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Evaluating the effects of sanitizing drinker lines in commercial broiler houses on the water quality and microbial content in biofilm layers

Abstract

The importance of water quality and its ability to cause major impacts, whether it is positive or negative, is often overlooked in the poultry industry. The microbial content of water is just one of many water characteristics that need to be monitored and regulated to ensure a flock is receiving good quality water. A field study was conducted to determine the effects of four different sanitizers under commercial broiler house conditions, with three replications over a three flock period. Each flock was grown for 56 days with a 2 week period between each flock. Four oxidative sanitizers were used and assigned to one of four commercial broiler houses at the Stephen F. Austin State University Broiler Research Center. Two water lines within each house were cleaned with the assigned product prior to placement for three consecutive flocks, leaving two water lines per house that were used as control groups. Water and swab samples were collected from all four water lines within each house after sanitizing on day 0, prior to flock placement and again on day 56, prior to the end of the flock. Total data points represent six sampling periods.

These samples were analyzed by the Pilgrim's Walker Creek Lab for aerobic plate count (APC), *Escherichia coli*, and yeast and mold counts (YM). Results showed that there was a significant decrease in the means of APC and YM counts over the period of three flocks, $P = 0.0009$ for swab results and $P = 0.0032$ for water results. There was a significant difference in the effects between two sanitizers compared to the others, $P = 0.0155$. There was a significant difference in the means of APC and YM counts according to the day in which samples were collected, $P = 0.0017$. No significant differences were observed for the means of *Escherichia coli* counts, which remained consistent throughout the entire trial at 10 colony forming units or less.

Degree Type

Thesis

Degree Name

Master of Science - Agriculture

Department

Agriculture

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Keywords

E. coli, Water Quality, Biofilms, Broiler, Sanitizer

EVALUATING THE EFFECTS OF SANITIZING DRINKER LINES IN
COMMERCIAL BROILER HOUSES ON WATER QUALITY AND MICROBIAL
CONTENT IN BIOFILM LAYERS

By

Kennedi Kay Achilles, Bachelor of Science in Agriculture

Presented to the Faculty of the Graduate School of

Stephen F. Austin State University

In Partial Fulfillment

Of the Requirements

For the Degree of

Master of Science

STEPHEN F. AUSTIN STATE UNIVERSITY

August 2019

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Acknowledgments

The author would like to express her appreciation to Dr. Bray and her academic and research advisors, Dr. John Micheal Mehaffey, Dr. Stephanie Jones, and Dr. Kathryn Kidd for their guidance and helpful suggestions in the preparation of this manuscript. Special thanks to Pilgrim's Pride and Dr. Dave McCreery for funding this research trial and Dr. Bray for allowing access to the facilities used in this trial. The author would like to thank the Houston Livestock Show and Rodeo for the grant that allowed for her graduate assistantship.

This manuscript would not have been made possible if it weren't for the support the author received from God, her mother Kelli Achilles, father David Achilles, and Gregory Martens. To the rest of her family and friends, the author cannot thank everyone enough for their continued support and motivation throughout this process.

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CHAPTER I

Introduction

The importance of water and its ability to cause major impacts, whether it is positive or negative, is often overlooked in the poultry industry. Many nutritionists believe water is the most important nutrient, however because it is consumed separately and not required in the diet it is hard to completely define water as a nutrient (Cheeke, 2005). This can cause major issues with poultry nutrition, because water is one of the essential nutrients required by poultry. It plays many important roles in the bird's bodily functions, including aiding with transportation of nutrients, lubrication of joints and organs, metabolism, and temperature regulation of the body (Fairchild and Ritz, 2009).

There are three sources of water for broilers, 1) drinking water, 2) water obtained through feed, and 3) metabolic water; however, the quality of the drinking water via the nipple drinkers of broiler houses is the focus of this research. Although there are many factors that affect water quality, the main concern in the poultry industry today is the transmission of pathogens through the drinker lines of poultry houses. Pathogens have the ability to inhabit the

biofilm layers within the drinker lines. Biofilms are thin films that develop inside water systems with the capability of harboring pathogens within these drinker lines, which increases the microbial content portion of water. The removal of these pathogens within drinker lines becomes increasingly difficult. The microbial content of water is just one of many water characteristics that needs to be monitored and regulated to ensure that a flock is receiving good quality water. Knowing the adverse effects that each characteristic can have and the levels at which they are harmful to a flock is necessary for producers to manipulate and regulate the microbial content of the water properly in order to ensure a healthy flock. This can be done simply by using effective sanitizers and following the protocols to administer them correctly. Sanitizing drinker lines have shown to minimize the microbial content of water (Watkins, 2006), with the most common sanitizer being chlorine based. Another sanitizer option is an oxidative sanitizer, a sanitizer with the ability to oxidize or steal electrons from other substances (Ryther, 2014).

This study evaluates the effectiveness of four different oxidative sanitizers as well as the microbial content in biofilm layers throughout the growing period of commercial broiler flocks. The collections of water and swab samples were taken before a new flock was placed and again once that flock was harvested for

processing over the three flock period. This will benefit the poultry industry by determining the extent at which certain microbes are able to repopulate biofilm layers, as well as evaluating which type of sanitizer is most effective with the removal of pathogens within the water lines.

Research Objective

The objective of this study was to examine and assess the effectiveness of four different oxidative sanitizers on the *Escherichia coli*, aerobic plate counts, coliforms counts, and yeast and mold counts. The effects of these sanitizers were assessed over a period of three commercial broiler flocks. Both water samples and swab samples were used to measure the effectiveness of each sanitizer before and after sanitization.

CHAPTER II

Literature Review

Water: The Importance of Quality

Water is arguably the most essential nutrient to all animals, it is imperative that the water being consumed by broiler flocks is free of toxic compounds and other contaminants. (Maharjan, 2016). Unless water is distilled it is not truly pure, as all water contains various forms of foreign substances that may affect palatability, function, and health. Water quality is important because water is involved in many bodily functions, including regulation of body temperature, digestion, nutrient transport, and excretion through urine (Jafari *et al.*, 2006). Furthermore, a broiler chick can survive after losing 98% of their body fat or 50% of their body protein, but a 10% loss of body water will cause physiological illness and a 20% loss will cause death (Pattison, 1993).

The characteristics of water that contribute to its quality include color, turbidity, hardness, iron, pH, total solids, nitrogen, poisonous metals, bacteria, sanitizers, and the maximum inclusion levels of these characteristics listed that is considered to be safe for consumption (North, 1981).The micro-element

component of the water provided to broiler flocks is attributed to the contents that accumulate in water as it drains from the surface and into the ground water that producers pull from (Bell, 2002). Each of these characteristics indicate water quality, they effect equipment and bird heath, and it is important to know how to properly manipulate each to ensure good water quality and production efficiency. The color of water is normally clear and indicates an increase in contamination if it changes. The turbidity of water is the suspension of particles like clay, sand, silt, or organic materials that alter the color of water and can cause faults in equipment. Hardness of water is simply the levels of calcium and magnesium in the water that create build ups in the water lines and other issues with equipment. Manganese levels and the amount of total solid materials suspended in the water does not contribute to bird health issues, but does lead to scale build up and equipment issues. Iron also contributes to equipment issues because it can be in the form of iron oxide and be a suspended solid particle or form rust water in lines. It is found that high iron levels can cause health issues as broiler chickens are vulnerable to iron intoxication (Suganya, *et al.*, 2016). The factors that contribute directly to bird health are nitrate levels, pH, toxic compound levels, and bacteria levels. The ideal pH of water provided to broilers should be between 6.0 and 6.8, however a flock can tolerate a pH range of 4 to 8. Nitrate levels can indicate decaying of organic material and that bacteria levels need to be

analyzed and the levels of toxic compounds, like selenium and arsenic, need to be kept at levels under 1ppm to maintain a healthy flock. Lastly, the bacteria levels in water need to be kept as low as possible. This has created a major issue within the poultry industry due to the difficulty of keeping these levels to a minimum. In addition to the bacteria content of water, other pathogenic contaminants that effect water quality and bird health include viruses, fungi, protozoa, and yeasts and molds. All of these aquatically transmitted pathogens are can be found inhabiting the organic build up within the drinker lines called biofilm layers. (Fairchild and Ritz, 2009)

Biofilm Layers

The formation of biofilms has been attributed to the continuous attachment of bacteria to the surfaces within waterways where communities begin to form and the start of inherent antimicrobial resistance is seen (Costerton, *et al.*, 1999). Biofilms play negative roles like the biological corrosion of pipelines, the development of infectious processes that are heightened by the increased resistance to disinfectants, and the colonization of equipment (Maksimova, 2014). The formation of biofilms allow for single celled organisms, such as bacteria, to form temporary multicellular habitats where survival is maintained

through group behavior (Kostakioti *et al.*, 2013). It is also known that “biofilms will acquire the chemical characteristics of the surrounding water environment and will accumulate microorganisms such as bacteria and viruses if they are present in the water and left untreated. These bacteria could be present from flock to flock unless the biofilm is completely removed. Biofilms can also reduce the effectiveness of medications, disinfectants, and make it difficult to conduct water analysis” (Fairchild, 2006).



Figure 1. Biofilm within PVC Drinker Line. 2012.

This being said, poor water quality and pipe materials can help to increase the pathogen populations. It is not wise to use plastic pipes for water because of their ability to maintain temperatures that happen to be very beneficial to the growth cycle of pathogens. So, within these biofilm layer pathogens you can find different species and variations of bacteria, protozoa, and viruses. Studies show that biofilms can be composed of a population that developed from a single

species or a community derived from multiple microbial species, and they can form on a vast array of abiotic and biotic surfaces (Hoffman, *et al.*, 2005). The possibility of any one of these infecting an entire flock is why the management of biofilm layers, as well as the quality of water provided, is a major concern with our industry. When addressing this issue it is crucial to gain an understanding of the types of bacteria, protozoa, and viruses that inhabit these drinkers. This will help to alter the sanitation routines, as well as give producers the knowledge they need to alter other management practices if specific pathogens are found and happen to be reoccurring. The biosecurity issue that arises because of these biofilm layers is something we need to address. Eliminating or reducing the layers can greatly reduce the mortality rates of a flock, which will financially benefit the producers and the industry.

Biofilm Pathogens

The pathogen populations within biofilm layers consists of bacteria, viruses, protozoa, and yeast and molds. Upon invasion of a host, bacteria are capable of rapid multiplication through binary fission, this will eventually be detected by the body, causing outward symptoms of disease which is then followed by higher mortality rates. When a virus finds a host it must then locate a host cell to invade before multiplication can begin. Though this might seem like a

good thing, a virus not being able to multiply freely as bacteria do, it is the reason they are so difficult to treat. Additionally, because viruses are so small in size they have the ability to bypass water filters with ease. It takes an electron microscope to be able to see a virus. Viruses are capable of producing disease symptoms that weaken the body's immune system allowing for further secondary infections to occur (Andersson and Banfield, 2008).

Protozoa are single-celled organisms that have shown high resistance to disinfectants and can rapidly multiply within the gut, causing tissue damage and toxins that result in the detriment of the health of the host (Moreng and Avens, 1985). Studies show that protozoa within biofilms work as mechanisms that allow for increased viral and bacterial growth (Donlan, 2002).

Once birds are infected the diseases can be spread from bird to bird very easily, this is due to the nature of confinement that is utilized in the poultry industry. As a result of this close confinement, when highly pathogenic diseases are contracted the whole flock will be infected quickly. To prevent the spread of these diseases from older to younger birds the industry utilizes a system that is explained as:

“The most practical program for broiler rearing has been to use the all-in, all-out system, in which only one age of broilers is on the farm at the same

time. All the chicks are started on the same day, and later sold on the same day, after which there is a period when no birds are on the premises. This lack of birds breaks any cycle of an infectious disease; the next group of birds has a 'clean start', with no possibility of contracting a disease from the older flocks on the farm" (North, 1981).

The bacteria and other pathogens that are of concern regarding this research include coliform bacteria, *Escherichia coli*, species including *E. coli* 0157, *Staphylococcus*, *Salmonella*, *Listeria*, and yeasts and molds. These are the specific categories used by the lab when analyzing the swab and water samples received to show the levels at which these pathogens and other material are inhabiting the water and biofilms for the broiler house drinker lines.

Bacteria, Yeasts and Molds

Bacteria are single-celled organisms that lack a nucleus, contain a strand of DNA, and replicated through binary fission (Schlegel, *et al.*, 1993). This method of replication creates exact copies of the original organelle and does not require a host cell to be completed, resulting in their ability to freely replicate

within water that leads to issues with rapid infection. There are many different types of bacteria, but these types of bacteria that prefer to inhabit aquatic pathways are called *Coliform* Bacteria (Vendrell and Atilas, 2013). Coliforms include all bacteria that are aerobes and facultative anaerobes, are gram-negative, non-spore forming, and can ferment lactose and produce carbon dioxide gas. Aerobic bacteria are strains that prefer environments with higher oxygen concentrations and anaerobic bacteria are those that prefer environments containing little to no oxygen. However, facultative anaerobes are able to thrive in environments with higher oxygen concentrations, however they are only able to do this for short period of time because after prolonged exposure to oxygen the over saturation leads to death (Rolfe, *et al.*, 1978).

Among the coliform bacteria there are two generally known subgroups that are referenced when testing water and preventing contamination, these are total coliform bacteria and fecal coliform bacteria. Total coliform bacteria are generally found in the environment in places like soil and vegetation. Fecal coliform bacteria are a group within the total coliforms that is found specifically in the gut and feces of warm-blooded animals (Coliform Bacteria and Drinking Water, 2016). One of the most well-known fecal coliforms is *Escherichia coli*, or *E. coli*. This is the most predominant of the fecal coliforms and is used as an indicator of

potential contamination when taking water samples because a positive result for *E. coli* is much more consequential than coliform bacteria alone due to the fact that it indicates that there is human and/or animal fecal contamination entering the water (Swistock, *et al.*, 2013). In addition to this, there are many diseases that can arise from an *E. coli* infection within a flock. A study conducted to examine the relationship between *E. coli* and colibacillosis, an infectious disease caused by *E. coli*, showed results suggested that combinations of *E. coli* strains that have identical pulsotypes are likely the cause of colibacillosis and the spread of antimicrobial resistance genes in avian pathogenic *E. coli* can be attributed to these pulsotypes having these genes and being integrated into their chromosomes (Ozaki, *et al.*, 2017).

Yeasts and molds are types of fungi in different forms. Yeast is a single celled growth form of fungus and mold is the filamentous form (Timoney, *et al.*, 1988). Fungal infections are typically caused by *Aspergillus fumigatus*, which typically grows on decaying organic material which then enters the bird where the fungus is able to reproduce within certain tissues of the bird. (North, 1981)

Salmonella spp. is a zoonotic foodborne disease that is distributed worldwide and is credited to be one of the most common pathogens causing bacterial foodborne diseases in humans (Yeh, *et al.*, 2017). Strains of *Salmonella*

that are of importance in the poultry industry include *S. pullorum* and *S. gallinarum* which are the causes for diseases like *bacillary white diarrhea* and *fowl typhoid* in chickens. (Timoney, *et al.*, 1988). It is difficult to be specific when discussing *Salmonella* as a result of there being over 2500 variations of *Salmonellae*. (Swayne, *et al.*, 2013) Generally, *Salmonellae* are aerobic and are free of special growth factors. *Salmonellae* are either named based off the disease they produce or the locations of their initial isolation. (Timoney, *et al.*, 1988) The poultry industry represents a large portion of reservoirs harboring *Salmonella*, resulting in broiler flocks contributing to and allowing for the growth of a foodborne illness that can be transmitted to humans (Gast, 2008). This is why salmonellosis has become a major public issue.

Listeria is a bacteria that is abundant in nature and generally occurs as septicemia or a localized encephalitis (Kahn and Line, 1991). *L. monocytogenes* is the strain most commonly found in poultry because it is able to rapidly colonize in poultry due to the fact that the birds do not show any clinical signs of infection, therefore making the bird a reservoir for replication and spread of disease (Kahn and Line, 1991). Although an infection from *L. monocytogenes* is usually not responsible for production losses and other problems with infection, broilers are

still carriers and can infect the litter, environment, and even humans and other animals (Bailey, *et al.*, 1989) .

Disinfection of Water and Oxidative Sanitizers

Sanitizing drinker lines have shown to minimize the microbial content of water (Watkins, 2006), with the most common sanitizer being chlorine based. It is known that routinely sanitizing between flocks solves the majority of microbial contamination issues (Maharjan, *et al.*, 2016), but there has been some dispute within the industry on over chlorination and the other oxidative sanitizers that could be utilized. An oxidative sanitizer can be explained as a sanitizer with the ability to oxidize or steal electrons from other substances (Ryther, 2014). Hydrogen peroxide based oxidative sanitizers are another option for water sanitation, which are becoming more popular topics of research. Hydrogen peroxide based sanitizers have been shown to be efficient substitutes for chlorine based sanitizers (Chlorine Dioxide Water Treatment Replacement). Chlorine based sanitizers are being used less and less because of its ability to react with organic matter which then created chlorinated byproducts that can cause health issues (Mead, 2005). Determining which type of sanitizer to use in a water system is based upon the microbial and mineral content and the buffering

capacity of the water, this can be determined using the oxidation-reduction-potential (ORP) (Tabler, *et al.*, 2013). ORP is explained as the electric potential required to transfer electrons from one compound or element, the oxidizer, to another compound, the reductant (Gómez-López, *et al.*, 2009). Using hydrogen peroxide as alternative to chlorine in water sanitation requires more research for greater certainty of efficiency in poultry production.

Other methods of sanitization used in the poultry industry is electrolyzed oxidizing water, this is sprayed onto eggs in the hatchery has proven to reduce broiler mortalities within the first two weeks of age (Fasenko, *et al.*, 2009).

CHAPTER III

Proposed Methods of Study

Animals and Housing

This study was conducted using four commercial broiler production houses in Nacogdoches, Texas between the months of January and August of 2019. Each house measured 43 feet in width and 500 feet in length. Houses were solid side walled and tunnel ventilated. Temperatures were kept around 90 degrees Fahrenheit to start and slowly decreased throughout the flock ending around 74 degrees Fahrenheit. An average of 19,600 birds were in each house during each flock. Birds were grown to eight to ten pounds before being harvested. Each house has 21,500 square feet of space equating to a stocking density of about 1.10 square feet of space per bird. Each house contained four drinker lines that were 220 feet in length made out of PVC pipe with nipple drinkers spaced approximately one foot apart down the entire line.

Drinker lines within each house were labeled A-D, moving across the house from north to south. Lines A and C were the experimental lines and lines B and D were the control lines. Only water lines on the front end of each house

were under experimental conditions. Water lines were not sanitized during each flock, between days 0-56. Water and feed were provided *ad libitum*.

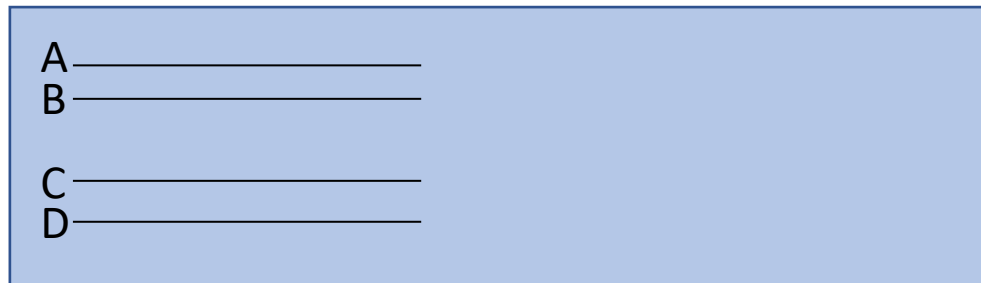


Figure 2. Drinker Line Assignments Per House

Experimental Treatment and Groups

All waterlines in each house were flushed with water for 15 minutes before experimental lines were sanitized. All waterlines were then shut off and a Qwik-Blend™ proportioner pump was set up to deliver approximately one gallon of sanitizer per 1600 linear feet of three-quarter inch waterline.

The Qwik-Blend™ proportioner pump delivers roughly 3% solution directly from the sanitizer containers into the water system.



Figure 3. A Qwik-Blend™ Proportioner Pump

The siphon end of the pump is inserted into the sanitizer container and ensured it was resting on the bottom of the container. Each spigot end of the Qwik-Blend™ proportioner pump is connected to the waterlines system which then slows water to flow in one hose, become mixed with a sanitizer, and then flow out of the other hose and into the water system. The experimental lines, lines A and C, were turned on and the sanitizer was flushed through. Once the sanitizer was

successfully administered throughout each of the experimental lines the water was then shut off and the sanitizer was left to sit within the lines for a minimum of 24 hours. The Qwik- Blend™ proportioner pump was removed from the water system and all the valves were returned to their original setting. After the 24 hour period had passed all waterlines were flushed once again for 15 minutes. Throughout this process the control lines, lines B and D, were flushed twice for 15 minutes each before samples were taken after sanitization of experimental lines. Water lines are cleaned between the harvest of the previous flock and the placement of the next flock. Water lines are not cleaned while birds are in house.

Data Collection:

Water Samples

Water samples were collected using a sterilized tool and container, sterile specimen cup, which is labeled with the farm name, date, and corresponding house and waterline. Samples were pulled directly from the drinker nipples after being thoroughly sterilized with a sanitizer wipe. Using a sterilized tool pressure was applied to the nipple head in an upward direction to release the water flow, the water will flow into a sterilized container placed closely under the water flow

so that the sides of the container do not touch the nipple. This placement is to ensure no air contaminants or contaminants on the drinker nipple and lines themselves compromise the sample being taken. Sample collections are a minimum of 0.05 to 1 cup of water. Sample containers were carefully be sealed and placed in the cooler for shipping.



Figure 4. Drinker Nipple Sanitization for Water Sample Collection



Figure 5. Water Sample Collection

Swab Samples

Swab samples were done using Quick Swabs-3M® and labeled with the farm name, date, and corresponding house and waterline. The end cap on each waterline was removed and the waterlines raised to stop water flow and the surrounding area of the pipe and pipe rim will be sterilized using sanitary wipes. The red pin on the end of the Quick Swab were snapped and pressure applied to the bulb to release the buffer solution into the end of the swab. The swabs were then removed cautiously to avoid the 'q-tip' portion from touching anything. Each swab was inserted 2 to 3 inches into the end of the line and then twisted several

times as it is removed to insure as much of the pipe is being sampled as possible. The swab was reinserted into the container it was in originally, sealed, and placed in the cooler for shipping.



Figure 6. End of Line Sanitization for Swab Sample Collection



Figure 7. Swab Sample Collection

Shipping Samples

Shipment of the samples were done using a cooler that is clean and dry. Both water and swab samples were placed in the cooler and covered with ice packs to insure the contents remain cold. Samples were then be received via same day or next day shipping through a third party shipment company. Sample shipments were delivered to the Walker Creek Lab in Pittsburg, Texas where they will be analyzed and processed.

Sample Testing

Swab samples were shaken for 30 seconds to remove the bacteria from the swab into the hydration solution. 1 mL of solution was poured into a 9 mL dilution blank, at -1 dilution. The sample was diluted from -2 through -3. Sample dilutions -1 through -3 were plated onto APC Petrifilm and sample dilutions -1 through -2 were plated onto Y/M Petrifilm and EC Petrifilm. The Petrifilms were incubated and results were recorded. Water samples were diluted through -3 and dilutions -1 through -3 were plated on APC Petrifilm and dilutions -1 through -2 were plated on Y/M Petrifilm and EC Petrifilm. Petrifilms were incubated and results were recorded.

Data Interpretations

The sample results were analyzed using Statistical Analysis System (SAS 9.4). The data was interpreted using one-way, analysis of variance (ANOVA) using the LS MEANS procedure. A P-value of 0.05 was set and any significant differences were observed when $P < 0.05$. Means were then separated using a Duncan's Multiple Range Test to determine the differences in means of aerobic plate counts and yeast and molds through each flock, between each day of sampling, and through each house.

CHAPTER IV

Results and Discussion

At the completion of the study, the results and statistical analysis were collected and evaluated by the researcher and poultry research director. Water sample results and swab sample results after statistical analysis showed significance in some areas and the completed determinations are as follows. The effects of each sanitizer, each drinker line sampled, each flock number, and each day of sampling were measured by the levels of *E. coli*, aerobic plate counts, and yeast and mold counts within each line. The effects of each sanitizer throughout each house, both control and treated lines, are indicated as sanitizer treatments A, B, C, and D. The differences in means between treated lines and control lines are indicated as the experimental groups treated or control. The differences in means between each flock are indicated as flock number 1, 2, or 3. The differences in means between the day of sampling is indicated as day 0 and day 56. All means were measuring using colony forming units (CFU) as the unit of measurement.

Water Results

The aerobic plate counts in the water samples showed significant differences between flock numbers, $P = 0.0032$, and day of sampling, $P = 0.0017$. The aerobic plate counts from the water samples also showed significant differences in the means between the flock numbers and the day of sampling. These differences are depicted in Table 1, Table 2, Table 3, and Table 4.

The results in Table 1. show that there are significant differences among the means of yeast and mold counts during flock 1 on day 56 for treated ($P = 0.064$) and control ($P = 0.0007$) lines. Table 1 also shows a significant difference in the means of yeast and mold counts in the control lines during flock two on day 0 ($P = 0.0001$) and day 56 ($P = 0.0018$) for Sanitizer A. Table 2 showed no statistical significance among the means of aerobic plate counts or yeast and mold counts over the three flocks on each day of sampling in both treated and control lines for Sanitizer B. Table 3 shows a significant difference among the means of aerobic plate counts ($P = 0.0001$) and yeast and mold counts ($P = 0.0086$) between the treated and control lines on day 56 during flock 3 for Sanitizer C. Table 3 also shows a significant difference among the means of aerobic plate counts in the control lines on day 0 ($P = 0.0029$) of flock 2 and day 56 (0.0001) of flock 1 for Sanitizer C. Table 4 does not show any significant

differences among the means of aerobic plate counts but shows significant differences among the means of yeast and mold counts during flock two for day 0 ($P = 0.0053$) and day 56 ($P = 0.0073$) for treated lines and on day 0 for the control lines ($P = 0.0063$) for Sanitizer D.

Table 1. Aerobic plate counts, yeast and mold counts, E. coli counts and coliform counts of water samples on day 0 and day 56 for Sanitizer A over a three flock period.

	Flock 1				Flock 2				Flock 3			
	Day 0		Day 56		Day 0		Day 56		Day 0		Day 56	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
APC	115420 ^a	2420 ^a	16500 ^a	19150 ^a	29350 ^a	13700 ^a	17050 ^a	15405 ^a	705 ^a	905 ^a	1490 ^a	1325 ^a
Y&M	3055 ^a	85 ^a	7800 ^b	9950 ^b	3600 ^a	15000 ^b	1300 ^a	9050 ^b	10 ^a	3605 ^a	120 ^a	25 ^a
E.Coli	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Coliforms	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

^{a-c}Row values with different superscripts differ significantly (P < 0.05).

¹APC is aerobic plate counts and Y&M is yeast and molds

Table 2. Aerobic plate counts, yeast and mold counts, E. coli counts and coliform counts of water samples on day 0 and day 56 for Sanitizer B over a three flock period.

	Flock 1				Flock 2				Flock 3			
	Day 0		Day 56		Day 0		Day 56		Day 0		Day 56	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
APC	37600 ^a	15850 ^a	61850 ^a	122300 ^a	17000 ^a	53200 ^a	8830 ^a	61150 ^a	12260 ^a	5325 ^a	610 ^a	2280 ^a
Y&M	55 ^a	195 ^a	465 ^a	870 ^a	1635 ^a	3150 ^a	20 ^a	1625 ^a	110 ^a	295 ^a	15 ^a	10 ^a
E.Coli	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Coliforms	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

^{a-c}Row values with different superscripts differ significantly (P < 0.05).

¹APC is aerobic plate counts and Y&M is yeast and molds

Table 3. Aerobic plate counts, yeast and mold counts, E. coli counts and coliform counts of water samples on day 0 and day 56 for Sanitizer C over a three flock period.

	Flock 1				Flock 2				Flock 3			
	Day 0		Day 56		Day 0		Day 56		Day 0		Day 56	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
APC	35740 ^a	68800 ^a	1346125 ^b	1509200 ^b	76000 ^a	1023500 ^b	840 ^a	10755 ^a	200 ^a	1900 ^a	4760 ^a	8750 ^a
Y&M	10 ^a	480 ^a	7505 ^b	15000 ^b	1640 ^a	3695 ^a	25 ^a	30 ^a	10 ^a	110 ^a	25 ^a	20 ^a
E.Coli	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Coliforms	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

^{a-c}Row values with different superscripts differ significantly (P < 0.05).

¹APC is aerobic plate counts and Y&M is yeast and molds

Table 4. Aerobic plate counts, yeast and mold counts, E. coli counts and coliform counts of water samples on day 0 and day 56 for Sanitizer D over a three flock period.

	Flock 1				Flock 2				Flock 3			
	Day 0		Day 56		Day 0		Day 56		Day 0		Day 56	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
APC	9405 ^a	1895 ^a	16950 ^a	33900 ^a	42950 ^a	91700 ^a	2860 ^a	18450 ^a	10 ^a	150 ^a	97750 ^a	10500 ^a
Y&M	10 ^a	220 ^a	740 ^a	1320 ^a	8000 ^b	7815 ^b	7665 ^b	950 ^a	10 ^a	25 ^a	225 ^a	10 ^a
E.Coli	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Coliforms	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

^{a-c}Row values with different superscripts differ significantly (P < 0.05).

¹APC is aerobic plate counts and Y&M is yeast and molds

Swab Results

Table 5 shows a significant difference among the means of aerobic plate counts during flock 2 in the control lines on day 0 for Sanitizer A ($P = 0.0007$). Table 6 shows a significant difference among the means of aerobic plate counts for the treated lines on day 0 in both flocks 1 ($P = 0.0242$) and 2 ($P = 0.0001$) for Sanitizer B. Table 6 also shows a significant difference among the means of yeast and mold counts in the treated lines during flock 2 on day 0 for Sanitizer B ($P = 0.0398$). Table 7 shows a significant difference among the means of aerobic plate counts during flock 2 on day 0 in both treated ($P = 0.0001$) and control lines ($P = 0.0585$) for Sanitizer C. Table 7 also showed a significant difference among the means of yeast and mold counts during flock 2 on day 0 in the control lines for Sanitizer C ($P = 0.0061$). Table 8 shows a significant difference among the means of aerobic plate counts ($P = 0.0016$) and yeast and mold counts ($P = 0.0001$) for day 0 of sampling during flock two in the treated lines for Sanitizer D.

Table 5. Aerobic plate counts, yeast and mold counts, E. coli counts and coliform counts of swab samples on day 0 and day 56 for Sanitizer A over a three flock period.

	Flock 1				Flock 2				Flock 3			
	Day 0		Day 56		Day 0		Day 56		Day 0		Day 56	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
APC	15105 ^a	7455 ^a	1540 ^a	27090 ^a	3985 ^a	126285 ^b	7100 ^a	5750 ^a	105 ^a	525 ^a	14500 ^a	6800 ^a
Y&M	10 ^a	10 ^a	10 ^a	10 ^a	160 ^a	235 ^a	10 ^a	10 ^a	15 ^a	25 ^a	15 ^a	10 ^a
E.Coli	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Coliforms	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

^{a-c}Row values with different superscripts differ significantly (P < 0.05).

¹APC is aerobic plate counts and Y&M is yeast and molds

Table 6. Aerobic plate counts, yeast and mold counts, E. coli counts and coliform counts of swab samples on day 0 and day 56 for Sanitizer B over a three flock period.

	Flock 1				Flock 2				Flock 3			
	Day 0		Day 56		Day 0		Day 56		Day 0		Day 56	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
APC	80500 ^b	50500 ^a	7000 ^a	3745 ^a	207500 ^b	1940 ^a	17650 ^a	19100 ^a	14900 ^a	1515 ^a	5850 ^a	7300 ^a
Y&M	310 ^a	15 ^a	385 ^a	70 ^a	600 ^b	205 ^a	90 ^a	10 ^a	35 ^a	55 ^a	10 ^a	10 ^a
E.Coli	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Coliforms	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

^{a-c}Row values with different superscripts differ significantly (P < 0.05).

¹APC is aerobic plate counts and Y&M is yeast and molds

Table 7. Aerobic plate counts, yeast and mold counts, E. coli counts and coliform counts of swab samples on day 0 and day 56 for Sanitizer C over a three flock period.

	Flock 1				Flock 2				Flock 3			
	Day 0		Day 56		Day 0		Day 56		Day 0		Day 56	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
APC	2100 ^a	46920 ^a	5035 ^a	1960 ^a	154400 ^b	67000 ^b	4657 ^a	705 ^a	195 ^a	745 ^a	13735 ^a	850 ^a
Y&M	50 ^a	35 ^a	15 ^a	10 ^a	185 ^a	815 ^b	10 ^a	20 ^a	10 ^a	85 ^a	10 ^a	15 ^a
E.Coli	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Coliforms	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

^{a-c}Row values with different superscripts differ significantly (P < 0.05).

¹APC is aerobic plate counts and Y&M is yeast and molds

Table 8. Aerobic plate counts, yeast and mold counts, E. coli counts and coliform counts of swab samples on day 0 and day 56 for Sanitizer D over a three flock period.

	Flock 1				Flock 2				Flock 3			
	Day 0		Day 56		Day 0		Day 56		Day 0		Day 56	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
APC	177750 ^a	1010 ^a	1665 ^a	2020 ^a	115500 ^b	23300 ^a	3895 ^a	12300 ^a	20 ^a	2405 ^a	5635 ^a	1700 ^a
Y&M	10 ^a	20 ^a	10 ^a	10 ^a	1655 ^b	10 ^a	10 ^a	65 ^a	15 ^a	30 ^a	55 ^a	80 ^a
E.Coli	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Coliforms	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

^{a-c}Row values with different superscripts differ significantly (P < 0.05).

¹APC is aerobic plate counts and Y&M is yeast and molds

Treated and Control Lines:

The means for the treated lines showed to be higher than the means of the control lines for both aerobic plate counts and yeast and mold counts in the water sample data. The opposite is true for the swab sample data, the means for aerobic plate counts and yeast and mold counts were higher in the control lines than in the treated lines.

***Escherichia coli* and Coliform Data:**

The data for the *Escherichia coli* and Coliform results in both swab and water samples were not statistically analyzed, the raw data showed no change in the *Escherichia coli* or Coliform counts throughout all three flocks in each set of sample results. The *Escherichia coli* and Coliform counts were consistently 10 CFU or less and never showed any change throughout the entire trial. Tables 1 through 8 depict the data for *Escherichia coli* and Coliform counts.

CHAPTER V

Summary and Conclusion

In summation, this study showed that all the oxidative sanitizers used were effective at reducing the aerobic plate counts and yeast and mold counts over the period of three flocks. The water sample results showed that there were significant differences between the means of both aerobic plate counts and yeast and mold counts according to which day the samples were collected. The results showed that the aerobic plate counts and yeast and mold counts were significantly greater on day 0 than on day 56. The opposite of this was true for the swab sample results, the aerobic plate counts and yeast and mold counts were significantly lower on day 0 than on day 56. This suggests that throughout the period of each flock, the flow of water from the pressure created by the birds drinking on the nipple drinkers caused the aerobic bacteria and yeast and molds to retreat into the biofilms within the lines.

The water sample results showed no significant differences between the effects of each sanitizer. The swab results did show significant differences in the effects of the sanitizers for both aerobic plate counts and yeast and mold counts.

The results showed *Escherichia coli* counts were consistently low and no significant differences were observed for swab samples and water samples.

In conclusion, the results of this study suggest that the initial application of the sanitizers effectively lower the microbial content within the drinker lines, however it can be assumed that throughout the growing period of each flock the microbes are able to grow in number within the biofilm layer of the drinker lines. Further research on the topic should be conducted to allow more concrete conclusions to be made.

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