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Evaluation of the Correlation Between the Oxidation Reduction Potential and Free Chlorine Residual for Different Chlorine Sources Used in Poultry Drinking Water Sanitation

Samantha Renee Cox
University of Arkansas, Fayetteville

Evaluation of the Correlation Between the Oxidation Reduction Potential and Free Chlorine Residual for Different Chlorine Sources Used in Poultry Drinking Water Sanitation

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Samantha Renee Cox
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_____
Dr. Susan E. Watkins
Thesis Director

_____
Dr. Fred D. Clark
Committee Member

_____
Dr. K. Jill Rucker
Committee Member

Abstract

A critical component in commercial poultry production is to ensure birds are provided clean, quality water. Multiple disinfectants can be utilized to optimize a good water quality program. The goal of these water disinfectants is to greatly reduce or eliminate the presence of all bacteria. In recent years, the oxidation-reduction potential (ORP) meter has been a tool utilized by the poultry industry to monitor chlorine efficacy in drinking water. An ORP reading of 650-750 millivolts (mV) has become the industry standard for assuring an acceptable sanitizing residual of free chlorine is present for controlling microbial contamination regardless of the actual amount of total or free chlorine or the water pH. A recent bench top evaluation of a new chlorine product revealed microbial contamination even when the oxidation-reduction potential read 650 mV. Given these results, it is beneficial for the poultry industry to re-evaluate the relationship between total chlorine residual, free chlorine residual, ORP value, and microbial levels. Additionally, the study determined if a new ORP standard should be the target and if this standard consistently correlates to free chlorine residual (ppm) even under scenarios of different water quality parameters. This study evaluated the efficacy of two different forms of chlorine, a liquid product (sodium hypochlorite) and a crystalline dry product (sodium dichloro-S-triazinetrione) at three concentrations (2, 4, and 8 oz/gal) commonly used for drinking water sanitation in the poultry industry. The objective was to determine the relationship between total and free chlorine residual, ORP, pH, and microbial content of the water when the chlorine products are utilized in water with a high microbial level typically found in unclean poultry drinking water lines. Results indicated both forms of chlorine were effective disinfectants for reducing aerobic bacteria present in the water. Additionally, the results of this experiment showed under field conditions where

microbiologically challenged water is present, an ORP reading of 700 mV is required to achieve an efficient microbial reduction/elimination and this is supported by at least 3 ppm of free chlorine.

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Dedication

I dedicate this thesis to parents,

Jim and Renee Cox,

and my brother,

Nicholas Cox

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Chapter I: Introduction

Ensuring birds have clean, good quality water is a critical component in commercial poultry production. Birds consume twice the level of water as compared to feed primarily because poultry have very little saliva and need water to hydrate and wash down what they eat. Water is a vital nutrient that affects almost every physiological function in the avian body including regulating body temperature, eliminating body waste, and digesting nutrients. Providing flocks with water, which is contaminated with pathogenic microorganisms and other contaminants, can lead birds to experience health related issues that results in overall poor flock performance. The quality of water is impacted by multiple factors such as pH, mineral composition, microbial contamination, and the amount of organic material found in the supply and distribution system. To ensure optimal quality of drinking water, each of these factors should be monitored and adjusted so they remain within acceptable ranges of less than 1000 colony-forming units per milliliter of water (cfu/ml).

For many years, the use of sub therapeutic levels of antibiotics in both feed and water could have been considered a temporary method for masking unacceptable water quality and other production issues. Antibiotics were given to poultry in low levels as a preventative tool for health issues like necrotic enteritis. Recent government bans on the use of antibiotics in food animal production as well as strong consumer demand to reduce all sub-therapeutic use of antibiotics has resulted in a dramatic decrease in the presence of these health-promoting tools in commercial poultry production targeted for the human food chain. The antibiotic free production trend means every factor of poultry production such as temperature, air, litter, feed, and water quality will have to be optimized in concentrated commercial production to assure performance is not compromised and remains economical.

It is important for the poultry industry‘s production personnel and farmers to evaluate and identify the drinking water quality challenges associated with their farms. These are necessary steps to improve or maintain the quality. Additionally, it is important to monitor the microbial quality during the production cycle to assure the quality of this required nutrient is never compromised. This is primarily accomplished by measuring the sanitizing residual at both the source of the drinking water and from the actual drinker line where the birds are consuming water.

There are multiple disinfectants utilized for optimizing a good water quality program including, chlorine, chlorine dioxide, and hydrogen peroxide. The goal of these water disinfectants is to greatly reduce or eliminate the presence of all bacteria with the belief if all bacteria are minimized then pathogenic bacteria have little opportunity to also be present. Chlorine products are the most common water disinfectants utilized in the poultry industry because they are affordable, available, and relatively easy to use. Sodium hypochlorite, also known as liquid bleach, is the most traditional drinking water sanitizer. When introduced into water supplies, chlorine can be present as hypochlorous acid or chloric ions depending on the pH. Factors such as pH, organic load, mineral content, and temperature of the water can affect the efficacy of chlorine as a sanitizer and compromise its ability to control pathogens. Because the efficacy of sanitizers are compromised when conditions are not ideal, it is important for the poultry industry to understand what specific guidelines, like pH and biofilm reduction, should be met to assure the birds are receiving clean quality water. Water supplies vulnerable to microbial challenge need a sanitizing residual containing adequate efficacy to control any potential pathogens present or pathogens at risk of being introduced into the system. Multiple birds share each drinker. Therefore, even a few sick birds in a flock is enough to introduce disease organisms into a drinker system and cause an infection to become seeded within a flock.

In recent years, the oxidation-reduction potential (ORP) meter has been a tool utilized by the poultry industry to monitor chlorine efficacy in drinking water. An ORP reading of 650-750 millivolts (mV) has become the industry standard for assuring an acceptable sanitizing residual of free chlorine is present for controlling microbial contamination regardless of the actual amount of total or free chlorine or the water pH. The portable ORP meter is an inexpensive (<$100) small hand-held device. This meter is simple to use and provides quick results when monitoring water quality. However, a recent bench top evaluation of a new chlorine product conducted in the Watkins Water Quality Lab at the University of Arkansas Poultry Science Department revealed microbial contamination even when the oxidation-reduction potential read 650 mV. Given these results, it is prudent to re-evaluate the relationship between total and free chlorine residual, ORP value, and microbial levels. Furthermore, researcher should determine if a new ORP standard should be the target and if this standard consistently correlates to a free chlorine residual (ppm) even under scenarios of different water quality parameters.

In recent years, the poultry industry has utilized a 650 mV ORP reading as the parameter for acceptable drinking water quality. This reading was believed to eliminate any microbial activity present and provide adequate chlorine sanitizing residual to eliminate any microbes which might contaminate the water system at the all the way to the drinker. Recent evaluations of a new chlorine product revealed microbial contamination was still present even when the ORP was 650 mV. For the past thirty years, the poultry industry has commonly used bleached mixed in doses of 2 to 8 ounces in a gallon of stock solution and then administered to the birds at a rate of one ounce to a gallon of water as the primary form of water sanitation. Since chlorine products used at low doses is the primary water sanitizer of choice for the industry, the purpose of this trial was to evaluate the most commonly used product, bleach, and a new more potent form of chlorine that is

easy to use, and determine if there is a difference in sanitizing effectiveness and to also correlate microbial kill level to ORP values.

The objective of this study is to evaluate the correlation between the oxidation-reduction potential, free chlorine residual, and total chlorine residual for different chlorines sources used in poultry drinking water sanitation. Furthermore, a second objective is to determine under a challenge water quality scenario if these tools can be used to predict when the water is at optimal quality for preventing or eliminating microbial contamination.

Chapter II: Review of Literature

2.1 Poultry Drinking Water Quality and Its Importance

Water is one of the most important nutrients for poultry. Because birds consume almost twice as much water as feed, it is crucial to optimize water quality to assure bird health and production are not compromised [1]. Good quality water will have acceptable ranges of pH as well as minimal microbial and mineral contaminants.

For many years, therapeutic and sub-therapeutic levels of antibiotics were used to help prevent or cure health challenges in meat production birds. Concerns about the use of antibiotics administered via feed or water to food animals may be contributing to antibiotic resistance in human pathogens which has led consumers to press for meat products produced with no antibiotics [2]. With these consumer demands, it becomes crucial to assure all production parameters, including water, are provided to food animals at the best quality possible to prevent or reduce health issues that have traditionally been controlled by the use of antibiotics. Unlike residential water supplies, water quality in commercial poultry operations is challenged by low flow rates (less than 5-10 gallons per minute), rise in the temperature of water during brooding, introduction of pathogens by the birds from sharing drinkers, as well as from products injected into the system.

Microbial contamination in poultry water systems is a continual challenge and, therefore, it is important to implement sanitizer treatment programs and management practices to reduce this risk. There are several water sanitizers available as well as approved for poultry drinking water systems that can economically and effectively minimize challenges.

2.2 Defining Water Quality

The Environmental Protection Agency (EPA) defines water quality as "the biological, chemical, and physical conditions of a water body. It is a measure of a waterbody's ability to support beneficial uses" [3]. The EPA establishes criteria for determining when the quality of water becomes unsafe for humans [4]. Additionally, the EPA sets standards for human drinking water quality and these standards are typically used as a guideline in animal agriculture [5]. Water is a natural environment for many forms of microorganisms. Therefore, water supplies can harbor many pathogens such as *Giardia*, *Salmonella,* and *E.coli* [4].

2.3 Physiological Requirements of Water

Water encompasses 70 to 80 percent of lean body mass by weight in birds [6]. Water serves as a vital nutrient that impacts almost every physiological function in the body including regulating body temperature [7]. Water can be absorbed into the body via the consumption of liquids or via the breakdown of carbohydrates through oxidation [8]. To maintain a balance between water consumption and physiological needs for water, excess water is filtered from the blood via the kidneys and excreted as urine [8]. How chickens absorb water, regulate body temperature by the use of water, and remove water from the body is covered in the following discussion.

2.3.1 Absorption of Water

Water is absorbed throughout the gastrointestinal tract [9]. The gastrointestinal (GI) tract begins at the mouth and continues through the crop, proventriculus, gizzard, and small and large intestine, and exits from the cloaca, which is the common opening for the digestive, reproductive and urinary tracts. One feature of the poultry digestive tract different from other species is the crop. The crop was designed to act as a storage pouch for food particles swallowed whole. These are softened by water prior to passing into the proventriculus where true digestion begins. Water flows through and from the GI tract with the aid of osmotic pressure working against a concentration gradient, or moving from low concentration of particles or solutes to areas of higher concentration [9]. The absorption of water in the large intestine is regulated by the presence of sodium and chloride ions. The sodium and chloride ions produce an osmotic gradient within the large intestine, which favors the absorption of water into the bloodstream [8]. In the small intestine, water is absorbed through the intestinal mucous membrane into the blood of the villi through osmosis. Water is passively transported, requiring no energy with the net flux of water associated to the net flux of sodium and potassium[9].

2.3.2 Regulation of Body Temperature

Poultry are "homoeothermic," meaning they can regulate their body temperatures to hold a constant temperature under a wide range of environmental conditions [7]. Water intake aids in thermoregulation of the body temperature. In order to maintain thermal equilibrium, water is lost via evaporation from the skin by passive diffusion or from the respiratory tract by panting [7]. At 2°C only 11 % of the heat loss was because of the evaporation of water [7]. Additionally, each time there was an increase in environmental temperature above 2°C there was also an increase in evaporative heat loss. When the environmental temperature reached 35° C, there was a 100 percent

heat loss through evaporation of water [7]. Because water is utilized to regulate body temperature, it is vital to continually provide sufficient water to birds, especially in hot environmental temperatures.

2.3.3 Removal of Water

The body maintains a balance between water intake and water output through excretion of urine from the kidneys. An excessive amount of dietary salt can influence the water intake and the amount of waste excreted [8]. Unlike pure water, salt is not easily excreted. When an excessive quantity of salt is accumulated, the amount of extracellular fluid is increased, causing edema. Extracellular fluid is increased because of the consumption of more water to cease thirst and to dilute the salt concentration to a normal concentration. Additionally, extracellular fluid is also increased because of the kidney reabsorbing large quantities, of water which reduces the amount of excreted urine [8].

2.4 Factors Which Impact Water Consumption

There are several factors that affect water consumption. Reference [9] states, "water consumption is influenced by several factors including size and age of the bird, environmental temperature, and type and amounts of food consumed." Monitoring daily water consumption can be a useful tool to measure performance in birds. Likewise, it is important to monitor daily water consumption because an increase or decrease in water consumption can be an indication of health problems within the flock [10]. Table 1 demonstrates the estimated annual drinking water usage for U.S. poultry farms in 2007 based on the different types of poultry produced.

Table 1. Estimated Drinking Water Usage for Poultry Farms in the United States

Sector	Number of Farms	Number of Birds	Water (mil gal)
Broilers	32,668	1,602,000,000	25,632
Layers and Breeders	145,615	349,772,000	1,399
Pullets	22,514	105,876,000	52,938
Turkeys	17,226	107,173,000	85,738

[11]

2.4.1 Taste

Taste can affect the amount of water a bird consumes [12]. The 316 taste buds of birds are dispersed mainly on top of the mouth towards the back [13]. Reference [13] stated the front of the tongue is cornified and comprised of a horny layer containing a few taste buds [8,9]. Reference [12] stated the taste buds in birds are situated so far back, by the time birds can taste something it is too late to stop the swallow process, as the material has nearly reached the oesophagus. Birds are able to distinguish two main tastes: sour and bitter. Because of this, birds prefer more acidic water than basic water and can drink water with a lower pH until the pH reaches about 1.5 [12]. An experiment was conducted to gather information on whether a change in the taste because of the addition of an acidified calcium bisulfate product affected water consumption by broiler chickens [14]. Results indicated there was no significant difference in the overall consumption of water in the flocks. However, the water consumption did decrease during the first week of age, and remained lower through 21 days of age, after which water consumption was increased for the remainder of the flock. Hence, the overall water consumption pattern was similar to the control

group [14]. These results indicate during the life of the flock, there may be times when taste may influence consumption. While many animals associate odors with taste, birds are minutely affected by the odors in their environment [14].

2.4.2 Water Temperature

The water temperature can also impact consumption of water in birds [12]. Birds prefer the temperature of water to be lower than their body temperature. It has been reported birds will suffer from acute thirst rather than consume water that is one or two degrees warmer than their body temperature [14]. However, there is no minimum low temperature observed that will cause birds to stop consuming water. Birds will readily consume water close to freezing temperature (32° F) [12]. Jones et al., (2007) stated while the birds have feathers, which provide them with insulating warmth, the decrease in consumption of warm water may be because of the restricted method of heat loss from the body.

2.4.3 Water Flow Rate

Low rates in water flow can also impact water consumption in birds [15][16]. It is important that poultry production facilities have sufficient volume of water to supply flock needs. This will be dependent on time of year, age, size, and capabilities of controlling the environment in the grow-out facility. Multiple barns on a farm can also compound water demand requirements, especially during times of peak water demand. Insufficient plumbing systems or pipe sizing can result in restrictions to water consumption [16]. Low water flow, particularly if the water has been warmed during the production cycle, can be because of mineral buildup in the lines with the primary culprits being calcium, magnesium, iron, and sulfate that can create deposits which restrict pipe diameter [17]. Cobb-Vantress, a leading genetic company for the production of commercial

broiler chicken lines, recommends the following static flow rates (Table 2) from the actual nipple drinkers [18].

Table 2. Recommended Water Flow Rates by Cobb-Vantress

Age (week)	Water Flow Rate Milliliter (ounce) / minute
Week 1	40 ml (1.35oz)/ min
Week 2	50 ml (1.69oz)/ min
Week 3	60 ml (2.03oz)/ min
Week 4	70 ml (2.37oz)/ min
Week 5	90 ml (3.04oz)/ min

[18]

2.4.4 Environmental Challenges

The environmental temperature can also affect water consumption in birds. Water intake can double or triple during times of elevated temperature as birds use water for cooling their body [19]. During heat stress, birds will pant to evaporate water from their respiratory system. The evaporated water needs to be replaced, which is why there is an increase in water consumption [19]. As temperatures increase over 21°C, broilers will increase their water consumption by 7 percent for every 1°C rise in environmental temperature [1]. A study conducted at the University of Georgia [19] investigated the water consumption of broiler chickens in response to different weather conditions. Results are provided in Table 3. The study found water consumption increased per each pound of feed consumed when the environmental temperature increased.

Table 3. Water Consumption Response to Different Weather Conditions

Weather Condition	Pounds of water per pound of feed
Cold Weather	1.55
Mild Weather	1.65
Hot Weather	1.75

[19]

Lighting programs, whether artificial or natural light, can also influence the amount of water consumed by birds [20]. Birds do not consume feed or water while the lights are off or during dark periods. If the lights are turned off for more than eight hours, it is not uncommon to see a decrease in daily water consumption. When the lights are turned on, particularly after an extended dark period, there is a peak in water consumption [20]. Additionally, water consumption is found to peak right before the lights are turned off at night when birds are given a consistent bedtime during their life [20]. In a natural lighting environment, these peaks would occur at dawn and at dusk. In artificial lighting the increase in consumption prior to the lights being turned off may be because of the fact that birds have become familiar with the lighting program [20]. Because birds can become familiar with the lighting program and adjust their behavior in preparation for the dark period, it is recommended that when there needs to be a change to the lighting program, one should always change the time the lights are turned on, rather than when the lights are shut off [20].

2.4.5 Feed Consumption

Feed and water consumption patterns are closely correlated. Reference [21] correlated the relationship between feed and water intake to be 0.98, meaning that when water intake changes, there is a 98 percent chance the feed intake will also change [21]. The temperature of the environment affects the energy requirement for the birds. To satisfy the energy requirement birds will consume more or less feed. When the environmental temperature is colder, birds will consume more feed. On the contrary, when birds are exposed to warmer temperatures, they will consume less feed, but more water. Feed consumption decreases by 1.5 % for each 1°C rise above the thermoneutral zone, 27°C and 37°C [1,16]. As a result of the decrease or increase in feed

consumption based on environmental conditions, the water intake will also increase or decrease because birds drink approximately twice the amount of water as the amount of feed consumed [1].

2.4.6 Bird Age

Consumption of water is also dependent on bird age with consumption increasing as birds age. Table 4 shows the cumulative daily consumption of water (gallons/1,000 birds) for broiler chickens and can be used as a water consumption guide for broilers [23]. As the birds age and water consumption increases, the amount of water as a percentage of body weight decreases [19].

At 22°C and one week of age, broilers should be consuming 10 gallons of water/ 1,000 birds per day. At eight weeks of age, broilers should be consuming 55 gallons of water/ 1,000 birds per day. As mentioned before, the temperature of the environment the birds are placed in will affect the amount of water consumed [19]. One week old broilers exposed to 32°C will consume 20 gallons of water/ 1,000 birds per day. Eight week old broilers at 32° C consume closer to 108 gallons of water per 1,000 birds per day [23].

Table 4. Cobb-Vantress Water Consumption Guide

Broiler Age	**Gal/1,000 birds/ day**	**Gal/1,000 birds/ day**
Weeks	22°C (70°F)	32°C (90°F)
1	10	20
2	16	31
3	25	49
4	33	65
5	40	72
6	46	90
7	51	100
8	55	108

[23]

2.5 Factors Which Affect Quality of Poultry Drinking Water

2.5.1 Alkalinity

Alkalinity refers to the amount of hydrogen ions absorbed without changing the pH, or in other terms, the ability to neutralize acid [24]. There are three chemical forms that contribute to alkalinity: bicarbonates (most common), carbonates, and hydroxides [25][24]. Alkalinity acts as a buffer in a solution, which means when acid is added to a solution, the pH will not become more acidic because of absorption of the excess amount of hydrogen ions, thus preventing the pH from fluctuating. Reference [24] provides an example of alkalinity. Stating, "if you add the same weak acid solution to two vials of water- both with a pH of 7, but one with no buffering power (e.g. zero alkalinity) and the other with buffering power (e.g. an alkalinity of 50 g/ml), - the pH of the zero alkalinity water will immediately drop while the pH of the buffered water will change very little or not at all [24]". The pH of a solution will alter when the buffering capacity is maximized. Alkalinity can occur naturally and is dependent on the type of soil and bedrock through which the water passes [24].

2.5.2 pH

The pH is the amount of hydrogen ions found in a solution [26]. It can affect how corrosive the water may be, the taste of the water, and the efficacy of chlorine treatments for sanitizing the water [26]. Acidity or basicness of water is determined by the pH. The pH scale ranges from 0 to 14 where a value of 7 is neutral, under 7 is acidic (hydrogen ion excess), and above 7 is basic (hydroxyl ion excess) [26] [17]. A difference in 1.0 signifies a factor of 10, since the pH scale is logarithmic [17]. To explain, a pH of 4.0 is 10 times more acidic than 5.0 pH. The average range of pH for natural waters is 6-9 [26].

Acidic water also occurs [17] and can have two different causes. The first, which is abnormal, happens when small amounts of mineral acids, like sulfuric and nitric, contaminate the water supplies. The second and more common way acidic water is created is when rain water absorbs carbon dioxide and then seeps into the groundwater table before it is dispelled [17]. Problems can arise when water pH falls below 6.8. Acidic water can cause corrosion to plumbing equipment such as galvanized metal pipes and pumps. If the piping is copper and corrosion occurs, there will be blue or green stains on the plumbing fixtures [17]. Copper corrosion can result in excess copper levels which may go above the safe drinking level for copper which EPA lists as 1 ml/l. Acidic water can also interfere with the removal of iron and manganese [17].

A high pH in natural waters is rarely a problem. However, a high pH can interfere with water treatments, such as treatment with chlorine, as the sanitizing effect of chlorine is found to be maximum when pH levels are below 6.5 [26] [17].

2.5.3 Contamination of Water

Pure water rarely exists in nature [26]. The minerals and organic material dissolved in water are what gives water its identity and characteristics. Minerals dissolved in water come from a variety of sources discussed below. The United States Environmental Protection Agency (EPA) considers anything dissolved in water as a contaminant and the quantities of contaminants are described as milligrams/liter (mg/L) or parts per million (ppm) [3].

2.5.3.1 Source Contamination:

Primary water sources for poultry include small streams, rivers, lakes, impoundments, and ground water [26]. The most common source for poultry production is groundwater or wells. It is important to know what challenges are prevalent in water sources. While water serves as a carrier for minerals, certain minerals can affect bird health and performance, such as sodium,

nitrates, and magnesium [26]. Water sources can also contain microorganisms, such as fecal coliforms or soil microbes that flow from the source into the poultry houses [26].

Stream water quality can vary by time and by geographical locations. The makeup of a stream is dependent on the change in climate, the quantity of rainfall, the variety of soils it comes in contact with or the sediments and rocks the water passes through as it enters the aquifer as run-off from the terrain. Also, human activity can influence the characteristics of a stream [26]. Lakes are typically more stable and have less variation in their composition as compared to streams. The composition of water that flows into a lake is the most important factor that can influence the water quality in the lake. Consequently, lakes tend to have a makeup similar to the streams and rivers flowing into them [26]. The amount of dissolved solids in lakes is also dependent on the constituents in the water flowing into the lake, the quantity of water flowing in and out of the lake, the volume of precipitation and evaporation, biological makeup of the lakes, and the arrangement of the lake. The concentration of the constituents is also affected by the physical location of the lakes and by the daily, seasonal, and annual changes the lakes regularly experience [26].

In the United States groundwater, commonly accessed through wells, is the most popular source for providing water to poultry. Similar to lakes and streams, ground water is also affected by the composition of the type of rocks, soils, and sediments it travels through before moving towards underground aquifers or pores [26]. The main source that affects constituents of groundwater is the amount of soluble products released during soil development and the breaking down of rocks over time. Groundwater is also affected by the amount of vegetation in an area [26]. Groundwater in an area with significant crop production can be affected by the amount of surface runoff and by the continuous buildup of elements in the surface soils. Wastewater used for industrial purposes can also affect the makeup of groundwater. Lastly, the flow rate

groundwater supplies to the surface is dependent on well depth, pump gallons per minute rating, and casing size [26].

2.5.3.2 Microbial Contamination of water:

Newly hatched chicks acquire passive immunity by the transfer of maternal antibodies from the yolk sac into the embryonic circulation which rapidly decreases during the first 3 days after hatch [27]. Baby chicks do not have a fully developed immune system for the first 2 weeks of life, thus making them susceptible to diseases [28] [29] [30] [31]. Therefore, drinking water quality for chicks should not be compromised as they may lack the defenses necessary to properly handle microbial rich water. It can potentially affect their health leading to both enteric and respiratory diseases resulting in a decrease in growth and performance.

Water can be a perfect host for many microorganisms such as fungi, protozoa, bacteria, and viruses [32]. Microorganisms use organic matter and nutrients found in the water system to grow. Many microorganisms need very little food to survive in water systems [32]. These microorganisms have the potential to proliferate and contaminate the entire water system, eventually reaching the intestines of the birds.

As mentioned previously, the Environmental Protection Agency (EPA) sets standards for drinking water quality used for human consumption, which are followed as a guideline to establish water quality standards in animal production and agriculture. These standards are demonstrated in Table 5.

Table 5. EPA Drinking Water Primary Standards and Treatments for Microorganism

Contaminant	Maximum Contaminant Level Goal (MCLG)	Maximum Contaminant Level (MCL) or Treatment Technique (TT)	Source of Contaminant of Drinking Water
Cryptosporidium	zero	TT- No limit information, include microorganism into watershed control provisions	Human and animal fecal waste
Giardia lambia	zero	TT- 99.9% removal/inactivation	Human and animal fecal waste
Heterotrophic plate count (HPC)	n/a	TT- No more than 500 bacterial colonies per milliliter	HPC measures a range of bacteria that are naturally present in the environment
Legionella	zero	TT- no limit, EPA believes if *Giardia* is removed/inactive, *Legionella* will be controlled	Found naturally in water; multiples in heating systems
Total Coliforms (including fecal coliforms and E.coli)	zero	5.0%	Coliforms are naturally present in the environment; as well as feces; fecal coliforms and E.*coli* only come from human and animal fecal waste
Turbidity	n/a	TT- < 0.3 Nephelometric Turbidity Unit (NTU) in at least 95% of the samples in any month	Soil runoff
Viruses (enteric)	zero	TT-99.9% removal/inactivation	Human and animal fecal waste

[4]

Even though drinking water without the presence of any microorganisms is ideal for bird consumption, achieving this can be difficult in field conditions. Table 6 displays the desirable levels of various microorganisms as well as the maximum acceptable levels that can be present in poultry drinking water [4] [5]. The microbial counts given in Table 6 are listed as colony forming units per ml of water (cfu/ml). A water system with less than 1,000 cfu/ml of total bacteria is considered a clean system. There is zero tolerance for organisms such as fecal coliforms,

Escherichia coli, and pseudomonas in water systems. It is unacceptable to have more than 50 cfu/ml of total coliforms in the water [4] [5].

Table 6. Drinking Water Quality Standards

Source	Good Quality	Maximum Acceptable Amount
	cfu/ml	
Main Water Supply	<100	<300
Total Bacteria Count	0	<1,000
Total Coliforms	0	50
Fecal Coliforms	0	0
Escherichia coli	0	0
Pseudomonas	0	0

[5]

Total plate count (TPC) bacteria is also called aerobic plate count (APC) or standard plate count or mesophilic count and is an indicator of the total bacterial load present. While this test does not identify the different types of bacteria, it does provide insight to the total level of bacteria in the water sample, which can indicate whether the water system has a light or heavy contamination load [33].

Presence of coliform bacteria in water samples is an indicator for potential disease causing bacteria in water [25][34]. Coliforms are a group of bacteria that are Gram-negative, rod-shaped, non-spore forming, and aerobic or facultative anaerobic bacteria [35]. These organisms are present throughout the environment and commonly found in soil, surface water, on plants, and on other living things. Most types of coliform bacteria are usually non-pathogenic to humans, but some can cause mild illnesses whereas a few can cause serious diseases [34]. Water samples are usually analyzed for total coliforms or for the presence of specific subgroups of coliform bacteria such as fecal coliforms and *E. coli* [25]. Coliform bacteria are used as indicator organisms in water samples as they are easy to detect if present in water, can normally live longer than other

pathogenic bacteria, are easy to isolate and enumerate in a water sample if present. Most of these bacteria are normally non-pathogenic and require no special safety procedures to handle [36]. Hence coliforms can be used as dependable indicators to determine whether the water has been securely processed for human consumption [25].

Escherichia coli (E.coli) is a major species under the group of fecal coliform bacteria and are normally found in the intestinal tracts of humans and warm blooded animals. Their presence indicates fecal contamination of the water supply[25]. Nonpathogenic fecal coliforms, including *Escherichia coli*, occur in human feces at 50 million coliforms per gram of feces. Additionally, untreated waste water typically contains more than 3 million coliforms per 100 milliliter of water [25]. This group of bacteria generally do not grow and reproduce in the environment and they constitute the majority of the fecal coliforms present in feces. Because of *E.coli* is prevalent in the feces, it is considered as the best indicator of fecal contamination as well as presence of pathogenic microorganisms in water systems[25]. Even though most of the strains of *E.coli* are non-pathogenic, a few strains are capable of producing toxins and diseases resulting in death [25].

Another class of bacteria usually found in poultry drinking water is the *Pseudomonas* species. These bacteria are ubiquitous in the environment and found in natural waters such as lakes and rivers [37]. There are around 191 species in the *Pseudomonas* genus, but the main species of concern is P. aeruginosa which grows in water especially at warmer temperatures[37] [38]. Pseudomonas species of bacteria are capable of adhering to the interior pipe walls, inside filters and on other surfaces where it will readily colonize and form large areas of biofilms. The biofilms produced can protect and harbor many other harmful bacteria such as Salmonella, Campylobacter, E.coli and Legionella [52] [53].

Microbial contamination can occur at the source of the water system, whether it is from an aquafer, a pond, or any other water source [26]. In the case of poultry barn water systems contamination can occur throughout the system. If a water supply becomes infected with microorganisms, regardless of where in the system, the microbes can readily and rapidly spread throughout the entire water system, starting from the source and proceeding into the mouth of the birds. Table 7 demonstrates a water system that may have an acceptable amount of microbes at the source, but by the time the water reaches the end of water line, the microbial level can increase to an unacceptable level [5] [39]. Microbial contamination of the water system can also occur by the action of the birds triggering the water nipples to obtain water. The beaks of the birds can contain numerous amounts of bacteria and when triggering the nipple they transfer microbes from the beak to the water system where the microbes can then wick back into the water lines [5].

Table 7. Examples of Aerobic Bacteria in Poultry Drinking Water (cfu/ml)

Farm	Source (i.e well)	End of Drinker Line
	cfu/ml	
A	2,700	26,600
B	600	282,000
C	0	4,775,000

[5] [39]

2.5.3.3 Mineral Contamination

It is important chickens get the appropriate amount of minerals, whether consumed in feed or water, to prevent harmful effects. [1][40]. A mineral analysis of water supplies can provide important information on the types and quantity of minerals present so treatment measures can be implemented if necessary or diets reformulated to compensate for elevated nutrient levels such as sodium and chloride.

2.5.3.3.1 Calcium

Calcium is an essential mineral for bone formation and egg shell formation, as well as aiding in blood clotting [1]. Deficiencies can lead to poor growth and rickets. An excess of dietary calcium can interfere with other minerals such as magnesium, manganese, phosphorus, and zinc making them less available for dietary needs [1]. Average calcium levels found in drinking water supplies is 60 mg/L [40]. To date maximum allowable level has not been established. However, treatment to the reduce calcium buildup may be necessary if levels exceed 51 mg/L or 3 grains of hardness [40] [17]. A water softener which replaces calcium with sodium or adjusting water pH to 5 or below with acidification can be used to prevent calcium deposits or scaling [17] [25]. Water softeners are an ion exchange process; meaning sodium ions are exchanged with calcium and magnesium ions in remove these minerals [17].

2.5.3.3.2 Chlorides

Chloride is required for growth, bone formation, egg shell quality, utilization of amino acids, and helps maintain an osmotic balance within the body [1]. Birds have a high tolerance for chlorides, unless it appears in combination with sodium which reduces tolerance level (Table 8) [40][41]. Overconsumption of chloride can have a negative effect on metabolism [42]. Average chloride levels in well water is 14 mg/L. The maximum acceptance level for chlorides in poultry is 250 mg/L [40] but reviews of field data indicate the tolerance level is closer to 150-200 mg/L. Chloride can be removed from the water system by the physical process of reverse osmosis or by the chemical process of anion exchange [43].

2.5.3.3.3 Copper

Copper is necessary for oxygen transport and binding in the blood. A shortage of copper can cause anemia in poultry, along with bone deformities [1] [44]. Too much copper can leave a

bitter taste in the water [1] [42]. Typical copper levels in source water are 0.002 mg/L. The maximum acceptable level for copper is 0.06 mg/L [40]. Copper can be removed from water through chlorination and filtration, reverse osmosis, or ion exchange [45].

2.5.3.3.4 Magnesium

Magnesium, in combination with phosphate and bicarbonates, is utilized in the body to maintain osmotic equilibrium and pH through the body [1]. A deficiency in magnesium can cause the birds to grow slowly with continued deficiency resulting in stunted growth and lethargic behavior [1]. If a magnesium deficient diet is provided to laying hens, a rapid decline in blood magnesium levels can occur resulting in the hen withdrawing magnesium from the bones. A continued deficiency results in a decrease in egg production, a comatose state, and death [1]. On the contrary, too much magnesium, particularly when combined with sulfates in the water, can lead to loose droppings [42]. The average amount of magnesium found in wells is 14 mg/L, while the maximum acceptable level is 250 mg/L [40]. However, magnesium at 50 mg/L can negatively affect broiler performance when combined with 50 mg/L per liter of sulfates [41]. Magnesium can also contribute to the formation of scale in watering systems [17]. Scaling is discussed more in detail in the 'Water Hardness' section.

2.5.3.3.5 Nitrates and Nitrites

Nitrates can be found in groundwater due to contamination caused by inorganic fertilizers and animal manure [46]. These minerals occur in groundwater because of contaminated runoff that seeps through the soil [42]. Nitrates can have a negative impact on poultry performance by reducing growth rate, feed conversion, and egg production [42] [47]. The only way to detect nitrates is through analysis because unlike most minerals, nitrates have no color, odor, or taste [42] [48]. It has been reported that 25 mg/L of nitrates can affect poultry [40]. However, as little as 4

mg/L per liter of nitrites will affect poultry performance [42]. The best way to remove nitrates and nitrites from the water is by detecting the source and then eliminating the contamination [17]. If this is not possible, they can be removed through reverse osmosis [17].

2.5.3.3.6 Sodium

Sodium is necessary for egg production, shell quality, bone development, and utilization of amino acids. Additionally, sodium plays a key role in maximization of bird growth [1]**.** Sodium deficiency results in poor growth and increase in adrenal gland weight, while laying hens will experience a decrease in egg production [1]. Conversely, an excessive amount, greater than 50 mg/L of sodium, can increase the amount of urine produced [42]. The average amount of sodium in well water is 32 mg/L. An evaluation of sodium levels and bird performance revealed birds were tolerant of up to 50 mg/L [40]. However, the maximum acceptable level changes when sodium is combined with other minerals such as chloride, sulfate, or bicarbonate [41]. Combinations of sodium with chloride and sulfate can result in detrimental effects to performance. Table 8 shows the levels of different combinations of minerals with sodium in drinking water results in detrimental effects on poultry [41].

Table 8. Chemical Combinations in Poultry Drinking Water

Chemical Combinations	Levels (mg/l)	Effect
Sodium Chloride	50 14	Detrimental to performance
Sodium Sulfate	50 50	Detrimental to performance
Sulfate Magnesium	50 50	Detrimental to performance
Sodium Bicarbonate	200 ≥ 500	No effect

[49]

2.5.3.3.7 Sulfides and Sulfates

Sulfides can cause the water to smell like rotten eggs [17]. This naturally occurring smell is derived from hydrogen sulfide gas. Certain bacteria present in water have the ability to reduce sulfates, sulfites, or sulfur to form hydrogen sulfide. These bacteria can produce black slime in water [17]. Along with causing taste and odor issues, hydrogen sulfide can be very corrosive. It can combine with other minerals, such as iron, and can cause water to turn black. Like manganese and iron, sulfur can also be removed through oxidation and filtration [17].

Sulfates can cause a laxative effect in birds. The maximum tolerance level for sulfates for poultry is 250 mg/L, while the average amount of sulfates found in well water is 125 mg/L. Sulfate can become more of a concern in water at a lower dose of 50 mg/l when combined with 50 mg/L of sodium [40].

2.5.3.3.8 Iron and Manganese

Manganese and iron are discussed together because they share many similarities. Both of these trace minerals are found in groundwater where rainfall, high in carbon dioxide, passes through igneous rock [17]. Higher concentrations of both minerals can cause a metallic taste and a red-brownish color in appearance [17][42]. These minerals dissolve in water and continue as bicarbonate in water until exposed to air [17]. When exposed to air, oxidation occurs causing the minerals to change from bicarbonate to insoluble hydroxide form. These insoluble hydroxide particles are what results in the reddish- brown color [17].

Manganese is necessary for poultry to ensure bone development and to maximize egg production and shell quality of layers [50]. A deficiency in manganese in chicks and poults can lead to perosis or slipped tendon [1]. Additionally, laying hens deficient in manganese can experience lower egg production, a decrease in egg shell strength, poor hatchability, and a decrease

in fertility [1]. An excessive amount of manganese for poultry consumption has not been determined. Iron is important because it plays a role in growth, performance, and reproduction [51]. A deficiency in iron for chickens and turkeys can result in anemia [1]. A source water iron level of 0.02 mg/L is considered average, with 0.03 mg/L considered to be an excessive amount for poultry production [40].

Reference [19] reported high levels of manganese (20ppm) and iron (600pm) did not have an impact on broiler health. However, there was equipment failure because of high iron concentrations. The mineral sediments caused water nipples to leak and fogging nozzles to clog, which could affect the performance of poultry by causing wet floors from leaking drinkers or inadequate cooling during hot weather [19].

Oxidation aids in the removal of iron and manganese [17]. There are four different methods utilized for iron and manganese removal: aeration; chlorination and filtration; ion exchange; and a slow sand filter. The pH of water plays a role in the removal of these minerals. Iron is typically removed at a pH value of 7.5 or higher, whereas manganese is difficult to eliminate at a pH value less than 8.5 [17].

2.5.3.4 Water Hardness

Water hardness is correlated to amounts of calcium, magnesium, sulfur, and bicarbonates found in water [17] [52]. Excessive amounts of these minerals form scale or mineral build-up and impacts equipment function by reducing the size of distribution pipes and impairing the activation of nipple drinkers. Reducing pipe volume can reduce water flow and water quantity to the birds [42]. Likewise, scale build-up can reduce the effectiveness of the sanitizer by acting as a barrier between the waterline and the sanitizer [48]. The water source also can have an effect on the level of hardness. Table 9 shows different hardness levels based on the amount of calcium carbonate as

illustrated by the U.S. Geological Survey [52]. Water hardness can be reduced by the use of a water softener.

Table 9. Hardness Values Classification by U.S. Geological Survey

Classification	Range (mg/l of $CaCO_3$)
Soft	0-60
Moderately Hard	61-120
Hard	121-180
Very Hard	>180

[52]

Table 10 displays water quality standards for the different minerals listed above [17] [40]. The table provides the average level of a contaminate as well as the maximum acceptable level. Additionally, the effect when maximum acceptable level has been reached is described along with treatment options.

Table 10. Water Quality Standards and Treatments

Water Quality Indicator	Levels Considered Average	Maximum Acceptable levels	Maximum Acceptable Levels Indicate	Treatment Options/Comments
pH	6.8- 7.5	5-8	-below 5 - metal corrosion above 8 - Water - sanitizers work poorly, “bitter” taste	Raise pH with soda ash, lime, or sodium hydroxide (NaOH) Lower pH with phosphoric acid, sulphuric acid and hydrochloric acid for strong alkalinity, citric acid and vinegar for weak alkalinity
Alkalinity	100 mg/l	300 mg/l	- Associated with bicarbonate, sulphates, and calcium carbonate - Can give water a bitter taste which makes it undesirable to the birds - High levels can make it difficult to lower the pH - Can be corrosive to cool cell pads	- Acidification - Anion Exchange de-alkalizer - Can be reduced by removing free CO2 (carbon dioxide) through aeration
Hardness	-Soft 0 - 75mg/l as CaCO2 -Somewhat hard 76 to 150 -Hard 151 to 300 -Very Hard >300	.	- Hardness causes scale which reduces pipe volume and drinkers hard are to trigger or leak (main factors are calcium and magnesium, but iron and manganese contribute small amount)	-Do not use water softener if water already high in sodium unless using potassium chloride instead of sodium chloride (salt) -Polyphosphates will sequester or tie-up hardness and keep in solution -Acidification to below pH of 6.5

Table 10. Water quality Standards and Treatments (Cont.)

Water Quality Indicator	Levels Considered Average	Maximum Acceptable levels	Maximum Acceptable Levels Indicate	Treatment Options/Comments
Calcium	60 g/l	.	-No upper limit for calcium, but if values are above 51 mg/l may cause scaling	-Treatment same as hardness
Chloride	50 mg/l	150 mg/l	-Combined with high Na levels, can cause flushing and enteric issues -Can promote Enterococcus bacterial growth	-Reverse osmosis, blend with non-saline water, keep water clean and use daily sanitizers such as hydrogen peroxide or iodine to prevent microbial growth
Copper	0.0002 mg/l	0.6 mg/l	-High levels can cause oral lesions or gizzard erosion	-Source is most likely from the corrosion of pipes or fittings
Iron	0.2 mg/l	0.3 mg/l	- Metallic taste -Iron deposits in drinkers may cause leaking -Can promote growth of bacteria such as E. coli and Pseudomonas	-Treatment includes addition of one of the following: chlorine, chlorine dioxide or ozone then filtration removal with proper sized mechanical filtration
Lead	0 mg/l	0.05 mg/l	-Can cause weak bones and fertility problems in broiler or turkey breeders	-Lead is not naturally occurring. Look for pipes, fittings or solder that contain lead -Water softeners and activated carbon can reduce lead

Table 10. Water quality Standards and Treatments (Cont.)

Water Quality Indicator	Levels Considered Average	Maximum Acceptable levels	Maximum Acceptable Levels Indicate	Treatment Options/Comments
Magnesium	14 mg/l	125 mg/l	-May cause flushing due to laxative effect particularly if high sulphate is present	-Treatment same for hardness
Manganese	0.01 mg/l	0.05 mg/l	-Black grainy residue on filters and in drinkers	-Similar to iron but can be more difficult to remove due to slow reaction time -Chlorination followed by filtration most effective in pH range of 8.5, needs extended contact time with chlorine prior to filtration unless using Iron X media -Ion exchange resin if pH is 6.8 or above -Greensand filters with pH above 8.0
Nitrates	1-5 mg/l	25 mg/l	-Poor growth and feed conversions -May indicate fecal contamination, test for coliform bacteria	-Reverse osmosis -Anion exchange

Table 10. Water quality Standards and Treatments (Cont.)

Water Quality Indicator	Levels Considered Average	Maximum Acceptable levels	Maximum Acceptable Levels Indicate	Treatment Options/Comments
Sodium	50 mg/l	150 mg/l	-With high Cl levels can cause flushing -Can promote Enterococcus bacterial growth	-Reverse Osmosis -Blend with non-saline water -Keep water clean and use daily sanitizers such as hydrogen peroxide or iodine to prevent microbial growth
Sulfates	15-40 mg/l	200 mg/l	-Flushing in birds -Rotten egg smell is hydrogen sulphide, by-product of sulphur-loving bacteria growth - this can cause air locks in water system as well as flushing in birds -Since sulphides can gas off, test results may underestimate actual level present	-Aerate water into a holding tank to gas off sulphur - Anion exchange (chloride based) - Treatment with oxidizing sanitizers then filtration -If a rotten egg odour is present, shock chlorination of well is recommended plus a good daily water sanitation program while birds are present
Zinc	.	1.5 mg/l	-Higher levels may reduce growth rates	-Look for locations where water may have come in contact with galvanized containers -Water softener and activated carbon will reduce adsorption

[17] [40]

2.6 Drinking Water Sanitation

Prior to the 1990's, when open style trough or bell plassons were the main drinkers used in the poultry industry, one could easily see how dirty the water was by merely looking at it. These open drinkers were easy targets for in-house contaminants like dust, feathers, feed, fecal and bedding material[53]. The industry converted to enclosed style drinkers, where the birds accessed the water supply by activating a small pendulum which allowed water to flow directly into the mouth of the bird from the enclosed pipe. The cleanness of the water increased and a direct correlation was found between the enclosed drinkers and a reduction plant condemnation because of diseases such as air saculitis. The enclosed systems led poultry producers to assume the water they were supplying the birds was clean, because there was no longer clearly visible contamination. However, this was not an accurate indicator [53]. Reference [53] stated these enclosed systems can provide a robust breeding ground for microbial contaminants such as fungi and bacteria that flourish on nutrient rich, slow moving warm water especially during brooding periods. Mineral buildup can occur in these closed systems leading to the development of biofilms that contributes to increased microbial contamination. Thus, it is very critical to have a successful water sanitation program in poultry production facilities to decrease or minimize the amount of microorganisms living in water. A water sanitation program also reduces the risk of many harmful diseases in the birds. If a high level of multiple types of microorganisms are present in a water supply, there is potential the water is harboring an infectious dose of a pathogens. Zero detection of microorganisms in the drinking water is ideal for bird consumption. Table 11 displays the microorganism parameters indicative of good quality drinking water. Additionally, the table suggests the maximum acceptable amounts of microorganisms in poultry drinking water [5]. The quantities given are in colony forming units per milliliter of water (cfu/ml).

Table 11. Poultry Drinking Water Quality Standards

Source	Good Quality	Maximum Acceptable Amount
	cfu/ml	
Main Water Supply	<100	<300
Total Bacteria Count	0	<1,000
Total Coliforms	0	50
Fecal Coliforms	0	0
Escheriachia coli	0	0
Pseudomonas	0	0

[5]

One key criteria for evaluating drinking water disinfectants for poultry operations is determining the time it takes to eliminate the targeted organisms and still provide a maintenance level of sanitizing residual to maintain microbial control throughout the entire water system under different operating conditions [54] [55]. The ideal disinfectant is environmentally friendly, produces minimal by-products as well as is easy to prepare and/or inject on-site plus safe to be used by poultry famers. Often the deciding factor for product selection in poultry operations is affordability. Achieving all these goals for establishing a water quality program on poultry farms with unique water quality makes the process challenging [55]. There are several disinfectants currently used in poultry production [54].

2.6.1 Chlorination

Chlorine is the most commonly used water disinfectant because of availability and low cost [54]. Chlorine is categorized as an oxidizer. As a water disinfectant, chlorine is used primarily in the form of sodium hypochlorite, calcium hypochlorite, and gas chlorine; all which are recognized as drinking water sanitizers by the Environmental Protection Agency (EPA) [56]. Recently, other forms of chlorine such as bleach crystals (99% sodium dichloro-s-triazinetrione dehydrate + 1 %

of other ingredients) have been approved for use in poultry drinking water [57]. As a drinking water sanitizer chlorine has both advantages and disadvantages. Chlorine is an effective, inexpensive biocide that is easy to use. It can oxidize minerals such as iron, manganese, and sulfates. Also it is capable of eliminating undesirable odor, color, and taste from water if used in appropriate levels [52] [56]. Chlorine based disinfectants leave a measurable residual in the form of free or total chlorine that can be monitored to ensure adequate levels of chlorine have been administered for microbial reduction/elimination. The EPA recommends 2-5 parts per million (ppm) of residual chlorine in drinking water [56].

The disadvantages of chlorine include its capacity to produce undesirable by-products and its loss of efficacy over time in the water system in unopened containers. Some forms of chlorine, such as gas chlorine, are considered hazardous and corrosive while other forms are considered non-hazardous but corrosive to metals and stainless steel [52] [56]. Another disadvantage is the sanitizing efficacy of chlorine is pH dependent. When chlorine is added to water it quickly hydrolyses to produce hypochlorous acid (HOCL) and/or hypochlorite ions (ClO-) depending upon the pH of water. Hypochlorous acid is produced when water has a lower pH (4-7) while hypochlorite ions are produced when pH of water goes above 7.5 [54] Hypochlorous acid is a stronger biocide than hypochlorite ion. Therefore, a chlorine disinfectant is more effective as a sanitizer when the water has a lower pH [43] [54].

The efficacy of chlorine is impacted by many other variables. When there is organic material in the water, microorganisms tend to be protected by this material which can create a barrio which does not allow the disinfectant to penetrate and act upon the organism [54]. The concentration of chlorine and the contact time are also factors which can affect the efficacy of chlorine and its disinfectant capabilities [54].

The main purpose of chlorine is to eliminate and prevent the growth of bacteria in the water. The inactivation of bacteria can occur through a number of mechanisms including inactivation of key enzymes, disruption of nucleic acids making them nonfunctional, and oxidative damage to cell wall or other vital cell components [52]. While it is important to know how bacteria are inactivated, it is more important to understand the factors that influence the rate and extent of inactivation [52]. These factors include the type and concentration of organisms being inactivated, the concentration of the disinfectant, the form in which disinfectant is present, contact time, temperature, pH, and interfering substances [52].

2.6.1.1 Sodium Hypochlorite

A chlorine based sanitizer used in poultry drinking water is sodium hypochlorite (NaClO) which is composed of elemental chlorine, sodium hydroxide, sodium chloride, and water [52],[58]. Liquid bleach (5.25% sodium hypochlorite concentrate) is the product commonly used for poultry water sanitization. This chlorine source is added at a rate of 2-8 ounces into a gallon of water to create a stock solution. Once the stock solution has been prepared, it is injected into the watering system with a metering pump. The metering pump pulls one ounce of the stock solution into each gallon of water that passes through it. The solution blends into the water flow via a mechanical or induction mixer. This is typically referred to as a 1:128 injector or one ounce added to every 128 ounces. A second method for injecting bleach is to add it straight into the water supply with a peristalic pump with an adjustable injection rate so smaller amounts can be dosed into the water supply. Sodium hypochlorite is easy to use in smaller watering systems [56] and with injection systems already in place for use with vaccinations or mediations. Other concentration levels typically available for water sanitation include 10 percent and 12 percent sodium hypochlorite.

A problem with using this form of chlorine in a water system is the significant loss of available chlorine from the sodium hypochlorite within a few days [52] [56]. Liquid bleach loses efficacy over time because it has a limited shelf life. Once a container of bleach is opened, 50% of the available chlorine can gas off in 3-4 months, resulting in a loss of chlorine efficacy [58]. Factors such as increased temperature, presence of heavy metals, pH, and exposure to light can all increase the degradation of sodium hypochlorite [56]. When degradation occurs, the available chlorine content reduces while the quantity of byproducts such as chlorates and bromates increase [52].

To optimize the stability of chlorine in sodium hypochlorite concentrates, basic pH stabilizers are added to achieve a pH of 12. This maximizes chlorite ions in the solution which are less prone to loss than hypochlorous acid. Therefore, adding such sodium hypochlorite to water raises the pH. This high pH can be corrosive to materials such as metals and stainless steel [56]. For optimal chlorine efficacy in water, an acid such as sulfuric acid, or carbon dioxide, should be injected with a second injector to lower the pH of the water to below 7 [52]. This helps in shifting chlorine from the basic pH chloric ion form to the more effective sanitizer form of hypochlorous acid.

2.6.1.2 Calcium Hypochlorite

Calcium hypochlorite ($Ca(ClO)_2$) comes in a solid form such as in granules, compressed tablets, and powder. These forms of calcium hypochlorite contain 65 percent to 75 percent available chlorine. One advantage is it does not lose as much available chlorine compared to sodium hypochlorite. Calcium hypochlorite only loses 5% of its available chlorine in a year. When dissolved, it creates a solution with a higher pH, however, when compared to sodium hypochlorite it produces a lower pH [56]. This oxidizer is more often used on small scale water

systems. The dry powder or tablet form must be dissolved into water to create a stock solution which is then injected into the water system. It is less practical for use on large scale systems such as municipal plants and wastewater plant as mixing and dissolving of a dry product is required [52],[56]. Using this type of product on a large scale system requires manual handling and loading into tanks or hoppers for solution preparation, which is not considered a cost-effective method [52]. For poultry farm use, a stock solution of calcium hypochlorite is injected into the water system in a similar manner as liquid bleach, with a metering pump where it is mechanically or indirectly mixed. Calcium hypochlorite has to be stored in a dry area, because it will react with moisture and heat [56].

2.6.1.3 Gas Chlorination

Gas chlorination is another strong oxidizer which is commonly used as a drinking water sanitizer [52],[56]. It can be produced a number of ways including the electrolysis of alkaline brine or hydrochloric acid, the reaction between chloride and nitric acid, or the oxidation of hydrochloric acid [56]. Gas chlorination has the disadvantage of being the most dangerous form of chlorine [58]. It is produced off-site and then transported in pressurized metal cylinders to the end user. The U.S. Department of Transportation categorizes chlorine gas as a poisonous gas. One advantage of gas chlorination is being more cost effective compared to sodium and calcium hypochlorite [56].

Gas chlorination works by the process of an injector using water flow through a venture to draw chlorine gas out of the tank into a stream of water to form a concentrated solution. This solution is then incorporated into the main water system through a diffuser or mechanical mixer [56]. When added to water, gas chlorine rapidly hydrolyzes within seconds creating the active

ingredient hypochlorous acid which is a strong biocide [43],[54]. The pH of water will decrease with the addition of gas chlorine [56].

2.6.1.4 Sodium dichloro-S-Triazinetrione (Dichloro)

Another form of chlorine that is EPA approved and approved as a water disinfectant is sodium dichloro-s-triazinetrione ($C_3H_4Cl_2N_3NaO_5$). This form of chlorine is available in two forms: dihydrate and anhydrous. Available chlorine in the dihydrate form (56%) is less than in the anhydrous form (62%). Sodium dichloro, similar to calcium hypochlorite, is typically sold as granules but is also available in a solid form. Sodium dichloro also claims to lower the pH more than the other forms of chlorine. Sodium dichloro is typically used as a pool disinfectant as it is capable of producing cyanuric acid. This is an advantage as cyanuric acid can act as stabilizer in water by stabilizing the amount of free chlorine available. The heat of the sun has great potential to degrade the amount of free chlorine, but because of the stabilization properties of cyanuric acid, free chlorine has a slower degradation. This disinfectant solution is prepared by dissolving the granules in water at the appropriate rate given depending upon the purpose for use.

2.6.1.5 Chlorine Dioxide

Chlorine dioxide (ClO2) is a strong biocide and germicide and approved by the EPA [56]. This oxidizing agent is produced by mixing sodium hypochlorite with hydrochloric acid. The chemical equation is as follows:

$2NaClO_2 + Cl_{2\,(g)} = 2ClO_{2\,(g)} + 2NaCl$

Chlorine dioxide is a strong oxidant and disinfectant [56]. It can remove taste and odor from water as well as oxidize minerals. Compared to chlorine, chlorine dioxide is more efficient in the removal of iron, manganese, and sulfide through oxidation from watering systems [52] [57]. Likewise, it is excellent for inactivating viruses like *Cryptosporidium* and *Giardia* [56]. In

aqueous solutions, chlorine dioxide exists as a dissolved gas and can be easily removed when mixed with air [52]. Likewise, it can degrade if left in the presence of light [52],[56]. Typically, chlorine dioxide is generated on-site requiring two injectors because it is not stable in larger concentrations and, hence, cannot be shipped [39],[56]. However, there are solutions of 3000 ppm chlorine dioxide, such as TriSan, that is stabilized and does not allow acidification activation on site. Because of its stabilization, this product can be shipped [59].

One advantage of chlorine dioxide is its effectiveness over the pH range of 6-10 [52],[25]. Similar to chlorine, the efficacy of chlorine dioxide decreases as the temperature of the solution decreases [56]. It is important to understand chlorine dioxide does not form significant disinfectant by-products such as trihalomethanes or haloaceic acids [52],[25] which are considered carcinogens [56]. When nitrogenous compounds react with chlorine dioxide, there is no production of chloramines that form trihalomethanes [25]. However, it does produce disinfectant byproducts such as chlorate and chlorite, primarily chlorite (50%-70%) [52],[25],[56]. Toxins can be produced by chlorate and chlorite residuals [25]. Therefore, EPA limits the amount of chlorite present in drinking water to 0.8 ppm [56].

2.6.2 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is an oxidizing bactericide not approved by regulatory agencies as the sole water treatment disinfectant. However, it is approved in combination with other treatments such as advanced oxidization process [56]. Hydrogen peroxide is available in concentrations of 16 percent, 20 percent, 35 percent, 50 percent, and 70 percent. Hydrogen peroxide is unstable and oxidization occurs when hydrogen peroxide is added to water because of production of large quantities of dissolved oxygen [60]

$$H_20_2 \longrightarrow H_2O + O^-$$

The oxygen produced is nascent oxygen, which is capable of oxidizing organic matter in water. It does degrade over time if not used, but at a slower rate (1% - 5% per year). In the water, degradation increases as water temperature and the impurities contained in the water increases. Hydrogen peroxide has the disadvantage of being flammable at high concentrations making it hazardous to handle [52].

2.7 Parameters Used to Measure the Efficacy of Sanitizers

2.7.1 pH

While pH is not a specific contaminant or chemical, it is a variable that impacts the quality of the water. The pH of water affects the efficacy of chlorine as a sanitizer [54]. When chlorine is added to water, it hydrolyses rapidly to produce two active ingredients: hypochlorous acid and/or hydrochloric acid. The pH and the concentration of chlorine will determine what form of chlorine will be present in water [43]. Hypochlorous acid has a strong biocide action produced with a lower pH value ranging from 4 to 7. When a pH value of 6.5 is reached, hypochlorous acid becomes a very effective disinfectant as 90% of the free chlorine is available as hypochlorous acid [54]. Hypochlorite ions, which have little sanitizing power, are produced when the pH value is greater than 7.5. The distribution between the hypochlorous acid and the hypochlorite ions is equal when the pH value is approximately 7.5 [54]. When the pH value goes above 9, hypochlorite ions dominate in their production [54] [43]. A disinfectant is more effective when the water has a lower pH value because of the amount of hypochlorous acid produced [54] [43]. Free available chlorine is defined as the total amount of hypochlorous acid and hypochlorite ions found in the water [52],[43]. The table 12 demonstrates the ratio of hypochlorous acid to chloric ions depending on the pH [48].

Table 12. Impact of pH on the Ratio of Hypochlorous Acid (HOCL) to Chloric Ion (OCL)

PH	% HOCL (Hypochlorus acid)	% OCL (Chloric Ion)
4	100	0
5	99	1
6	96	4
7	75	25
7.4	52	48
7.5	48	52
8	22	78
9	7	9

[48]

2.7.2 Residual Chlorine

EPA recommends a chlorine residual of 2 to 4 parts per million (ppm) for drinking water supplies for optimal microbial control with the recommendation that 85% of the free chlorine present be in the HOCl form [56]. Free chlorine, combined chlorine and total chlorine are all considered chlorine residuals [52]. Free chlorine is defined as the amount of hypochlorous acid and chlorite ions found in a solution. Combined chlorine is the amount of chloramines found in a solution. Chloramines are produced when chlorine reacts with ammonia nitrogen in the water [52] [61]. Lastly, total chlorine is the sum of free chlorine and combined chlorine in a solution after the chlorine demand has been met [25] [52] [61].

Chlorine demand can be described as the amount of chlorine in water that reacts with various constituents in water such as organic and inorganic matter, minerals (iron and manganese), metals, and various other constituents [61]. It is the difference between the amount of chlorine added and the amount of chlorine remaining after being consumed by the constituents listed above. It is important to supply enough sanitizer to meet the chlorine demand while producing enough residual chlorine for microbial elimination. If the chlorine demand is excessive, action should take

place to determine what is causing the excessive demand to reduce cost in sanitizer product and the formation of by-products.

Monochloramine, dichloramine, and trichloramine are considered as chloaramines [43]. Compared to free chlorine, these chloramines hydrolyze at a slower rate and can have a higher efficacy at greater pH values around 10. While combined chlorine is less potent, it is more unrelenting in the water lines and can be maintained in the for a longer period of time than free residual chlorine [52]. However, when compared to combine residual, free residual chlorine is the best way to determine disinfection in a water system because of high germicidal power [52].

While total chlorine measures all the chlorine present, including the amount bound with minerals or microbes, free chlorine is the residual which still has oxidizing capability [52]. In poultry operations, detecting sufficient chlorine residual at the end of the water lines can indicate adequate sanitizer has passed through the system. It can also can help determine the correct amount of sanitizer needed to eliminate microbial populations [52].

Reference [62] reported a relationship between chlorine residual and the temperature of the water [62]. When the temperature of the water ranged from 44°F - 60°F, the loss of combined residual was low. However, when the temperature of the water was warmer ranging from 68°F - 73°F a quick loss of residual occurred [62].

2.7.3 Oxidation-Reduction Potential:

The oxidation-reduction potential (ORP) is a measurement of the efficacy of various chlorine residuals as a germicide and is measured in millivolts [52],[63]. Utilizing an electrode, the ORP reads the amount of potential electrons in the water, detecting whether the chlorine species is sufficient to meet the demand for microbial elimination [52]. Oxidation describes the process where oxidizers gain electrons and, hence, these ORP values are positive [63],[64]. Chlorine,

chlorine dioxide, peroxide, bromine, and ozone are all considered to be oxidizers [64]. Reduction describes the process where electrons are lost. Therefore, these ORP values are negative [63],[64]. Sodium sulfite, sodium bisulfate, and hydrogen sulfite are considered to be reducers [64]. Oxidizers are good water sanitizers because they are pull electrons away from the bacteria cell membrane, which causes the membrane to be unstable. When the bacterial membrane becomes unstable, cell death occurs because of the destruction of the makeup of the membrane [63].

An advantage of ORP is the capability to provide immediate results to assess the disinfection potential [63]. ORP has the ability to measure the efficacy of chlorine residuals as germicide without regard for non-germicidal residuals [52]. It allows the applied disinfectant to be assessed rather than the applied dose. Therefore, it measures the efficacy or activity of the disinfectant rather than assessing the efficacy based on the dose [63].

Research shows higher ORP values (>665) can result in higher germicidal effects by killing pathogenic bacteria, such as *E.coli O157:H7* and *Salmonella*, within 30 seconds [63]. These values are shown below in Table 13. Other species of microorganisms, such as spoilage yeast, can be eliminated at a ORP value of 650mV to 700mV after a contact time of couple minutes or less [63]. A lower ORP value, such as 250 mV, can be an indication of a heavy organic load which will affect the amount of chlorine available or necessary for disinfection [48].

Table 13. Summary of Results from Various Lab Simulations and Commercial Hydrocooler Survey Studies

Pathogen/Indicator	Survival in seconds (s) or hours (h) at ORP (mV)		
	< 485	**550< X <620**	**> 665**
E.coli O157: H7	> 300 s	< 60 s	< 10 s
Salmonella spp.	> 300 s	> 300 s	< 20 s
Listeria monocytogenes	> 300 s	> 300 s	< 30 s
Thermotolerant coliforms	> 48 h	> 48 h	< 30 s

[63]

The pH and free chlorine residual has an effect on the ORP [63]. Hypochlorous acid is predominant in water with a lower pH of 4 - 7. On the contrary, hypochlorite ions are predominant in water with a higher pH of greater than 7.5. Because hypochlorous acid has more sanitizing power than hypochlorite ions, it has higher free chlorine residual [54]. ORP will increase with more available chlorine because there is more potential for chlorine to disinfect [63].

2.7.4 Microbial Analysis

Good quality water has minimal to zero microbial contamination [33]. Quality water meets the poultry recommendation levels provided in Tables 14 and 15. All levels are based on the EPA's standards for human water consumption [5]. Values are measured in colony forming units per ml of water (cfu/ml). Table 14 displays the level of microorganisms as well as acceptable levels for a drip sample of water to be considered good quality. Table 15 demonstrates the level of microorganisms found in 1 ml of buffered water after a hydrated sterile swab was used to wipe the inside of a water line [5]. While there are no definite acceptable levels for yeast and mold, it is important to understand the presence of these organisms could be an indication of biofilm presence [5]. It is important to note there is zero tolerance for microorganisms such as fecal coliforms, *E.*

coli, and *Pseudomonas* in water systems [4],[5]. Not only should the water have minimal microbial contamination, but it also should meet the standards for other factors that affect water quality including mineral contamination, pH, alkalinity, and hardness. The water should be considered unacceptable as a drinking water source if it contains more microorganisms than the maximum acceptable levels shown in Table 14. [5].

Table 14. Acceptable Levels for Microorganisms in Water Drip Samples

Microorganism	Good Quality	Max. Acceptable Level
	cfu/ml	
Aerobic Plate Count	0	< 1,000
Total Coliforms	0	< 50
Escherichia coli	0	0
Yeast	0	-
Mold	0	-

[5]

Table 15. Acceptable Level for Microorganisms in Swab Samples

Microorganism	Good Quality	Max. Acceptable Level
	cfu/ml	
Aerobic Plate Count	0	< 5,000
Total Coliforms	0	< 50
Escherichia coli	0	0
Yeast	0	-
Mold	0	-

[5]

A drip water sample is taken from a clean, sanitized nipple drinker near the end of the water line. A swab sample of water systems is a sample taken using a sterile sponge hydrated in buffered water that is inserted into the end of the waterline [5]. A swab sample is ideal for detecting if there is any biofilm buildup in the water lines. Biofilms can act as a barrier to protect microorganisms from disinfectants [65]. A drip sample may indicate whether the waterline has lower levels of

microbial contamination, while a swab sample may indicate higher levels of microbial contamination. Therefore, it is always beneficial to gather a swab sample, as drip samples may not accurately reflect the amount of microbial contamination present [33],[5]. However, both drip and swab samples are important components in performing a quality assurance microbial analysis to determine the bacterial load in water lines. Once the water samples are properly taken, they should be submitted to a microbiological laboratory to determine the amount of microbes present [33],[5].

Once microbial contamination is detected in water lines a water quality program with an effective sanitizer should implemented [39]. The type of water sanitation program and type of sanitizer used is dependent on the level of microbial contamination as well as the mineral content, pH, and any other factors that affect the quality of the water [5]. The best way to measure the efficiency of a water sanitizer is to analyze a drip and swab sample of water before and after the application of water sanitizer in the lines [33].

To reduce or eliminate microbial populations harmful to the bird's performance a responsive chemical agent is needed [54]. The amount of chlorine needed to achieve a biocide effect should be evaluated and amounts may be increased depending upon the evaluation. If the contact time is long enough and the dosage level is appropriate, biocides with oxidizing power will quickly eliminate all microorganisms that come in contact with free chlorine molecules [43].

The poultry industry has long accepted an oxidation reduction potential (ORP) reading of 650 mV to provide assurance that there was no microbial activity in a drinking water supply and there was adequate free chlorine residual present to quickly reduce the impact of any microbial challenge that might be potentially introduced post sanitation. Recent evaluations of a new chlorine product revealed microbial contamination was still present even when the ORP was 650 mV. These

results indicate it would be beneficial to re-evaluate the relationship between chlorine residual, ORP reading and microbial levels, and determine if a new ORP standard should be the target and if this standard can be correlated to a free chlorine residual (ppm).

The purpose of this study is re-evaluate the relationship between chlorine residuals, ORP reading, and microbial levels. Furthermore, determine if a new ORP standard should established and if this standard can be correlated to a free chlorine residual. The objectives that guided this study include:

1. Evaluate the efficacy of a commonly used water sanitizer disinfectant, sodium hypochlorite and a new chlorine product, sodium dichloro-d-triazinetrione, when these products are introduced into microbial rich water collected from poultry drinking water lines.
2. Determine how chlorine residual and ORP reading are correlated to determine if under typical poultry drinking water conditions if one or both of these measurements are adequate for determining sanitizer efficacy.

Chapter III: Material & Methods

This bench-top experiment was conducted to evaluate the correlation between the oxidation-reduction potential and microbial elimination using typical poultry farm drinking water sanitation products and usage levels. The products used in this evaluation were liquid bleach, (LB- 8.25% sodium hypochlorite) with 7.85% concentration of available chlorine and bleach crystals, (BC- sodium dichloro-s-triazinetrione) with 55% concentration of available chlorine. Three different levels of BC and LB were evaluated for their efficacy of treating contaminated poultry drinking water with the goal of not exceeding the EPA's recommendation of 2-4 ppm of chlorine

residual and to evaluate the products at levels commonly utilized in the poultry industry for drinking water disinfection. Untreated water served as the control.

Approximately 10 gallons of water was collected from poultry drinker lines from the University of Arkansas Poultry Research Farm and blended to create a uniform mixture. There were no birds present at the time of water collection. The water was stored in a five-gallon container at room temperature. The water was thoroughly blended. One ml samples were collected and plated in duplicate on 3M APC Petrifilm^{TM} and incubated for 48 hours at 35^{o} C. Results of the preliminary microbial analysis showed the water contained an aerobic bacteria level of approximately 100,000 cfu/ml.

3.1 Treatments

De-chlorinated tap water was used to make the three different stock solutions for each product. The stock solutions prepared for each treatments (TRT) are shown below in TABLE 16. Each TRT is described in two ways. First as the amount of product mixed in ounces to a gallon (or 1,893 ml) of water to prepare the stock solution at the rates commonly utilized in commercial poultry production operations. Then, as the smaller amount used in this benchtop test to make only 1/4th of the first solution. The second TRT explanation for each TRT is shown as grams (g) either for the BC or milliliters (ml) for LB blended into 473.25 ml of de-chlorinated tap water.

Table 16. Test Stock Solution Preparation Ratios and Mixing Rates

Treatment	Product	Stock Solution Ratios (Product/gal of water)	Mix Ratios for Stock Solutions (Product/water)	Actual Mix Rates for Test Solution Preparations
1	BC	2oz/gal	2.48g/1893ml	0.62g/473.25ml
2	BC	4oz/gal	5.0g/1893ml	1.25g/ 473.25ml
3	BC	8oz/gal	8.0g/1893ml	2.0g/473.25ml
4	LB	2oz/gal	30ml/1893ml	7.5ml/473.25ml
5	LB	4oz/gal	59ml/1893ml	14.7ml/473.25ml
6	LB	8oz/gal	118ml/1893ml	29.5ml/473.25ml
7	Control	-	0/1893ml	0/473.25ml

3.2 Stock Solution

Prior to initiation of the trial, two 5 liters of tap water was collected in 5000 ml Erlenmeyer flasks and left exposed to air for 2 days. The water was blended and a 5 ml aliquot was collected and tested with the HACH Pocket Colorimeter Kit for and total chlorine test kit, which confirmed there was no chlorine present. Next 473.25 ml of the de-chlorinated tap water was placed into 1000 ml beakers. This was repeated five more times. The treatments were then prepared in the water utilizing the one-fourth reduced concentration levels listed for each treatment. Each treatment was mixed for 15 seconds with a 10 ml pipette. The pipette remained with its respective treatment and was used for adding the stock solution to the test water at one ounce of stock solution added to 128 ounces of drinking water (1:128). This is the injection rate is commonly used with poultry house drinking water treatment injectors, also known as medicators.

3.3 Medicator Rate of 1:128

The microbial rich test water was blended and aliquots of 384 ml of microbial rich water were placed in 21, 1000 ml beakers. For each aliquot, an initial 1 ml of water was removed and plated for determination of aerobic bacteria. Immediately after mixing each stock solution for 15 seconds, each stock solution was then added to its respective three replicates of 384 ml of test water at a rate of 3 ml to simulate a 1:128 injection rate. After addition of the treatments, each replicate was gently stirred for 10 seconds to thoroughly blend. Post application of the treatments, samples was analyzed for aerobic bacteria, oxidation-reduction potential, free and total chlorine residual, and pH was measured for each replicated prior to addition of treatments (PRE) at 0, 15, 30, and 240 minutes.

3.4 Sampling Times

The sampling times of 0, 15, 30, and 240 minutes were chosen to correspond to potential real world water flow rates observed through the life of a flock. The 0 minute sampling time represents the time at which birds are reaching their target weight. At this point water is fast flowing through the waterlines as there is increased consumption of water by the birds. When the rate of water flow increases disinfectants in the water have minimal time to react with the different microbial organisms present in water. The 240 minutes sampling time represents the time when birds are in the brood period. At this period water is slow moving in waterlines as the water consumption is reduced. This time point would provide information on the presence or absence of chlorine in water after 240 minutes as well as the amounts at which is present if it has not dissipated.

3.5 Microbial Plating

At each sampling time, 10 ml of water was removed with a sterile 10 ml pipet and placed in a sterile container for neutralization of the chlorine residue using the neutralization procedure below. After neutralization, 1 ml of each treatment was directly pipeted using a sterile pipet onto 3M APC Petrifilm^{TM}. Additionally samples were serially diluted at a rate of 1 ml to 9 ml of sterile phosphate buffer saline. Each sample was diluted up to 3 times with each dilution plated on the agar plates in duplicate (APC Petrifilm^{TM}) and incubated at 35^{o}C for 48 hours. The most probable number was determined for each sample.

3.6 Neutralization Procedure

This process is performed to neutralize the bactericidal effect of the sanitizers and thus, to obtain an accurate reading of microorganisms at the established time points. The neutralization solution is made by dissolving 2.0 g of Sodium Thiosulphate (2.0% w/v) in 100 ml of sterile phosphate buffer saline (PBS). The neutralizing solution prepared is used at the rate of 500 µl per 10 ml of the sample solutions.

3.7 Oxidation- Reduction Potential

The oxidation-reduction potential (ORP) was measured using HM Digital 200-ORP meter. To obtain the ORP reading, the ORP meter was dipped into the water sample, swirled lightly for 5 seconds, and then the reading in millivolts was recorded 15 seconds after the swirling. Once the reading was determined, the ORP meter probe was rinsed with distilled water and wiped with a soft tissue before the next sample.

3.8 Chlorine Residual (Free and Total)

Free and total chlorine was measured using Pocket Colorimeter TM II Cat. No. 58700-12 from HACH test kit. To obtain free chlorine residual one tube was filled using a pipet with the

sample water to the 5 ml mark in the tube and inserted into the left opening of the color wheel comparator. A second tube was filled with the same sample water similar to the first tube to which a DPD Free Chlorine Reagent Powder Pillow was added. After the addition of powder pillow the tube was swirled to mix and inserted into the right opening of the comparator. The free chlorine residual was read within one minute of adding the reagent by holding the comparator against a light source. The color disc was rotated until the colors in the front windows matched and the results were recorded in mg/L.

To obtain total chlorine residual, one tube was filled with the sample water to the 5 ml mark and inserted into the left opening of the comparator. A second tube was filled with the same sample water up to the mark. One DPD Total Chlorine Reagent Powder Pillow was added to the second tube, swirled to mix, and inserted into the right opening of the comparator. The total chlorine residual was read within 3-6 minutes after adding the reagent by holding the comparator against daylight or a fluorescent light source. The color disc was rotated until the colors in the front windows match and the results were recorded in mg/L.

3.9 pH Reading

The pH was recorded using Fisher Scientific Accumet Basic pH Meter (model number: 201400). The probe was standardized by dipping it in a buffer reference standard solution of 7. To obtain a pH reading, 15 ml of the sample solution was placed into a 50 ml tube, the pH probe was placed into the tube for 2 minutes at which time the meter reading would be stabilized, and then the reading was recorded. The pH readings for samples were obtained before treatment and at 0, 15, 30, and 240 minutes post-treatment. The pH probe was rinsed with distilled water and wiped with a soft tissue between samples.

3.10 Statistics

Each replicate of treated test water served as the experimental unit. APC results were converted to Log10 to normalize the data prior to statistical analysis. The independent factors were treatment and time. The dependent factors were APC, ORP, free chlorine residual, total chlorine residual and pH. Results were analyzed using the PROC GLM Procedure of SAS (SAS Inst, Inc., Cary, NC, 2016). The factorial of Time by Treatment was analyzed and results were considered significant at the $P<0.05$ level. Significantly different means were separated using PDIFF option in SAS. Correlations were generated with CORR procedure in SAS.

Chapter IV: Results

4.1 Aerobic Plate Count

The Aerobic Plate Count (APC) results are shown in Table 16. Because a significant interaction (P value = 0.001) was observed between treatment and time, the results will be covered by comparing the changes in APC for the treatments across the different sampling times. The pre-treatment results showed similar APC levels for all treatments including the control (~4.3 Log10). At 0 minute post-treatment, APC levels were lower than the control for all treatments with TRT 2 (BC: 4oz/ 1 gal), 3 (BC: 8 oz/ 1 gal), 5 (LB: 4 oz/ a gal) and 6 (LB: 8 oz/1 gal) experiencing the greatest reductions in APC (~3 logs). Control APC levels remained similar to pre-treatment levels for all sampling times. The lower dosage level treatments, treatment 1 (BC: 2 oz/ 1 gal), and treatment 4 (LB: 2 oz/ 1 gal), had APC values lower than the control but were still higher than the other treatments, indicating lower efficacy for immediate microbial reductions. By 15 minutes post-treatment, all treatments had similar APC levels which were almost 3 logs lower than the control with treatment 6 (LB: 8 oz/ 1 gal) APC levels even lower (3.69 log reduction as compared to control at the same time). This trend held through the 30 minute sampling time and by the 240

minute sampling time, the APC results for treatments 1, 2, 3, 4 and 5 were still similar to the 30 minute results but treatment 6 had furthered dropped to a non-detectable level. These APC results indicate there was an immediate impact of the sanitizer on APC levels for treatments 2, 3 and 5 with no further reductions over time. The lower dosage treatments, 1 and 4, required at least 15 minutes of contact time before additional reductions in APC levels were observed. Then, the results were similar for the remaining sample times. Treatment 6 continued to see a drop in APC levels through the final sampling time and was the only treatment that effectively eliminated all aerobic bacteria but it did take more than 30 minutes to achieve this.

Table 17. Treatment by Time Affect for Aerobic Plate Count (APC)

	APC Log10 (cfu/ml)				
	PRE	Post-Treatment (minutes)			
TREATMENT	**-**	**0**	**15**	**30**	**240**
1: Cry: 2oz/1gal	4.31_{a}	3.0_{c}	1.37_{d}	1.45_{d}	1.36_{d}
2: Cry: 4oz/ 1gal	4.32_{a}	1.34_{d}	1.38_{d}	1.37_{d}	1.3_{d}
3: Cry: 8oz/1gal	4.34_{a}	1.39_{d}	1.4_{d}	1.34_{d}	1.09_{de}
4: LB: 2oz/1gal	4.35_{a}	3.23_{c}	1.37_{d}	1.45_{d}	1.32_{d}
5: LB: 4oz/1gal	4.3_{a}	1.4_{d}	1.36_{d}	1.37_{d}	1.2_{d}
6: LB: 8oz/1gal	4.3_{a}	1.33_{d}	0.66_{e}	0.73_{e}	0.00_{f}
Control	4.39_{a}	4.56_{a}	4.35_{a}	3.82_{b}	4.5_{a}
SEM			0.168		
P Value			0. 0001		

4.2 Oxidation Reduction Potential

The Oxidation Reduction Potential (ORP) results are shown in Table 17. Similar to the APC results, there was a significant interaction between time and treatment (P= 0.0001) which indicated change in ORP for the different treatments over time. Initial pre-treatment ORP levels were similar for all treatments including the control. At 0 minutes post-treatment, results were similar for all treatments, except TRT 6 and the control. TRT 6 showed a higher ORP than all the

others and control having a lower ORP than all treatments except TRT 1. For TRTs 2, 3, 4, and 5, ORP readings peaked at 0 minutes post-treatment with similar values and remained similar throughout the experiment until the 240 minute sampling time when lower readings were observed. For TRT 1 and TRT 6, the ORP values remained higher than all the other treatments for all sampling periods except the 240 minutes sampling time. At 240 minutes, TRT 1 had a lower ORP than the other treatments except control. TRT 1 and TRT 2 had similar ORP values as the control at this sampling time.

After addition of treatments, with the exception of TRT 1 there were no significant changes in ORP until 240 minutes. TRT 1 experienced a significant change with ORP increasing from 466mV to 493mV at 30 minute post-treatment. The ORP for TRT 6 did not significantly decrease over time similar to other treatments. Treatments with higher doses of chlorine, TRT 3 and 6, experienced higher ORP values over time while the treatments with lower dosages, TRT 1 and 4, experienced lower ORP values over time until 240 min post-treatment at which time TRT 1 and the Control had the lowest ORP values but TRT 1 was still similar to TRT 2, 3, 4 and 5.

Table 18. Treatment by Time Effect for Oxidation Reduction Potential (ORP)

	ORP (mV)				
	PRE	Post-Treatment (minutes)			
TREATMENT	-	0	15	30	240
1: Cry: 2oz/1gal	299_{l}	475_{cdef}	466_{ef}	493_{cd}	373_{jk}
2: Cry: 4oz/ 1gal	295_{l}	514_{cd}	506_{cd}	511_{cd}	403_{ij}
3: Cry: 8oz/1gal	300_{l}	535_{c}	530_{c}	526_{cd}	429_{ghi}
4: LB: 2oz/1gal	301_{l}	497_{cd}	496_{cd}	504_{cd}	415_{hi}
5: LB: 4oz/1gal	304_{l}	509_{cd}	505_{cd}	509_{cd}	436_{ghi}
6: LB: 8oz/1gal	307_{l}	734_{a}	721_{ab}	722_{ab}	697_{b}
Control	309_{kl}	455_{fg}	441_{gh}	461_{efg}	359_{jkl}
SEM			12. 32		
P Value			0.0001		

4.3 pH

The pH values for the different treatments over time are shown in Table 18. Similar to APC and ORP, there was a significant interaction (p= 0.0063) treatment and time interaction. The pre-treatment results showed similar pH values for all treatments and the control pH levels remained similar to the pre-treatment levels for all the sampling times. The pH values significantly decreased for all treatments at 0 minute post-treatment, expect for the lower dosed treatments, TRT 1 and 5. However, TRT 2 and 3 had the greatest reductions in pH (0.49 and 0.67 respectively) at 0 minute post-treatment. At 15 min post-treatment, pH increased (0.32) for TRT 4 while the other treatments remained similar to the levels at 0 min post-treatment. For all the treatments, the pH values remained more or less the same at 15 and 30 min post-treatment. However, pH for TRT 1 increased at 240 min post-treatment, while the pH for all the other treatments remained the same.

Table 19. Treatment by Time Affect for pH

		pH			
	PRE	**Post-Treatment (minutes)**			
TREATMENT	**-**	**0**	**15**	**30**	**240**
1: BC: 2oz/1gal	8.00_{abcd}	$7.85_{cdefghi}$	$7.88_{bcdefghi}$	7.75_{ghijk}	7.99_{abcdef}
2: BC: 4oz/ 1gal	8.06_{abc}	7.57_{klm}	7.62_{jklm}	7.68_{ijkl}	$7.79_{defghij}$
3: BC: 8oz/1gal	7.99_{abcde}	7.32_{n}	7.30_{mn}	7.48_{lmn}	7.61_{jklm}
4: LB: 2oz/1gal	8.03_{abc}	7.73_{hijk}	8.05_{abc}	8.07_{abc}	8.12_{a}
5: LB: 4oz/1gal	8.05_{abc}	$7.94_{abcdefgh}$	7.77_{fghijk}	$7.97_{abcdefg}$	8.04_{abc}
6: LB: 8oz/1gal	8.06_{abc}	7.71_{ijk}	7.74_{hijk}	$7.77_{efghijk}$	$7.87_{cdefghi}$
7: Control	8.07_{abc}	8.11_{a}	8.1_{ab}	8.10_{a}	8.14_{a}
SEM			0.07		
P Value			0.006		

4.4 Free Chlorine Residuals

The free chlorine residual did not show a significant time by treatment interaction (P = 0.2744). However, there was an interaction between sampling times (P= 0.001) and between treatments (P = 0.001). Table 19 shows the effect of treatments on free chlorine residual while Table 20 shows the effect of time.

There was a difference between treatments with TRT 6 showing the highest free chlorine residual (3.0ppm) while TRT 4 had the lowest (0.6ppm) free chlorine residual. TRTs 1, 2, 3, 4 and 5 all had similar results for the free chlorine residuals, while the control had a residual of 0ppm. Table 20 indicates the highest free chlorine residual was observed at post-treatment sampling times of 0, 15, and 30 minutes. The lowest amount of free chlorine residual (0.97ppm) was recorded at 240 min post-treatment.

Table 20. Difference in Free Chlorine Residuals between Treatments

TREATMENT	Residual (ppm)
1: BC: 2oz/1gal	0.78_{b}
2: BC: 4oz/ 1gal	1.31_{b}
3: BC: 8oz/1gal	1.02_{b}
4: LB: 2oz/1gal	$0.6b_{c}$
5: LB: 4oz/1gal	1.19_{b}
6: LB: 8oz/1gal	3_{a}
Control	0_{c}
SEM	0.263
P Value	0.001

Table 21. Difference in Free Chlorine Residuals between Times

Time	Residual (ppm)
Pre	0_{c}
0	1.44_{ab}
15	1.61_{a}
30	1.6_{a}
240	0.97_{b}
SEM	0.222
P Value	0.001

4.5 Total Chlorine Residual

To measure total chlorine residual, the HACH Pocket Colorimeter test kit was used. The maximum reading which can be displayed by the colorimeter wheel is 3.5ppm. Since most of the samples tested had a total chlorine residual greater than 3.5ppm, the results are not shown but for correlations analysis was listed as greater than 3.5 ppm.

4.6 Correlations

APC and ORP were found to have a strong negative correlation (-0.7). As ORP increases, APC decreases. Likewise, as free chlorine increased, the APC levels decreased (-0.6). However,

the correlation between the amount of total chlorine residual and APC levels was stronger (-0.9) than the correlation between free chlorine residual and APC levels (-0.6). The ORP for the different treatments was found to have a positive correlation with free and total chlorine residual, whereas the pH did not experience strong correlations with any other parameters.

Table 22. Correlation of the different Parameters Measured

	APC	ORP	pH	Free
APC	-	-0.7	0.5	-0.6
ORP	-	-	-0.4	0.7
pH	-	-	-	-0.4
Free	-	-	-	-

Chapter V: Discussion

This trial evaluated the efficacy of two different forms of chlorine, a liquid product, LB, (sodium hypochlorite) and crystalline dry product, BC, (sodium dichloro-S-triazinetrione) at three different concentrations commonly used for drinking water sanitation in the poultry industry. The objective was to determine if there is a relationship trend between total and free chlorine residual, ORP, pH, and microbial content of the water when the chlorine products are utilized in water with a high microbial level typically found in unclean poultry drinking water lines.

The results indicate chlorine, in both forms, was an effective disinfectant in reducing aerobic bacteria with both products having the most efficacy at 4 oz/gal stock solution dosage level when immediately evaluated post-treatment application (0 min sampling time). By the 15 min sampling time, BC and LB at the 2 oz/gal stock solution rate had achieved similar APC reductions of three logs or 99.99% as the other treatments and for all treatments except LB at the 8 oz/gal

stock solution rate, no further significant APC reductions were noted. Only LB at the highest application rate continued to show further reductions in APC until zero APC detection at the 240-minute sampling time. The control APC levels remained similar to the pre-test levels, indicating the aerobic bacteria present were a stable population. Since this water was collected from poultry drinking water lines, this indicates that the microbial population developed under these conditions was somewhat stable at least from the time of collection (5 days prior to initiation of the trial) and through the 240 minute sampling time. This helps confirm poultry drinking water systems, when left dormant and full of water with no sanitizer present, can develop APC levels of over 10,000 cfu/ml even if the initial source is municipal water.

The correlation between APC and ORP was -0.7 indicating as ORP increases, APC decreases. This is supported by the fact prior to treatments pre- ORP values prior to treatments were in the range of 300 mV and immediately after treatment all ORP values increased from ~300 mV to ~ 545. Even the Control ORP increased although not quite as high as all treatments except BC at the 2 oz/gal rate. This could have been associated with some residual chlorine coating on the ORP meter between samplings at the 0 time which was not removed by rinsing and wiping. It remained higher throughout all sampling periods but it is unclear as to why. This does indicate with the control APC levels in the range of 3.8 to 4.5 logs throughout the sampling periods and the control ORP ranging from 359 to 455 mV, these ORP levels are clearly not a good indicator APC levels are less than 1000 cfu/ml. At the 0 min sampling time, the BC and LB treatments of 2 oz/gal had ORP values under 500 mV and because the APC levels were still in the 3 log range, this would indicate utilizing an ORP of 500 mV or less is not a good indicator there is no aerobic bacteria present. Only the LB treatments of 8 oz/gal which gave ORP readings over 700 mV had APC

levels of less than 1 log by 15 minutes. The results of this trial indicate that a minimum ORP reading of 700 mV is desirable as an indicator of no aerobic bacteria present.

Other research confirms pathogenic bacteria such as foodborne pathogens were destroyed within 30 seconds of application of a disinfectant capable of producing an ORP greater than 665mV [63] [66]. With the increase in treatment stock concentration from 4 to 8 ounces, the ORP readings in this trial jumped from the 500 mV range to 700 plus mV. Additional experiments should be conducted to eliminate the low end treatment of a 2 oz stock solution and focus on 4, 6 and 8 oz stock solutions. Interestingly, the BC has a stronger chlorine level than LB but it was the LB, which gave the best results in terms of APC reduction, free chlorine residual and ORP reading at the highest treatment level. Our results are in agreement with work done reference [67] where chlorine was found to be a potent disinfectant to control biofilm growth. This could be an indicator crystal bleach products do not dissolve and release the chlorine as rapidly or effectively as liquid bleach. This observation should be taken into consideration when selecting water sanitizers where contaminated water supplies are possible and water utilization means water has limited contact time with sanitizers prior to consumption by the birds.

However, the correlation between free chlorine residual and ORP was 0.7 indicating that as free chlorine increases, ORP increases. Furthermore, the correlation between free chlorine residual and APC was -0.6 indicating as free chlorine increases, APC levels decrease. Throughout the post sampling times (0 - 240 min), the BC and LB treatments of 4oz/gal had ORP values under 510 mV and free chlorine residuals were in the range of 1.1-1.3 ppm. This does make the case that if high microbial content water is an issue or a potential concern in poultry water lines, then utilizing free chlorine residuals of 1.1-1.3 ppm or less, may not be an adequate indicator there is sufficient free chlorine available to achieve a total reduction in aerobic bacteria. The desired ORP

reading (700-750 mv) that was correlated in this trial with a complete aerobic bacteria elimination. This is also supported by the fact that APC levels were still present (1.3 log) throughout the same time period for the BC and LB treatments of 4oz/gal. Only the liquid bleach treatment of 8 oz/gal gave an ORP reading over 700 mV and the free chlorine residual was 3.0 ppm. Additionally, when the free chlorine residual was 3.0 ppm there was ~4 log reduction in APC levels. This indicates free chlorine residual of 3 ppm or greater is needed to achieve a 700-750 mV ORP.

Our studies also found ORP and pH is negatively correlated; however, the correlation was not very strong (-0.4). Similarly, the APC counts and pH also showed a weak positive correlation (0.5). Reference [63] showed a decrease in pH raised the amount of hypochlorous acid and thereby, increased the ORP value. Reference [68] study showed that the pathogens in water can be inactivated at a wider range of pH (2.6-7) if there is sufficient residual chlorine. The results of this trial place less emphasis on pH and more on free chlorine residual and ORP value for determining aerobic bacteria reduction.

Chapter VI: Conclusion

Our results indicate the chlorine is an effective disinfectant for poultry water sanitation and that the product most commonly used by the industry, liquid sodium hypochlorite is effective as a water sanitizer when challenged with a microbial population that is typical of a poultry drinking water system. Traditionally used levels of 2 and 4 ounces of chlorine products in stock solutions that are administered at a rate of 1 ounce to 128 ounces of drinking may be not quite adequate for rapid reduction of microbial populations and more work needs to be done to determine if higher concentrations should be utilized, particularly in challenge situations. Out of the different parameters tested to determine the efficacy of the disinfectants used in this study, ORP was found to be the most important and dependable parameter. Based on our study we recommend an ORP

value between 700-750 mV under practical field conditions to achieve an efficient microbial reduction/elimination and this can be supported by at least 3 ppm of free chlorine.

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