airborne *E. coli* to understand ratios of resistance and to which classes of antibiotics *E. coli* resists the most readily at different spatial and temporal measurements over grow-out.

CHAPTER 2

LITERATURE REVIEW

REVIEW OF PARTICULATE MATTER IN COMMERCIAL POULTRY

Introduction

Particulate matter (PM) from agricultural practices is one of the leading causes of atmospheric PM (Shen et al. 2022). Specifically, from a poultry house, this can include feces, dust, feed, feathers, dander, and more. PM can vary in sizes from 1, 2.5, 4, to 10 microns, with PM_{10} being the particle size with the ability to enter the respiratory tract, and the smaller sizes, such as $PM_{2.5}$, being of particular concern to humans and animals due to their penetrating abilities into air sacs and lungs. Poultry house air is recommended to have less than 5 milligrams per cubic meter (mg/m^3) total particulate solids at bird level. Up to 8 mg/m^3 can be tolerated with ideal conditions where birds are not being stressed by other air quality factors such as ammonia and high humidity (University of Kentucky Agricultural Extension, 2009). Several studies have shown that particulate matter levels in a broiler house can exceed the recommended levels for the course of an entire grow-out if not properly ventilated (Redwine, et al., 2002; Viegas, S., et al., 2013; Yasmeen, Roheela, et al., 2019). Even in poultry houses where particulate matter did not exceed poultry production guidelines, it is suggested that personal protection equipment be used by handlers to reduce health effects from particulate matter exposure (Seo, Hyo-Jae, et al., 2020).

Particulate matter exposure to humans has long been studied in agricultural, manufacturing, and everyday settings such as wood burning stoves for cooking. First studies regarding potential health hazards for agricultural workers were published back in 1977 and continue to be a focus of concern (Donham, K. J., et al., 1977; C. Arden Pope, III, et al., 1995; Hartung, J., and J. Schulz, 2011; Viegas, S., et al., 2013; Losacco, Caterina, and Antonella Perillo, 2018). The World Health Organization (WHO) has air quality guidelines with particulate matter of both sizes 2.5 and 10 microns being one of the six criteria pollutants. The air quality guidelines outline long-term and 24-hour PM exposure for both sizes. For an eight-hour workday, the Occupational Safety and Health Administration (OSHA) standards set total particulate matter levels to 15 mg/m^3 and respirable particles to 5 mg/m^3 (OSHA, 2006).

Table 1. Recommended short-term (24-hour) air quality guideline level and intermit targets for $PM_{2.5}$

Recommendation	$PM_{2.5}$ (µg/m³)
Interim target 1	75
Interim target 2	50
Interim target 3	37.5
Interim target 4	25
AQG level	**15**

Table 2. Recommended annual air quality guideline level and interim targets for $PM_{2.5}$

Recommendation	$PM_{2.5}$ (µg/m³)
Interim target 1	35
Interim target 2	25
Interim target 3	15
Interim target 4	10
AQG level	**5**

Table 3. Recommended short-term (24-hour) air quality guideline level and intermit targets for PM_{10}

Recommendation	PM_{10} (µg/m³)
Interim target 1	150
Interim target 2	100
Interim target 3	75
Interim target 4	50
AQG level	**45**

Table 4. Recommended annual air quality guideline level and interim targets for PM_{10}

Recommendation	PM_{10} (µg/m³)
Interim target 1	70
Interim target 2	50
Interim target 3	30
Interim target 4	20
AQG level	**15**

Tables 1, 2, 3, and 4 obtained from WHO Global Air Quality Guidelines, 2021, under the Creative Commons Attribution-Noncommercial-Share Alike 3.0 Intergovernmental Organization license.

Despite ambitious WHO guidelines of an annual exposure level of $PM_{2.5}$ being less than 5 $\mu g/m^3$, there is evidence to support that adverse health effects and increased risk of mortality can be observed at levels lower than 10 $\mu g/m^3$ (Pinault, Lauren, et al., 2016). Particulate matter, especially the finer particles, can be difficult to control and remediate because of the low levels that must be achieved to see significant reductions in adverse health effects (WHO Global Air Quality Guidelines, 2021). Poultry workers have been shown to suffer from higher rates of respiratory problems (Viegas, S., et al., 2013) which could have direct ties to elevated particulate matter levels.

In commercial poultry, the particulate matter levels can be much higher than ambient outdoor exposure recommendations, and studies have attempted to understand how much workers are being exposed to during activity using personal particulate matter monitors (Seo, Hyo-Jae, et al., 2020. Further work needs to be performed to gain a better understanding of short-term and long-term exposure problems workers may face.

Associated Illnesses and Risks from Particulate Matter Exposure

When dealing with associated risks for PM exposure, short-term and long-term adverse health effects are considered for handlers, while only short-term effects are considered for birds due to the short life span in commercial poultry.

For humans, short-term PM exposure can result in minor adverse health effects such as irritation with the eyes, nose, and throat. This may include sneezing, coughing, or labored breathing. There has also been positive association shown between short-term exposure to $PM_{2.5}$ and PM_{10} with all-

cause mortality rates. There is also positive association shown between $PM_{2.5}$ and PM_{10} with respiratory, cardiovascular, and cerebrovascular mortality, even from a few hours to weeks of exposure, and this risk can increase with susceptible workers (Du, Yixing et al., 2016; Orellano, Pablo, et al., 2020).

Long-term exposure to $PM_{2.5}$ and PM_{10} in humans has been shown to have positive association with all-cause mortality and cardiovascular disease. (Chen, Jie, and Gerard Hoek, 2020). In addition, long-term exposure to high levels of PM can cause respiratory illnesses such as asthma, allergic alveolitis, organic dust toxic syndrome, lung cancer, and chronic obstructive pulmonary disease (Hartung, J., and J. Schulz, 2011; Viegas, S., et al., 2013).

Although birds typically have a short lifespan, the constant exposure to particulate matter can have several adverse health effects on the bird. Broiler chickens were shown to have elevated levels on lung inflammatory factors and injury at high levels of particulate matter exposure. There is also correlation between high PM exposure, inflammatory response, microbial changes in the lungs, and metabolic problems that ultimately cause a significant decrease in average daily weight gain (Shen, Dan, et al., 2021).

These exposures to PM have been shown to cause adverse effects in respiratory function in both humans and birds alike. Physiologically, this can weaken the immune system by damaging cilia which allows for pathogens such as bacteria and viruses to be more likely to colonize within the respiratory system (Hofstetter, Dan, and Gino Lorenzoni, 2020).

REVIEW OF PHYSICAL PARAMETERS (TEMPERATURE, RELATIVE HUMIDITY, LITTER MOISTURE, AND AMMONIA) ON PARTICULATE MATTER

Introduction

The environment within a poultry house is complex and dynamic system with many different measurable effects. The stocking density, age of the birds, and ventilation can affect particulate matter concentrations, but other measurable effects that change throughout the course of the grow-out may also have significant impacts on particulate matter concentrations.

Temperature Effect on Particulate Matter Concentration

Temperature is an important factor in commercial poultry that must be consistently monitored. When the birds are very young, temperatures must be elevated up to 32^{o}C for the chicks to get the warmth they are unable to produce on their own. As the birds grow larger and can self-sufficiently produce heat, elevated temperatures can become an issue for health reasons caused by heat-stress such as oxidative stress, immune repression, and intestinal damage (Lin, Hai, et al., 2006; Quinteiro-Filho, W. M., et al., 2010). It is recommended for temperatures to between 18^{o}C and 24^{o}C when birds are matured, but high stocking density, ventilation and circulation, and seasonal differences can often cause temperature to rise above recommended levels.

Higher temperature within the poultry house may cause elevated levels of particulate matter due to decrease in moisture in the house. Dry poultry litter, feed, feathers, and dander are more likely to become airborne due to lower weight and high amounts of moisture. With other air pollution parameters, temperature is typically considered a confounding variable (Ren, C., and S.

Tong, 2006). Increased temperatures are associated with human health risks (Basu, R., and J. M. Samet, 2002; Curriero, Frank C., et al., 2002), and some studies suggest that there is connection between the adverse health effects of particulate matter and elevated temperatures (Roberts, Steve, 2004; Ren, C., and S. Tong, 2006).

Relative Humidity Effect on Particulate Matter Concentration

Depending on the season, ventilation, and age of the bird, relative humidity varies greatly during a grow-out. The ideal relative humidity range should be kept between 50-70% (Reece, F. N., and B. D. Lott, 1982). Relative humidity higher than 70% can contribute to lower levels of particulate matter due to a high equilibrium moisture content (Cambra-López, María, et al., 2010). At this level, particles can contain condensed water with cause aggregations and precipitation (Takai, H., et al., 1998). High relative humidity levels can also cause wet litter and elevated ammonia concentrations. Relative humidity lower than 50% can lead to higher levels of particulate matter due to reduction of poultry litter moisture and other surface solids that may become aerosolized.

Poultry Litter Moisture Effect on Particulate Matter Concentration

Poultry litter can be a significant cause of particulate matter in a poultry house. Particulate matter from livestock can contain up to 90% organic matter and can contribute up to 20% of total dust related emissions (Kabelitz, Tina, et al., 2020). The type of litter (pine shavings, peanut hulls, sawdust, etc.), litter moisture content, and amount of litter used can all influence levels of particulate matter (Aarnink, A. J. A., and H. H. Ellen, 2007). Material with low levels of

moisture is light enough to become aerosolized and lifted from surfaces by circulating air, bird, or human activity. At a certain level of moisture, it becomes too heavy for the material to become airborne. One study of bedding material of laboratory animals and found that high levels of particulate matter were linked to lower levels of moisture (Kaliste, E., et al., 2004). Another study suggests that poultry litter moisture can play a significant role in particulate matter levels (Wathes, C. M., et al., 1997). When using poultry litter as manure, one study found the optimal range for moisture to be between 30-45% to reduce particulate matter emissions (Kabelitz, Tina, et al., 2020). However, care must be taken when treating poultry litter as a particulate matter source because high levels of litter moisture (>25%) can lead to problems such increased microbial activity. Wet litter can also cause dermatitis and reduce insulation and cushioning (Dunlop, Mark W., et al., 2016).

Ammonia Effect on Particulate Matter Concentration

Gaseous ammonia has been shown to increase particulate matter concentration due to the formation of salts such as ammonium sulfate, ammonium nitrate, and ammonium chloride. It has also been shown to be an important contributor of aerosol nucleation, which can increase concentrations as well. Typically, these increases are in the presence of sulfuric acid, nitric acid, hydrochloric acids, and sulfur-rich environments (Zhang, Yang, et al., 2008; Gong, Longwen, et al., 2013). Ammonia has the potential to increase particulate matter concentrations, but that association is of little concern in the poultry house itself due to the absence of the salt-forming acids required. Nevertheless,

atmospheric ammonia released from agriculture practices could be a concern for broader scale particulate matter problems as described by the positive association between particulate matter produced by nucleation and high ammonia levels in Atlanta (McMurry, PH, et al., 2005).

REVIEW OF AIRBORNE BACTERIA IN COMMERCIAL POULTRY

Introduction

Airborne bacteria play a major part in the air quality problems that commercial poultry houses face. Bacteria have the capability to attach to particles in the air causing high concentrations that transfer throughout the house (Pal, Amrit, et al., 2021). There have been studies that show the airborne microbial profiles in a poultry house (Sauter, E. A., et al., 1981; Plewa, K., and E. Lonc, 2011; Zhang et al., 2019) along with studies that give suggestions on ways to reduce culturable bacteria from poultry houses (Zheng et al., 2014, Chai et al., 2018), but few studies show the culturable concentrations over the course of a grow-out and how spatial changes may affect these levels. Potential bacterial contaminations can come from poultry litter, feces, water, feed, hatcheries, or handlers with the possibility of becoming airborne. Airborne bacteria must have the ability to survive as well, and certain factors such as relative humidity and temperature may play a part in the viability of these microbes (Cox, C. S., and Christopher M. Wathes, 1995). A higher temperature can cause the airborne bacteria to desiccate, while a higher relative humidity can allow these microbes to absorb water to survive longer. The survival of airborne bacteria can also be influenced by the particulate matter concentration and particle characteristics, such as size, surface area, and dry bed porosity. Although bacteria are recovered from PM, the role of PM in pathogen transmission is not yet fully understood (Adell, Elisa, et al., 2014).

Airborne Bacteria Presence in Poultry House

As next-generation sequencing techniques like 16S rRNA gene sequencing and high-throughput sequencing becomes more accessible, so does the ability to fully understand what exactly is in the air at any given time. Sampling previously may have been restricted to culturable bacteria where only bacteria that grew on selective media would be able to be quantified and further studied. Bacteria that were below limits of detection or unable to grow on specific media often went unnoticed. Table 5 below shows a range of bacteria that have been identified in poultry compiled from several studies using culturing methods (Sauter, E. A., et al., 1981; Plewa, K., and E. Lonc, 2011) and genomic DNA extraction of filtration membranes with high-throughput sequencing (Zhang, Jianlong, et al., 2019). This table reveals several beneficial and pathogenic airborne bacteria in the poultry environment.

Beneficial bacteria in the poultry setting can take on many different forms. They can reside in the gastro-intestinal tract to aid in digestion and nutrient uptake. Some beneficial bacteria produce antimicrobial metabolites that make it more difficult for pathogenic bacteria to colonize (Adil, S., and S. N. Magray, 2012). Finally, there are certain beneficial bacteria that aid in the breakdown of feathers, feces, dander, and other detritus. Genera of these beneficial bacteria include *Nocardiopsis, Bifidobacterium*, certain types of *Clostridium, Ruminococcus, Lactobacillus, Bacteroides, Bacillus,* and *Streptococcus,* all of

which are listed in Table 5 from airborne sampling events (Pan, Deng, and Zhongtang Yu, 2014; Jha, R, et al., 2020).

Pathogenic bacteria like *Salmonella* sp., *E. coli, Clostridium perfringens, Listeria monocytogenes, Campylobacter* sp., and *Klebsiella pneumoniae* can produce harmful endotoxins that can cause diarrhea, fever, vomiting, abdominal cramps, and in some extreme cases can be fatal (Adil, S., and S. N. Magray, 2012; Scallan, E., et al., 2013). Table 5 shows that in several airborne microbial sampling events in a poultry house, many of these pathogens are present.

Although little research was available on the presence of naturally occurring *Salmonella* sp., it was shown to have the ability to become aerosolized and attach to particulate matter when inoculated into the poultry litter at varying moistures (Adell, Elisa, et al., 2014; Pal, Amrit, et al., 2021). The ability for *Salmonella* sp. to be airborne is concerning for several reasons. As previously described, high levels of particulate matter and other poor air quality conditions in a poultry house can weaken immune systems in handlers in birds, which may make outbreaks more likely, especially when handlers are not wearing proper personal protective equipment. Airborne *Salmonella* sp. can also be a concern when considering poultry production as well. Although there are safety guidelines in place to reduce the risk of *Salmonella* sp. outbreaks, it has been shown to have the ability to be airborne in processing facilities as well (Whyte, P., et al., 2001). Although few studies have reported the presence of naturally occurring airborne *Salmonella* sp., it has the capacity to become aerosolized which should

be considered for future scientific experiments involving *Salmonella* sp. in the poultry house as well as for the safety of commercial handlers.

Table 5. Genera and species of common airborne bacteria in a broiler house across studies

Genus	**Species**	**Source**
Aerococcus	*vividans*	Sauter, E. A., et al., 1981; Zhang, Jianlong, et al., 2019
Alcaligenes	*paradoxus*	Sauter, E. A., et al., 1981
Arcobacter	not reported	Zhang, Jianlong, et al., 2019
Bacillus	*alvei, brevis, cereus, megatherium, stearothermophilus, pantohenticus, mycoides*	Sauter, E. A., et al., 1981; Plewa, K., and E. Lonc, 2011
Bacteroides	not reported	Zhang, Jianlong, et al., 2019
Bifidobacterium	not reported	Zhang, Jianlong, et al., 2019
Clostridium	*bifermentans, perfringens, noyvi, sordellii*	Sauter, E. A., et al., 1981

Corynebacterium	*pseudotuberculosis, xerosis*	Sauter, E. A., et al., 1981
Enterobacter	*agglomerans*	Sauter, E. A., et al., 1981; Plewa, K., and E. Lonc, 2011
Enterococcus	not reported	Zhang, Jianlong, et al., 2019
Escherichia	*coli*	Sauter, E. A., et al., 1981; Zhang, Jianlong, et al., 2019
Faecalibacterium	not reported	Zhang, Jianlong, et al., 2019
Klebsiella	*pneumoniae*	Plewa, K., and E. Lonc, 2011; Zhang, Jianlong, et al., 2019
Lactobacillus	not reported	Zhang, Jianlong, et al., 2019
Listeria	*monocytogenes*	Sauter, E. A., et al., 1981
Methylophilus	not reported	Zhang, Jianlong, et al., 2019
Micrococcus	*candidus, luteus, roseus, vividans*	Sauter, E. A., et al., 1981

Peptococcus	*asacharolyticus*	Sauter, E. A., et al., 1981
Planococcus	*citreus*	Sauter, E. A., et al., 1981
Proteus	*mirabilis, morganii, rettgeri, vulgaris*	Sauter, E. A., et al., 1981; Plewa, K., and E. Lonc, 2011
Pseudomononas	*fluorescens, aeruginosa, chloraphis, maltophila, diminuta, putida, syringae*	Sauter, E. A., et al., 1981; Plewa, K., and E. Lonc, 2011
Ruminococcus	not reported	Zhang, Jianlong, et al., 2019
*Salmonella**	*entiritidis, typhimurium*	Adell, Elisa, et al., 2014; Pal, Amrit, et al., 2021
Serratia	*plymuthica, marcescens*	Plewa, K., and E. Lonc, 2011
Shigella	*boydii*	Plewa, K., and E. Lonc, 2011
Staphylococcus	*lentus, epidermis, aureus, sciuri, roseum, saprophyticus,*	Sauter, E. A., et al., 1981; Plewa, K., and E. Lonc, 2011

Streptococcus	*avium, faecalis, lactis, mitis* *pyogenes*	Sauter, E. A., et al., 1981;

**Salmonella* sp. represented by these studies were NOT found naturally occurring. They were inoculated into the poultry litter and included into this table to show the capability of *Salmonella* sp. becoming airborne.

REVIEW OF AIRBORNE AND LITTER-BOUND *E. COLI* AND COLIFORMS IN COMMERCIAL POULTRY

Introduction

Coliforms and *E. coli* are often considered indicator organisms in certain environments because it shows conditions that may be favorable for enteric pathogens like *Escherichia, Salmonella, Shigella,* and *Campylobacter* (Halkman, H. B. D., and A. K. Halkman, 2014). Coliforms are Gram-negative, facultatively anaerobic, lactose fermenting bacteria of several genera of the family Enterobacteriaceae including *Enterobacter, Escherichia, Klebsiella,* and *Citrobacter* (Carl A. Batt, Pradip Patel, 2014) which can include total and fecal coliforms. Fecal coliforms like *E. coli*, are coliforms that naturally occur in the intestines of chickens while other coliforms are associated with plant material.

Escherichia coli is a Gram-negative, rod-shaped facultative anaerobe that is in the intestines of birds and spread widely through feces. Birds in a poultry house are continuously exposed to *E. coli* contaminated poultry litter, feces, dust, and water. Damage to bird's disease resistance can allow for more pathogenic strains of *E. coli* to infect the bird (Charlton, B. R., et al., 2006)

Although the presence of indicator bacteria cannot be used to signal the presence of pathogens, the correlation with indicator bacteria when pathogens are present is strong. Understanding levels of *E. coli* and coliforms as indicator bacteria in poultry litter and air can help monitor potential levels of enteric pathogens and be used as a reliable indication of potential risk of contamination.

This understanding can help understand when and where in the poultry house risk factors are the highest (Wu, J., et al., 2011).

Pathogenic E. coli in Commercial Poultry

Most *E. coli* are harmless and an important part of a healthy gastro-intestinal tract, but certain types of *E. coli* carry virulence genes and antibiotic resistance that can be harmful to humans and animals. Enterotoxigenic *Escherichia coli* (ETEC) produce toxins stimulate the lining of the intestines which can cause diarrhea, dysentery, cramps, and fever (Qadri, F., et al., 2005). Shigatoxigenic and verotoxigenic *E. coli* (STEC), (VTEC), are strains of *E. coli* that produce Shiga toxin and verotoxin. These toxin producing strains are commonly known as enterohemorrhagic *E. coli* (EHEC) and are responsible for ailments such as bloody diarrhea and hemolytic uremic syndrome (Esperandio, Vannesa, and Ye Nguyen, 2012).

Both EHEC and ETEC have been found in commercial poultry settings, and when tested for antibiotic resistance, these pathogenic strains of *E. coli* often carry antibiotic resistance to wide range of drugs. The toxigenic effects and antibiotic resistance of pathogenic strains of *E. coli* pose a threat to the poultry industry along with animal and human safety (Lee, Gi Yun, et al., 2009; Bashar T, Rahman M, et al., 2011; El-Rami, Fadi, et al., 2012)

Avian pathogenic *Escherichia coli* (APEC) are a class of pathogens that cause avian colibacillosis, one of the biggest diseases harming global poultry industries. It is also a public health concern as it is one of the most common avian diseases that is communicable to humans (Lutful Kabir, S. M, 2010). APEC

commonly belong to serotypes O1, O2, and O78 among others. Infection models have identified virulence genes present in many APEC, but do not occur invariably between all strains. This makes APEC more difficult to identify and control as there may be several mechanisms that effect pathogenicity (Dziva, Francis, and Mark P. Stevens, 2008).

Table 6. Virulence Factors/Genes and Function of Avian Pathogenic *E. coli*

Virulence Factor/Gene	Function	Source
traT; *iss*	Serum survival	Dziva, Francis, and Mark P. Stevens, 2008
K and O antigens	Anti-phagocytic activity	Dziva, Francis, and Mark P. Stevens, 2008
F1 and P fimbriae	Colonization and upper respiratory adhesion	Dziva, Francis, and Mark P. Stevens, 2008; Nakazato, Gerson et al., 2009
ibeA	Invasion	Dziva, Francis, and Mark P. Stevens, 2008
tsh	Temperature sensitive hemagglutination	Dziva, Francis, and Mark P. Stevens, 2008; Nakazato, Gerson et al., 2009
fyuA; irp-2; iucA; fepC	Iron acquisition systems	Nakazato, Gerson et al., 2009

Col plasmids Ia, Ib, E1, E2, E3, I, K, B, and V	Bacterial growth inhibition from same or relation species	Nakazato, Gerson et al., 2009
iutA; cvaC; stx1; stx2; vat	Enterotoxin, verotoxin (Shiga-toxin), and nectrotoxin production	Blanco, J. E., et al., 1997; Nakazato, Gerson et al., 2009; Sharif, H, et al., 2018

Airborne E. coli and Coliforms in Commercial Poultry

Inhalation of airborne bacteria has been recognized as an important factor contributing to respiratory problems within agricultural communities for decades. Testing the presence of these bacteria has undergone several methodologies to best represent the aerobiome. Often, sedimentation is used to allow the particles to fall directly on selective media for culturable growth and enumeration. Other forms of sampling such as drawing air through a vacuum with a filter may also be used.

One study cultured total airborne bacteria in a layer house to identify the total ration of Gram-negative bacteria, including coliforms and *E. coli* present in the house. It was found that of the total culturable airborne bacteria, Gram-negative bacteria made up approximately 2%. Of that 2% Gram-negative bacteria, 82.7% were identified to be *E. coli* (Zucker, B. A., et al., 2000).

Another culturable bacteria study found levels reaching 2.36 10^2 Colony Forming Units per cubic meter (CFU/m^3) for airborne *E. coli* in a poultry house. Furthermore, *E. coli* was detected up to 100 meters downwind of the poultry house showing that airborne bacteria can travel great distances. (Duan, H., et al., 2007). The same research group continued the study into the next year. Studying broiler houses again, they were able to culture and enumerate airborne *E coli* within a poultry house at levels ranging from 9-63 CFU/m^3. Using Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC PCR), it was shown that 34.1% of isolated indoor *E.* coli had 100% similarity to *E. coli* isolated from feces. Furthermore, 54.5% of *E. coli* recovered up to 200 meters downwind of the poultry houses were found to have 100% similarity to *E. coli* found from indoor air or feces. (Duan and Chai, 2008).

Although there has been concern about airborne bacteria, there remains large gaps in the research in this area. For example, there are discrepancies between sampling methods, so there is no true standard in the industry for measurements. Additionally, much of the culturable bacteria data for poultry houses are not recent.

Litterborne E. coli and Coliforms in Commercial Poultry

The poultry litter in a broiler house can act as a significant reservoir for *E. coli*, coliforms, and other bacteria. Feces and feed can also contribute to the microbiome of the litter. One study from four different broiler farms in Bangladesh found *E. coli* present in 87.50% of poultry litter samples and 37.50% of feed samples (Islam, M. M., et al., 2014). A similar study tested five commercial and

five local poultry farms and found *E. coli* prevalence in 90% of the litter samples tested (Abdu, Habibu and Adamu Ibrahim, 2021). Another study enumerated litterborne *E. coli* on two different farms in several houses with litter treated with and without Poultry Litter Treatment (PLT) (Jones-Hamilton Company, Wood County, Ohio, USA). *E. coli* concentrations in the house treated with PLT at week 0 were non-detectable but increased as the grow-out progressed. Poultry litter that was untreated showed elevated levels of *E. coli* throughout grow-out. PLT was shown to reduce the level of total litter bacteria, including coliforms, at week 0, but it was not significantly reduced in later stages of grow-out (Pope, M. J., and T. E. Cherry, 2000).

One study used 16S rRNA and functional gene markers to determine the microbial composition in four separate farms in Northeast Georgia with varying bird ages and litter ages. Of the 10^9 Colony Forming Units per gram of litter (CFU/g), 10^5, or 0.01%, were coliform bacteria. Total Gram-negative enteric bacteria including *E. coli* and other coliforms made up 0.11% of total aerobic bacteria (Lu, J., et al., 2003).

REVIEW OF BACTERIAL ROUTES OF INOCULATION IN COMMERCIAL POULTRY

Introduction

With high concentrations of microbes in poultry houses, there are many opportunities for bacteria to colonize within the system of a chicken. Once pathogenic bacteria colonize in the bird, it can not only cause health issues such as colibacillosis but can also multiply to high concentrations that can be excreted and spread to other birds in the house (Cox, N. A., et al., 1996). Bacteria can be introduced into the system orally through drinking water, feed, or pecking at litter and other birds. Bacteria can be introduced through the navel in young birds or the cloaca when birds sit on litter and feces. Bacteria can also be introduced in airborne bacteria through the trachea, lungs and air sacs, and mucosal membranes in the nose and eyes when the birds are young (Cox, N. A., et al., 1996).

Typically, when looking at routes of inoculation, success is determined by the colonization of the target bacteria. Bacteria can pass through a bird without colonization due to the concentration, lack of certain virulence factors, natural immunity, or competitive exclusion from other microbes. Often the cecum, two pouches at the junction of the small and large intestine, are analyzed as a reservoir for bacteria that are colonized in a chicken (Beery, J. T., et al., 1985). They are often used due to their specific role in productivity, health, and high microbial concentration used to aid undigested food (Stanley, Dragana, et al.,

2015). Higher concentrations of bacteria in the ceca can cause greater levels of bacteria to be shed into the feces which can exacerbate the problem.

Oral, Cloacal, and Respiratory Routes of Bacterial Inoculation

Excluding respiratory routes, previous studies comparing oral to intracloacal suggest that the oral-fecal route is the primary route of infection with higher concentrations of colonized bacteria within the bird (Kallapura, G., et al., 2014). Conversely, intracloacal challenge can yield the highest percentage of birds colonized with bacteria but at lower concentrations (Cox, N. A., 2020). In recent, more attention has been given to bioaerosols in commercial poultry facilities and how increased vulnerabilities can lead to airborne pathogenic transfer. Intratracheal and respiratory challenge is being recognized as a major contributor to pathogenic transfer with evidence supporting higher rates of colonization than oral-challenge with greater concentrations of bacteria in the ceca and cecal-tonsils. (Kallapura, G., et al., 2014; Kallapura, G., M.H. Kogut, et al., 2014; De Cort, W., et al., 2015).

Understanding routes of inoculation are important because certain bacteria, like *Salmonella* sp. that are pathogenic to humans are commensal in poultry and can spread through an entire flock of birds without any problematic visual signs (Pal, Amrit, et al., 2021). Knowing how bacteria transfers and colonizes can help shape remediation efforts by focusing on reduction to the most sensitive areas. There remains a gap in the current studies that focus on indicator bacteria concentrations at different times and locations in a poultry house and how they link to pathogenic transfer.

REVIEW OF ANTIBIOTIC RESISTANT PRESENCE IN COMMERCIAL POULTRY

Introduction

Commercial broiler production has conventionally used large amounts of antibiotics for both growth promotion and to therapeutically treat bacterial infections. Antibiotics used to treat disease may be administered for a short time at higher concentrations, while growth promoting antibiotics can be used as feed additives at a lower concentration throughout the grow-out regardless of the presence of disease or pathogens (Chhedi Lal Gupta, et al., 2021). Certain antibiotics, like fluoroquinolones, were used to treat respiratory diseases in poultry. Evidence showed fluoroquinolone-resistant zoonotic pathogens like *Campylobacter* sp. increasing. Because of the important role fluoroquinolones play in medicine, this led to a ban of use of this drug for food animal production in the USA and EU (Chhedi Lal Gupta, et al., 2021). Other countries like China and Brazil continue to use this class of antibiotics, and they have over 40% antibiotic resistant *E. coli* from poultry versus below 5% in the USA (Roth, Nataliya, et al., 2019). There is an epidemic increase in antibiotic resistant bacteria in health care systems, and food animals have been linked to the increase in resistance. Due to organizations such as the American Veterinary Medical Association advocating for antimicrobial stewardship, antibiotic usage in the hatchery has decreased from 93% to 17%, feed added tetracycline decreased by 95%, and water-soluble penicillin decreased 21% between 2013-2017. Antibiotic usage in the poultry industry in America is trending downwards, and that can be partially attributed to

“Raised Without Antibiotics” production, which is estimated to exceed 50% of annual poultry production in the USA, including for this study (Singer, Randall S., et al., 2020).

Commensal Bacteria Role in Antibiotic Resistance in Commercial Poultry

Antibiotics are not solely selective against pathogens and can also affect and cause selection in commensal bacteria. Treating pathogenic bacteria with antibiotics may solve the immediate concern, but commensal bacteria can acquire antibiotic resistance and act as reservoirs for resistance that may continue to spread these genes via horizontal gene transfer (Juricova, H., et al., 2021). In one study under “Raised Without Antibiotics” production, whole genome sequencing revealed that a commensal *E. coli* population was the main reservoir for a plasmid carrying antibiotic resistance that was horizontally transferred to *Salmonella* Heidelberg. This implies that simply reducing antibiotics is insufficient in eradicating antibiotic resistance in the poultry house (Oladeinde, Adelumola, et al., 2021).

Prevalence of Antibiotic Resistance in Commercial Poultry

One study compared the prevalence of antibiotic resistant bacteria that persisted in retail poultry meat from conventional, organic, and “raised without antibiotics” production. Although there were differences between levels of multidrug resistance, or resistance to three or more antibiotics, there was not difference between prevalence of antibiotic resistant *E. coli* (Davis, Gregg S., et al., 2018). Another study on a farm using therapeutic antibiotics to treat disease showed that airborne and litterborne bacteria increased significantly before the

birds were placed to late stages of the production, and overall resistance of the bacteria tested trended upwards as the grow-out progressed (Brooks, J. P., et al., 2010). Metagenomic analysis allows for greater insight into antibiotic resistant genes. For instance, a recent study showed that airborne bacteria in a poultry house carried a larger variety of antibiotic resistant genes compared to animal feces, but the airborne bacteria resistome is correlated significantly with fecal resistomes, but feces are not the sole contributor (Luiken, Roosmarijn E. C., et al., 2020).

Antibiotic resistant bacteria can be found in abundance in the air and poultry litter. Although there are trends that show antibiotic usage can increase levels of antibiotic resistance, complete removal of drugs from the production system does not guarantee that antibiotic resistant bacteria will disappear and could persist either through resistance reservoirs, plasmid transfer for ulterior purpose, or other mechanisms not fully understand. Increased accessibility to next-generation sequencing will allow for a closer look at the prevalence of antibiotic resistant genes and their origins.

REVIEW OF PARTICULATE MATTER, AIRBORNE BACTERIA, AND LITTERBORNE REMEDIATION IN COMMERCIAL POULTRY

Introduction

As previously discussed, particulate matter and bioaerosols are not only a concern for animal caretaker, consumer, and animal safety, but they also can affect the income and efficiency of producers by reducing the average daily weight gain of chickens. Because of this, there have pushes towards reducing particulate matter levels, airborne bacteria, and litterborne bacteria.

Particulate Matter Reduction in Commercial Poultry

One method of particulate matter reduction is spraying oil, water, or an emulsion into the air. Oil sprays were shown to reduce particulate matter levels between 23-80% in a swine barn. This depended on the oil droplet size and application rate (Zhang, Y., et al., 1996; Nonnenmann, M. W., et al., 2004). In poultry, a 2% canola oil emulsion significantly reduced PM_1-PM_{10} by 42-49% in a laying house, and an average reduction of 47% in a broiler house particulate matter levels (Ikeguchi, A, 2002). A similar study showed spraying canola oil in a broiler house reduced PM_{10} concentrations by 44-82% depending on dosage. It also reduced $PM_{2.5}$ concentrations up to 48% (Aarnink, A. J. A., et al., 2008).

Another method of particulate matter reduction is electrostatic precipitation. A device imparts a negative charge to airborne particles which causes them to precipitate. Experiments using varying voltages to test the reduction of particles in the air with efficiency ranging from a 37-79% particulate

matter reduction depending on the charge applied to the air with max efficiency at -30 kV (Chai, Ming, et al., 2009).

Airborne Bacteria Reduction in Commercial Poultry

To reduce airborne bacteria, some research has directed focus on the air by spraying slightly acidic electrolyzed water (SAEW) and tap water into the air. The SAEW at pH 6.0 and 80 mg/L of available chlorine was at a constant dosage per day. Like the particulate matter reduction studies, the control water and the SAEW both showed reduction of PM > 7 microns, but only the SAEW treatment showed a significant decrease in airborne bacteria attached to the particulate matter (Zheng, W., et al., 2014). Another study using varying dosages of SAEW at different pH levels of 3, 5, and 7 showed that a lower dosage of all pH levels showed a significant decrease in airborne bacteria, but the lower pH levels showed greater efficiency in reducing airborne and litterborne bacteria. This study also showed that higher volumes of spray resulted in higher total airborne bacteria due to the litter moisture increase which promoted bacterial growth (Chai, Lilong, et al., 2018).

Litterborne Bacteria Reduction in Commercial Poultry

The poultry litter is not only a source for high levels of ammonia but can also facilitate the growth of high concentrations of bacteria that may become airborne. Poultry Litter Treatment (PLT) is a commonly used litter acidifier designed to lower pH to help control ammonia and lower litterborne microbes. This application, typically applied before birds are placed on the litter, has been shown to be effective at significantly reducing total litter microbes and *E. coli*

concentrations (Pope, M. J., and T. E. Cherry, 2000). Other studies have tested litter amendments such as superphosphate and charcoal while still analyzing PLT efficiency and found that while superphosphate and charcoal lowered litter moisture and pH like PLT, they did not significantly lower *E. coli* and *Salmonella* Typhimurium in the litter like PLT (Soliman, E. S., et al. 2018).

Air and house quality improvement is ongoing and pressing in the poultry industry, and new treatments are frequently being used and tested to determine what is the most effective. Additional efforts not mentioned include ventilation changes, filtration and biofiltration, litter moisture control, built-up litter management, different litter bedding material, changes in diet, and immunizations. Currently, there is no single strategy extensively studied that provides reduction in ammonia, particulate matter, and airborne bacteria (Wood, D. J., and B. J.van Heyst, 2016). There are gaps in the research understanding how these treatments may work together and their effect on the particulate matter and airborne bacteria concentrations. This research will help shed light on the problem so remediation efforts and resources can be focused on factors, locations, and times which may be most problematic.

CHAPTER 3

PARTICULATE MATTER AND AIRBORNE BACTERIA CONCENTRATIONS IN BROILER HOUSE INFLUENCED BY GROW-OUT DAY, SPATIAL CHANGES, AND HOUSE PARAMETERS [1]

[1] G. Zock, A. Oladeinde, S. Aggrey, J. Johnson, Y. Guo, D. Cudnik, J. Lawrence, and L. Chai. To be submitted to *Poultry Science*.

ABSTRACT

Broiler houses introduce high levels of particulate matter (PM) and airborne bacteria which may lead to health concerns and pathogenic transfer in birds and handlers. Other broiler house factors such as litter moisture, litter pH, litter *E. coli* abundance, temperature, and relative humidity may play a role in air quality problems. While studies have shown compositional profiles of PM and bacteria in broiler houses, there is little data on temporal or spatial changes in broiler house particulate matter and aerobiome. The purpose of this study was to determine the relationship between PM concentrations, airborne bacteria concentrations, and house factors at different time points, heights, and locations of a broiler house. One-hundred and fifty Cobb-500 broiler chicks were raised in floor-pens on reused pine shavings for 49 days following RWA production conditions. A DustTrak DRX (TSI Inc) was used to measure airborne PM sizes of 1, 2.5, 4, and 10 micrometers. Open petri dishes with ECC CHROMagar$^{(TM)}$ were placed in the broiler house to quantify levels of airborne coliforms and *E. coli*. Bacterial and PM sampling took place at heights representing bird level (height 1) and human level (height 2). Bacterial sampling also took place at upper room circulation (height 3). Sampling events took place for 12 minutes per location each week for 7 weeks. Poultry litter was collected weekly and tested for litter moisture, litter pH, and litter *E. coli* abundance. Temperature and relative humidity were monitored daily for bird health but only recorded during sampling events. All data and samples were collected simultaneously during weekly sampling events. PM concentrations were significantly different at bird height and

human height for sizes 1, 2.5, 4, and total. There were no significant differences in PM concentrations between bird height and human height for PM_{10}. PM total sizes consisted of 18-25% $PM_{2.5}$ and 33-50% PM_{10} regardless of height or day of sampling. No airborne *E. coli* was detected at day 0, however, *E. coli* was measured on day 7 at 2.16 Log CFU/m^3 for height 1 and 3. Airborne *E. coli* concentrations remained elevated for days 14-49, but there was no significant difference between heights ($P > 0.05$). In contrast, coliform bacteria concentration decreased at all heights from day 14-63. There were significant differences in airborne coliform concentrations at height 1 and 2 ($P = 0.0161$) and height 1 and 3 ($P = 0.0062$). There were positive correlations found between pairwise comparisons of PM total concentrations and airborne *E. coli* concentrations (r = 0.7494), poultry litter moisture and airborne *E. coli* concentrations (r = 0.6118), and poultry litter *E. coli* concentration and airborne *E. coli* concentration (r = 0.7721). There was a negative correlation found between pairwise comparisons of litter moisture and particulate matter concentration (r = -0.5296). Results demonstrate PM_1, $PM_{2.5}$, and PM_4 and total PM concentrations are higher at bird height than human height. There is no significant difference in airborne *E. coli* populations at bird height, human height, and upper ventilation levels ($P = 0.8593$). It can be concluded that particulate matter levels are still at harmful levels for humans, airborne *E. coli* is present during the grow-out when birds are present, and remediation efforts must take care to monitor poultry litter levels when attempting to reduce PM and airborne bacteria.

INTRODUCTION

In a broiler house, air quality is a multifaceted problem that has many contributing factors. Particulate matter makes up the physical component of the air, while bacteria, molds, and viruses much up the biological components of the air. Other house parameters such as temperature, relative humidity, ammonia, and CO_2 can have large impacts on air quality and must be properly monitored and maintained. The poultry litter used in a house can also contribute to air quality problems depending on the type of material, quantity, size of material, moisture, and bacterial concentrations. Other factors such as stocking density, ventilation, and season can play significant roles in air quality. This study focuses on particulate matter, airborne *E. coli* and coliforms, poultry litter moisture, poultry litter pH, poultry litter *E. coli* abundance, temperature, and relative humidity as focuses of air quality.

PM is comprised of matter of different sizes ranging from 1, 2.5, 4, and 10 microns in diameter. PM_{10} and below are respirable particles, as these are the particle sizes that can enter the respiratory tract. Larger categories of particulate matter sizes contain the classes below it. For instance, $PM_{2.5}$ concentrations contain particles sized 2.5 microns in diameter and below, including $PM_{1.}$ PM_{10} concentrations contain particles sized 10 microns in diameter and below, including $PM_{1,}$ $PM_{2.5}$, PM_4, and so on. The smaller sizes such as $PM_{2.5}$ and PM_1 can be more dangerous as they have an increased ability to penetrate the lungs or air sacs of humans and birds which can cause respiratory distress which may

make them more susceptible to pathogenic transfer (Chen, Jie, and Gerard Hoek, 2020).

PM from agricultural practices is one of the leading causes of atmospheric PM (Shen et al. 2022). Specifically, from a poultry house, this can include feces, dust, feed, feathers, dander, and more. Poultry house air is recommended to have less than 5 milligrams per cubic meter (mg/m^3) total particulate solids at bird level. Up to 8 mg/m^3 can be tolerated with ideal conditions where birds are not being stressed by other air quality factors such as ammonia and high humidity (University of Kentucky Agricultural Extension, 2009). For an eight-hour workday, the Occupational Safety and Health Administration (OSHA) standards set total particulate matter levels to 15 mg/m^3 and respirable particles to 5 mg/m^3 (OSHA, 2006). Despite seemingly low levels of particulate matter for safety recommendations, there is evidence to support that adverse health effects and increased risk of mortality can be observed at levels lower than 10 $\mu g/m^3$ (Pinault, Lauren, et al., 2016). Even in poultry houses where particulate matter did not exceed poultry production guidelines, it is suggested that personal protection equipment be used by handlers to reduce health effects from particulate matter exposure (Seo, Hyo-Jae, et al., 2020). There is positive association shown between $PM_{2.5}$ and PM_{10} with respiratory, cardiovascular, and cerebrovascular mortality, even from a few hours to weeks of exposure to elevated particulate matter concentrations, and this risk can increase with susceptible workers (Du, Yixing et al., 2016; Orellano, Pablo, et al., 2020). Constant exposure to high levels of particulate matter can have also have several adverse health effects on

chickens. Broiler chickens were shown to have elevated levels on lung inflammatory factors and injury to high levels of particulate matter exposure. There is also correlation between high PM exposure, inflammatory response, microbial changes in the lungs, and metabolic problems that ultimately cause a significant decrease in average daily weight gain (Shen, Dan, et al., 2021).

Higher temperature within the poultry house may cause elevated levels of particulate matter due to decrease in moisture in the house. Dry poultry litter, feed, feathers, and dander are more likely to become airborne due to lower weight and high amounts of moisture. With other air pollution parameters, temperature is typically considered a confounding variable (Ren, C., and S. Tong, 2006).

Relative humidity higher than 70% can contribute to lower levels of particulate matter due to a high equilibrium moisture content (Cambra-López, María, et al., 2010). At this level, particles can contain condensed water with cause aggregations and precipitation (Takai, H., et al., 1998). High relative humidity levels can also cause wet litter and elevated ammonia concentrations. Relative humidity lower than 50% can lead to higher levels of particulate matter due to reduction of poultry litter moisture and other surface solids that may become aerosolized.

Poultry litter can be a significant cause of particulate matter in a poultry house. The type of litter (pine shavings, peanut hulls, sawdust, etc.), litter moisture content, and amount of litter used can all influence levels of particulate matter (Aarnink, A. J. A., and H. H. Ellen, 2007). Material with low levels of

moisture is light enough to become aerosolized and lifted from surfaces by circulating air, bird, or human activity. At a certain level of moisture, it becomes too heavy for the material to become airborne.

Airborne bacteria play a major part in the air quality problems that commercial poultry houses face. Bacteria have the capability to attach to particles in the air causing high concentrations that transfer throughout the house (Pal, Amrit, et al., 2021). Beneficial bacteria, including *Nocardiopsis, Bifidobacterium*, certain types of *Clostridium, Ruminococcus, Lactobacillus, Bacteroides, Bacillus,* and *Streptococcus* can all be present within a poultry house. These bacteria can reside in the gastrointestinal tract to aid in digestion and nutrient uptake. Some beneficial bacteria produce antimicrobial metabolites that make it more difficult for pathogenic bacteria to colonize (Adil, S., and S. N. Magray, 2012). Finally, there are certain beneficial bacteria that aid in the breakdown of feathers, feces, dander, and other detritus. Pathogenic bacteria like *Salmonella* sp., *E. coli, Clostridium perfringens, Listeria monocytogenes, Campylobacter* sp., and *Klebsiella pneumoniae* can produce harmful endotoxins that can cause diarrhea, fever, vomiting, abdominal cramps, and in some extreme cases can be fatal (Adil, S., and S. N. Magray, 2012; Scallan, E., et al., 2013). Most bacteria previously listen has recovered from across several studies from air sampling events (Sauter, E. A., et al., 1981; Plewa, K., and E. Lonc, 2011; Pan, Deng, and Zhongtang Yu, 2014; Zhang, Jianlong, et al., 2019; Jha, R, et al., 2020). This study focuses on airborne coliforms and *E. coli* as sentinel organisms to model for pathogenic bacteria.

Coliforms and *E. coli* are often considered indicator organisms in certain environments because it shows conditions that may be favorable for enteric pathogens like *Escherichia, Salmonella, Shigella,* and *Campylobacter* (Halkman, H. B. D., and A. K. Halkman, 2014). Coliforms are Gram-negative, facultatively anaerobic, lactose fermenting bacteria of several genera of the family Enterobacteriaceae including *Enterobacter, Escherichia, Klebsiella,* and *Citrobacter* (Carl A. Batt, Pradip Patel, 2014) which can include total and fecal coliforms. Fecal coliforms like *E. coli*, are coliforms that naturally occur in the intestines of chickens while other coliforms are associated with plant material.

Escherichia coli is a Gram-negative, rod-shaped facultative anaerobe that is in the intestines of birds and spread widely through feces. Birds in a poultry house are continuously exposed to *E. coli* contaminated poultry litter, feces, dust, and water. Damage to bird's disease resistance can allow for more pathogenic strains of *E. coli* to infect the bird (Charlton, B. R., et al., 2006).

Although the presence of indicator bacteria cannot be used to signal the presence of pathogens, the correlation with indicator bacteria when pathogens are present is strong. Understanding levels of *E. coli* and coliforms as indicator bacteria in poultry litter and air can help monitor potential levels of enteric pathogens and be used as a reliable indication of potential risk of contamination. (Wu, J., et al., 2011).

With the understanding that poultry houses can have air quality conditions that are not favorable to humans and birds, there have been many pushes for remediation to address high PM and airborne bacteria concentrations. One

method of particulate matter reduction is spraying oil, water, or an emulsion into the air. Similarly, to reduce airborne bacteria, some research has directed focus on the air by spraying slightly acidic electrolyzed water (SAEW) and tap water into the air. Low dosages of low pH SAEW was shown to be effective at reducing airborne bacteria (Zheng, W., et al., 2014). Although these methods can be effective at decreasing particulate matter levels, it can cause litter moisture to increase (Aarnink, A. J. A., et al., 2008). Higher levels of litter moisture can lead to excess bacterial growth which may cause elevated levels of airborne bacteria (Chai, Lilong, et al., 2018). Another method of particulate matter reduction is electrostatic precipitation. A device imparts a negative charge to airborne particles which causes them to precipitate. Experiments using varying voltages to test the reduction of particles in the air with efficiency ranging from a 37-79% particulate matter reduction depending on the charge applied to the air with max efficiency at -30 kV (Chai, Ming, et al., 2009). This method can cause airborne bacteria to precipitate onto litter, feed, waterers, and birds.

Some remediation techniques turn to poultry litter amendments as a source of air quality improvement. The poultry litter is can also facilitate the growth of high concentrations of bacteria that may become airborne. Poultry Litter Treatment (PLT) is a commonly used litter acidifier designed to lower pH to help control ammonia and lower litterborne microbes. This has been shown to be effective at reducing litter bound *E. coli* concentrations (Pope, M. J., and T. E. Cherry, 2000).

Air and house quality improvement is ongoing and pressing in the poultry industry, and new treatments are being used and study to determine what is the most effective. Unfortunately, there is no “one size fits all” approach to improving air quality, and the compounding effects of different approaches are not well understood. Sometimes one method may be improving air quality in one aspect, such as lowering particulate matter concentration, but it is worsening conditions in another way, such as increasing litter moisture.

Despite previous studies profiling PM and airborne bacteria concentrations in a house, there are gaps in the research profiling how these values change over the course of the grow-out and at different locations in the house. The purpose of this study is to 1) determine the particulate matter profile in the poultry house for PM sizes 1, 2.5, 4, and 10 microns at different spatial and temporal measurements over grow-out, 2) determine airborne *E. coli* and coliform concentrations at different spatial and temporal measurements over grow-out, and 3) measure house and litter parameters such as temperature, relative humidity, litter pH, litter moisture, and litter *E. coli* concentrations to determine correlative effects on the concentrations of PM and airborne *E. coli.* The central hypotheses to this portion of the study are 1) grow-out day will have a significant effect on the PM and airborne bacteria concentrations, 2) airborne *E. coli* concentrations will be significantly affected by PM concentrations, and 3) house and litter factors will have significant correlations with airborne *E. coli* concentrations.

MATERIALS AND METHODS

Chick Placement and Husbandry

One-day-old Cobb-500 chicks (n = 150) were purchased from a commercial hatchery in Cleveland, Georgia. They were placed in a broiler house approximately 458 cm wide and 610 cm in length. On one long-side of the room, 6 pens of 180 cm x 116 cm were constructed and held 20-25 Cobb 500 broiler chickens per pen. Figure 1 shows an aerial view of the experimental poultry house used. Chicks were raised on used poultry litter composed of pine shavings. Used litter had two previous, consecutive flocks of birds raised to 49 days with three weeks of downtime between flocks. Poultry litter was treated with PLT 24 hours before bird placement according to consumer guidelines. Birds were raised for 49 days from day of hatch under Raised Without Antibiotic (RWA) production. Broiler chicks were provided ad libitum intake of water and feed without the presence of antibiotics at any stage of development. Chicks were fed a starter, grower, and finished diet, and the feed was synthesized by the University of Georgia poultry research center feed mill. Husbandry and management follower commercial broiler chicken industry guidelines. After 49 days, all broiler chickens were euthanized, and all experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Georgia.

Particulate Matter Concentration Determination

The room was divided into three heights to represent bird height (10 – 50 cm), human height (170 cm), and upper fan level (214 cm), represented as H1,

H2, and H3. The room was divided into three widths to represent proximity to the birds at 150 cm (closest), 250 cm, and 383 cm represented as W1, W2, W3. The room was divided into three lengths to represent proximity to the ventilation fan at 100 cm (furthest), 400 cm, and 500 cm (closest) represented at L1, L2, L3.

For particulate matter sampling, spots were selected at bird and human height (H1 and H2) at the closest bird proximity (W1) across the length of the house (L1, L2, L3) for a total of 6 sampling spots (H1 W1 L1, H1 W1 L2, H1 W1 L3, H2 W1 L1, H2 W1 L2, H2 W1 L3). Figure 2 provides a visual representation of the house with particulate matter sample sites being represented by a “Red X”. Sampling events took place weekly starting at Day 0 (birds were placed) to Day 63 (two weeks after birds were culled). Samples were taken during times of tissue collection to have a representative idea of times during high human and bird activity which may pose the most risk for contamination. A DustTrak DRX Aerosol Monitor 8533 (TSI. Shoreview, Minnesota, USA) was placed at selected sampling location to record particulate matter at micron sizes PM_1, $PM_{2.5}$, PM_4, PM_{10}, and the total PM concentration for twelve minutes per sampling event. Sampling events recorded concentrations every ten seconds. The first six samples were removed from each sampling event to ensure minimal interference from human handling. This results in 66 readings of each PM size for each location. Average PM concentrations were taken for each size, and a relative ratio was created using the average PM concentration for a given size divided by the average PM concentration of the total sizes.

Airborne E. coli and Coliforms Concentration Determination

The room was divided into three heights to represent bird height (10 – 50 cm), human height (170 cm), and upper fan level (214 cm), represented as H1, H2, and H3. The room was divided into three widths to represent proximity to the birds at 150 cm (closest), 250 cm, and 383 cm represented as W1, W2, W3. The room was divided into three lengths to represent proximity to the ventilation fan at 100 cm (furthest), 400 cm, and 500 cm (closest) represented at L1, L2, L3.

For airborne *E. coli* and coliform concentration sampling, nine sampling spots were selected at each of the three heights (H1, H2, H3) at the closest proximity to the birds (W1) at each length of the house (L1, L2, L3).

a. H1 W1 L1, H1 W1 L2, H1 W1 L3

b. H2 W1 L1, H2 W1 L2, H2 W1 L3

c. H3 W1 L1, H3 W1 L2, H3 W1 L3

Another three sampling spots were selected at each height (H1, H2, H3) furthest from the birds (W3) closest to the door (L1) near a small, circulating fan.

d. H1 W3 L1, H2 W3 L1, H3 W3 L1

This resulted in a total of twelve passive airborne bacteria sampling locations.

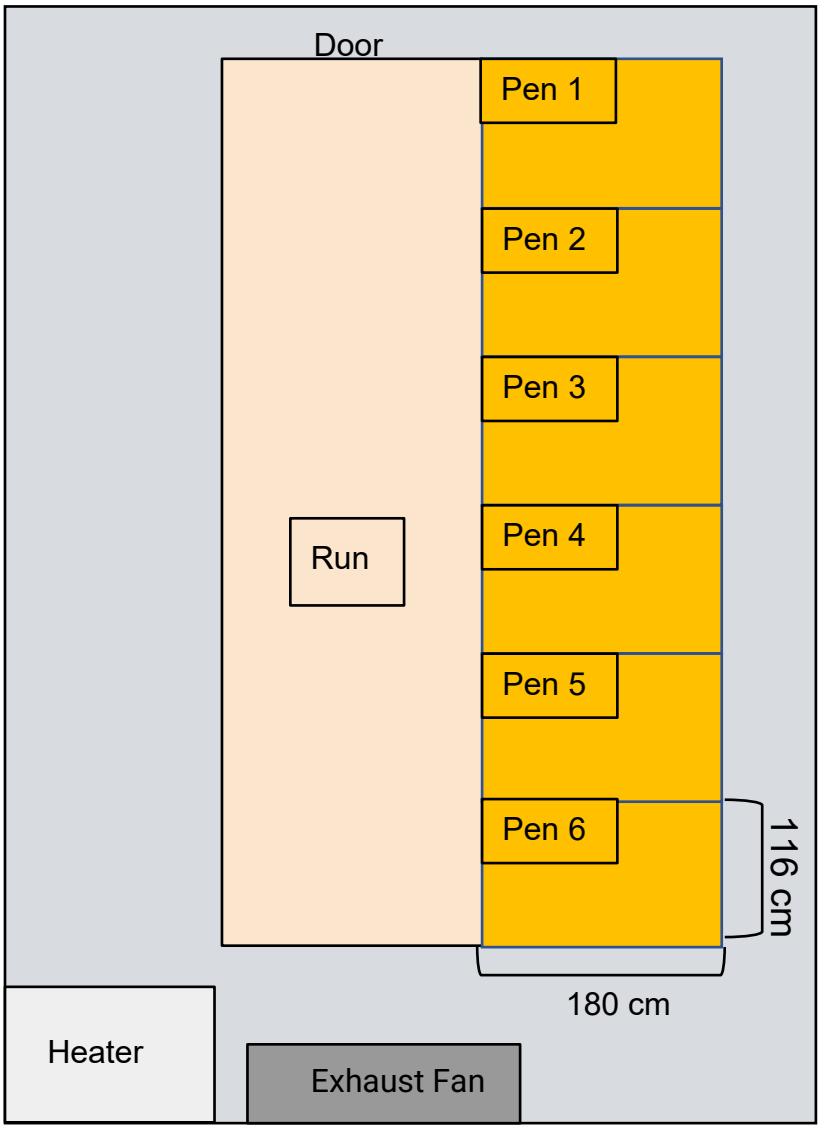

Figure 1. *Aerial View of Experimental Broiler House.* This figure represents an aerial view of the experimental broiler house. Pens were 116cm by 180cm. Each pen contained between 20-25 birds. At the back of the poultry house opposite of the door was the exhaust fan. Adjacent to the exhaust fan was a heating unit.

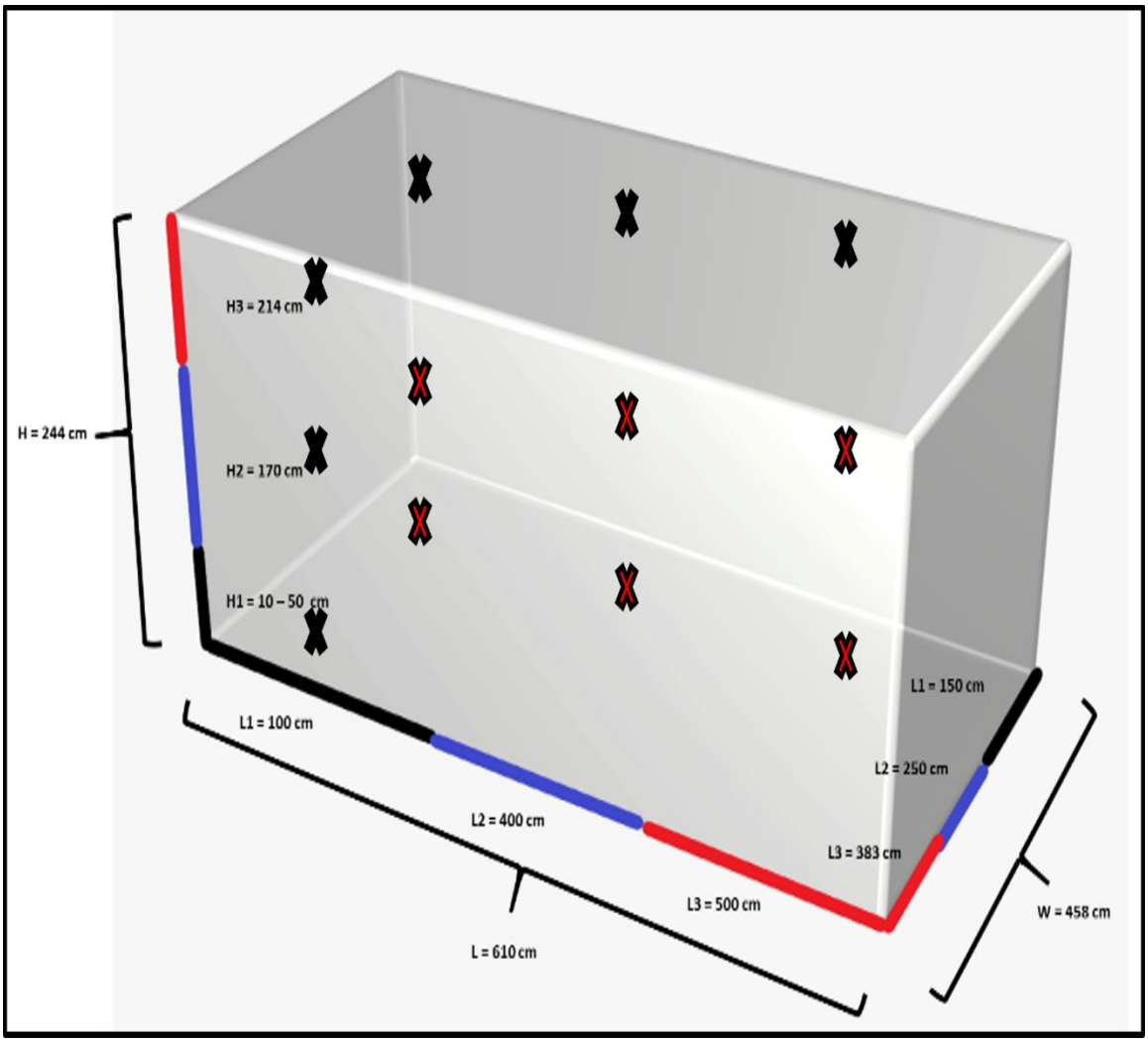

Figure 2. *Visual Representation of Experimental Poultry House*. The figure shows the model poultry house total height (264 cm) divided into three heights (10-50 cm, 170 cm, and 214 cm). Total width (458 cm) was divided into three sub-widths (150 cm, 250 cm, and 383 cm). Total length (610 cm) was divided into three sub-lengths (100 cm, 400 cm, and 500 cm). The six “Red X” represent the six locations of particulate matter sampling. The twelve “X” (red and black) represent the twelve locations of airborne bacteria sampling.

Sampling events took place weekly starting at Day 0 (birds were placed) to Day 63 (two weeks after birds were culled). Samples were taken during times of tissue collection to have a representative idea of times during high human and bird activity which may pose the most risk for contamination. A total of four petri dishes of CHROMagar™ ECC (CHROMagar. Paris, France) and Brain Heart Infusion agar (BHI) (Thermo Fisher Scientific. Waltham, Massachusetts, USA) in replicate were placed at each sampling site to allow for passive sedimentation to collect on the surface of the dish. Plates were incubated overnight at 37°C. *E. coli* and coliform colonies were identified using **Figure 2** and quantified using the Omeliansky formula (Guiamet, Patricia, et al., 2011; Awad, A. H., and H. A. Mawla, 2012):

$$N = 5a * 10^4 * bt$$

Where *N* is CFU/m^3; a is the number of colonies per petri dish; b is the petri dish surface area in cm^2; t is time of exposure in minutes.

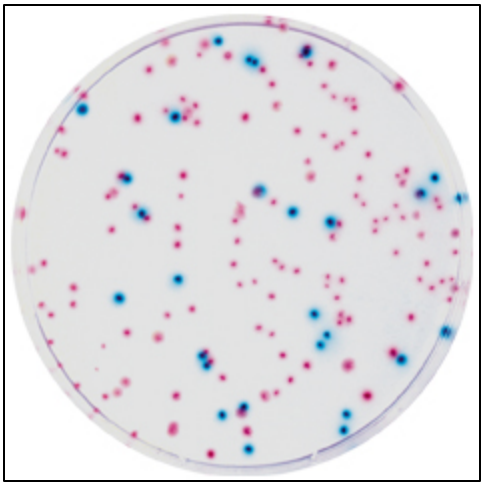

Figure 3. *CHROMagar Plate with E. coli and coliform colonies*. Catalogue image from (CHROMagar. Paris, France) showing *E. coli* colonies in blue and coliform colonies in pink.

Determination of Broiler House Temperature and Relative Humidity

Temperature and relative humidity were recorded weekly using an AcuRite Temperature and Humidity sensor (AcuRite. Lake Geneva, Wisconsin, USA). Sensor was placed closest to the door above the pens when viewing Figure 1. Temperature and relative humidity were continuously monitored for the health of the birds, but data was recorded weekly at the time of sampling events.

Determination of Poultry Litter E. coli concentration, moisture, and pH

Between 300-400 grams of litter were taken from each pen weekly over the course of the grow-out. A grab sample was taken based on the model in Figure 4**.** Samples were taken from seven locations in each pen, including the corners and under the waterers. The six pens from Figure 1 were assigned numbers, and three odd or even-numbered pens were samples weekly. For

example, on day 0, we sampled pens 1, 3, and 5, whereas on day 7 we sampled pens 2, 4, and 6. Poultry litter samples were mixed thoroughly within the sampling bag to homogenize the sample.

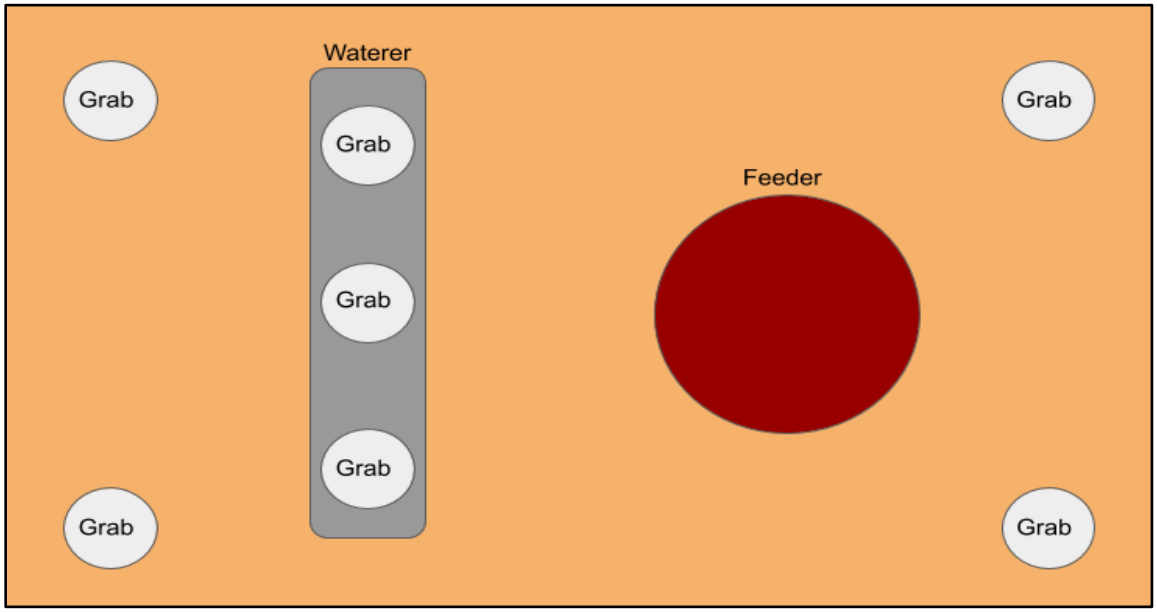

Figure 4. *Pen overview with grab sample locations for poultry litter.* Seven grab locations were sampled for each pen. A grab sample was taken in each corner in addition to three grab samples under the waterer.

For bacterial analysis of *E. coli* concentration in the poultry litter, 10 g of poultry litter was mixed with 50 mL of 1x Phosphate Buffered Saline (PBS) (ThermoFischer Scientific. Waltham, Massachusetts, USA) in a Nalgene bottle containing a handful of acid-washed glassed beads covering the bottom of the bottom (S800242, ThermoFisher Scientific). Sample bottles were placed on a Boekel mechanical wrist shaker (Boekel Scientific. Feasterville-Trevose, Pennsylvania, USA) at 450 rpm for 10 minutes and allowed to rest upright for 5 minutes after shaking. Poultry litter eluate was removed from solid components

and serially diluted in 1x PBS and plated onto CHROMagar to selectively grow *E. coli* and coliforms. Plates were incubated overnight at 37° C, and colony forming units were calculated and adjusted to the dry weight of the poultry litter. To determine the dry weight of the poultry litter, gravimetric analysis was performed. Approximately 1 g of poultry litter was weighed, dried overnight in an 87° C oven, and weighed again to determine the percent moisture lost and the dry weight of poultry litter. Using a pH probe (ThermoFischer Scientific. Waltham, Massachusetts, USA), litter pH was recorded by calibrating to standards and placing into poultry litter eluate.

Statistical Analysis

Statistics were performed using JMP 15 (JMP, SAS Institute Inc., Cary, NC). The significance level was set to $\alpha = 0.05$. Anderson-Darling normality testing was performed to determine whether datasets were normally distributed. If data was normally distributed, Tukey's honest significance tests were used to compare paired means. If data was not normally distributed, a Wilcoxon signed rank test was used to compare means.

RESULTS

Particulate Matter 1 (PM_1) Concentration

PM_1 concentrations ranged from 0.056 mg/m^3 on day 56 to 2.404 mg/m^3 on day 14. Day 0 concentration was significantly different than concentrations on days 7, 14, 21, 28, 35, 42, 49, and 56 ($P < 0.05$). Day 7 concentration was significantly different than concentrations on all other days ($P < 0.05$). Day 14 concentration was significantly different than concentrations on all other days. ($P < 0.05$). Day 21 concentration was significantly different than concentrations on all other days ($P < 0.05$). Day 28 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 35 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 42 was significantly different than concentrations on all other days ($P < 0.05$). Day 49 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 56 was significantly different than concentrations on all other days ($P < 0.05$). Day 63 was significantly different than concentrations on days 7, 14, 21, 28, 35, 42, 49, and 56 ($P < 0.05$) (Fig. 5).

There was a significant difference between PM_1 concentration at bird height and human height ($P = .0012$) (Fig. 6).

Particulate Matter 2.5 ($PM_{2.5}$) Concentration

$PM_{2.5}$ concentrations ranged from 0.058 mg/m^3 on day 56 to 2.437 mg/m^3 on day 14. Day 0 concentration was significantly different than concentrations on days 7, 14, 21, 28, 35, 42, 49, and 56 ($P < 0.05$). Day 7 concentration was significantly different than concentrations on all other days ($P < 0.05$). Day 14 concentration was significantly different than concentrations on all other days. (P

< 0.05). Day 21 concentration was significantly different than concentrations on all other days ($P < 0.05$). Day 28 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 35 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 42 was significantly different than concentrations on all other days ($P < 0.05$). Day 49 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 56 was significantly different than concentrations on all other days ($P < 0.05$). Day 63 was significantly different than concentrations on days 7, 14, 21, 28, 35, 42, 49, and 56 ($P < 0.05$) (Fig. 7).

There was a significant difference between $PM_{2.5}$ concentration at bird height and human height ($P = 0.0017$) (Fig. 8).

The significant results for $PM_{2.5}$ concentration mirror the significant results for PM_1 concentrations.

Particulate Matter 4 (PM_4) Concentration

PM_4 concentrations ranged from 0.063 mg/m^3 on day 56 to 2.565 mg/m^3 on day 14. Day 0 concentration was significantly different than concentrations on days 7, 14, 21, 28, 35, 42, 49, and 56 ($P < 0.05$). Day 7 concentration was significantly different than concentrations on all other days ($P < 0.05$). Day 14 concentration was significantly different than concentrations on all other days. ($P < 0.05$). Day 21 concentration was significantly different than concentrations on all other days ($P < 0.05$). Day 28 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 35 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 42 was significantly different than concentrations on all other days ($P < 0.05$). Day 49 was significantly different than days 0, 14,

21, 42, 56, and 63 ($P < 0.05$). Day 56 was significantly different than concentrations on all other days ($P < 0.05$). Day 63 was significantly different than concentrations on days 7, 14, 21, 28, 35, 42, 49, and 56 ($P < 0.05$) (Fig. 9).

There was a significant difference between PM_4 concentration at bird height and human height ($P = 0.0050$) (Fig. 10).

The significant results for PM_4 concentration mirror the significant results for PM_1 and $PM_{2.5}$ concentrations.

Particulate Matter 10 (PM_{10}) Concentration

PM_{10} concentrations Values ranged from 0.11 mg/m^3 on day 56 to 3.762 mg/m^3 on day 14. Day 0 concentration was significantly different than concentrations on concentrations on all other days ($P < 0.05$). Day 7 concentration was significantly different than concentrations on all other days ($P < 0.05$). Day 14 concentration was significantly different than concentrations on all other days. ($P < 0.05$). Day 21 concentration was significantly different than concentrations on all other days ($P < 0.05$). Day 28 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 35 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 42 was significantly different than concentrations on all other days ($P < 0.05$). Day 49 was significantly different than days 0, 14, 21, 42, 56, and 63 ($P < 0.05$). Day 56 was significantly different than concentrations on all other days ($P < 0.05$). Day 63 was significantly different than concentrations on days 7, 14, 21, 28, 35, 42, 49, and 56 ($P < 0.05$) (Fig. 11).

There was no significant difference between PM_{10} concentration at bird height and human height (P = 0.0695) (Fig. 12).

Particulate Matter Total (PM Total) Concentration

PM total concentrations ranged from 0.29 mg/m^3 on day 56 to 10.418 mg/m^3 on day 14. Day 0 concentration was significantly different than all other days (P < 0.05). Day 7 concentration was significantly different than concentrations on day 0, 14, 21, 42, and 56 (P <0.05). Day 14 concentration was significantly different than all other days (P < 0.05). Day 21 concentration was significantly different than all other days (P < 0.05). Day 28 was significantly different than days 0, 14, 21, 42, 56, and 63 (P < 0.05). Day 35 was significantly different than days 0, 14, 21, 42, 56, and 63 (P < 0.05). Day 42 was significantly different than all days but 14 (P < 0.05). Day 49 was significantly different than days 0, 14, 21, 42, 56, and 63 (P < 0.05). Day 56 was significantly different than all other days (P < 0.05). Day 63 was significantly different than all other days (P < 0.05) (Fig. 13).

There was a significant difference between Total PM concentration at bird height and human height (P = 0.0030) (Fig. 14).

Airborne E. coli Concentration

Day 0 concentration was significantly different than concentrations at day 14, 21, 28, 35, 42, and 49 (P < 0.05). Day 7 concentration was significantly different than concentrations at day 14, 21, 28, 35, 42, 49, 56, and 63 (P < 0.05). Day 14 concentration was significantly different than concentrations at day 0, 7, 35, 56, and 63 (P < 0.05). Day 21 concentration was significantly different than

concentrations at days 0, 7, 42, 56, and 63 ($P < 0.05$). Day 28 concentration was significantly different than concentrations at days 0, 7, 35, 42, 56, and 63 ($P < 0.05$). Day 35 concentration was significantly different than concentrations at day 0, 7, 14, 28, 49, 56, and 63 ($P < 0.05$). Day 42 concentration was significantly different than concentrations at days 0, 7, 21, 28, 56, and 63 ($P < 0.05$). Day 49 concentration was significantly different than concentrations at days 0, 7, 35, 56, and 63 ($P < 0.05$). Day 56 concentration was significantly different than concentrations at days 7, 14, 21, 28, 35, 42, and 49 ($P < 0.05$). Day 63 concentration was significantly different than concentrations at day 7, 14, 21, 28, 35, 42, and 49 ($P < 0.05$) (Fig. 15).

There were no significant differences in concentrations between bird height, human height, and upper ventilation levels ($P = .8593$) (Fig. 16).

Average airborne *E. coli* were undetectable at day 0 but rose to 2.380 Log CFU/m^3 on day 14 and peaked at 2.640 on day 28. *E. coli* levels dropped to 2.122 on day 49 when birds were culled, and there was a steep drop of airborne *E. coli* with levels becoming undetectable two weeks after birds were culled. No significant differences were found between airborne *E. coli* concentrations at different locations ($P > 0.05$).

Airborne Coliform Concentration

Average Airborne coliform concentrations had concentrations at the lowest level of detection at 1.86 Log CFU/m^3 to 2.63 Log CFU/m^3 on day 14. Day 0 airborne coliform concentration was significantly different than concentrations at days 7, 21, 28, 35, 42, 49, 56, and 63 ($P < 0.05$). Day 7 airborne coliform

concentration was significantly different than concentrations at days 0, 35, 42, 56, and 63 ($P < 0.05$). Day 56 airborne coliform concentration is significantly different from days 14, 21, 28, 49, and 63 ($P < 0.0161$). Day 63 airborne coliform concentration is significantly different from days 14, 21, and 28 ($P < 0.0062$) (Fig. 17).

There was a significant difference between airborne coliform concentrations at bird height and human height ($P < 0.05$). There was also a significant difference between airborne coliform concentrations at bird heigh and upper ventilation levels ($P < 0.05$) (Fig. 18).

Poultry Litter E. coli Concentration

Poultry litter *E. coli* concentration ranged from 3.75 Log CFU/g on day 7 to 7.47 Log CFU/g on day 49. Day 0 litter *E. coli* concentration was not significantly different than any other day. Day 7 litter *E. coli* concentration was significantly different than days 21, 28, 35, 42, and 49 ($P < 0.05$) (Fig. 19).

Poultry Litter Moisture

Poultry litter moisture ranged from 10% on days 0, 7, 56, and 63, to 36% on day 21. Day 0 litter moisture was significantly different than day 49 litter moisture ($P = 0.0282$). Day 7 litter moisture was significantly different than day 49 litter moisture ($P = 0.0282$). Day 56 litter moisture was significantly different than day 49 moisture ($P = 0.0282$). Day 63 moisture is significantly different than day 49 moisture ($P = 0.0282$) (Fig. 20).

Poultry Litter pH

Poultry litter pH ranged from 6.76 on day 0 to 7.16 on day 42. (Fig. 21). No days were significantly different than each other during the grow-out (P = 0.2512) (Fig. 21).

Temperature

Temperature ranged from 31.81 ^{o}C on day 0 to 21.11 ^{o}C on day 35. No days were significantly different than each other during the grow-out (P = 0.4289) (Fig. 22).

Relative Humidity

Relative humidity ranged from 76.5% on day 49 to 21.75% on day 0. No days were significantly different than each other during the grow-out (P = 0.4289) (Fig. 23).

Correlative Effects on Particulate Matter Concentration and Airborne E. coli Concentration

There were positive correlations found between pairwise comparisons of PM total concentrations and airborne *E. coli* concentrations (r = 0.7494), poultry litter moisture and airborne *E. coli* concentrations (r = 0.6118), and poultry litter *E. coli* concentration and airborne *E. coli* concentration (r = 0.7721). There was a negative correlation found between pairwise comparisons of litter moisture and particulate matter concentration (r = -0.5296).

DISCUSSION AND CONCLUSION

PM_1, $PM_{2.5}$, and PM_4 mean concentrations had no significant differences between sizes ($P < 0.05$), which means that $PM_{2.5}$ and PM_4 readings were primarily made up of particles sized PM_1 or smaller. Mean values for PM_1, $PM_{2.5}$, and PM_4 ranged from 0.148 mg/m^3 on day 56 to 1.096 mg/m^3 on day 14. PM_{10} is where differences in size were displayed. Mean PM_{10} concentrations ranged from 0.279 mg/m^3 on day 56 to 1.668 mg/m^3 on day 14. Mean concentrations PM_1, $PM_{2.5}$, and PM_4 to PM_{10} ratio varied between 46.09% - 62.58%. This means that up to 53.91% of average PM_{10} concentration was made up of particles sized between 4-10 microns in diameter. Mean PM Total concentrations ranged from 0.792 mg/m^3 on day 56 to 3.968 mg/m^3 on day 14. Mean PM Total concentrations did not reach above the recommended guidelines of 5.000 mg/m^3, but at individual points of measurement, levels as high as 26.400 mg/m^3 were recorded. Mean PM Total concentrations were made up of 18.04% - 25.32% of PM_1, $PM_{2.5}$, and PM_4. Mean PM Total concentrations were made up of 33.16 – 50.72% PM_{10} concentrations. This means that up to 66.84% of mean PM Total concentrations were made up of non-respirable particles sized greater than 10 microns in diameter. Mean levels of PM_1, $PM_{2.5}$, PM_4, and PM total were significantly higher at bird level than human level ($P = 0.0017$). This can be explained because the birds have higher levels of activity that can agitate fine particles causing them to become airborne. Additionally, heavier particles contributing to mean PM total levels could be more likely to stay closer to the ground. The ventilation unit and circulating were also much higher than bird level

which could prevent particles closer to the ground from being lifted and vented out of the house. There was no significant different between heights for mean PM_{10} levels (P = 0.0695). A potential explanation for this is PM_{10} includes particle sizes that can include mold, pollen, and dust. It is a coarser size, and airborne bacteria have found to be more likely to adhere to coarse sized particles (Gong, Jing, et al., 2020). Mean airborne *E. coli* concentrations were found to have no significant differences between bird height, human height, and upper ventilation. The size, particulate make-up, and support that some bacteria are evenly distributed at different heights could support this data. Particulate matter data from other studies show similar mean PM concentrations over the course of the grow-out. PM concentrations can vary greatly depending on other air quality factors but based on other studies with PM levels lower than recommended guidelines, the results we gathered seemed reasonable. In one study, mean $PM_{2.5}$ concentrations ranged from 0.0921 mg/m^3 to 0.272 mg/m^3. Mean PM_{10} concentrations ranged from 0.128 mg/m^3 to 0.316 mg/m^3 (Zhang JianLong, et al., 2019). Some studies show a consistent increase in mean PM concentrations over the course of the grow-out from beginning to end, (Winkel, Albert, et al., 2015; Zhang JianLong, et al., 2019), but our results showed an increase from days 0-14 followed by a decrease at day 21. Mean PM concentrations increased from days 21-42 followed by a decrease at day 49. Mean PM concentrations decreased further during weeks of litter downtime at days 56 and 63. One possible explanation for this could be the due to the litter moisture. Litter moisture was low for days 0, 7, and 14. Mean moisture increased from 11.6% on day 14 to

28.0% on day 21. From days 21 and 42, litter moisture decreased from 28.0% to 13.9%. Finally, litter moisture increased from days 42 to 49 from 13.9% to 21.5%. These results would be consistent in showing that increased litter moisture can reduce particulate matter levels (Wathes, C. M., et al., 1997; Kaliste, E., et al., 2004; Kabelitz, Tina, et al., 2020).

Mean airborne *E. coli* concentrations ranged from non-detectable levels on days 0 and 63 to 2.640 Log CFU/m^3 on day 28. Mean coliform concentrations ranged from 0.078 Log CFU/m^3 on day 56 to 2.223 Log CFU/m^3 on day 0. One explanation for this difference is that coliforms consist of fecal coliforms like *E. coli* and non-fecal coliforms including *Citrobacter, Enterobacter,* and *Klebsiella.* Bacteria, like *E. coli* can enter dormant stages in which cells are still viable but non-culturable. *E. coli* conditions for survival in litter and air are less favorable than in the intestinal system causing low levels at day 0, 56, and 63 (van Elsas, Jan Dirk, et al., 2010). It is possible that fecal coliforms like *E. coli* remained mostly dormant in early stages while non-fecal coliforms remained active. As the presence and activity of the birds increased, non-fecal coliforms could have decreased while fecal coliforms, including *E. coli* increased. There was no significant difference between mean airborne *E. coli* concentrations at bird height, human height, and upper ventilation level ($P = 0.8593$). There was a significant difference between average coliform concentrations between bird and human height ($P < 0.05$) and bird height and upper ventilation ($P < 0.05$). One explanation for this is that airborne *E. coli* have found to be more likely to adhere to coarse sized particles (Gong, Jing, et al., 2020), and there was no significant

difference between heights for mean PM_{10} concentrations (P = 0.0695). Although *E. coli* is a type of fecal coliform, other genera of coliforms may have different airborne viabilities or ability to adhere to different sizes of particulate matter that could explain the discrepancy. The factors that influence adhesion to different sized particles is poorly understood (Gong, Jing, et al., 2020). Results of mean airborne *E. coli* increasing to higher levels in the middle of the grow-out and decreasing after birds were culled is consistent with other broiler studies showing similar sinusoidal trends for airborne bacteria concentrations (Vučemilo, M., et al., 2007). One study has shown that of the total culturable bacteria, aerobic Gram-negative bacteria makes of 0.02% on average in broiler houses. This resulted in 1.15 Log CFU/m^3 total Gram-negative airborne bacteria, with airborne *E. coli* making up 0.901 Log CFU/m^3. Although the values we recovered during days 14-49 were higher than these numbers, concentrations of airborne bacteria can vary greatly, and these results are not unreasonable (Zucker, B. A., et al., 2000).

There were positive correlations found between pairwise comparisons of PM total concentrations and airborne *E. coli* concentrations (r = 0.7494), poultry litter moisture and airborne *E. coli* concentrations (r = 0.6118), and poultry litter *E. coli* concentration and airborne *E. coli* concentration (r = 0.7721). There was a negative correlation found between pairwise comparisons of litter moisture and particulate matter concentration (r = -0.5296). These are consistent with other research that shows that airborne bacteria concentrations can increase as PM concentrations increase (Chen, Cao, et al., 2014; Xie, Zhengsheng, et al., 2018)

Litter moisture can increase levels of litter bound bacteria, and an increase in litter bound bacteria and airborne bacteria concentrations is consistent with other studies (Pal, Amrit, et al., 2021). Finally, an increase in litter moisture reducing particulate matter concentrations is consistent with results from other studies (Wathes, C. M., et al., 1997; Kaliste, E., et al., 2004; Kabelitz, Tina, et al., 2020).

Limitations of this study include the sample-sizes for collected data being small in some instances. For example, temperature and relative humidity represent one data point per week. Litter moisture, litter pH, and litter *E. coli* concentrations represent 3 data points per week except on day 49 where n = 6. This caused issues when performing comparison of means between weeks. For example, average litter moisture on day 0, 21, and 49 was found to be 12.3%, 28.0%, and 21.5%, respectively. Despite day 21 having higher average moisture than day 0, it was not found to be significantly different from day 0 moisture, whereas day 49 was. This could be because day 49 has more data points which increases the accuracy for significant tests. If sampling events had higher replications, it would improve the confidence of results. Another limitation of the study is that this data only represents results from one flock of broilers over the course of the grow-out. It would be beneficial to see the replicability and consistency of results and how other factors, such as season and poultry litter age would impact the results. We were in the process of replicating this study for a second flock, but COVID-19 complications caused early field termination. Research was conducted in an experimental facility with significant differences to a commercial poultry facility such as number of birds, litter amount, stocking

density, and ventilation. These results may be used as a model and can guide similar research to take place in a commercial facility. Another limitation was limited amount of instrumentation available. For example, only one DustTrak DRX was available for collection of PM data, so we were limited by how many sampling spots within the house we were able to do during a sampling event. In the future, more locations and heights can be tested with increased access to instrumentation. Finally, limitations existed on the ability of statistical analysis. More advanced methodology can be used in the future to determine significance of causal effects versus correlative effects.

Overall, results from this study were consistent with results from other studies. The major conclusions from this chapter include that PM_1, $PM_{2.5}$, PM_4, and PM Total are significantly higher at bird height than human height ($P < 0.05$). There was no significant difference found between bird height and human height for average PM_{10} concentration ($P = 0.0695$) The concentrations of all average PM concentrations are significantly higher during days of broiler grow-out versus days 0, 56, and 63 ($P < 0.05$). There is no significant difference between mean airborne *E. coli* concentrations at bird height, human height, and upper ventilation levels ($P = 0.8593$). There were significant differences between mean coliform concentrations between bird height and human height along with bird height and upper ventilation ($P < 0.05$). The mean concentration of airborne *E. coli* is significantly higher on days 14-49 compared to days 0, 56, and 63 ($P < 0.05$). Finally, there were positive correlations found between pairwise comparisons of PM total concentrations and airborne *E. coli* concentrations (r = 0.7494), poultry

litter moisture and airborne *E. coli* concentrations (r = 0.6118), and poultry litter *E. coli* concentration and airborne *E. coli* concentration (r = 0.7721). There was a negative correlation found between pairwise comparisons of litter moisture and particulate matter concentration (r = -0.5296). This shows that grow-out day and location in the house can play a significant impact on the levels of PM and airborne bacteria concentrations. Specifically, days 14-49 and bird height are more likely to have significantly higher levels of mean PM and airborne bacteria concentrations. The correlations also show that remediation efforts should focus on reducing particulate matter levels and litter bound *E. coli* levels while not increasing litter moisture levels.

FIGURES

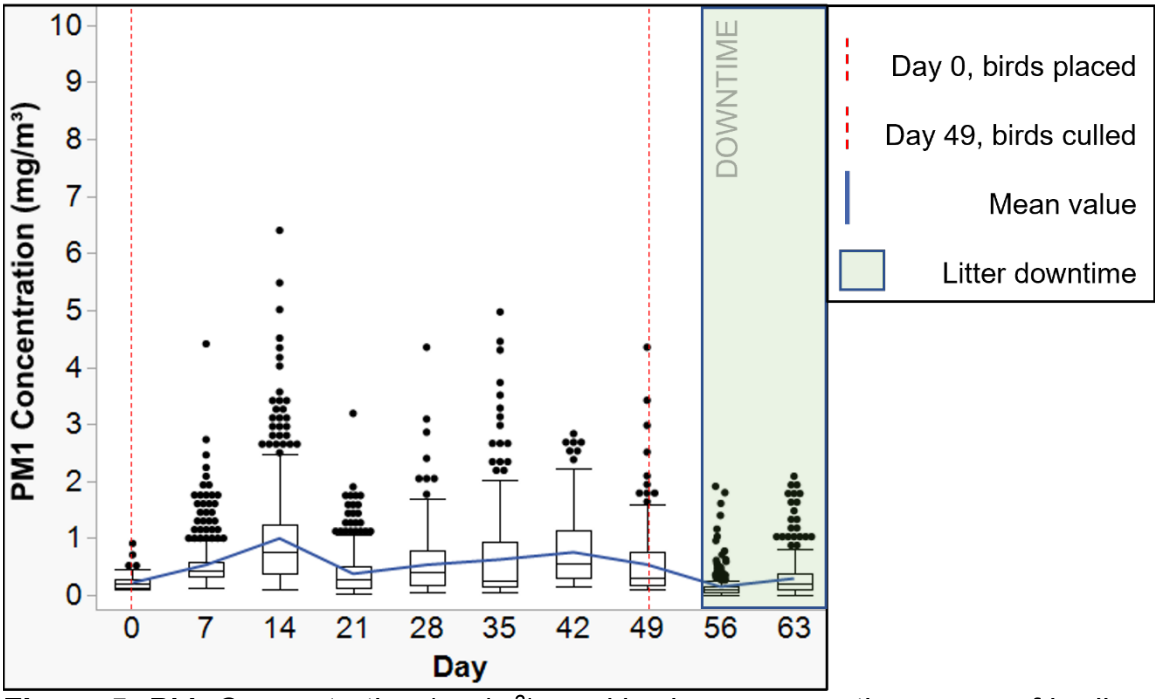

Figure 5. *PM_1 Concentration (mg/m^3) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from six different house locations with 66 data points per location (n = 6) for a total of n = 396 data points per sampling event except at day 0 where n = 198. Results are reported in (mg/m^3). Values ranged from 0.29 mg/m^3 on day 56 to 10.418 mg/m^3 on day 14. Error bars represent standard error. Significance was set to ($P \leq 0.05$).

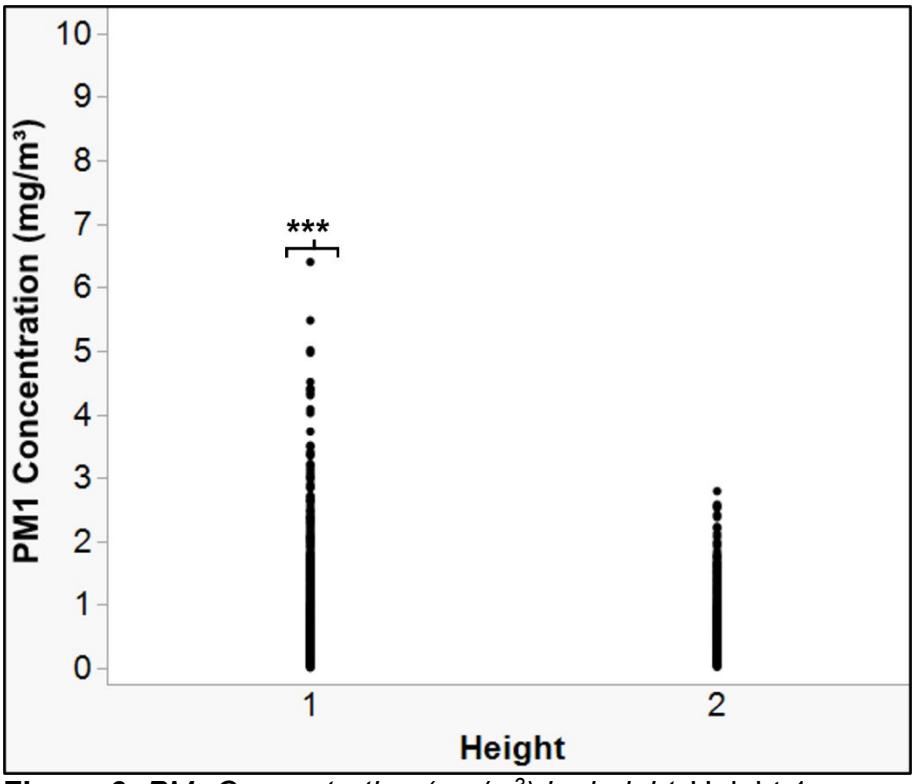

Figure 6. *PM_1 Concentration (mg/m^3) by height.* Height 1 represents the height of the bird at approximately 10-50 cm. Height 2 represents the height of an average human at approximately 170 cm. Height 1 shows n = 1980 data points. Height 2 shows n = 1783 data points. Significance was set to ($P \leq 0.05$). There was a significant difference between PM_1 concentration (mg/m^3) and height (P = 0.0012) shown by asterisks.

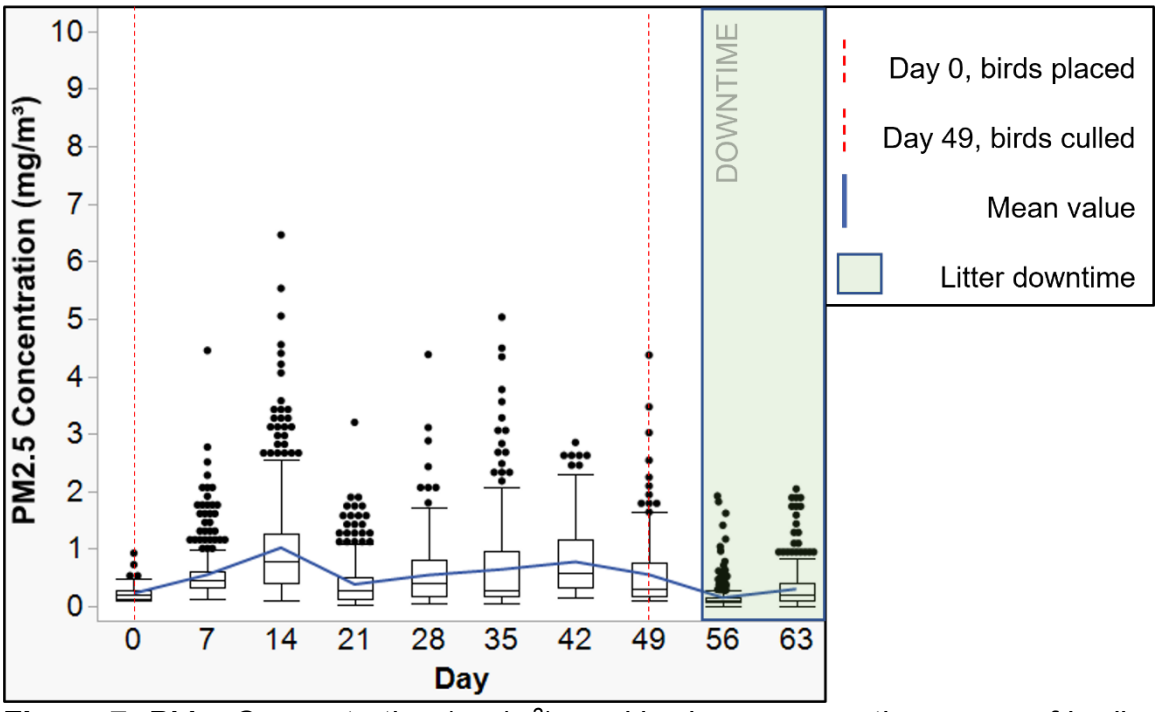

Figure 7. *$PM_{2.5}$ Concentration (mg/m^3) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from six different house locations with 66 data points per location (n = 6) for a total of n = 396 data points per sampling event except at day 0 where n = 198. Results are reported in (mg/m^3).. Values ranged from 0.29 mg/m^3 on day 56 to 10.418 mg/m^3 on day 14. Error bars represent standard error. Significance was set to ($P \leq 0.05$).

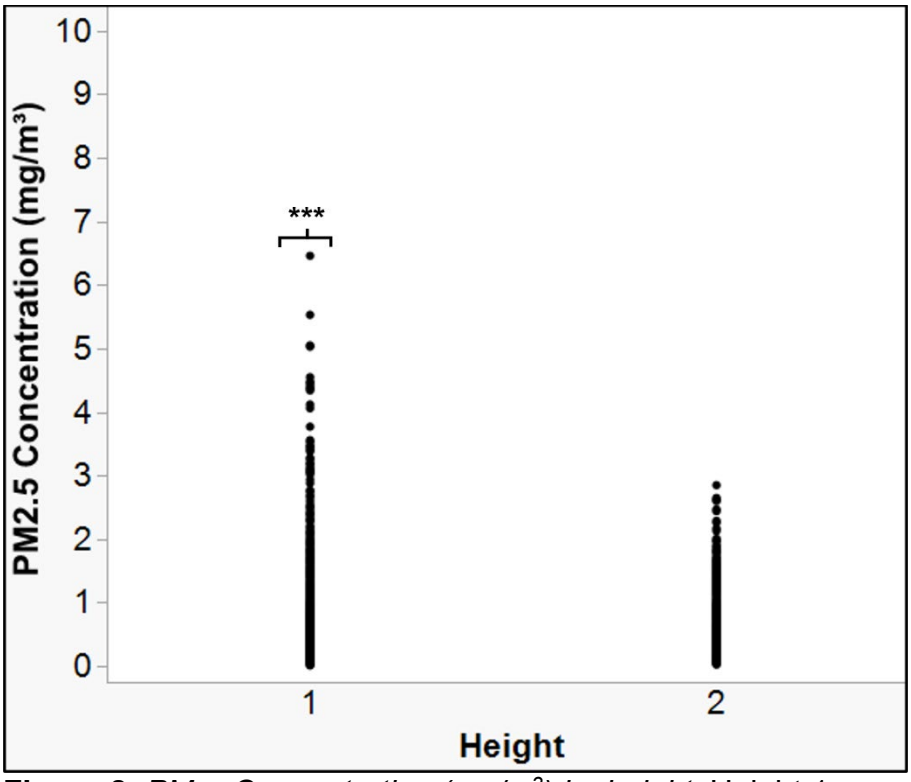

Figure 8. *$PM_{2.5}$ Concentration (mg/m^3) by height.* Height 1 represents the height of the bird at approximately 10-50 cm. Height 2 represents the height of an average human at approximately 170 cm. Height 1 shows n = 1980 data points. Height 2 shows n = 1783 data points. Significance was set to ($P \leq 0.05$). There was a significant difference between $PM_{2.5}$ concentration (mg/m^3) and height (P = 0.0017) shown by asterisks.

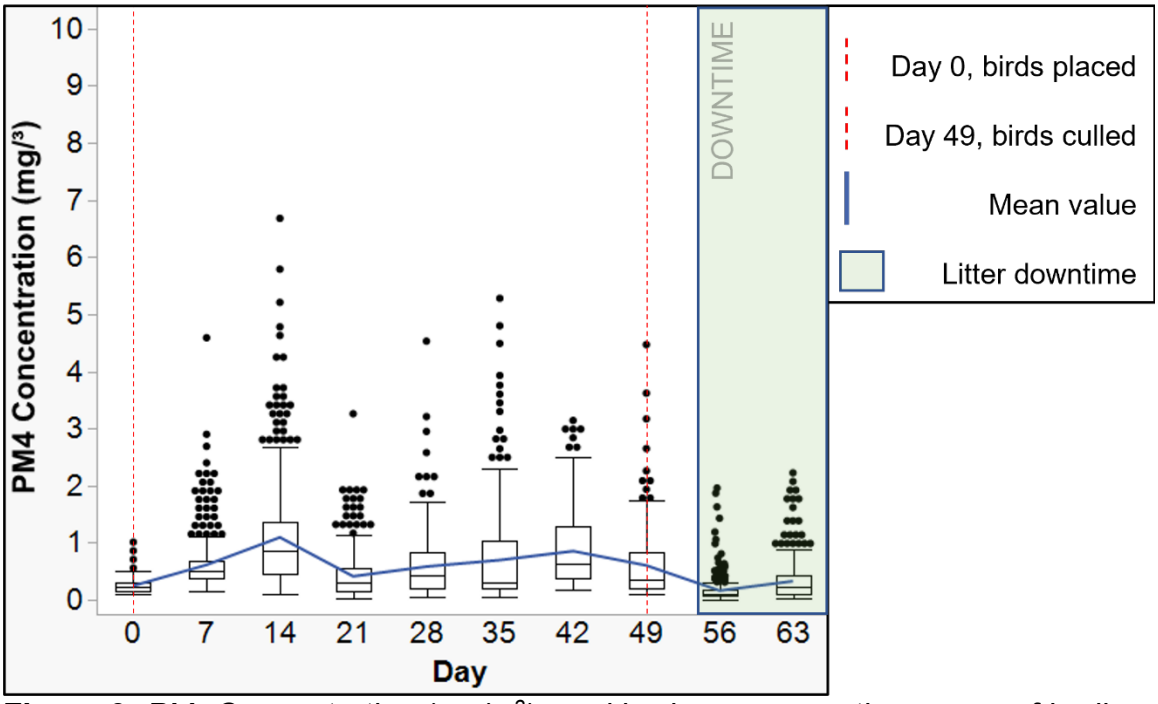

Figure 9. *PM_4 Concentration (mg/m^3) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from six different house locations with 66 data points per location (n = 6) for a total of n = 396 data points per sampling event except at day 0 where n = 198. Results are reported in (mg/m^3). Values ranged from 0.29 mg/m^3 on day 56 to 10.418 mg/m^3 on day 14. Error bars represent standard error. Significance was set to ($P \leq 0.05$).

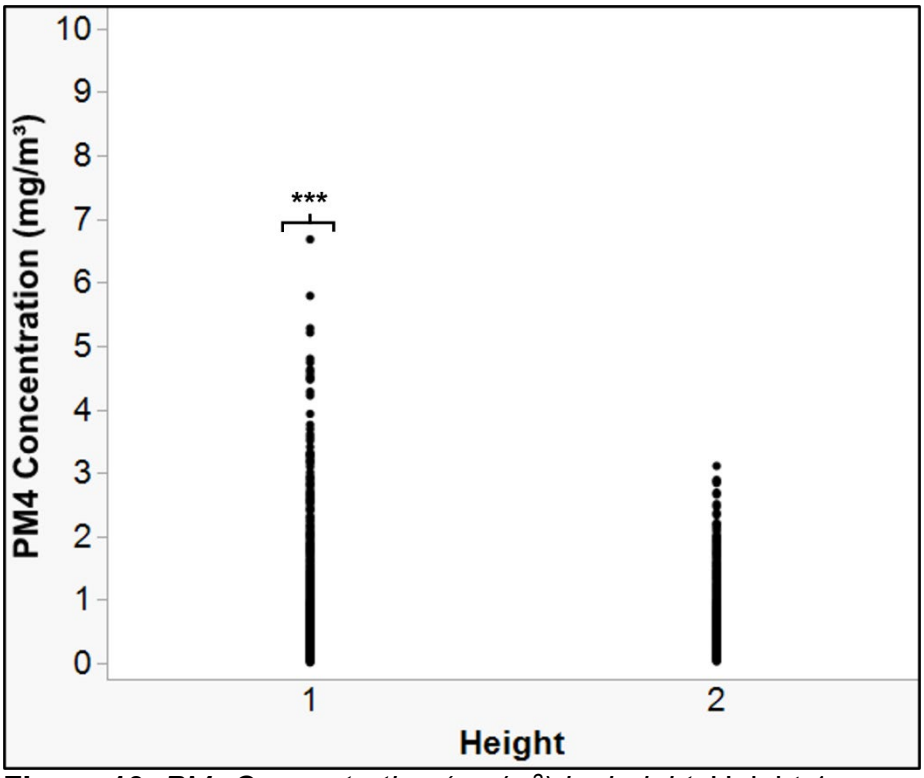

Figure 10. *PM_4 Concentration (mg/m^3) by height.* Height 1 represents the height of the bird at approximately 10-50 cm. Height 2 represents the height of an average human at approximately 170 cm. Height 1 shows n = 1980 data points. Height 2 shows n = 1783 data points. Significance was set to ($P \leq 0.05$). There was a significant difference between PM_4 concentration (mg/m^3) and height ($P = 0.0050$) shown by asterisks.

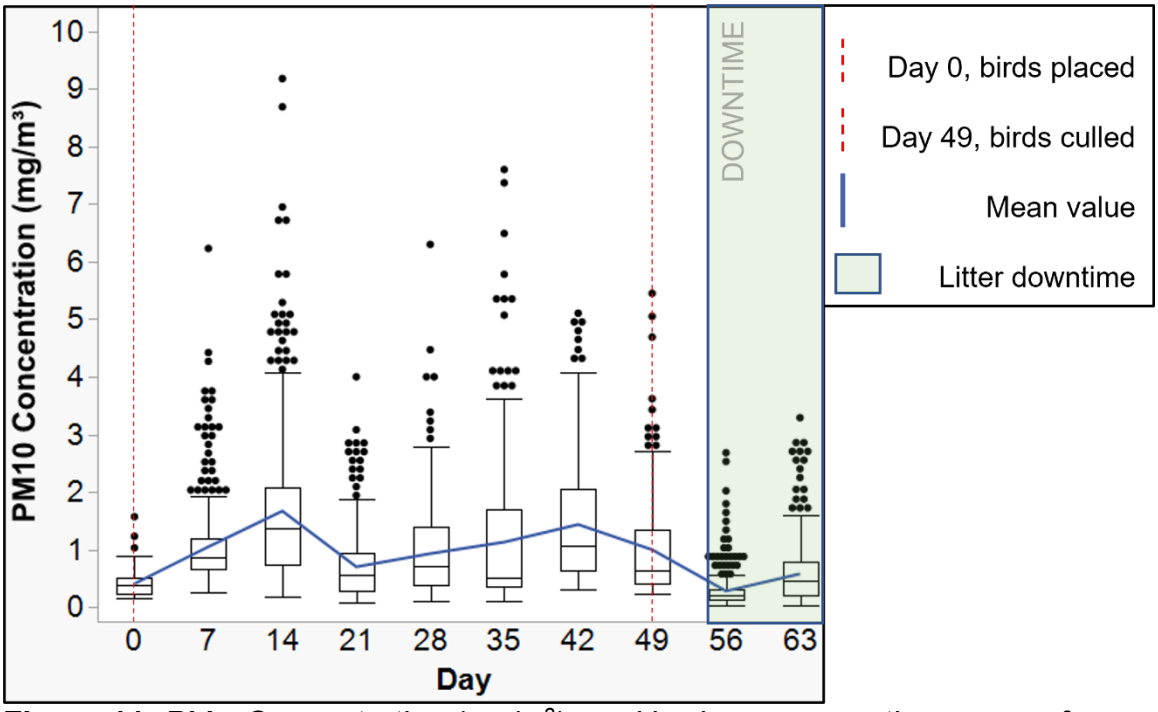

Figure 11. *PM_{10} Concentration (mg/m^3) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from six different house locations with 66 data points per location (n = 6) for a total of n = 396 data points per sampling event except at day 0 where n = 198. Results are reported in (mg/m^3). Values ranged from 0.29 mg/m^3 on day 56 to 10.418 mg/m^3 on day 14. Error bars represent standard error. Significance was set to ($P \leq 0.05$).

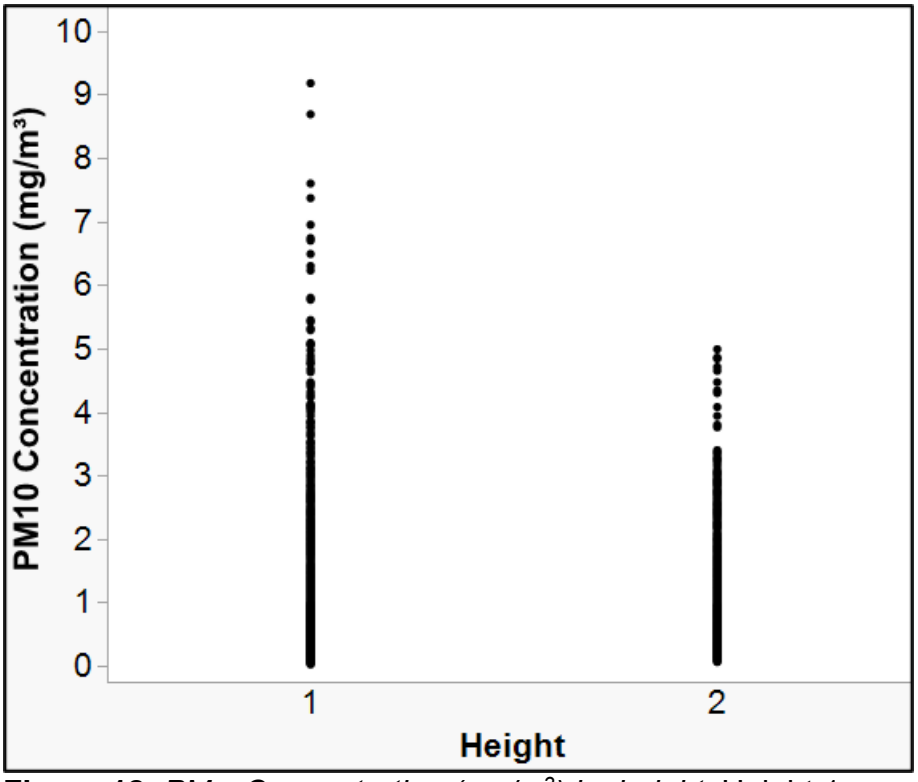

Figure 12. *PM_{10} Concentration (mg/m^3) by height.* Height 1 represents the height of the bird at approximately 10-50 cm. Height 2 represents the height of an average human at approximately 170 cm. Height 1 shows n = 1980 data points. Height 2 shows n = 1783 data points. Significance was set to ($P \leq 0.05$). There was no significant difference between PM_{10} concentration (mg/m^3) and height (p = 0.0695).

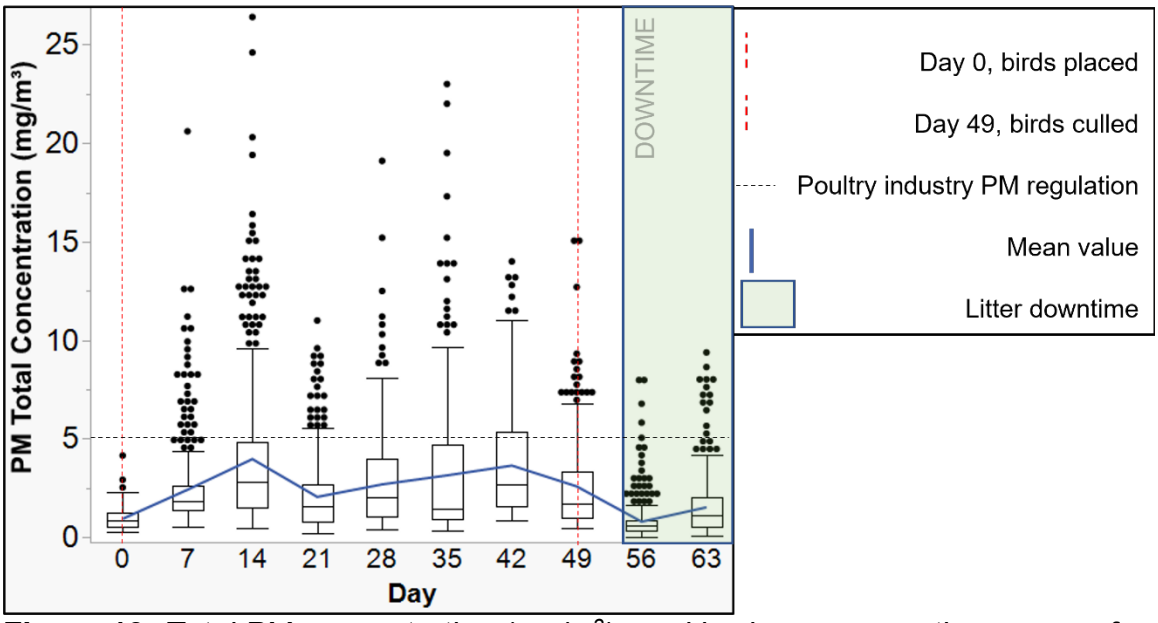

Figure 13. *Total PM concentration (mg/m^3) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from six different house locations with 66 data points per location (n = 6) for a total of n = 396 data points per sampling event except at day 0 where n = 198. Results are reported in (mg/m^3). Values ranged from 0.29 mg/m^3 on day 56 to 10.418 mg/m^3 on day 14. Error bars represent standard error. Significance was set to ($P \leq 0.05$).

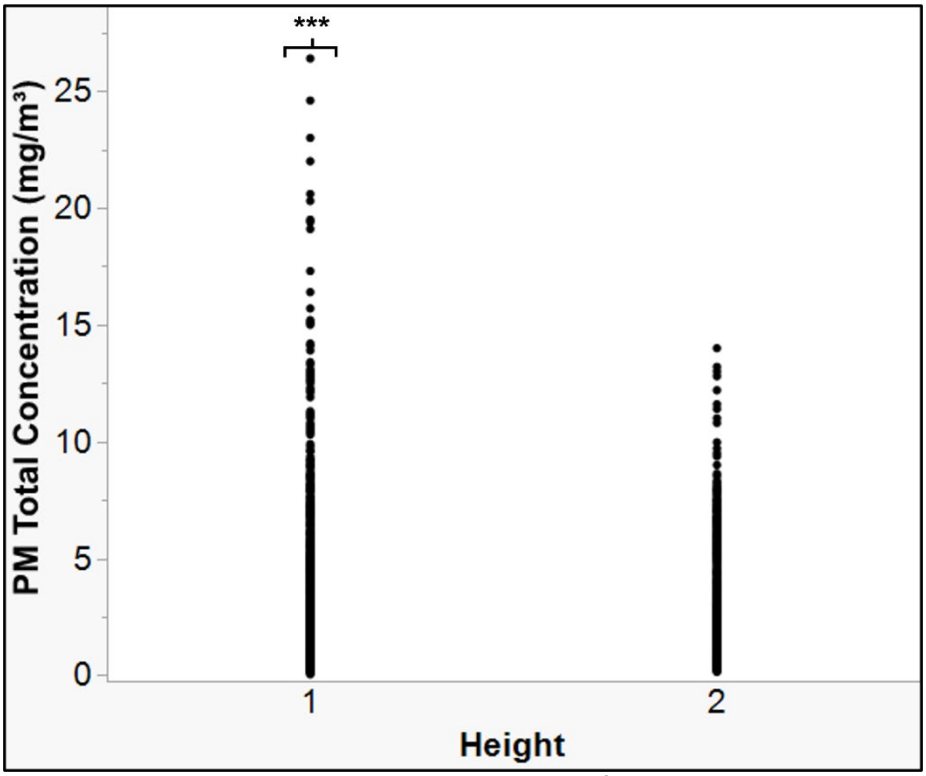

Figure 14. *Total PM Concentration (mg/m^3) by height.* Height 1 represents the height of the bird at approximately 10-50 cm. Height 2 represents the height of an average human at approximately 170 cm. Height 1 shows n = 1980 data points. Height 2 shows n = 1783 data points. Significance was set to ($P \leq 0.05$). There was a significant difference between PM Total concentration (mg/m^3) and height ($P = 0.0030$) shown by asterisks.

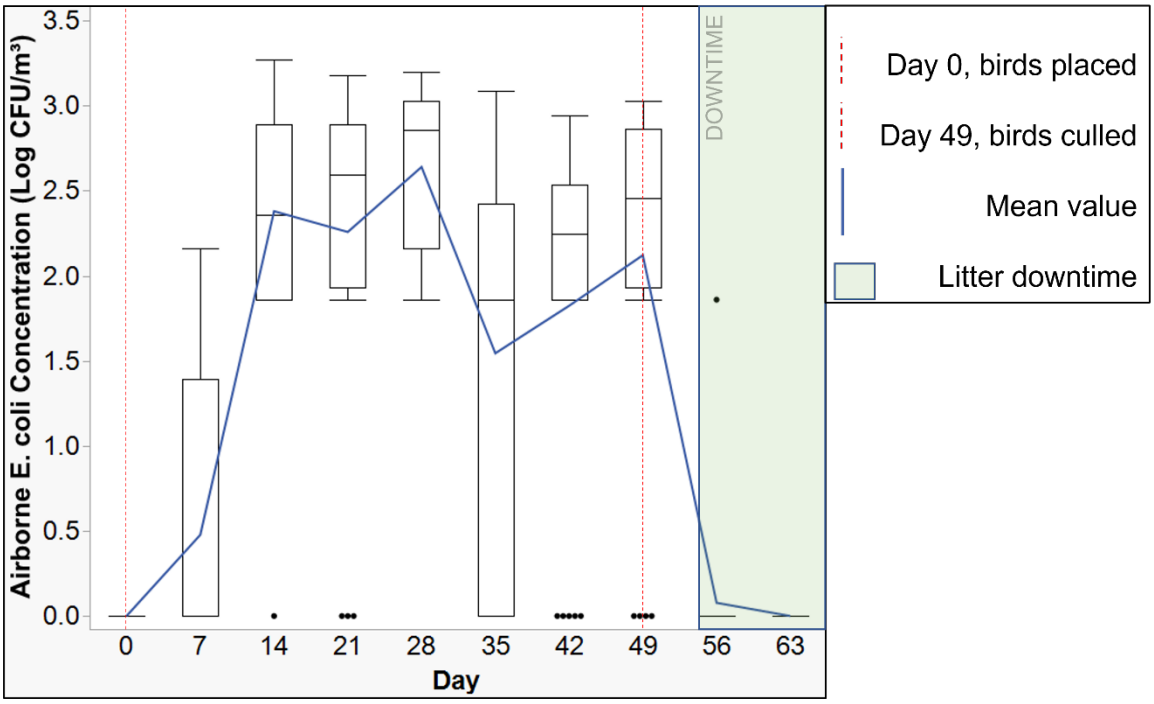

Figure 15. *Airborne E. coli concentration (Log CFU/m^3) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from six different house locations with two data points per location (n = 12) for a total of n = 24 data points per sampling event except at day 0 where n = 6. Results are reported in (Log CFU/m^3). Error bars represent standard error. Significance was set to ($P \leq 0.05$).

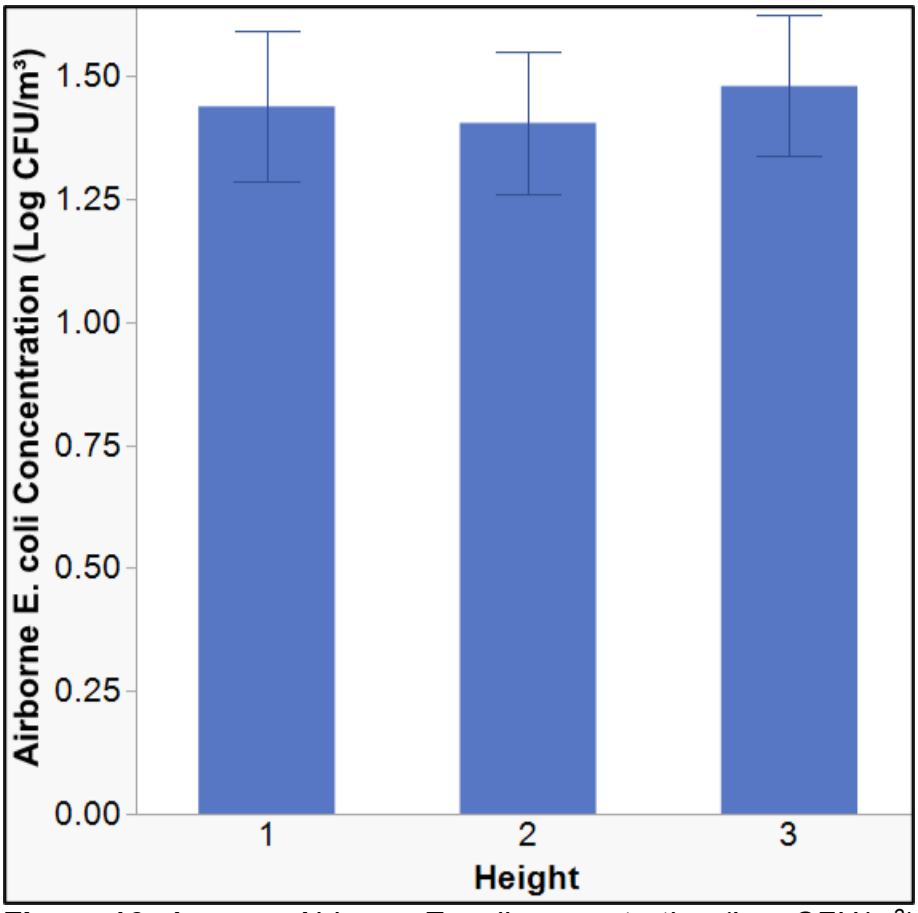

Figure 16. *Average Airborne E. coli concentration (Log CFU/m^3) by height.*

Height 1 represents the height of the bird at approximately 10-50 cm. Height 2 represents the height of an average human at approximately 170 cm. Height 3 represents the upper ventilation level at 217 cm. Height 1 shows n = 79 data points. Height 2 shows n = 73 data points. Height 3 shows n = 73 data points. Error bars represent standard error. Significance was set to (P = 0.05).

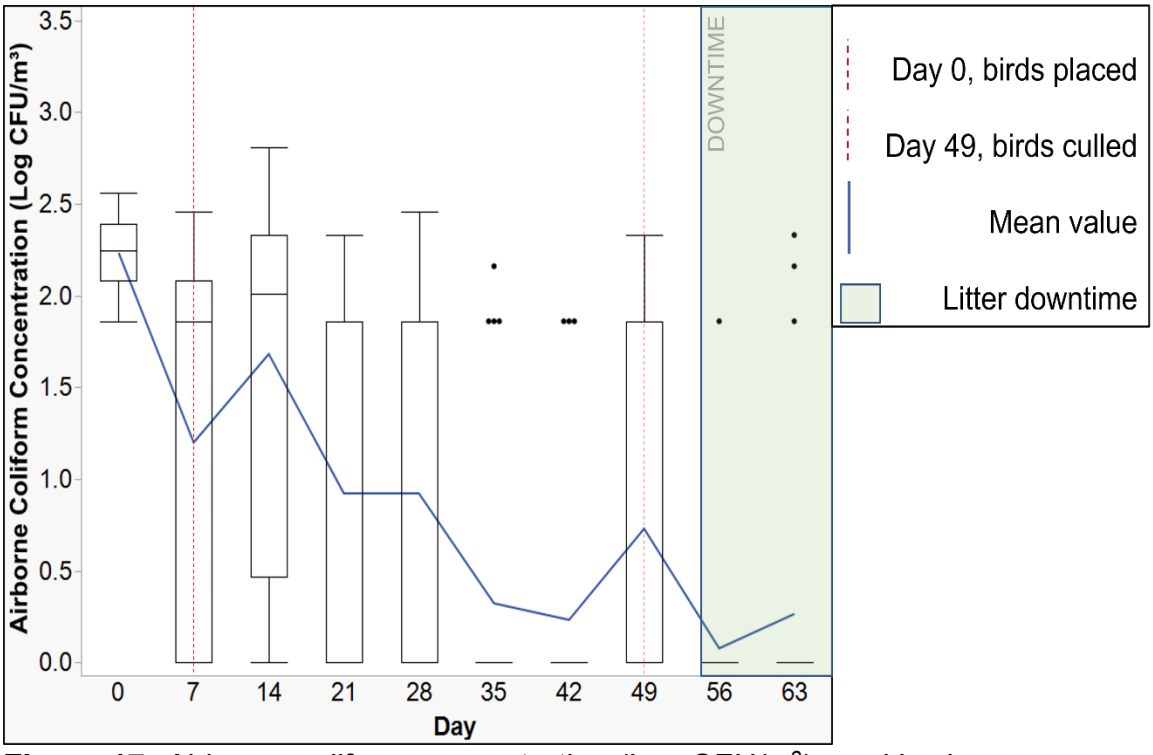

Figure 17. *Airborne coliform concentration (Log CFU/m^3) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from six different house locations with two data points per location (n = 12) for a total of n = 24 data points per sampling event except at day 0 where n = 6. Results are reported in (Log CFU/m^3). Error bars represent standard error. Significance was set to ($P \leq 0.05$).

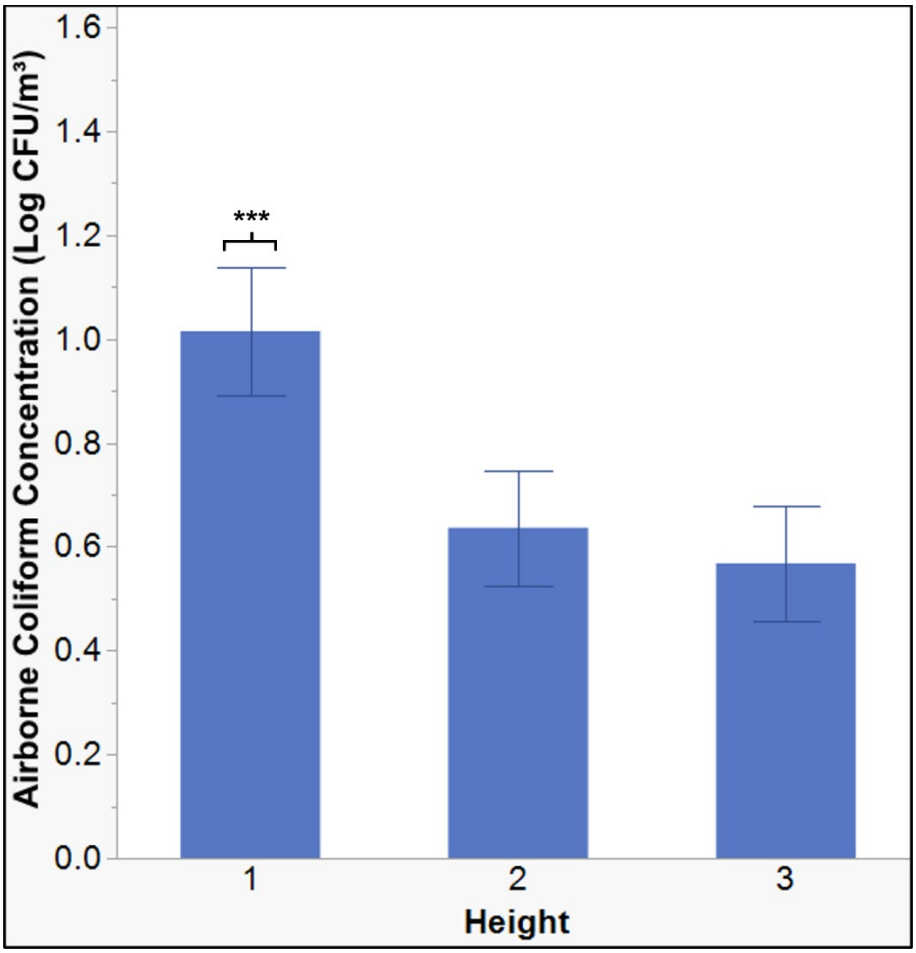

Figure 18. *Average Airborne coliform concentration (Log CFU/m^3) by height.* Height 1 represents the height of the bird at approximately 10-50 cm. Height 2 represents the height of an average human at approximately 170 cm. Height 3 represents the upper ventilation level at 217 cm. Height 1 shows n = 79 data points. Height 2 shows n = 73 data points. Height 3 shows n = 73 data points. Error bars represent standard error. Significance was set to (P = 0.05). ‘

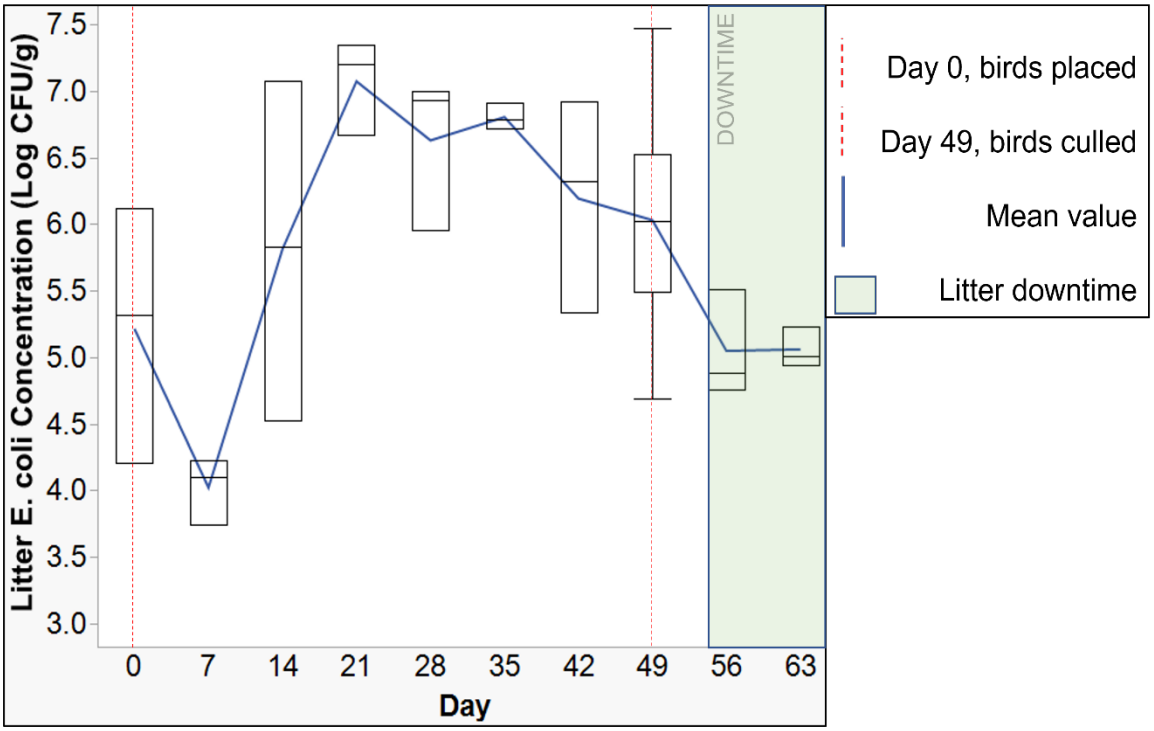

Figure 19. *Litter E. coli Concentration (Log CFU/g) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from three different house locations with one data point per location or a total of n = 3 data points per sampling event except at day 0 where n = 6. Results are reported in (Log CFU/g). Error bars represent standard error. Significance was set to ($P \leq 0.05$).

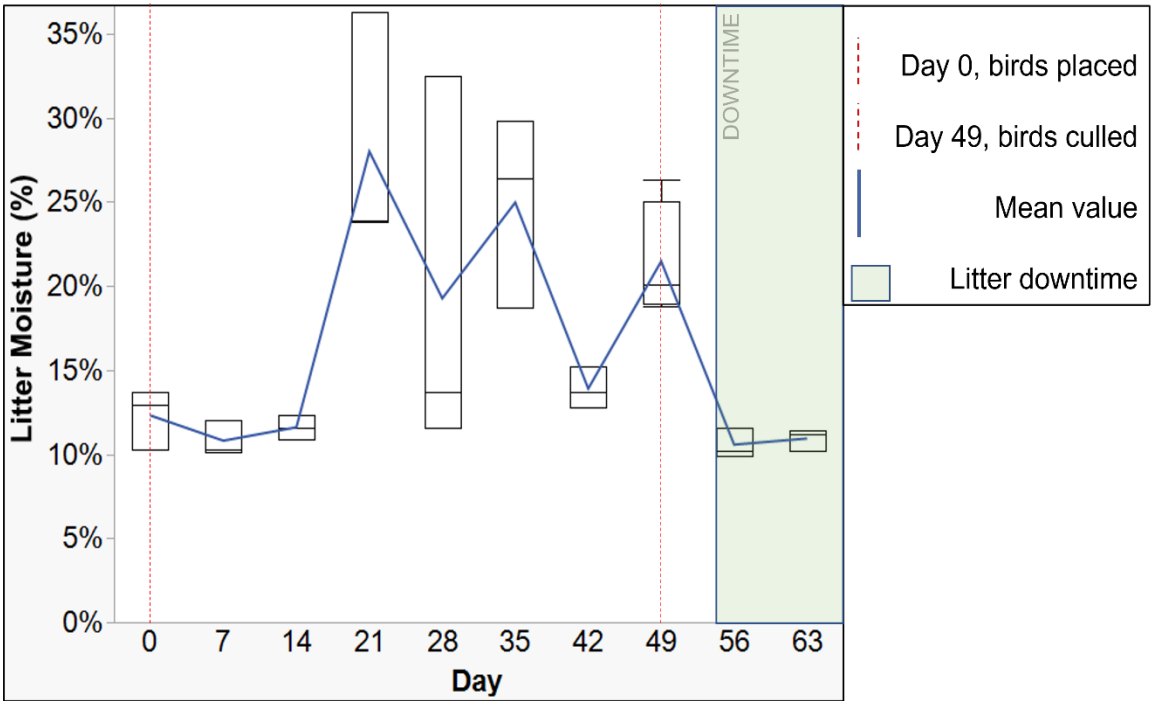

Figure 20. *Litter Moisture (%) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from three different house locations with one data point per location or a total of n = 3 data points per sampling event except at day 0 where n = 6. Results are reported in (%). Error bars represent standard error. Significance was set to ($P \leq 0.05$).

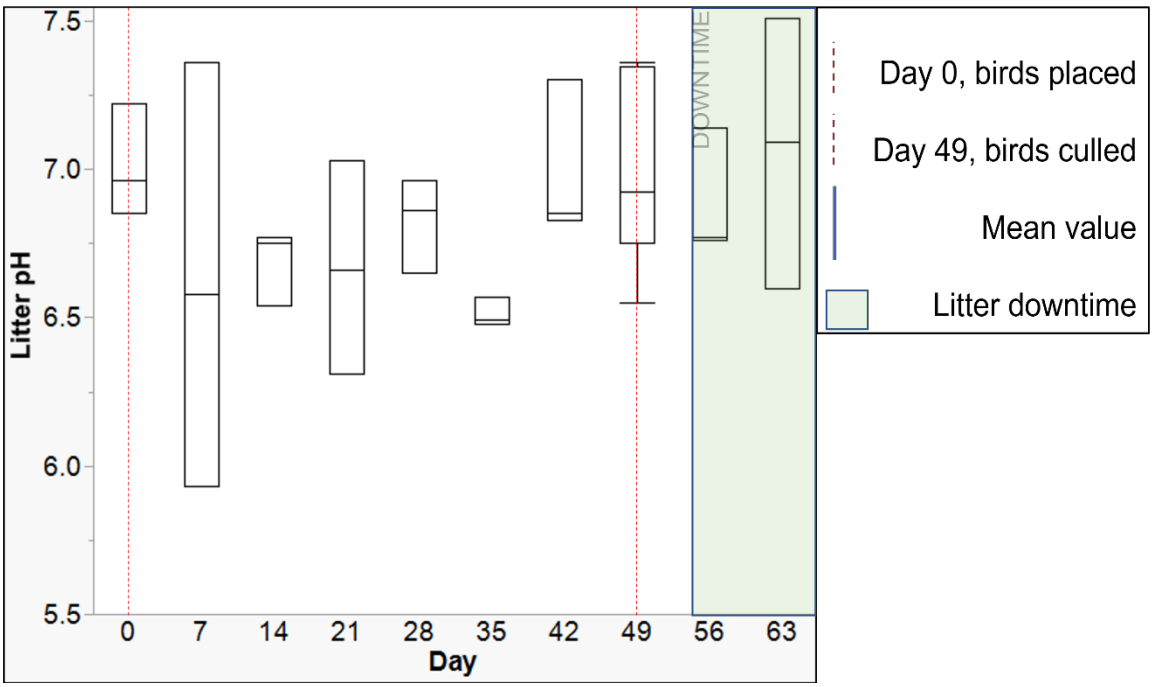

Figure 21. *Litter pH weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent average values from three different house locations with one data point per location or a total of n = 3 data points per sampling event except at day 0 where n = 6. Results are reported in pH units. Error bars represent standard error. Significance was set to ($P \leq 0.05$).

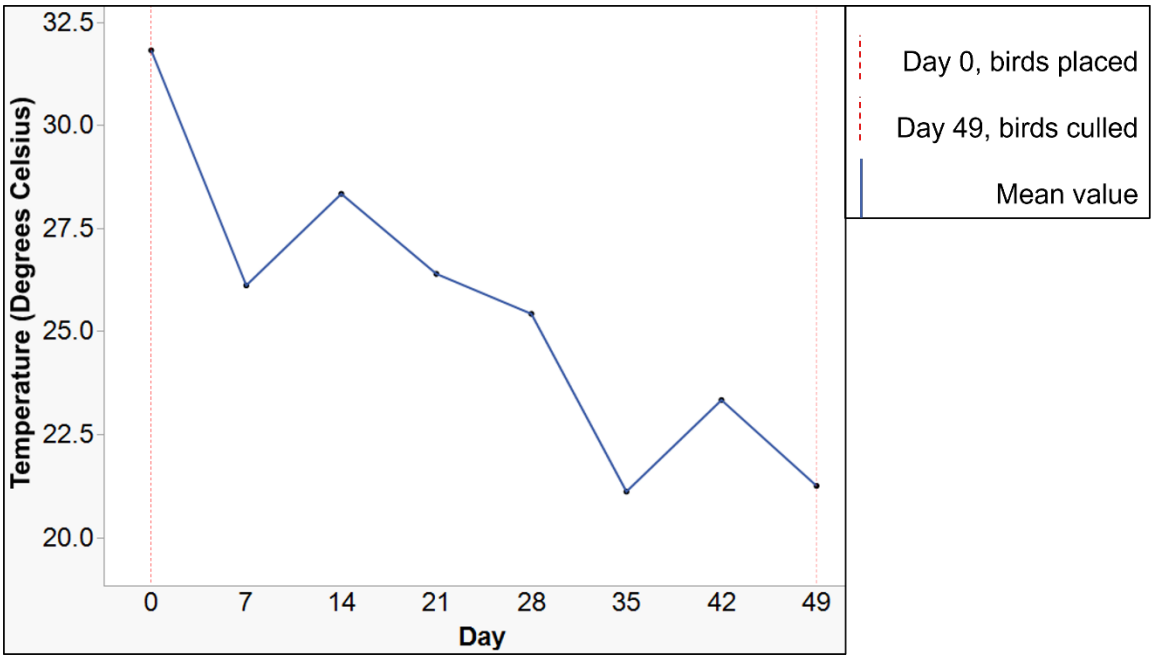

Figure 22. *Broiler house Temperature (Degrees Celsius) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent one sensor reading at the time of sampling events, so n = 1 for each data point. Results are reported in Degrees Celsius. Significance was set to ($P \leq 0.05$).

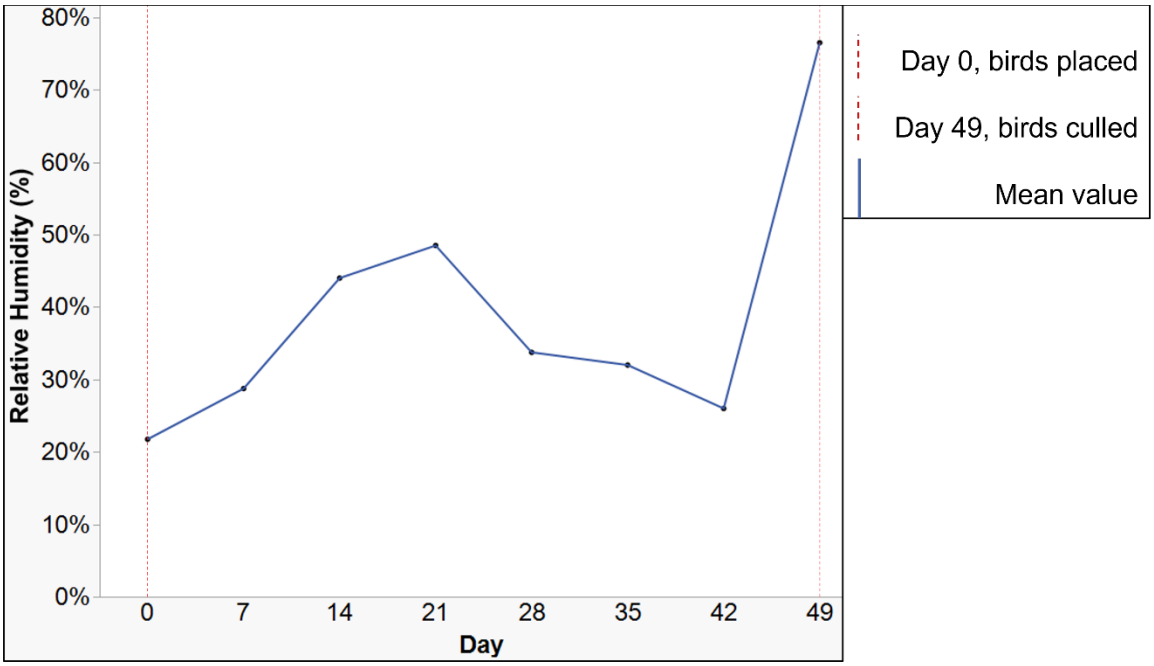

Figure 23. *Broiler house Relative Humidity (%) weekly changes over the course of broiler chicken grow-out.* Weekly datapoints represent one sensor reading at the time of sampling events, so n = 1 for each data point. Results are reported in (%). Significance was set to ($P \leq 0.05$).

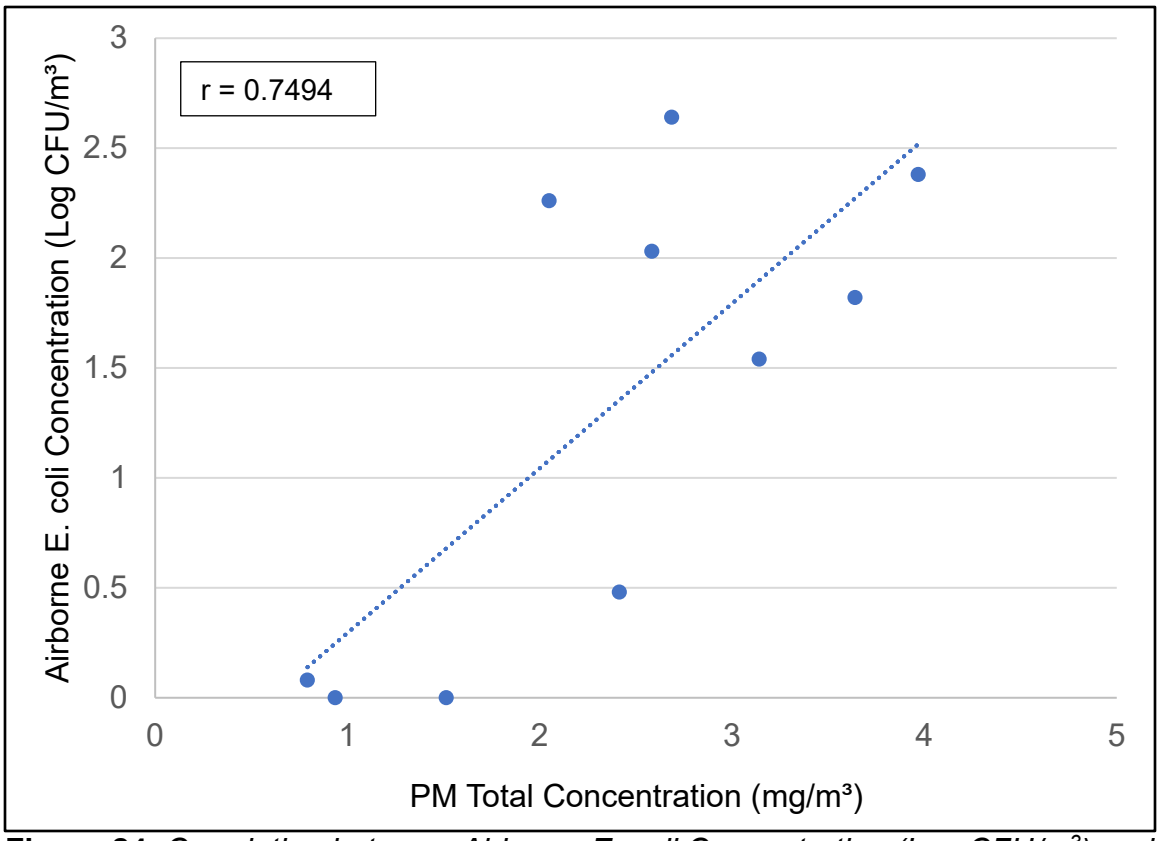

Figure 24. *Correlation between Airborne E. coli Concentration (Log CFU/m^3) and PM Total Concentration (mg/m^3).* For correlations, weekly averages for airborne *E. coli* concentration (Log CFU/m^3) and PM total concentration (mg/m^3) were calculated for pairwise comparisons. The correlation coefficient was found to be r = 0.7494.

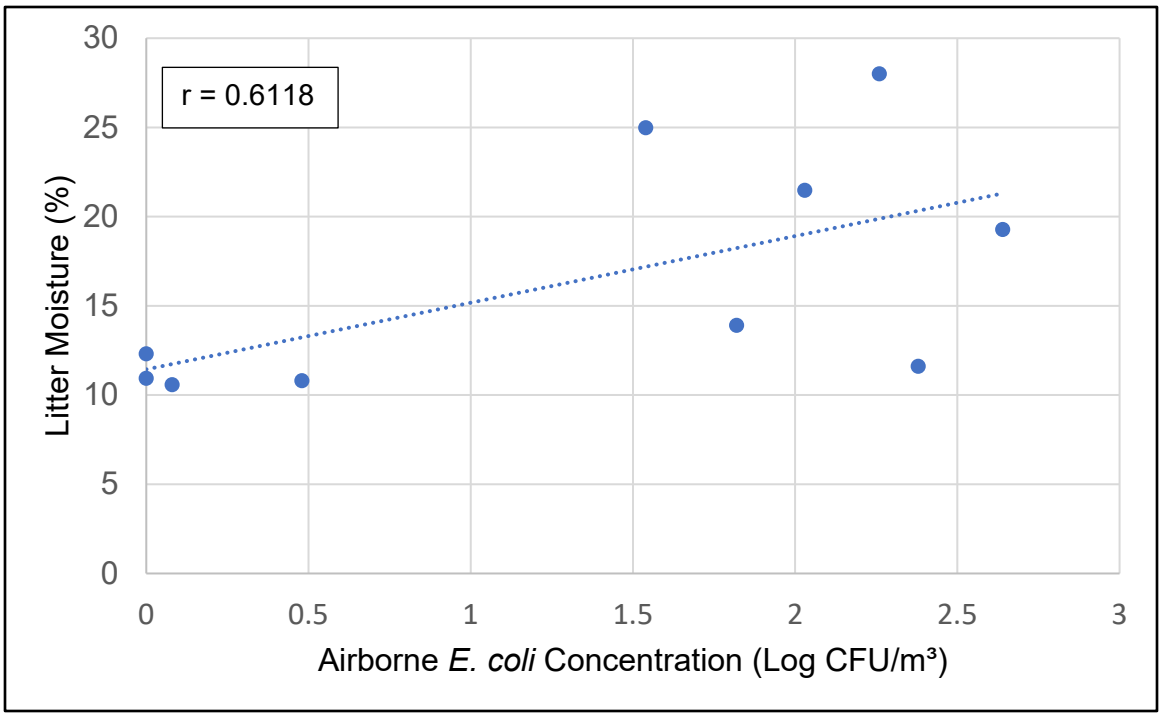

Figure 25. *Correlation between Litter Moisture (%) and Airborne E. coli Concentration (Log CFU/m^3)* For correlations, weekly averages for airborne *E. coli* concentration (Log CFU/m^3) and litter moisture (%) were calculated for pairwise comparisons. The correlation coefficient was found to be r = 0.6118.

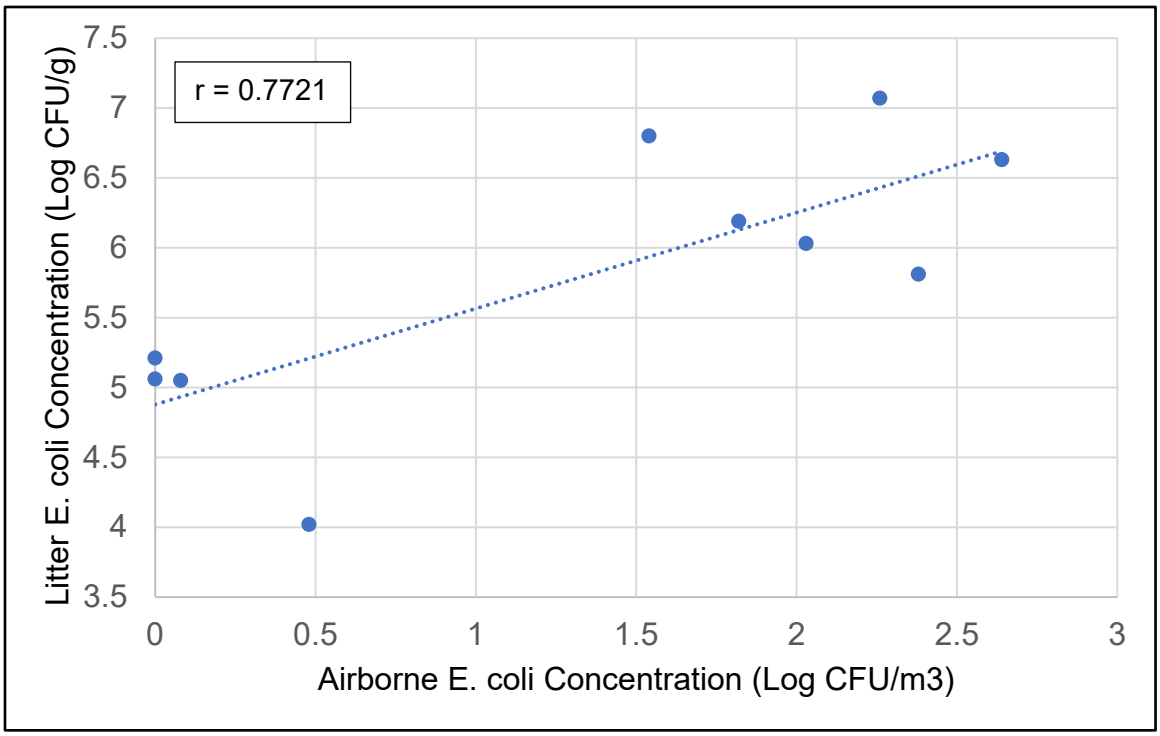

Figure 26. *Correlation Between Litter E. coli Concentration (Log CFU/g) and Airborne E. coli Concentration (Log CFU/m^3).* For correlations, weekly averages for airborne *E. coli* concentration (Log CFU/m^3) and litter *E. coli* concentration (Log CFU/g) were calculated for pairwise comparisons. The correlation coefficient was found to be r = 0.7721.

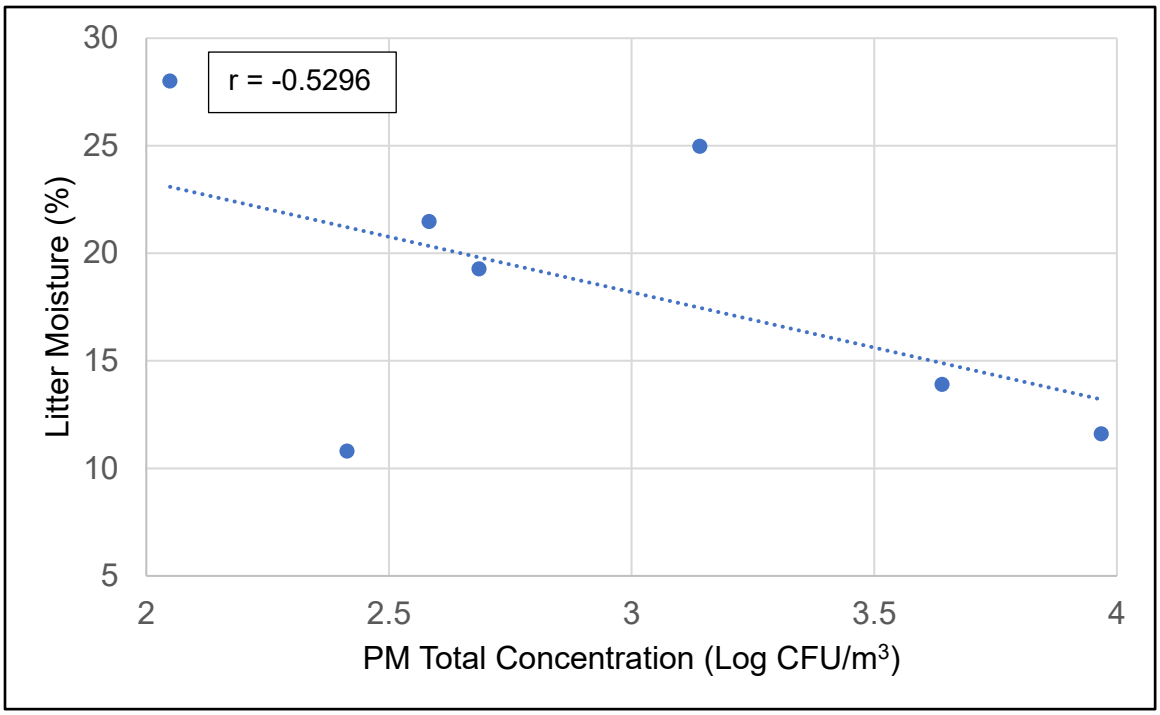

Figure 27. *Correlation between Litter Moisture (%) and PM Total Concentration (mg/m^3).* For correlations, weekly averages for airborne PM Total Concentration (mg/m^3) and litter moisture (%) were calculated for pairwise comparisons. The correlation coefficient was found to be r = -0.5296.

CHAPTER 4: ANTIBIOTIC RESISTANT PROFILE OF AIRBORNE *E. COLI* RECOVERED DURING BROILER CHICKEN GROW-OUT UNDER "RAISED WITHOUT ANTIBIOTICS" PRODUCTION[2]

[2] G. Zock, A. Oladeinde, S. Aggrey, J. Johnson, Y. Guo, D. Cudnik, J. Lawrence, and L. Chai. To be submitted to *Poultry Science*.

ABSTRACT

Commercial broiler production has conventionally used large amounts of antibiotics for both growth promotion and the therapeutic treatment of bacterial infections. The general shift towards antibiotic-free live broiler production indicates the need for active research on AB resistant commensal bacteria that may persist in broiler houses under “Raised Without Antibiotics (RWA)” production. This study analyzed airborne *E. coli* isolates recovered at different time points and locations in the broiler house over the course of a broiler grow-out. CHROMagar plates we placed open at twelve sampling locations in duplicate (n = 24) for twelve minutes weekly for 7 weeks. Air particles passively settled on the plate and were incubated overnight. Up to two airborne *E. coli* isolates were recovered per plate for a total of 228 over the course of the grow-out. One-hundred and twenty-nine selected airborne *E. coli* isolates from unique time points, sampling locations, and CHROMagar plates were used to determine the antibiotic resistant profile. Using broth microdilution, a bacterial suspension of *E. coli* at a known concentration was prepared and placed into wells with varying increasing concentrations of antibiotics. A Gram-Negative Sensitre™ plate with 14 different antibiotics was used to determine the minimum inhibitory concentration of each drug on airborne *E. coli* isolates. Of the 129 isolates, 89 were susceptible to all antibiotics tested. Eleven isolates displayed resistance to one drug, 16 isolates displayed resistance to two drugs, and 13 isolates displayed resistance to 3 or more drugs. Of the 14 drugs tested, the most common resistance was to Tetracycline which appeared in 28 of the 40 isolates.

There was no significant difference between susceptible or antibiotic resistant airborne *E. coli* and the height at which they were recovered ($P > 0.05$). No antibiotic resistant airborne *E. coli* isolates were found on day 0, 56, or 63. On day 21, 9 of the 40 antibiotic resistant isolates were recovered. Results demonstrate that antibiotic resistant airborne bacteria persist in a poultry environment despite being RWA production. Furthermore, they are present at all heights within the broiler house for the majority of the grow-out.

INTRODUCTION

Commercial broiler production has conventionally used large amounts of antibiotics for both growth promotion and to therapeutically treat bacterial infections. Antibiotics used to treat disease may be administered for a short time at higher concentrations, while growth promoting antibiotics can be used as feed additives at a lower concentration throughout the grow-out regardless of the presence of disease or pathogens (Chhedi Lal Gupta, et al., 2021). There is an epidemic increase in antibiotic resistant bacteria in health care systems, and food animals have been linked to the increase in resistance. Fluoroquinolones, for example, were used to treat respiratory diseases in poultry. As a result, fluoroquinolone-resistant zoonotic pathogens like *Camplyobacter* sp. increased. Because of the important role fluoroquinolones play in medicine, this led to a ban in use of this drug for food animal production in the USA and EU (Chhedi Lal Gupta, et al., 2021). Other countries like China and Brazil continue to use this class of antibiotics, and they have over 40% antibiotic resistant *E. coli* from poultry versus below 5% in the USA (Roth, Nataliya, et al., 2019). There has been a major push in the poultry industry towards “Raised Without Antibiotics” production which is estimated to exceed 50% of annual poultry production in the USA, including for this study (Singer, Randall S., et al., 2020). This means no antibiotics would be used at any point during the grow-out of broilers.

Simply removing antibiotics may not be enough to eradicate the problem of antibiotic resistant bacteria. Antibiotic resistant bacteria can be found in abundance in the air and poultry litter. Although there are trends that show

antibiotic usage can increase levels of antibiotic resistance (Brooks, J. P., et al., 2010), complete removal of drugs from the production system does not guarantee that antibiotic resistant bacteria will disappear. Genes may persist either through resistance reservoirs in commensal bacteria, plasmid transfer for ulterior purpose, or other mechanisms not fully understand. Antibiotics alone are not the sole reason for the development, spread, and persistence of antibiotic resistant genes. One potential source of continued antibiotic resistance is the presence and addition of heavy metal into feed for disease control and quick growth. There is evidence to support that antibiotic resistant genes were co-selected due to the presence of heavy metals such as zinc, copper, and cadmium (Mazhar, Sohaib H., et al., 2021). In one study under “Raised Without Antibiotics” production, whole genome sequencing revealed that a commensal *E. coli* population was the main reservoir for a plasmid carrying antibiotic resistance that was horizontally transferred to *Salmonella* Heidelberg (Oladeinde, Adelumola, et al., 2021).

Most *E. coli* are harmless and an important part of a healthy gastro-intestinal tract, but certain types of *E. coli* carry virulence genes and antibiotic resistance that can be harmful to humans and animals. *Escherichia coli* (ETEC) produce toxins stimulate the lining of the intestines which can cause diarrhea, dysentery, cramps, and fever (Qadri, F., et al., 2005). Shigatoxigenic and verotoxigenic *E. coli* (STEC), (VTEC), are strains of *E. coli* that produce Shiga toxin and verotoxin. These toxin producing strains are commonly known as enterohemorrhagic *E. coli* (EHEC) and are responsible for ailments such as

bloody diarrhea and hemolytic uremic syndrome (Esperandio, Vannesa, and Ye Nguyen, 2012). Avian pathogenic *Escherichia coli* (APEC) are a class of pathogens that cause avian colibacillosis, one of the biggest diseases harming global poultry industries. It is also a public health concern as it is one of the most common avian diseases that is communicable to humans (Lutful Kabir, S. M, 2010). EHEC, ETEC, and APEC have been found in commercial poultry settings, and when tested for antibiotic resistance, these pathogenic strains of *E. coli* often carry antibiotic resistance to wide range of drugs. The toxigenic effects and antibiotic resistance of pathogenic strains of *E. coli* pose a threat to the poultry industry along with animal and human safety (Lee, Gi Yun, et al., 2009; Bashar T, Rahman M, et al., 2011; El-Rami, Fadi, et al., 2012).

Despite previous studies showing antibiotic resistant airborne bacteria in a poultry house, there are gaps in the research profiling how these values change over the course of the grow-out and at different locations in the house. Additionally, the literature is sometimes unclear whether antibiotics have been used during the grow-out of the broiler's life. The purpose of this study is to 1) determine the antibiotic resistant phenotype of recovered airborne *E. coli* isolates 2) determine ratios of drug resistance and to which classes of antibiotics *E. coli* resists the most readily 3) determine how spatial and temporal changes effect levels of antibiotic resistant airborne *E. coli*. The central hypothesis to this portion of the study is that antibiotic resistant *E. coli* will persist despite Raised Without Antibiotics production.

MATERIALS AND METHODS

Airborne E. coli Isolation and Recovery

E. coli recovered on CHROMagar (Paris, France) plates as described in "*Airborne E. coli and Coliforms Concentration Determination* in Chapter 3 on Page xx*"* were selected for isolation. Agar plates were placed in duplicate at each sampling location for 12 minutes and incubated at 37° C overnight. Each replicate had up to two random *E. coli* colonies selected for isolation. Colonies were re-struck onto fresh CHROMagar plates and incubated overnight. The next day, isolated colonies from the re-struck plates were once again struck onto fresh CHROMagar plates and incubated overnight. This was repeated until discrete, uncontaminated *E. coli* colonies were able to be identified. One uncontaminated colony was struck onto Sheep's Blood Agar (SBA) (Thermo Fisher Scientific. Waltham, Massachusetts, USA) and incubated overnight for final growth and decontamination. Approximately five colonies were then taken from the SBA plates and placed in Luria-Bertani Brother with 30% glycerol (30% LB Glycerol) (Thermo Fisher Scientific. Waltham, Massachusetts, USA) for downstream analysis and long-term storage.

Antibiotic Susceptibility Testing of Airborne E. coli Isolates

E. coli isolates were removed from long-term storage and struck from 30% LB Glycerol onto fresh SBA plates and allowed to incubate overnight. A single isolated colony was struck once more onto fresh SBA plates and incubated overnight to insure there are not contaminants. Isolates from this plate were used in a Gram-Negative Antibiotic-Susceptibility Testing (AST) panel against 14

antibiotics. Isolates from these plates were also used for downstream DNA analysis for consistency with the AST. A Gram-negative Sensitire™ panel (CMV#AGNF, ThermoFisher Scientific) was prepared for *E. coli* isolates according to manufacturer instructions. Isolated colonies were selected from SBA and resuspended in 5 mL of demineralized water. Suspension was adjusted to a 0.5 McFarland equivalent using the Sensitre nephelometer. Ten µL of the suspension was transferred to a tube containing Sensitre Cation-Adjusted Mueller-Hinton Broth with TES. Fifty µL of solution was dispensed into each well of the Gram-negative Minimum Inhibitory Concentration (MIC) panel. Plates were incubated at 35 ±1ºC for 18 hours. Plates were visually inspected to determine the concentration of antibiotic to display the MIC to determine whether an isolate is susceptible or resistant to the antibiotic tested.

RESULTS

At each sampling event, duplicate CHROMagar plates were placed at each of the 12 sampling locations across the house for a total of 24 CHROMagar plates per week. If *E. coli* were present on the plates after incubation, up to two separate colonies were selected randomly per plate. Two-hundred and twenty-eight airborne *E. coli* colonies were isolated over the course of the 49 day broiler grow-out and 14 day litter downtime. One-hundred and twenty-nine representative airborne *E. coli* isolates were chosen from different days and locations in the house for antibiotic susceptibility testing (AST). Each *E.* coli isolate tested was selected from a unique day, sampling location, and CHROMagar plate.

The standard Gram-negative Sensitire AST panel tests for 14 antibiotics. Resistance to 10 antibiotics were found across airborne *E. coli* isolates (Table 7).

Testing revealed 89 (~69%) of *E. coli* were susceptible to the 14 antibiotics in the panel. Forty airborne *E. coli* isolates (~31%) exhibited resistance to at least one antibiotic tested. Of the 40 airborne *E. coli* isolates displaying antibiotic resistance, 11 (27.5%) displayed resistance to only one drug. Sixteen isolates (40%) displayed resistance to two drugs. Thirteen isolates (32.5%) displayed multidrug resistance to either tetracyclines, fluoroquinolones, beta-lactams, aminoglycosides, or sulfonamides (Table 7; Fig. 29; Fig 32).

Airborne *E. coli* isolates were recovered from bird level, human level, and upper ventilation levels. There were no significant differences found between

isolates displaying antibiotic resistance and the height at which they were recovered (Fig 28).

The most common drug resistance displayed in airborne *E. coli* isolates was Tetracycline, which was found in 28 of the 40 resistant isolates (70%). Ampicillin and Streptomycin were the next most common drugs resisted with resistance being displayed in 17 of the 40 resistant isolates for each drug (42.5%). Gentamicin and Nalidixic acid followed as the next most resisted drugs with resistance being displayed in 10 of the 40 isolates for each drug (25%). Four isolates displayed resistance to Sulfizoxazole (10%). Three isolates displayed resistance to Ceftriaxone (7.5%). Two isolates each displayed resistance to Amoxicillin–Clavulanic Acid and Cefoxitin (5%). Only one isolated displayed resistance to Chloramphenicol (2.5%). No recovered airborne *E. coli* isolate displayed resistance to Azithromycin, Ciprofloxacin, Meropenem, or Trimethoprim– Sulfamethoxazole.

The classes of antibiotics that were resisted belonged to Tetracyclines, Aminoglycosides, Beta-Lactams, Fluoroquinolone, or Sulfonamides. The most resisted class was Tetracyclines with 28 of 40 isolates displaying resistance (70%). Aminoglycosides were the next most resisted with 24 of 40 isolates displaying resistance (60%). The Beta-lactam class was resisted by 17 of the 40 isolates displaying resistance (42.5%). The Fluoroquinolone class was resisted by 10 of the 40 isolates displaying resistance (25%). The Sulfonamide class was resisted by 4 of the 40 isolates displaying resistance (10%) (Fig. 32).

No antibiotic resistant airborne *E. coli* isolates were recovered on day 0, 56, or 63. Four antibiotic resistant isolates were determined from day 7. Six antibiotic resistant isolates each were determined from day 14 and 21. Nine antibiotic resistant isolates were determined on day 28. Five antibiotic resistant isolates were determined on day 35. Seven antibiotic resistant isolates were determined on day 42. Three antibiotic resistant isolates were determined on day 49 (Fig. 31).

DISCUSSION AND CONCLUSION

It has been demonstrated that antibiotic resistant bacteria persist in poultry broiler houses despite the push for Raised Without Antibiotics production and the absence of antibiotics during a broiler grow-out (Mazhar, Sohaib H., et al., 2021; Oladeinde, Adelumola, et al., 2021). This study aimed to determine the antibiotic resistant phenotype of airborne *E. coli* populations. We also aimed to determine how spatial and temporal changes during grow-out affected levels of antibiotic resistant airborne *E. coli*. Two-hundred and fifty-eight airborne *E. coli* isolates were recovered during the broiler grow-out from different locations and heights. One-hundred and twenty-nine airborne *E. coli* isolates were selected for antibiotic susceptibility testing, and 40 isolates were determined to be resistant to one, two, or three or more antibiotics.

Other studies have recovered *E. coli* from litter, feed, water, and air to determine the overall resistance profile. It was found that ampicillin resistance was found in 55.81% of samples, and gentamicin resistance was found in 46.51% of samples (Md. Jannat Hossain, et al, 2020). No other antibiotics tested coincided with the 14 antibiotics tested in our study. Antibiotic resistant airborne *E. coli* in our study displayed resistance to ampicillin and gentamicin in 42.5% and 25% of samples, respectively. Gentamicin resistant frequency was higher in out study, and this could be contributed to the fact that the isolates used in their study did not solely come from airborne samples. *E. coli* can carry different antibiotic resistant genes and plasmids depending on their source (Md. Jannat Hossain, et al, 2020). Another study looked at the distribution of antibiotic

resistant genes in the chicken gut and found the three most common classes of antibiotics resisted to be tetracyclines, macrolides, and aminoglycosides (Juricova, H., et al., 2021). The two most common classes of antibiotics resisted in our study were tetracyclines and aminoglycosides. This suggests that that a major source for antibiotic resistant *E. coli* isolates could be a result of feces dropped from broilers.

In *E. coli*, tetracycline resistance is regulated by genes that are associated with plasmids that often carry other antibiotic resistance genes, heavy metal resistance genes, and pathogenic factors like toxins (Diarrassouba, F., et al., 2007). Tetracyclines were commonly used in agriculture as a feed additive for growth promotion in poultry which surpassed quantities used of all other antibiotic classes (Granados-Chinchilla, Fabio, and César Rodríguez., 2017). Despite being banned as a feed additive in some countries, like Canada, high levels of tetracycline resistance persist in commercial poultry. One study found 74% of recovered *E. coli* isolates were resistant to tetracyclines, which is comparable to the 70% of antibiotic resistant airborne *E. coli* resistant to tetracyclines in our study. Tetracycline is one of the few antibiotics used to treat *E. coli* airsacculitis which may have given rise to high levels of resistance (Singer, R. S., and C. L. Hofacre., 2006). Furthermore, tetracycline resistance was reported in 87% of avian pathogenic *E. coli* (Zhao, Shaohua, et al., 2005). This is cause for concern as *E. coli* can act as reservoirs for these virulence factors and transfer them via horizontal gene transfer to other bacteria.

A study determining the antibiotic resistance profiles for airborne and litter bound *E. coli* on a farm using antibiotics for therapeutic reported several results that supported observations in our study. For example, airborne *E. coli* concentrations were very low when there were not birds present and increased during flock grow-out. Consequently, antibiotic resistant presence started low in recovered airborne *E. coli* isolates and increased during flock grow-out. Antibiotic resistant *E. coli* from the poultry litter remained stable throughout their experiment despite flock presence, so this suggests that antibiotic resistant bacteria may not persist well in the harsh aerosol environment and may require constant aerosolization of antibiotic resistant litter *E. coli* (Brooks, J. P., et al., 2010). Overall, antibiotic resistance levels of recovered airborne *E.* coli were found to be higher in their study (66%) compared to ours (31%), but this can be contributed to the fact that the broiler houses use antibiotics in their rearing process (Brooks, J. P., et al., 2010). Although we did not find differences in presence of antibiotic resistant airborne *E. coli* between measured heights, we did see that the number of isolates increased when the flock was present compared to day 0, 56, and 63. This result makes sense because airborne *E. coli* concentrations were significantly different by flock day, and there was no significant difference between airborne *E. coli* concentrations and height.

Limitations of this study are that data only represents results from one flock of broilers over the course of the grow-out. It would be beneficial to see the replicability and consistency of results and how other factors, such as season and poultry litter age would impact the results. We were in the process of

replicating this study for a second flock, but COVID-19 complications caused early field termination. Research was conducted in an experimental facility with significant differences to a commercial poultry facility such as number of birds, litter amount, stocking density, and ventilation. These results may be used as a model and can guide similar research to take place in a commercial facility.

Future studies may consider comparing the phenotypic antibiotic resistance of airborne *E. coli* to *E. coli* recovered from other sources such as the poultry litter, feed, water, chicken ceca, and meconium to understand levels of antibiotic resistance from all sources in the house. Whole genome sequencing can also be performed on selected antibiotic resistant *E. coli* isolates to determine antibiotic resistant genes, plasmids, and other virulence factors that may be present. Sequencing results will also be able to source where airborne antibiotic *E. coli* is coming from within the house by comparing to the genome of other recovered *E. coli*.

In conclusion, antibiotic resistant airborne *E. coli* remains to be present despite Raised Without Antibiotics production. There was no significant difference number of antibiotic resistant airborne *E. coli* between bird height, human height, and upper ventilation level ($P = 0.7594$) which indicates that antibiotic resistant airborne *E. coli* is present at all locations of the broiler house. No antibiotic resistant isolates were determined on days 0, 56, and 63, but levels increased from days 7-28 and decreased from days 28-49. Antibiotic resistant *E. coli* in poultry environments is still a public health concern despite declines in antibiotic usage.

TABLES

Table 7. *Fourteen antibiotics, corresponding class, and the presence of resistance in airborne E. coli isolates.*

Antibiotic	Class	Resistance
Amoxicillin–Clavulanic Acid (Amc)	**Penicillin (β-lactam)**	**Yes**
Ampicillin (Amp)	**Penicillin (β-lactam)**	**Yes**
Azithromycin	Macrolide	No
Cefoxitin (Fox)	**Cephalosporin (β-lactam)**	**Yes**
Ceftriaxone (Axo)	**Cephalosporin (β-lactam)**	**Yes**
Chloramphenicol (Chl)	**Aminoglycoside**	**Yes**
Ciprofloxacin	Fluoroquinolone	No
Gentamicin (Gen)	**Aminoglycoside**	**Yes**
Meropenem	Carbapenem	No
Nalidixic acid (Nal)	**Fluroquinolone**	**Yes**
Streptomycin (Str)	**Aminoglycoside**	**Yes**
Sulfizoxazole (Sox)	**Sulfonamide**	**Yes**
Tetracycline (Tet)	**Tetracycline**	**Yes**
Trimethoprim– Sulfamethoxazole	Sulfonamide	No

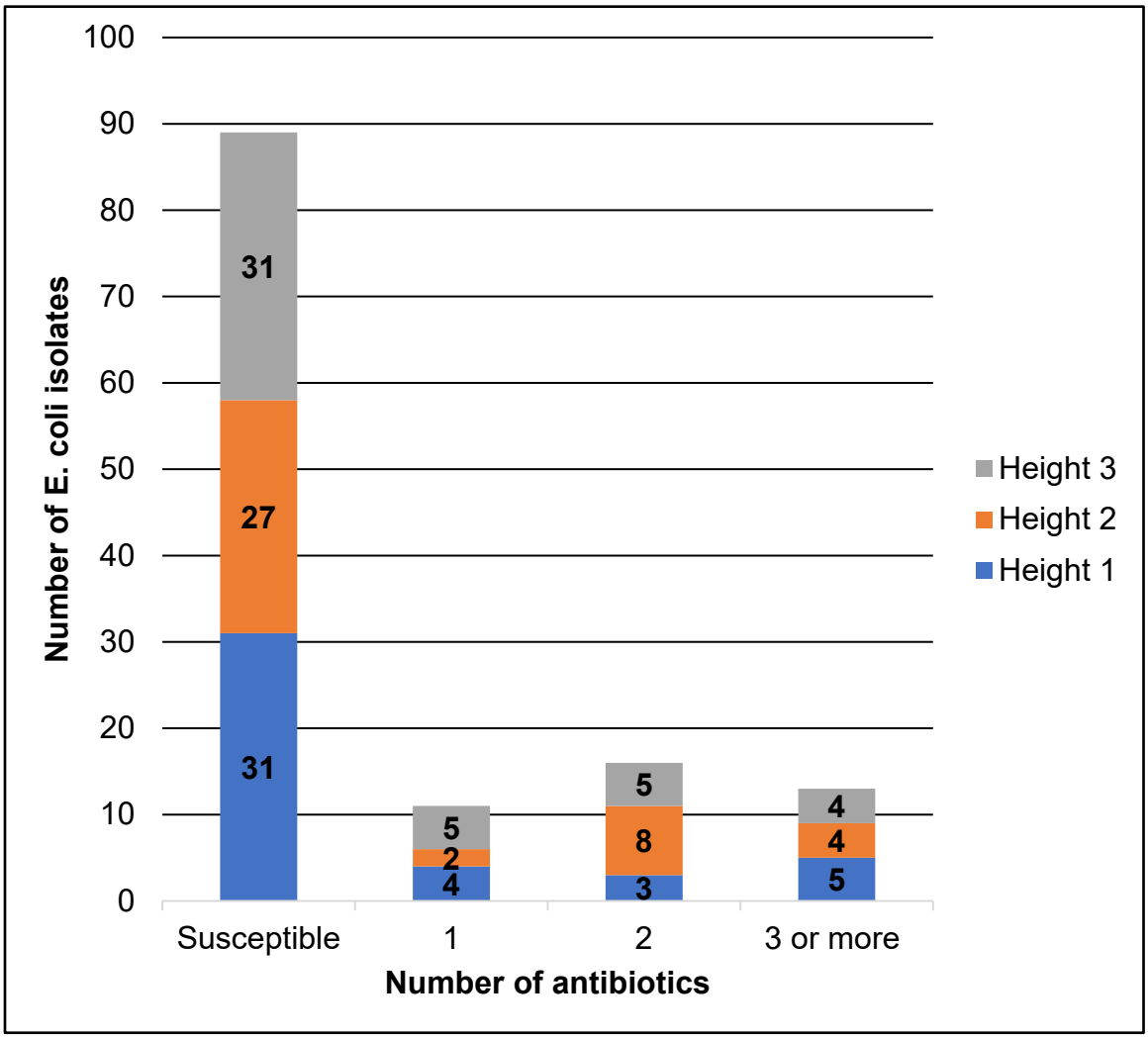

Figure 28. *Airborne E. coli isolates at different heights of the broiler house versus the number of antibiotics resisted.* Over the course of the broiler grow-out, 228 *E. coli* isolates were recovered from different timepoints, heights, and locations. One-hundred and twenty-nine representative isolates for chosen for antibiotic susceptibility testing. This figure shows the susceptibility or resistance to one, two, or 3 or more antibiotics from the height isolates were recovered.

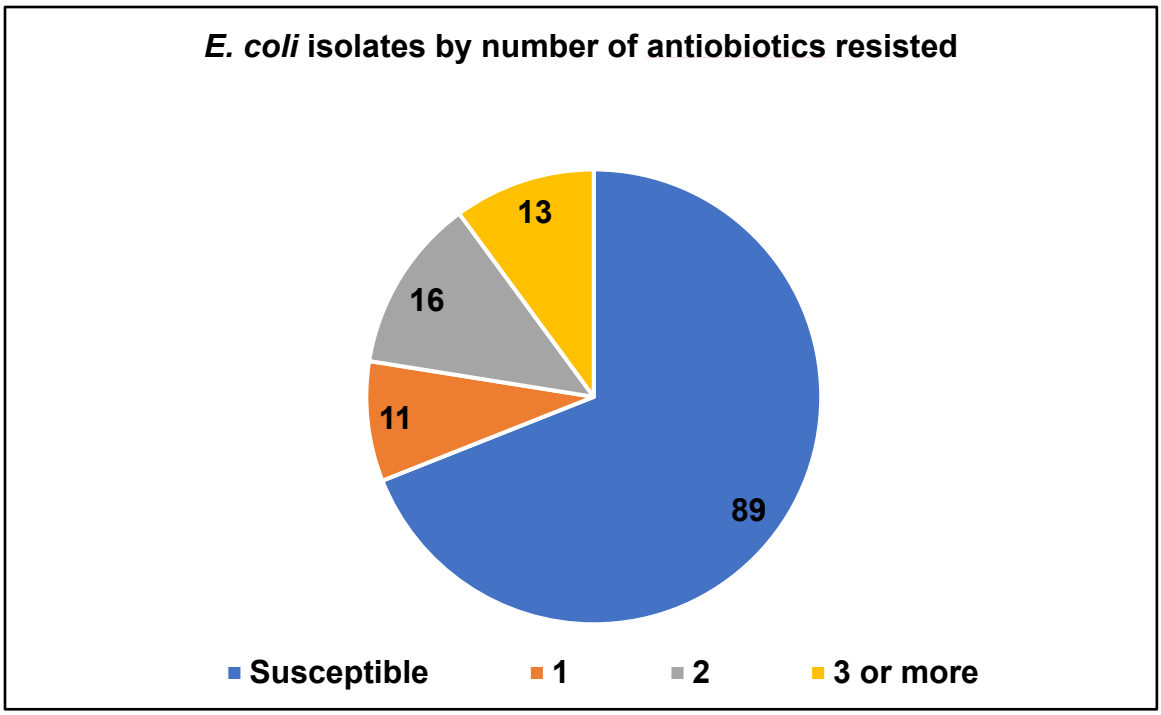

Figure 29. *Ratio of airborne E. coli isolates that are susceptible to antibiotics versus resistant to one, two, or 3 or more antibiotics. .* Over the course of the broiler grow-out, 228 airborne *E. coli* isolates were recovered from different timepoints, heights, and locations. One-hundred and twenty-nine representative isolates for chosen for antibiotic susceptibility testing. This figure shows the ratio of antibiotic resistant airborne *E. coli* isolates.

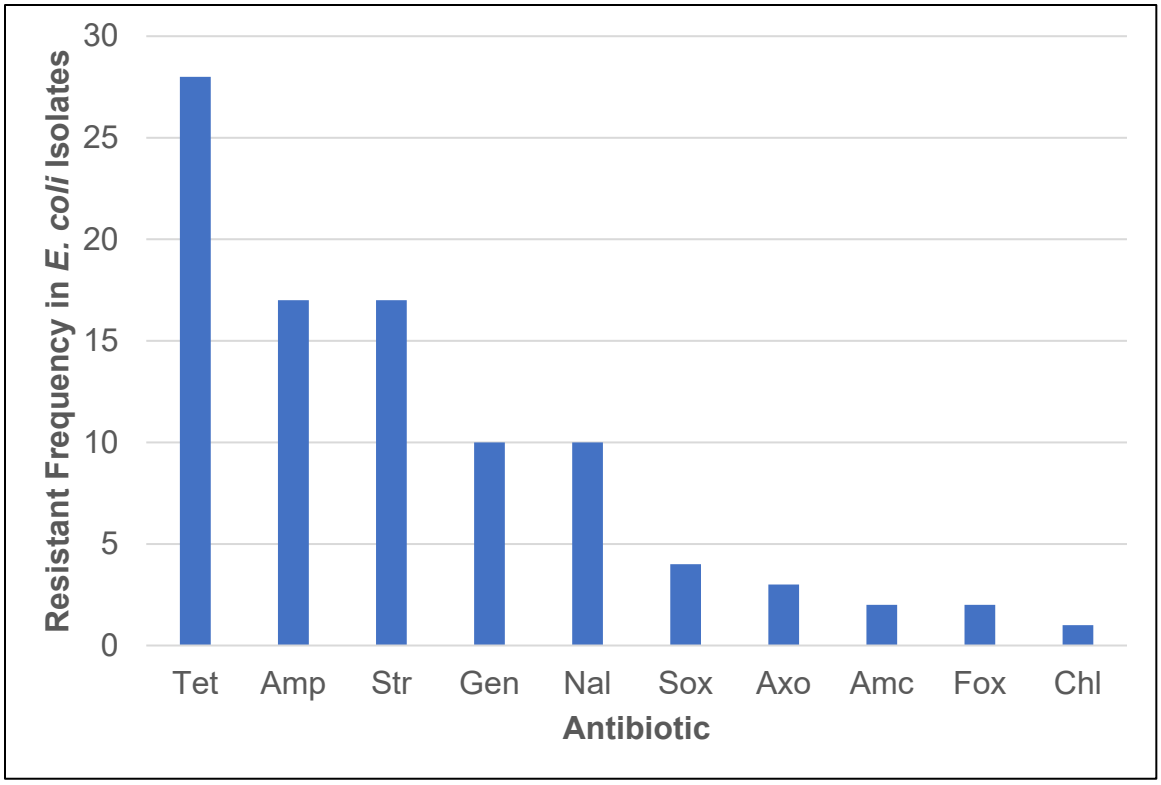

Figure 30. *Frequency of antibiotic resistance seen in airborne E. coli isolates displaying drug resistance.* Of the 129 airborne *E. coli* isolates tested for antibiotic susceptibility testing, 40 isolates displayed resistance to one, two, or three or more antibiotics. This figure shows the frequency at which resistances to specific drugs appear across airborne *E. coli* isolates. Tet = Tetracycline, Amp = Ampicillin, Str = Streptomycin, Gen = Gentamycin, Nal = Nalidix acid, Sox = Sulfizoxazole, Axo = Ceftraxone, Amc =Amoxicillin-Clavulanic Acid, Fox = Cefoxitin, Chl = Chloramphenicol.

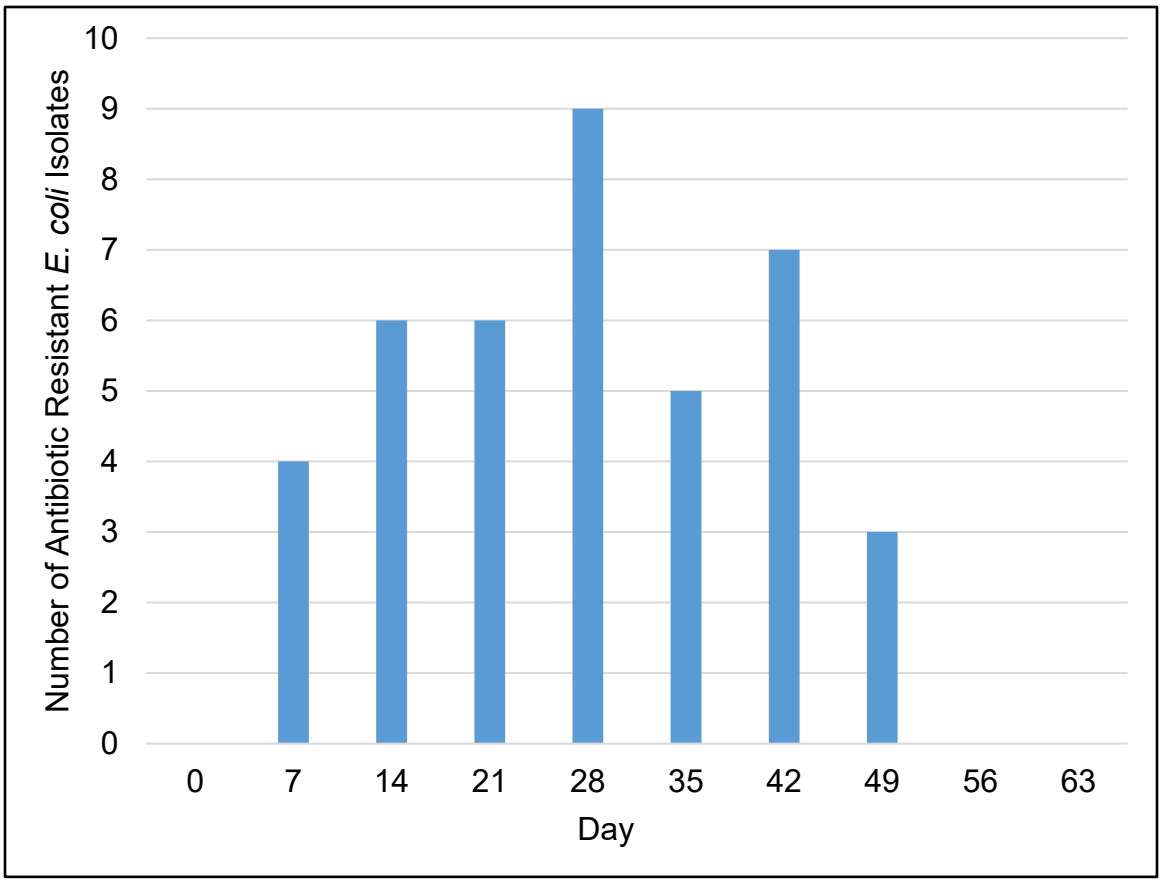

Figure 31. *Number of Antibiotic Resistant E. coli Isolates by grow-out day.* Of the 129 airborne *E. coli* isolates tested for antibiotic susceptibility testing, 40 isolates displayed resistance to one, two, or three or more antibiotics. This figure shows the frequency at which these antibiotic resistant *E. coli* isolates were recovered by day.

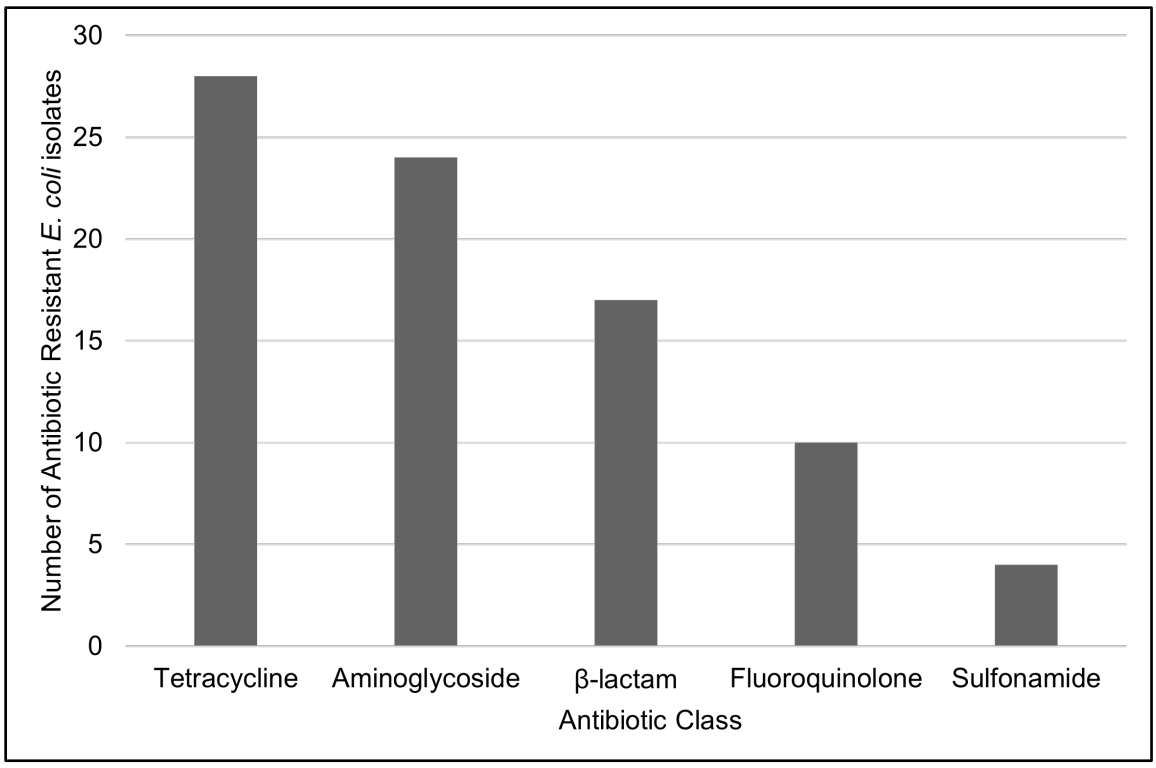

Figure 32. *Frequency of antibiotic class seen in airborne E. coli isolates displaying drug resistance.* Of the 129 airborne *E. coli* isolates tested for antibiotic susceptibility testing, 40 isolates displayed resistance to one, two, or three or more antibiotics. This figure shows the classes of antibiotics and the frequency at which antibiotic resistant airborne *E. coli* resister these classes.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Air quality relating to broiler production has been a concern for years, particularly regarding poultry health. Poor air quality can also be a major issue for handlers as well. Poultry workers have been shown to suffer from higher rates of respiratory problems which could have direct ties to elevated particulate matter levels (Viegas, S., et al., 2013). Bacteria can also play an impact in air quality as they can adhere to particles and transfer through the house with potential pathogenic transfer to birds and handlers (Pal, Amrit, et al., 2021). Factors such as stocking density, ventilation levels, and time of year can all affect air quality in the broiler house. Furthermore, factors such as ammonia, relative humidity, temperature, litter moisture, litter pH, and litter bacteria abundance all contribute to the complex air quality problems and may have compounding effects on particulate matter and airborne bacteria concentrations.

Particulate matter from agricultural practices is one of the leading causes of atmospheric PM (Shen et al. 2022). Specifically, from a poultry house, this can include feces, dust, feed, feathers, dander, and more. For an eight-hour workday, the Occupational Safety and Health Administration (OSHA) standards set total particulate matter levels to 15 mg/m^3 and respirable particles to 5 mg/m^3 (OSHA, 2006). Exposure guidelines for poultry recommend that average total particulate matter concentrations reach no higher than 5 mg/m^3 (University of Kentucky Agricultural Extension, 2009). Worker exposure to high levels of particulate

matter can cause throat and eye irritation, respiratory problems, asthma, and damage the immune system (Viegas, S., et al., 2013). Chickens raised in houses with high concentrations of PM have an increased risk of respiratory disease and increased mortality (Cambra-López, María, et al., 2010). Damage to the immune systems and respiratory ailments can elevate the risk of infection from pathogenic bacteria found within the broiler house.

A broiler house is full of different types of microorganisms including fungi, bacteria, and viruses. Many of the bacteria are mostly harmless and can even be beneficial within the poultry environment. Specific bacteria can aid digestion and nutrient uptake, competitively exclude pathogens, and decompose waste organic matter (Sauter, E. A., et al., 1981). Pathogenic bacteria can contaminate a poultry house and produce harmful endotoxins that can cause diarrhea, fever, vomiting, abdominal cramps, and in some extreme cases can be fatal (Adil, S., and S. N. Magray, 2012; Scallan, E., et al., 2013). Both beneficial and pathogenic bacteria have the capacity to aerosolize and become airborne.

This study focuses on the presence of airborne coliforms and *E. coli* as a sentinel for pathogenic presence. Coliforms and *E. coli* are often considered indicator organisms in certain environments because it shows conditions that may be favorable for enteric pathogens like *Escherichia, Salmonella, Shigella,* and *Campylobacter* (Halkman, H. B. D., and A. K. Halkman, 2014). Coliforms are Gram-negative lactose fermenting bacteria that span several genera (Carl A. Batt, Pradip Patel, 2014). Total coliforms include fecal and non-fecal coliforms.

Fecal coliforms like *E. coli*, are coliforms that naturally occur in the intestines of chickens while other coliforms are associated with plant material.

Escherichia coli is a Gram-negative facultative anaerobe that is often commensal in the intestines of birds and spread widely through feces. Birds in a poultry house are continuously exposed to *E. coli* contaminated poultry litter, feces, dust, and water. Damage to a bird's disease resistance can allow for more pathogenic strains of *E. coli* to infect the bird (Charlton, B. R., et al., 2006). Most *E. coli* are harmless and an important part of a healthy gastro-intestinal tract, but certain types of *E. coli* carry virulence and antibiotic resistant genes that can be harmful to humans and animals. (Lutful Kabir, S. M, 2010).

Certain strains of virulent *E. coli* contain genes that classify them as avian pathogenic *E. coli* which causes colibacillosis. This is one of the leading causes of morbidity and mortality in broiler chickens (Diarrassouba, F., et al., 2007). Furthermore, colibacillosis is a public health concern as it is one of the most common avian diseases that is communicable to humans (Lutful Kabir, S. M, 2010). Antibiotics are now commonly used to treat avian colibacillosis which may lead to an increase in antibiotic resistant *E. coli* (Bass, L., et al, 1999). Furthermore, avian pathogenic *E. coli* commonly carry resistance to antibiotics such as tetracycline (Zhao, Shaohua, et al., 2005).

With an epidemic increase in antibiotic resistance, there has been a push remove antibiotics as a feed additive for growth promotion, and now many countries only use antibiotics as therapeutic treatment for disease. Additionally, Raised Without Antibiotics production is estimated to exceed 50% of annual

poultry production in the USA today (Singer, Randall S., et al., 2020). Despite reductions in antibiotic usage and removal from husbandry practices completely, antibiotic resistant bacteria continue to persist. Antibiotics can affect commensal bacteria alongside pathogenic bacteria, and as a result, commensal bacteria can act as reservoirs for antibiotic resistant genes that can be transferred to other bacteria via horizontal gene transfer (Juricova, H., et al., 2021). It has been shown that commensal *E. coli* acts as one of the main reservoirs of a plasmid carrying antibiotic resistance that can be transferred to *Salmonella* Heidelberg via horizontal gene transfer (Oladeinde, Adelumola, et al., 2021). Furthermore, other factors, such as heavy metal resistance, could be a co-selector for antibiotic resistant genes. The addition of heavy metals such as zinc and copper into feed for growth promotion and microbial control may cause bacteria to acquire plasmids that carry heavy metal resistant genes and antibiotic resistant genes on the same plasmid (Mazhar, Sohaib H., et al., 2021).

This project examined particulate matter, airborne coliform, and airborne *E. coli* concentrations over the course of a broiler chicken grow-out. Different locations and heights in the house were also measured to determine how concentrations differ spatially. Temperature, relative humidity, litter moisture, litter pH, and litter *E. coli* abundance were also measured to determine correlations between these factors and PM and airborne bacteria concentrations. Furthermore, this project recovered airborne *E. coli* isolates at different locations within the house over the course of the grow-out to determine the antibiotic resistant profile against 14 different drugs. All results can be used to determine

what days of the grow-out and locations in the house may be most problematic for air quality. Results can also be used to help focus remediation efforts based on factors influencing particulate matter and airborne bacteria concentrations. Based on previous research, we hypothesized that grow-out day and height would have a significant impact on PM and airborne bacteria concentrations. We also hypothesized that other house environmental factors would be significantly correlated with the change in concentrations over the course of the grow-out. Finally, we hypothesized that antibiotic resistant *E. coli* would persist despite Raised Without Antibiotics production.

Chapter 3 determined the particulate matter and airborne bacteria profile over the course of the grow-out at different locations in the house. We also analyzed other house parameters such as relative humidity, temperature, litter pH, litter moisture, and litter *E. coli* abundance to determine correlations with PM and airborne bacteria concentrations. Results from this study demonstrated that PM_1, $PM_{2.5}$, PM_4, and PM Total concentrations were significantly higher at bird height than human height. Concentrations of all PM concentrations are significantly higher during days of broiler grow-out versus days 0, 56, and 63. Airborne *E. coli* concentrations are significantly higher during days of broiler grow-out versus days 0, 56, and 63. There was no significant difference between airborne *E. coli* concentrations and different heights. There was a significant difference between airborne coliform concentrations and different heights. There were positive correlations between PM total concentration and airborne *E. coli* concentration, litter moisture and airborne *E. coli* concentration, and litter *E. coli*

concentration and airborne *E. coli* concentration. There was a negative correlation between litter moisture and PM total concentration.

Chapter 4 determined the antibiotic resistant profile of recovered airborne *E. coli* isolates over the course of the grow-out at different locations in the house. Results showed that a majority (~69%) of recovered airborne *E. coli* isolates were pan susceptible to all 14 antibiotics tested. Approximately 31% of recovered airborne *E. coli* isolates displayed antibiotic resistance to 1, 2, or 3 or more antibiotics. There was no significant difference between antibiotic resistant *E. coli* isolates and the height at which they were recovered. Antibiotic resistant airborne *E. coli* isolates were not determined on days 0, 56, and 63, showing that flock grow-out day plays an important factor in levels of antibiotic resistant airborne *E. coli*. The three most common antibiotics that isolates displayed resistance to were tetracycline, ampicillin, and streptomycin. The three most common classes of antibiotics that isolates displayed resistance to were Tetracyclines, Aminoglycosides, and Beta-lactams.

In conclusion, there are significant differences in PM and airborne bacteria concentrations for days of the flock grow-out, where days 7-49 had higher concentrations than days 0, 56, and 63 when no birds were present. Height can play a significant role in certain levels of PM and airborne bacteria concentration and should be taken into consideration for air quality assessment. Litter moisture and litter *E. coli* concentrations had positive correlative effects on PM and airborne bacteria concentrations. Finally, antibiotic resistant airborne *E. coli* persist at all heights of the broiler house despite Raised Without Antibiotics

production with differences shown between day of flock grow-out. Elevated levels of PM concentration, airborne *E. coli* concentrations, and persistence of antibiotic resistant airborne *E. coli* continue to pose a risk to animal and human safety with increased adverse health effects and potential pathogenic transfer into food systems. Remediation efforts should focus on lower levels of PM, airborne bacteria, and litter bacteria concentrations without affecting levels of litter moisture.

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