THE IMPACT OF WATER ANTIMICROBIAL APPLICATIONS PRE-PROCESSING AND SCALDING PROTOCOLS ON POULTRY PROCESSING WASTEWATER LOAD FOR BROILERS

by

CAITLIN ELIZABETH HARRIS

(Under the Direction of Brian H. Kiepper)

ABSTRACT

Pre-harvest *Salmonella* interventions have the potential of reducing pathogen contamination entering poultry processing plants and in US processing plants, 26 L of water is used to process each bird. Poultry processing wastewater (PPW) loading exiting scalder tanks was evaluated: hard vs. soft scalding, scalding immersion time and temperature separately, and presence of residual blood. Results indicate immersion time impacted PPW more than temperature and PPW significantly decreased with use of sequential tanks. Five experiments evaluated cetylpyridinium chloride (CPC) and acidic drinking water treatments for water usage and *Salmonella* retention following feed and water withdrawal. Results indicate CPC and most acidic water treatments were not effective in *Salmonella* reduction for crops and ceca post withdrawal. In conclusion for both studies, immersion time appears to a major indicator for predicting PPW loading in scalder tanks and 50 ppm hydrogen peroxide with citric acid (pH= 5.0) may be a potential pre-harvest *Salmonella* intervention.

INDEX WORDS: first processing, poultry processing wastewater, scalding protocols, pre-processing, feed withdrawal, *Salmonella* interventions

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CAITLIN ELIZABETH HARRIS

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CAITLIN ELIZABETH HARRIS

Major Professor:	Brian H. Kiepper
Committee:	Brian J. Jordan
	R. Jeff Buhr

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
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DEDICATION

To my family and friends, thank you for your love and support.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

There have been many studies performed for establishing concentrations (mg/L) of common wastewater analytical parameters generated by processing plants (Porges, 1950; Hamm, 1972; Singh et al., 1972). Although this information is useful, being able to determine the actual mass of contaminants in poultry processing wastewater (PPW) coming from different operations within the processing plant would be beneficial for determining which areas contribute the largest quantities of material in the PPW stream. Only one other study has been located that attempted to determine the loading (g/kg^{lwt}) of wastewater parameters from scalding broiler carcasses (Plumber, 2012). Therefore, the first goal of thesis is to further investigate this topic. Chapter 2 consists of a study evaluating scalding tank poultry processing wastewater loading following the slaughter and scalding of commercially raised broilers. The scalding protocols that were evaluated were: hard vs. soft scalding (experiment 1), scalding immersion time and temperature individually (experiment 2), and the presence of residual blood (experiment 3).

Consumption of raw poultry products has been determined as an important *Salmonella* transmission route and prior research has indicated that both the ceca and crops of broilers are important alimentary tract areas for the colonization of *Salmonella* species (Mughini-Gras et al., 2014; Snoeyenbos et al., 1982; Hargis et al., 1995). Feed withdrawal is the total time (8 to 12 hours) birds are off feed prior to slaughter and during this time, *Salmonella* contamination in both laying hen and broiler crops increases (Corrier, D. E., 1999; Ramirez et al., 1997;

Humphrey et al., 1993). There has been some success with adding acidic treatments to drinking water during feed and water withdrawal to reduce *Salmonella* contamination (Byrd et al., 2001). Therefore, the second goal of this thesis was to further investigate the effect of antimicrobial drinking water treatments of *Salmonella* retention in the crops and ceca in broilers subjected to feed and water withdrawal. Five trials were performed evaluating: *Salmonella* retention of CPC (experiment 1), water usage of CPC at varying concentrations (experiment 2), water usage of CPC and hydrogen peroxide treatments (experiment 3), *Salmonella* retention of hydrogen peroxide treatments (experiment 4), and *Salmonella* retention of hydrogen peroxide and sodium bisulfate treatments (experiment 5).

LITERATURE REVIEW

U.S. Poultry Industry

The US broiler chicken industry is one of the most productive sectors of agriculture in the country which has transformed from a loose system of local, independent businesses originating in the 1920s to a successful and highly productive vertically-integrated system (National Chicken Council, 1999). In the early 1900s, western poultry production consisted of small dual-purpose flocks that were used for both meat and egg production (Barbut, 2002). During this time, the most common type of chicken consumed in American households were “fowls”, a.k.a. spent laying hens, because capons (i.e., castrated domesticated cockerels) and broilers (i.e., young meat-type chickens) were considered expensive luxuries (Bugos, 1992). Beginning in the 1930s the sole consumption of fowl shifted because the commercial scale broiler industry was created and began to flourish on the Delmarva Peninsula, which popular lore credits Mrs. Wilmer Steele with starting (Bugos, 1992). The per capita consumption of poultry in the US has drastically increased since the 1960s and the current annual chicken meat consumption is now higher than

for both pork and beef (National Chicken Council, 2017a; Ollinger and Madison, 2000). The term "poultry" can refer to groups of avian species (e.g. chickens, turkeys, and ducks) that are raised for meat and egg consumption; however, for the purpose of this thesis, the term will be used specifically for 6 to 7 week old broiler chickens because over 95% of poultry slaughtered in the US are broilers (USDA-NASS, 2017).

The US industry is the world's largest poultry producer with a little over 9 billion chickens slaughtered in 2017, and the world's 2nd largest exporter of poultry meat with approximately 18% of production exported (USDA-NASS, 2018; USDA-ERS, 2017). In the US, Georgia is the largest in broiler production and slaughtered almost 15% (1.26 billion birds) of the national total chickens in 2016, followed by Alabama (1.07 billion), Arkansas (980 million), North Carolina (780 million), and Mississippi (726 million) (USDA-NASS, 2018). The concentration of chicken meat production in southeastern US began during World War II (WWII) in1942. Prior to this time, the Delmarva peninsula area produced a large portion of the poultry produced in the US. This geographical shift occurred during WWII due to government requirements that Delmarva growers sell the meat they produced to military bases, which allowed southern growers to fill the national consumer market void (Ollinger and Madison, 2000). Not only did WWII shift where US poultry was produced, it also stimulated the country's consumption of poultry. Poultry was not rationed during WWII because it was considered a secondary part of the American diet, which resulted in increased poultry consumption over beef and pork during much of the early 1940s (Ollinger and Madison, 2000). After WWII poultry per capita consumption continue to increase. The annual rate of broilers slaughtered increased 510% from 1.5 billion slaughtered in 1960, to 7.8 billion slaughtered in 1998 (Ollinger and Madison, 2000).

Commercial poultry operations can be split into two main categories: live production and processing. Production involves the rearing and management of live flocks and includes the following components: breeding, hatching, grow-out, and feed manufacturing for both breeder and broiler flocks (Sams and Alavardo, 2010). Since the implementation of vertical integration in the 1950-60s, integrators gained the ability to better control bird quality and improve uniformity which allowed for the development of highly automated processing facilities (Sams and Alavardo, 2010; Ollinger and Madison, 2000). Processing operations begin with transport of birds to the processing plants and includes all the steps involved in the conversion of live birds into meat (Kiepper, 2003). While the main goal of processing is to produce meat for human consumption, non-food uses of poultry byproducts (i.e. feathers), raw materials for pet/livestock feed, and waste management are also important components of processing (Sams and Alavardo, 2010).

U.S. Poultry Processing

Chickens are processed in highly-automated plants that slaughter birds, remove inedible parts from the carcasses, and preserve/package the edible portions of meat that are then distributed to customers (Sams and Alavardo, 2010). Processing plants have increased in size (250,000 to 350,000/shift/day), line speeds and efficiency because of the implementation of vertical integration, advancements in technology, and improved bird uniformity (Bugos, 1992). From 1972 to 1992, plants with over 400 employees have increased from being responsible for 25% of total chicken/turkey production to over 80% of US production (Ollinger and Madison, 2000). While 50 years ago the maximum line speed was approximately 2,000 birds/hr, the implementation of automated evisceration and cut-up lines allowed for over 6,000 birds/hr to be processed per line in 2002 (Barbut, 2002; Barbut, 2016). This number has increased further with

modern plants able to process 13,500 birds/hr on a single line (Barbut, 2016). The shift from smaller slaughter and whole carcass packaging plants to more automated and specialized facilities can also be attributed to changes in consumption of poultry products. In 1962, over 87% of the poultry products were sold as whole carcasses. However, by 1997 only about 13% of products consumed were sold as whole carcasses (Ollinger and Madison, 2000). The amount of poultry that is exported has also drastically changed and the US is now the second largest exporter of poultry products behind Brazil (Davis et al., 2013). According to the USDA Economic Research Service (Davis et al., 2013), the amount of poultry products exported increased 65% from 1997 with 3.3 million metric tons being exported in 2012.

Currently, over 9 billion chickens are slaughtered annually in the US with a total live weight of 56 billion pounds (USDA-NASS, 2018). Poultry processing operations can be divided into 6 general categories: pre-processing (feed withdrawal through unloading), first processing (slaughter through chilling), second processing (parts, deboning, and portion control), third processing (margination and coating), cook plants, and rendering (Smith, 2014). Each processing plant is unique in the products it produces to meet the needs of their customers, so further processing steps also vary between plants. For this thesis, only pre-, first, second, and third processing will be discussed.

Pre-Processing

Prior to arriving at the processing plant and beginning first processing, there are several final steps of live production that must be completed, including: feed withdrawal, catching/cooping of birds, transportation to processing plant, live holding and unloading/shackling. The goal of pre-processing is to prepare the live birds for processing and plays an important role in influencing carcass and meat quality (Northcutt and Buhr, 2010).

According to Fletcher (1999), these pre-slaughter steps are short-term factors (<12 hours) that affect quality, compared to long-term factors like nutrition and management during live production.

The first step in pre-processing is feed and water withdrawal. Feed withdrawal is the total time that the birds are off feed prior to slaughter (including time in the broiler house without feed, transportation to processing plant, and in the live holding area) and typically it is recommended to last 8 to 12 hours (Smith, 2014; Northcutt and Buhr, 2010). During withdrawal, the alimentary tract is emptied of ingesta and fecal material, which then results in reduced fecal contamination within the processing plant (Northcutt and Buhr, 2010; Wabeck, 1972). Proper feed withdrawal to empty the digestive tract became even more important with the implementation of the Hazard Analysis and Critical Control Point (HACCP) system by USDA (1996). HACCP states that there is a "zero tolerance" performance standard for fecal contamination that is visible on carcasses entering the chiller, which encouraged producers to ensure that feed withdrawal is done properly (USDA, 1996). Although the common recommendation is for 8 to 12 hours, recommendations for feed withdrawal length have spanned from 4 to 21 hours with the ideal time being 10 hours to lower the chance of carcass contamination as well as reduce carcass yield losses (Northcutt, 1999; Lyon et al., 1991; Wabeck, 1972). Because the withdrawal timeline also includes the time it takes for transportation and time spent in live haul, it has been recommended for feeders to be raised 2 to 5 hours prior to catching (Northcutt and Buhr, 2010, Wabeck, 1972). Although birds are taken off feed for 2 to 5 hours, the water should not be withdrawn at the same time as feed because it helps with the clearing of feed from the crop (Northcutt and Buhr, 2010). According to National Chicken Council Welfare Guidelines (2017b) for broilers, feed withdrawal should be no more than 18

hours and the water lines should not be raised for water withdrawal for more than an hour prior to catching. Proper feed withdrawal is not only dependent on the amount of time the birds are off feed, but also: health of broilers, feeding program, lighting program, environmental temperatures, and excitement during catching and cooping (Northcutt, 2000; Buhr et al., 1998).

Feed and water withdrawal does not only affect meat quality and carcass yield, but also has a chemical, physical, and microbiological impact in the crops of birds (Hinton et al., 2000b). Previous research has identified the ceca as the main site of *Salmonella* colonization within the chicken, although the effect of feed withdrawal on *Salmonella* contamination in the ceca is inconclusive (Snoeyenbos et al., 1982). Along with ceca, the crop is another part of the alimentary tract that has been identified as an important site for *Salmonella* (Hargis et al., 1995). Those points are important to note because previous research demonstrated that *Salmonella* contamination in the crops increases after birds were subjected to feed withdrawal in both laying hens and broilers (Corrier, D. E., 1999; Humphrey et al., 1993; Ramirez et al., 1997). There are several reasons why *Salmonella* contamination is believed to increase in birds once feed is withdrawn. The first reason was suggested by Corrier et al. (1999), who stated that the behavior of broilers to peck at contaminated floor litter after the feeders are raised could result in increased *Salmonella* contamination is the crops of broilers. In Buhr et al. (2017), results indicated that the *Salmonella* prevalence in broiler crops (when subjected to pre-transport feed withdrawal) depends not only on the *Salmonella* status of the individual broiler prior to feed withdrawal, but also on the *Salmonella* status of the litter The second reason is that once the crop is empty of feed (i.e. approximately 6 hours after feed withdrawal begins), the lactic acid-producing bacteria population decreases because there are no longer fermentable carbohydrates that the bacteria require (Hinton et al., 2000a). Because lactic acid production decreases, the pH

in the crops will increase, the number of salmonellae and Enterobacteriaceae increases because the growth is not inhibited by a lower pH (Hinton et al., 2000a). This reason was further enforced by Hilton et al. (2000b) determining that providing a glucose-based cocktail during feed withdrawal will help maintain the lactic acid-producing bacteria population, therefore inhibiting the growth of *Salmonella* and Enterobacteriaceae.

After the first hours of feed withdrawal in the growout house, broilers are caught and placed into dump-coops to be transported to the processing plant. Prior to catching, the feed and water lines need to be raised to harvest birds. In the US, the standard for catching birds is for "catching crews" consisting of 6 to 10 individuals to manually catch and coop broilers in the house (Northcutt and Buhr, 2010). In a house with 20,000 birds, it typically takes 2 to 3 hours to catch and 3 to 4 trucks to transport all the birds (Northcutt and Buhr, 2010). Crew members typically catch broilers by the legs and the number of birds a crew member can carry at once depends on the weight of the birds (National Chicken Council, 2017b). Although there are strict welfare guidelines for how birds are manually caught, this step is often when injuries (e.g. bruising and broken bones) occur that can negatively affect carcass quality (National Chicken council, 2017b, Northcutt and Buhr, 2010). Mechanical harvesting systems are also a potential option for catching birds, but these systems are typically less economically feasible to use and are not commonly employed in the US (Northcutt and Buhr, 2010).

Once the birds are cooped, they are then transported to the processing plant to a live haul area and after that, the birds are unloaded and shackled in preparation for slaughter. As previously stated, birds are typically placed into dump-coops that, once removed from the truck trailer, allow birds to be "dumped" into a conveyor belt (Northcutt and Buhr, 2010). This is the standard in the US for unloading birds and the conveyor belt transfers the birds into the plant

where they are immediately hung on shackles by employees to begin first processing (Northcutt and Buhr, 2010).

First Processing: Slaughter through Chilling

First processing begins with the receiving and shackling of broilers from farms and although there may be variations in the operations of different plants, the general steps are similar for all plants (Barbut, 2002). The steps for first processing include: stunning/bleeding, scalding, defeathering, evisceration, and chilling (Sams and McKee, 2010). For first processing, traditionally one kill line transports birds through stunning/bleeding, scalding and defeathering at a speed of 140 birds per minute (bpm), which typically supplies carcasses to 2 evisceration lines, which run at a rate of 70 bpm or 1 line at 140 bpm (Sams and McKee, 2010).

Stunning is the first step in first processing to prepare broilers for humane slaughter through exsanguination. There are multiple methods in which stunning can be performed including: chemical, mechanical, or electrical (Fletcher, 1999). In the US, electrical stunning is the most predominant method used and stunning is typically performed using a combination of both direct current (DC) water bath, at approximately 15 volts, and alternate current (AC) low voltage-high frequency plate, at approximately 37 volts (Bourassa et al., 2017). Compared to the US, many other countries use higher voltages and amperages, so the birds are killed by electrocution and not exsanguination (Smith, 2014). In the past, the main purpose of stunning was to immobilize birds and render them unconscious in preparation for mechanical neck cutting or decapitation (Fletcher, 1999). More recently, the perception of stunning purpose has shifted from immobilization to welfare, because stunning is viewed as a way to reduce distress connected with exsanguination; although, stunning is not mandated for poultry in the Humane Methods of Slaughter Act (1978). Stunning also helps reduce convulsions post-exsanguination,

although stunning may cause carcass and meat defects if the birds are under-stunned, causing incomplete cutting, or over-stunned, causing broken clavicles or hemorrhages from capillaries and arteries (Fletcher, 1999; Sams and McKee, 2010). Once the birds are stunned, the next step is slaughter.

Once exiting the stunning cabinet, birds are immediately bled. Because the US requires birds processed for human consumption to be slaughtered via exsanguination, this is typically done by an automated killing machine that cuts the jugular veins and carotid arteries on either or both sides of the neck (Smith, 2014). Although automated killing machines are used, there must be a back-up plant individual after the automated machine to manual kill birds that are missed by the machine. Once the veins/arteries are cut, the carcasses enter a blood tunnel where the blood drains for 2 to 3 minutes. During this bleed-out time, 30 to 50% of blood is lost (about a 4% total yield loss), which causes the death of the bird (Smith, 2014; Sams and McKee, 2010). Proper cutting and bleed-out is also important for carcass quality because if birds are not bled adequately, blood in the skin will cause skin discoloration during defeathering and the carcass will be condemned as a "cadaver" (Smith, 2014). It takes approximately 2.5 to 5 minutes for all the steps from unloading to bleed-out to occur prior to the carcasses to entering the scalder tanks (Fletcher, 1999).

The next step of first processing is scalding the carcasses to aide in feather removal. Scalding is the process of immersing the carcasses in tanks containing air-agitated hot water, which helps loosen feather follicles that keep feather quills in place (Smith, 2014; Sams and McKee, 2010; Kaufman et al., 1972). Besides air-agitated immersion scalding, other methods of scalding include scalding via a combination of water-steam-air at atmosphere pressure because it reduces cross-contamination; but for this thesis, only immersion scalding will be discussed

because it is most commonly used in the US (Kaufman et al., 1972). Scaling protocols include factors such as tank set-up, immersion time, and scalding temperature. For tank set-up, it is common for processing plants to have a multi-stage system that has multiple tanks, which can allow for multiple temperatures, and counter-current flow (i.e., carcasses flow through tanks in the opposite direction of water stream) is often used (Smith, 2014; Sams and McKee, 2010). Although single tank systems can be used as well, multi-stage is the most effective for reducing bacterial loads in the tanks (Nunes, 2013; Cason et al., 1999).

For immersion time/scalding temperature, there are two protocols that are commonly used: soft and hard scalding. The soft method scalds carcasses at a lower temperature (e.g. 53°C/128°F) for a longer immersion time (e.g. 120 s) (Sams and McKee, 2010). This method preserves the cuticle when the carcasses are defeathered, which does not remove the associated skin color. But this method is not desirable when applying batters or breading, so soft scalding is typically utilized when the processors wish to produce whole carcass products (Smith, 2014). The other scalding protocol is hard scalding, which is a combination of higher water temperature (e.g. 62-64°C/145-148°F) with a shorter immersion time (e.g. 45 s) (Sams and McKee, 2010). With the higher temperature, the feathers are more easily removed, and the cuticle is taken off as well. Skin-on poultry products produced using hard-scalding methods are more often coated with breading or batter because the coating will adhere better to skin with the cuticle removed (Smith, 2014). As previously mentioned, scalding prepares the carcasses for feather removal, which is the next step in first processing.

Defeathering is performed using picking machines and as the name suggests, it is the process of removing feathers from carcasses post-scalding. Picking machines contain rows of metal plates with ribbed picker fingers attached, which are made from rubber (Smith, 2014). The

rows of flexible fingers rotate rapidly as the carcasses pass by and remove the feathers from carcasses. This step usually lasts less than 2 minutes (Sams and McKee, 2010). Picking also results in a total yield loss of approximately 6.5%. This is another point in which carcass quality can be affected if the process is not done properly. Because blood present is no longer an issue at this step because of bleed-out, picking cannot cause bruising, but it can cause broken bones or skin tears if the picker is adjusted too close to the carcasses (Sams and McKee, 2010). Oppositely, if the picker is adjusted too wide, the machine may not remove all the feathers. Filoplumes are hair-like feathers that are difficult to remove by the picker, so plants will sometimes singe the filoplumes off with a flame (Sams and McKee, 2010; Parry, 1995). Picking is another point where cross-contamination is an issue because the pressure from picker fingers can cause the release of fecal material from the cloaca, which transfers to other carcasses (Nunes, 2014). After leaving the pickers, the heads and feet are removed, and carcasses must be transferred to another area for evisceration according to USDA Food Safety Inspection Service (FSIS) rules (Smith, 2014).

Evisceration is a combination of machinery whose purposes are to remove the viscera, both edible and inedible, from the carcass cavity (Sams and McKee, 2010). The organization of the evisceration equipment and process may vary between processing plants, but the goal of preparing carcasses for chilling is the same overall (Smith, 2014). First, the preen gland is removed from the tail base, a circular knife removes the cloaca/attached colon, and a cut must be made from the cloacal opening to the posterior tip of the breastbone (Smith, 2014; Parry, 1995). After this opening step, the viscera are removed from the body cavity and the carcass/viscera are USDA inspected according to the Poultry Products Inspection Act (1957). Once the carcasses/viscera are inspected, the viscera pack is removed and typically the hearts, gizzards,

and livers are recovered as edible giblets (which are chilled and packed for sale) while the remaining viscera is transported to a central collection area (Barker, 2004). Finally, carcasses will go through washers that wash both the inside and outside of the carcasses simultaneously as a final measure in removing contaminants prior to chilling (Nunes, 2013; Parry, 1995). These inside/outside washer cabinets have high pressure nozzles that not only spray the inside and outside of carcass with water, but also often incorporate antimicrobial agents (e.g., chlorine, peracetic acid) (Barbut, 2002).

The final step in first processing is chilling the carcasses. Chilling is an important step in processing because cooling the carcasses minimizes the growth of bacteria and the Modernization of Poultry Slaughter Inspection; Final Rule (USDA, 2014) requires that poultry processors have chilling procedures listed in their HACCP programs. Chilling not only impacts the control of pathogenic microorganisms, it also serves to increase the shelf-life of poultry carcasses through spoilage bacteria control. There are two main types of chilling: water and air chilling. Because chilling in the US is almost entirely water chilling versus Europe who typically air chill, water chilling is the only method that will be discussed here (Sams, 2001).

Like scalding tanks, chiller tanks are also commonly set-up as counter-flow systems where the water-flow and carcass-flow are going in opposite directions. There are several types of chiller tanks, but in general, the tanks are trough-like and contain an auger, rakes, or paddles to move the carcasses slowly through the tanks. The goal of water (or immersion) chilling is for the carcasses to reach a deep muscle temperature of $\leq 4°C$ as quickly after evisceration as possible (Barbut, 2002). To reach this temperature range and ensure a cleaner product, a pre-chiller stage is often used to pre-chill the carcasses to 30-35°C in 10 to 15 minutes. Because the

carcasses are approximately 38°C before chilling, the lipids in the skin are still fluid and water can easily penetrate the skin (Sams, 2001).

After exiting the pre-chiller, the carcasses are moved into a main chiller tank where the carcasses continue to cool in clean chilled water or a mixture of clean water and added ice. In this chilling tank, the water temperature is typically 4°C at the beginning and 1°C at the end of the tank, and carcasses remain in this tank for 45 to 60 minutes. At this point, the skin lipids will solidify as the carcasses reach 4°C or less, trapping the water that was absorbed into the carcasses during the pre-chiller step. As previously mentioned, chilling is also important from a microbial standpoint because water chilling helps to remove bacteria from the carcasses to reduce the carcass bacteria load. Along with the washing of bacteria off carcasses, various antimicrobial products, such as chlorine or peracetic acid, are also added to the chillers in the US to further decrease the bacterial load (Sams, 2001). Chilling is the last step of first processing and once carcasses have completed this step, the carcasses can either be marketed as whole-carcasses or continue to be processed to produce other poultry products.

Second and Third Processing

Further processing is any additional steps that go beyond producing whole carcasses to produce value-added products (Baker and Bruce, 1996). These operations can be divided into second and third processing. Before the 1970s, most of the poultry sold in the US was in the form of whole carcasses, but now, less than 10% of the carcasses in the US left the processing plant as fresh or frozen whole carcasses. This is due to a shift during the 1980s where there was in increase in the use of tray packs of carcass parts that include boneless, skinless poultry products (Fletcher, 2004). With the increase in further processing (especially boneless skinless

legs meat), these newer poultry products helped increase per capita broiler consumption from 46.2 lbs. in 1981 to 89.9 lbs. in 2016 (National Chicken Council, 2017a).

The second processing operations including cutting, deboning, and portioning, can be performed by manual cutting or by using mechanical equipment. It is common that processing plants will have both manual and mechanical cut-up and deboning operations on second processing lines. Not only can secondary processing produce common products like breast quarters, wings, drumsticks, or thighs, it can also produce products like portion-control cuts or mechanically deboned meat (MDM) (Fletcher, 2004; Baker and Bruce, 1996). Portion-control cuts are made by computerized portioning systems that can produce thousands of uniform products each hour often using high-pressure, water-jet cutting. These products are often also further processed (i.e. marinated or breaded) and sold to fast-food or restaurant customers as breast fillets, nuggets, or "boneless" wings (Fletcher, 2004). MDM, also known as mechanically separated meat, is produced from the skeletal frames and bone residues where the high-pressure machine will separate the adhering muscle tissues from the bones and connective tissue. The resulting product has a fine texture, is pink in color, and is typically used in inexpensive products like hotdogs (Fletcher, 2004; Baker and Bruce, 1996).

Third processing involves additional operations after second processing and typically includes: marination, cooking, coating, and individually quick frozen (IQF) (Smith, 2014; Smith and Acton, 2001; Baker and Bruce, 1996). As previously stated, the set-up and operations vary from plant to plant, and it is often common that the third processing operations are performed after the distribution of the uncooked products (Smith and Acton, 2001). Common value-added products that are produced in third processing are fully or partially cooked patties, nuggets, tenders, and filets (Smith, 20l4).

Marination is the incorporation of liquids into meat prior to cooking, and marinating is typically one of the first steps in third processing. Not only does marinating increase the water-holding capacity of the meat, it can also improve yield, tenderness and flavor (Smith, 2014). Coating has multiple definitions and can include anything from spraying water on individual products prior to freezing to produce IQF products, to the battering or breading of meat (Smith, 2014). Finally, cooking is an operation that has become more common with some fast-food customers only accepting fully-cooked products. Cooking methods use air, steam, oil, or water to apply heat to the raw products and common ways in which these products are cooked in further processing include using ovens or fryers (Smith and Acton, 2001).

Wastewater

Wastewater, also referred to as sewage, is the spent water remaining after it is used in homes, public institutions, and commercial or industrial establishments, and may contain groundwater, organic/inorganic substances, or industrial wastes (Sincero and Sincero, 2003). Before the 1940s, the municipal wastewater produced in the US was mainly from domestic or home sources, but with the increase in industrial establishments the amount of industrial wastewater has drastically increased. With the increase of wastewater from industrial sources, the characteristics of wastewater has also changed to have higher contents of heavy metals and organic compounds (Tchobanoglous et al., 2003).

Wastewater pollution that enters the environment can be categorized into point versus non-point sources. Non-point wastewater pollution comes from diffuse sources and is carried by runoff and deposited into bodies of water. Examples of non-point pollutants include: fertilizers, sediments from construction sites, bacteria from livestock manures, and oil or toxic chemicals from urban runoff. Point sources include confined or finite areas like pipes, channels, and

containers (USEPA, 2017a; U. S. Congress, 1972). Point source wastewater can be also be broken down into two categories: sanitary vs. non-sanitary. Sanitary wastewater is associated with human use and includes waste as well as the wastewater is coming from homes. This wastewater is also referred to as domestic wastewater or domestic sewage. Non-sanitary wastewater is sewage produced from manufacturing processes and is also referred to as industrial wastewater/sewage (Sincero and Sincero, 2003).

The first major legislation regarding water pollution in the US was passed in 1948 and was called the Federal Water Pollution Control Act (USEPA, 2017b). The main goal of this legislation was to regulate the pollution entering US surface water and there have been multiple amendments to this act since its creation. In response to major pollution issues in surface water like Lake Erie, Chesapeake Bay, Potomac River, and the Cuyahoga River in Cleveland, Ohio, the 1972 amendment was passed. This amendment was the first comprehensive clean water legislation enacted in the US in hopes of reducing water pollution and it is commonly referred to as "The Clean Water Act" today (Sincero and Sincero, 2003; U. S. Congress, 1972). This legislation enforced many large changes including: creating structure for regulating pollutant discharging and giving EPA (which was established in 1970) the authority to set industrial wastewater standards (USEPA, 2017b). The 1972 amendment prohibited point-source pollution to be discharged into navigable waters, unless the individual is given a permit from the National Pollutant Discharge Elimination System (NPDES) (U. S. Congress, 1972).

Beginning in the 1970's, there have been incentives given by the EPA to wastewater treatment authorities to charge non-residential customers based on the burden they place on publicly owned treatment facilities (POTWs). This charge is often called a surcharge, which is determined by the volume of various pollutants (e.g. biochemical oxygen demand, total

suspended solids, and ammonia) in the wastewater stream the customers are producing. Using the concentration (i.e., mg/L or ppm) of the pollutants and the volume of water being sent to the treatment facilities, a load per unit time (e.g., lbs./day) can be calculated using the following equation:

Flow (MGD) x Concentration (mg/L or ppm) x 8.34 = Load (Lbs./day)

Where: Flow = wastewater flow (in million gallons per day)

Concentration = of pollutant (in milligrams per liter or parts per million)

8.34 = Lbs. of 1 gallon of water, and

Load = mass of a pollutant contained in the wastewater stream per day

This equation can also be used to calculate load in kg/day by using the conversion factor of kg of 1 gallon of water (3.785) rather than the conversion factor of lbs. of 1 gallon of water (8.34). Once the load of a pollutant is determined, then a per pound monetary charge can be applied and an associated surcharge can be calculated. From a survey of 71 local wastewater treatment facilities, 60 of those facilities used the same surcharge calculation and it included the following variables: volume of water discharged per month, concentration of a specific characteristic, the allowable concentration of the characteristic, a unit conversion factor, and the cost factor for the characteristic (Garcia et al., 2016).

Poultry Processing Wastewater

On average, poultry processing plants use approximately 26 L (or 7 gal) of water per bird for processing after the implementation of the Hazard Analysis and Critical Control Point Final Rule (HAACP) in 1998. Prior to HACCP implementation, the average water use per bird was 5.4 L less (Northcutt and Jones, 2004). With an average of a little over 9 billion birds processed in 2017, the annual water use in the US for poultry processing is approximately 234 billion L, or 61

billion gallons (USDA-ERS, 2017; Northcutt and Jones, 2004). Within the processing plant, this water use is primarily for scalding, bird washing before/after evisceration, chilling, and cleaning/sanitizing processing equipment and facilities (USEPA, 2002). Another important role of water is to transport offal (e.g., feathers and viscera) from the different operations of the plant for collection using mechanical rotary screens. These screen systems are usually comprised of primary and secondary screens that filter particulates from the stream that are greater than 0.5 millimeters (i.e., 500 microns) in size (Kiepper, 2008). The PPW that has been screened can be recycled to areas of the plant that doesn't require potable water. In a survey of 72 poultry processing plants, 38.5% indicated that they recycle PPW (Northcutt and Jones, 2004).

A majority of the material or waste in the PPW stream comes from various operations in the plant: live haul, slaughter, defeathering, evisceration, carcass washing, chilling, cut-up, rendering, further processing, and sanitation. The waste produced from these operations will typically include: blood, feathers, viscera, soft tissue, fecal material, external debris, oil, fat, and sanitation compounds. In the PPW stream, much of the waste is made up of biodegradable organic compounds, fats, and proteins and because of this, PPW typically has high concentrations of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), nitrogen, and phosphorus (USEPA, 2002). Because the type and set-up of operations, as well as the wastewater treatment, in poultry plants can vary from plant to plant, the composition of wastewater also differs between plants (Zhang et al., 1991).

Since the 1950's, there has been an effort to determine the concentrations of organics, particulates, and nutrients in the PPW streams (Kiepper, 2009; Merka, 2001; Singh et al., 1973; Hamm, 1972; Porges, 1950). Compared to domestic wastewater, the concentration of BOD, COD, TSS, nitrogen, and phosphorus is higher in PPW, even after a screening process (USEPA,

2002). Because of the difficulty in calculating the volume of water in each operation, there have been few studies attempting to calculate the loading, or mass of pollutants, that operations contribute to the PPW stream (Plumber, 2012; Merka, 1991). There have also been few studies to look at the proximate composition of the waste produced by PPW and Kiepper et al. (2008) determined that fat made up over half of the dry-weight of screened waste, followed by protein, ash, and fiber.

Wastewater Analytics

As previously stated, the contamination in PPW is typically characterized by the concentrations of organic material, solids/particulates, and nutrients. The standard methods for analyzing organic content can be used to determine the concentrations or organic matter in wastewater, treated effluents, and determining the efficacy of treatment processes. The most common parameters used to measure the concentration of organic material in wastewater are biochemical oxygen demand (BOD), chemical oxygen demand (COD), and oil and grease (O&G). For determining particulates in PPW, the most common parameters measured are total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), and total volatile solids (TVS). Total Kjeldahl nitrogen (TKN) and phosphorus (P) are the most common parameters measured for determining nutrient content (APHA, 2005). In a survey of 71 local wastewater treatment facilities, all but 2 facilities used BOD, COD, or a combination of both to determine organic content. Other common parameters used included TKN, total phosphorus, ammonia, and fats, O&G (Garcia et al., 2016). For the purpose of this thesis, only BOD, COD, TS, and TSS will be discussed in detail.

BOD is the measure of the relative oxygen requirements of the microbial population in a water sample and is measured by determining the molecular oxygen used by microbes for the

degradation of organic material (carbonaceous demand) during an incubation period. BOD relies on the principle that the more organic material a water sample contains, the more oxygen the microbes will demand to mitigate the organic material. As well as determining the molecular oxygen used, BOD can also be used to measure the amount of oxygen used to oxidize sulfides and ferrous iron. Nitrogenous demand can interfere with BOD calculation, so typically an inhibitory compound will be added to the water samples, which eliminates the issue of nitrogen oxidation. There are many methods to measure BOD that vary in incubation periods and can be used to determine oxygen uptake rate, but a 5-day test, oxygen consumed after incubation for 60 to 90 days, and continuous oxygen uptake are the three methods described in the American Public Health Association Standard Methods (2005). The 5-day method, described section 5210B, includes adding the water sample (kept at or below 4°C) to an airtight container and measuring the dissolved oxygen concentration both before and after the 5-day incubation at 20°C ± 1°C (APHA, 2005). In poultry processing plants, the uncollected blood, digestive tract contents, soluble fats, and fecal material are the main BOD contributors to the PPW stream (USEPA, 2002). In a study performed by Merka (1991), it was determined that the BOD concentration post-secondary screening was 2,178 mg/L; which is about 6 times higher than the maximum concentration allowable for surcharge-free discharge from local wastewater treatment facilities, calculated by Garcia et al. (2016).

COD is another method to determine organic matter concentration and unlike BOD, this method measures an oxidation agent that reacts with the organic material in a water sample (APHA, 2005). When the oxidation agent is added to the sample, it will be reduced based on the amount of organic material in that sample. This method is commonly used to determine the amount of pollutants in wastewater or surface waters. There are also three methods for COD

determination described by the American Public Health Association, including: open reflux method, titrimetric method, and colorimetric method. Out of the three, the colorimetric method is commonly used and consists of adding potassium dichromate ($K_2Cr_2O_7$) to a sample that is then placed in a block digester set at 150°C for 2 hrs. During this digestion period, the dichromate ion will oxidize any COD material and produce chromic ion (Cr^{3+}) in the sample, causing a color change that can be measured using a spectrophotometer (APHA, 2005).

One benefit of calculating COD rather than BOD is the fact that the colorimetric method can be performed in 3 hours, compared to a 5-day test. Also compared to BOD, the COD concentration is typically higher for the same sample because it includes the slowly biodegradable organic compounds like cleaning solvents (Barbut, 2002; USEPA, 1975). Both BOD and COD are important calculations for effluents discharged into the environment. If there is too much organic material in the effluent, degradation of the material by microbes can deplete the dissolved oxygen to a level that cannot support aquatic life (USEPA, 2002). The Merka (1991) study determined the effluent COD concentration from a poultry processing plant prior to wastewater treatment was 3,772 mg/L, which is slightly less than 5 times higher than the maximum concentration allowable for surcharge-free discharge from local wastewater treatment facilities, calculated by Garcia et al. (2016).

Particulate solids are defined as material that is either dissolved or suspended in water, and meat processing facilities tend to produce wastewater that has a relatively large amount of organic solids, like feathers or fecal material (APHA, 2005; USEPA, 2002). Because the solids in PPW have high nitrogen content and high oxygen demand, reducing the amount of solids in effluent also will help to maintain surface waters (USEPA, 2002). For determining the amount of solids in a sample, the American Public Health Association (2005) has 6 standard methods: TS,

TDS, TSS, Fixed and volatile solids, settleable solids, and total, fixed, and volatile solids in solid and semisolid samples. TS is the sum of both TDS and TSS; as well as the sum of both fixed and volatile solids. Therefore, if 2 of the characteristics have been determined (i.e. TSS and TS, or TS and fixed solids), then the concentration of the remaining characteristic can be calculated (USEPA, 2002).

As the name suggests, TS is the residue remaining once a sample has been evaporated in a drying oven (Barbut, 2016). This method is performed by adding a sample into a heat clean crucible, allowing the sample to evaporate at a temperature approximately 2°C less than boiling, and then increasing the temperature to 103 to 105°C for drying the residue material. By comparing the weight of the crucible before and after drying, the amount of residue remaining in the crucible is used to determine the TS concentration (APHA, 2005).

TSS has a method similar to TS, except that the water sample is first filtered through a glass-fiber filter and the residue remaining on the filter after the drying processes is the TSS. These filters, which have a specific pore size (i.e., approximately 2.0 microns) are placed onto a filtration apparatus, which helps vacuum filter the water sample when added to the apparatus. Once the sample has been filtered, the filter is then transferred to an aluminum weighing dish and then dried in the over at 103 to 105°C. The weight of the filter/weighing dish is measured before and after the sample is filtered, and those weights are used to determine the concentration of TSS from the sample (APHA, 2005).

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CHAPTER 2

IMPACT OF SCALDING DURATION AND SCALDING WATER TEMPERATURE ON BROILER PROCESSING WASTEWATER LOADING[1]

[1]C. E. Harris, K. A. Gottilla, L. N. Bartenfeld, D. V. Bourassa, R. J. Buhr, and B. H. Kiepper. Submitted to *Journal of Applied Poultry Research (JAPR)*

ABSTRACT

A series of 3 experiments were performed to evaluate scalding tank poultry processing wastewater (PPW) loading following the slaughter and scalding of commercially raised broilers: hard vs. soft scalding protocols (experiment 1), scalding immersion time and temperature individually (experiment 2), and the presence of residual blood (experiment 3). Similar processing methods were used for each experiment, which was conducted in a pilot plant containing 3 triple-pass scald tanks (740 L each) that were air agitated. After the scalding of each 20-carcass batch, 1 L PPW samples were taken from each scald tank and analyzed for chemical oxygen demand (COD), total solids (TS), and total suspended solid (TSS) concentrations, which were then used to calculate PPW loading (g/kg broiler live weight). For experiment 1, the mean COD loading in tank 1 for the Soft scald protocol (1.834 g/kg^{lwt}) was significantly higher that the Hard scald protocol (1.510 g/kg^{lwt}), which was significantly higher than either protocol in tanks 2 or 3. For experiment 2, the mean TS loading in long immersion time/tank 1 (2.514 g/kg^{lwt}) was significantly higher that the short immersion time/tank1 (1.857 g/kg^{lwt}), which was significantly higher than either immersion time in tanks 2 or 3. In all three experiments, the use of sequential scalder tanks significantly impacted the mean PPW loading for both organic and solid pollutants (COD, TS, and TSS). As an example, in experiment 1, mean PPW loadings were reduced 62 to 69% over the series of three scalding tanks. Residual blood following a 120 s bleed time did not impact PPW loading compared to non-bled carcasses. These results indicate that scalder immersion time appears to be a major indicator for predicting PPW loading in scalders, which shows a significant decrease when sequential scald tanks are utilized.

DESCRIPTION OF PROBLEM

Poultry Processing Wastewater

In 2016, the US poultry processing industry slaughtered approximately 8.8 billion broilers and on average, US broiler processing plants use approximately 26 L of potable water per carcass during processing (USDA-NASS, 2017; Northcutt and Jones, 2004). Much of this water is used for four processing purposes: scalding, chilling, carcass washing/rinsing, and plant sanitation. The resulting process water after use and/or reuse generates the processing plant's wastewater stream (Northcutt and Jones, 2004). Poultry processing wastewater (PPW) is the total accumulation of process waters containing residual blood, feathers and other offal removed from carcasses during processing, in addition to organic debris from cleaning of the live haul area through the cutup areas of the processing plant (Kiepper et al., 2008). PPW data is typically presented in the form of concentration (mg/L) for common wastewater analytical parameters. This is because, although valuable, determining the actual mass of contaminants in PPW requires accurate water volume measurements, which is extremely difficult to isolate and measure in an operating processing facility. Being able to determine the actual loading (i.e. mass) of organics and other pollutants in PPW for various areas of processing operation and individual pieces of equipment in processing plants would help to better identify areas and equipment that contribute the largest quantities of contaminants in the PPW stream, and areas and equipment that contribute little contamination, but consume large quantities of water.

Broiler Processing Scalding

Once broiler carcasses are stunned and bled, typically the next step is immersion scalding in hot, air-agitated water to aid in the release of the feather quills from the feather follicles in the skin, which enables defeathering without damage to the skin surface (Kaufman et al., 1972). The

traditional “hard scald” method uses water temperatures from 60 to 66°C and immersion times of 45 to 90 s, while the traditional “soft scald” method uses lower water temperatures from 51 to 54°C and longer immersion times of 120 to 210 s (Owens et al., 2010; Buhr et al., 2014). The scald method chosen by poultry processors depends on the desired market product requirements, the weight and age of broilers, and the number of scald tanks used (Buhr et al., 2014). Most US commercial broiler processing plants today use hard scalding because the higher immersion temperature aides in the removal of the outer skin surface cuticle layer, which improves the adhesion of batter and breading to the skin surface (Suderman and Cunningham, 1980). Commercial scalding can be performed in a single or a series of water-filled tanks, but research has shown that the use of more than one immersion scalding tank that are arranged in series and in a counter-current flow design where carcasses flow in one direction, while water flows in the opposite direction, helps to reduce the carcass microbial load as carcasses progress from dirty to cleaner tanks (Cason et al., 1999). Previous research looking at wastewater overflow from scalder tanks in commercial operations showed that it contained highly contaminated PPW and had the highest concentration of chemical oxygen demand (COD=2,268 mg/L) and total solids (TS=1,635 mg/L) when compared to the PPW effluent coming from 7 different areas in commercial processing plants (Hamm, 1972).

The objective of this series of 3 experiments, was to evaluate the effects of hard or soft scalding protocols (immersion scald time and temperature), the use of multiple sequential scalder tanks, and the presence of residual carcass blood on PPW loadings of COD, TS, and total suspended solids (TSS).

MATERIALS AND METHODS

Three experiments were performed assessing the contribution to PPW loading from each of 3 successive scalder tanks. In experiment 1, the effect of traditional hard vs. soft scalding, as well as the use of 3 successive scalder tanks on PPW loading was evaluated. For experiment 2, scalder immersion time and scalder temperature were evaluated to determine if either of these scalder protocol factors affected PPW loading independently. In experiment 3, the effect of residual blood post-bleeding on scalder PPW loading was evaluated.

On each processing day, the pilot plant (USDA-ARS U.S. National Poultry Research Center, Athens, GA) water and steam supply lines were flushed for 5 mins and then the 3 scalding tanks were filled with 740 L of water and heated with direct steam injection to the scalder temperature set points. After the scald water temperatures were attained and prior to scalding the first batch of carcasses on each trial date, a 1 L representative water sample was taken from the center of each scald tank to serve as a background level for all subsequent treatment samples collected that day. For every experiment, male broilers were selected and obtained 1 h prior to processing from a standard commercial dump coop at a commercial processing plant live haul area. Selected broilers had been feed and water withdrawn, were approximately 42 days of age, and were transported to the pilot processing plant in solid bottom plastic coops (89 cm long, 60 cm wide, 26 cm high; Pakster Athens, TN; 10 broilers/coop). The broilers and standard operating procedures used in this study were covered by an animal use proposal approved by the US National Poultry Research Center IACUC PMSPRU-03-2016-A. The vast majority of the scalded carcasses resulting from these experiments were subsequently defeathered and utilized for additional meat quality research that is not described in this manuscript.

Experiment 1

In experiment 1, there were 3 replicate trial days utilizing 40 birds each day (120 total). On each trial date, broilers were randomly assigned to be either hard scalded or soft scalded (20 carcasses/batch each, the maximum number of broiler carcasses required to fill all of the shackles in one of the triple tank scalders at one time when spaced on 6-inch/15.2-cm centers). Broilers were individually weighed, shackled, shanks/shackles wet with water, electrically stunned using a brine stunner (model LD-7001, Simmons Engineering Company, Dallas, GA) set at 15 V pulse DC for 10 s, and bled for 2 min following the carotid arteries and jugular veins being cut using an automatic rotary blade (model SK-5, Simmons Engineering Company). Each batch of carcasses was scalded in a triple tank system (model SGS-3CA, Stork-Gamco Inc., Gainesville, GA) where each scald tank (740 L) was set at the same temperature depending on protocol temperature. For the hard scalding, broiler carcasses were scalded at 60°C for 90 s total immersion time and for soft scalding, broilers were scalded at 53°C for 120 s immersion, both with low air agitation to minimize water loss from the tanks (Buhr et al., 2014). No additional water was added after the initial scalder tank filling. Immediately after the last carcass had exited a scalding tank, a representative 1L sample of water from each scald tank was collected from the center of the tank and placed on ice. The water in each scalding tank was then emptied, the tanks rinsed with tap water, refilled, and reheated between each batch (2 batches/day) of 20 carcasses.

Experiment 2

For experiment 2, there were 4 replicate trial days utilizing 80 broilers each day (320 total). On each trial date, broilers were assigned to one of four scalding protocols (20 birds each). Two of the protocols were the traditional hard scald/short (HS, 60°C for 90 s) or soft scald/long (SL, 53°C for 120 s) protocols. The remaining two protocols took the reciprocal of the

immersion times, creating a hard scald/long (HL) immersion time (60°C for 120 s) and a soft scald/short (SS) immersion time (53°C for 90 s). Broilers were weighed, shackled, shanks/shackles wet, electrically stunned, and bled for 2 min using the same methods as described in experiment 1. Again, prior to the first batch each day, a 1 L representative sample was taken from each scald tank and immediately after the last carcass had exited a scalding tank for each batch and placed on ice until samples were analyzed. The water in each scalding tank was emptied, the tanks rinsed, refilled, and reheated between each batch (4 batches/day) of 20 carcasses.

Experiment 3

In experiment 3, there were 3 replicate trial days utilizing 40 broilers each day (120 total). On each day, broilers were assigned to one of two scalding protocols: hard scald and bled (HB) or hard scald and not bled (HNB) (20 birds each). Broilers were individually weighed and shackled. The HB broilers were stunned and bled using the same method previously described for experiments 1 and 2. The not-bled (HNB) broilers were stunned and then electrocuted using the contact metal plate, that was added following the brine stunner, at 120 V AC for 5 s (Bourassa et al., 2017). Since these carcasses were not to be bled, to assure IACUC compliance with standard operating procedures for the pilot plant, the HNB carcasses were then cervically dislocated while shackled to assure that no cadavers would occur in the absence of bleeding. For both bled and non-bled protocols, carcasses were hard scalded (60° for 90 s) as described in experiments 1 and 2. Again, a 1 L representative water sample was taken from each scald tank prior to the first batch and immediately after the last carcass had exited a scalding tank following each batch and all were placed on ice until sample analysis. The water in each scalding tank was

emptied, the tanks rinsed, refilled, and reheated between each batch (2 batches/day) of 20 carcasses.

Analytical Methods

All scalder water background and PPW samples were analyzed in triplicate for COD (chemical oxygen demand method 5220D), TS (total solids method 2540B) and TSS (total suspended solids method 2540D) (APHA, 2005). Using the sample concentrations (mg/L), the loading values (g/kg live weight (lwt)) were determined for each sample by multiplying the scalder tank volume (740 L) by the concentration, dividing by 1000, and then dividing by the total broiler live weight in kg (20 broilers) for each treatment. The data point remained as reported if the background concentration was below detectable limit (BDL). If the background level loading was detectable from the initial control water samples on any trial dates, the final loading values were adjusted by subtracting the background loading value and was done for all 3 experiments.

Statistical Analysis

Each of the triplicate concentration data points for COD, TS and TSS, were used to calculate a loading value. Then the three loading values were averaged to produce a single loading data point for statistical analysis. All loading data points were then subjected to statistical analysis using SAS JMP Pro 13. For all experiments, differences in means were considered significant at $P \leq 0.05$. Experiment 1 was analyzed as a 2 x 3 factorial design with scalder protocol (Hard, Soft) and successive scald tanks (1, 2, 3) as main effects. For experiment 1, two-way ANOVA was used for the scalding protocol and successive scalder tank effects to determine if interaction between the main effects was significant. If interaction between the factors was significant for any variable (i.e. COD), then the resulting 6 treatments were analyzed

individually using one-way ANOVA. On the other hand, for experiment 1, if the interaction between factors was not significant for any variable (i.e. TS and TSS), then data from the 3 replicate trials were analyzed using Student's t-test for the scalding protocol effect and one-way ANOVA for the effect of successive scalder tank.

Experiment 2 was analyzed as a 2 x 2 x 3 factorial design with scalder temperature (Hard, Soft), scalder duration (Short, Long), and successive scald tanks (1, 2, 3) as main effects. For experiment 2, full factorial two-way ANOVAs and a three-way ANOVA were used for the scalding temperature, scalding duration, and successive scalder tank effects to determine if interaction between the main effects was significant. For experiment 2, if any two-way interactions were identified (i.e. TS based on Immersion Time/Scald Tank), then the main effects in question were analyzed as coupled treatments. If no significant interactions between factors was found, then one-way ANOVA was used for the 4 replicate trials to determine the effect of scalding temperature, scalding duration or successive scald tanks on PPW loading (g/kg^{lwt}).

Experiment 3 was analyzed as a 2 x 3 factorial design with scalder/bleed protocol (HB, HNB) and successive scald tanks (1, 2, 3) as main effects. For experiment 3, two-way ANOVA was used for the scalding/bleed protocol and successive scalder tank effects to determine if interaction between the main effects was significant. No significant interactions were found between factors in experiment 3, therefore data from the 3 replicate trials were analyzed using Student's t-test for the bled vs. not bled effect and one-way ANOVA for the effect of successive scalder tanks on loading (g/kg^{lwt}). Significant means for all experiments were separated using Tukey's HSD test.

RESULTS

Experiment 1

The mean pre-slaughter live body weight for all 120 commercially obtained broilers in experiment 1 was 2.102 kg. There was a significant interaction for COD loadings between scald protocol and successive scald tanks (P=0.0340), thus the COD loadings in experiment 1 were analyzed as 6 individual treatments with the 2 factors combined. Mean COD loadings for each of the 6 treatments are presented in Table 2.1 and a probability table for COD loadings based on the two-way ANOVA output is presented in Table 2.2. Results from experiment 1 showed that the Soft Scald/Tank 1 treatment had a significantly higher mean COD loading (1.834 g/kg^{lwt}) than any other treatment. This was followed by the mean COD loading (1.510 g/kg^{lwt}) produced by the Hard Scald/Tank 1 treatment, which was significantly lower than the Soft Scald/Tank 1 treatment for mean COD loading but was significantly higher than the remaining 4 treatments. The Soft Scald/Tank 2 treatment had a mean COD loading of 0.672 g/kg^{lwt}, which was not significantly different from the Hard Scald/Tank 2 treatment mean COD loading (0.488 g/kg^{lwt}) but was significantly higher than the remaining 2 treatments. While the Hard Scald/Tank 2 mean COD loading (0.488 g/kg^{lwt}) was not significantly higher than the Hard Scald/Tank 3 treatment mean COD loading of 0.220 g/kg^{lwt}, it was significantly higher than the mean COD loading produced by the Soft Scald/Tank 3 treatment (0.152 g/kg^{lwt}). The Hard Scald/Tank 3 and Soft Scald/Tank 3 treatments were not significantly different from each other.

For TS and TSS mean loadings in experiment 1, the interaction between scalder protocol and successive scald tanks was not significant (P=0.1185 and P=0.6883), thus the main effects were analyzed independently. Mean TS and TSS loading values for scalding protocols and successive tanks are presented in Table 2.3. Mean loadings for TS (1.509 and 1.753 g/kg^{lwt}) and

TSS (0.266 and 0.324 g/kg^{lwt}), were not significantly different between hard and soft scalding protocols. However, both TS and TSS mean loadings in the successive scald tanks, as presented in Table 2.3 and Figure 2.1, did have significant differences. With both TS and TSS, the mean loadings in Tank 1 (TS = 2.634 g/kg^{lwt} and TSS = 0.517 g/kg^{lwt}) were significantly higher than the corresponding mean loading values in Tanks 2 (TS = 1.261 g/kg^{lwt} and TSS = 0.206 g/kg^{lwt}) and Tank 3 (TS = 0.999 g/kg^{lwt} and TSS = 0.162 g/kg^{lwt}). However, meaning loading for TS and TSS were not significantly different between Tanks 2 and 3. Mean TS loading values were reduced by 52% from Tank 1 to Tank 2, and an additional 10% reduction in mean TS loading was seen in Tank 3 (62% mean TS loading reduction in total over the three tanks). Meanwhile, mean TSS loading values were reduced by 60% from Tank 1 to Tank 2, and an additional 9% reduction was seen in Tank 3 (69% mean TSS loading reduction in total over the three tanks). Although hard scalded carcasses lose the skin cuticle layer during defeathering and thus could be expected to have a higher PPW loading impact, results from experiment 1 suggest that perhaps the longer immersion time for the soft scalding protocol (120 s vs. 90 s) could possibly be the cause of significantly higher PWW loading in the successive scalding tanks.

Experiment 2

The mean pre-slaughter live body weight for all 320 commercially obtained broilers in experiment 2 was 2.405 kg. There was a significant interaction for mean TS loadings between scald duration and successive scalding tank (P=0.0155), thus these main effects remained coupled for statistical analyses. However, this was the only significant main effects interaction, thus main effects (i.e., scald temperature, immersion time, and successive scald tank) for the PPW variables (COD, TS and TSS), with the exception of the mean TS loading for scald duration*successive scalding tank, were analyzed independently.

Table 2.4 shows the mean TS loadings based on scalding temperature (i.e., Hard or Soft) and the 6 scalding immersion time/scald tank treatments, which accounts for the main effects interaction. For scald temperature, there were no significant differences between the mean TS loading for hard scald temperature (1.290 g/kg^{lwt}) compared to the soft scald temperature (1.246 g/kg^{lwt}), which resulted in overall TS loading mean of 1.268 g/kg^{lwt}. The mean TS loadings based on the scalding immersion time/scald tank treatments showed that the Longer Immersion Time/Tank 1 treatment had the highest mean TS loading (2.514 g/kg^{lwt}) compared to any of the other treatments. The second highest in mean TS loading was the Shorted Immersion Time/Tank 1 treatment with 1.856 g/kg^{lwt}, which was significantly lower than the Longer Immersion Time/Tank 1 treatment, but was significantly higher than all other treatments. The four remaining treatments: Shorter Immersion Time/Tank 2 (1.060 g/kg^{lwt}), Longer Immersion Time/Tank 3 (0.859 g/kg^{lwt}), Longer Immersion Time/Tank 2 (0.772 g/kg^{lwt}), and Shorter Immersion Time/Tank 3 (0.545 g/kg^{lwt}) were not significantly different from each another.

There were no significant interactions between scalding temperature, scalding immersion time and successive scalder tanks for COD or TSS (P=0.5359), so effects were analyzed separately. The mean PPW loading values for scalding temperature, scalding immersion time and successive tanks are presented in Table 2.5. For the scalding temperature effect, there were no significant differences for mean COD loadings between the Hard scald temperature (0.483 g/kg^{lwt}) and the Soft scald temperature (0.465 g/kg^{lwt}). Likewise, there were no significant differences for mean TSS loadings between the Hard scald temperature (0.212 g/kg^{lwt}) and the Soft scald temperature (0.216 g/kg^{lwt}). For the scalding immersion time effect, there were no significant differences for mean COD loadings between the Long scald immersion time (0.513 g/kg^{lwt}) and the Short scald immersion time (0.436 g/kg^{lwt}). Likewise, there were no significant

differences for mean TSS loadings between the Long scald immersion time (0.222 g/kg^{lwt}) and the Short scald immersion time (0.205 g/kg^{lwt}).

As presented in Table 2.5 and Figure 2.2, mean PPW loading was significantly higher in Tank 1 compared to tanks 2 and 3 for COD and TSS (both at P<0.0001). The mean COD loading for Tank 1 (0.958 g/kg^{lwt}) was significantly greater than for Tank 2 (0.297 g/kg^{lwt}) or Tank 3 (0.167 g/kg^{lwt}), which were not significantly different from each other. Likewise, the mean TSS loading for Tank 1 (0.371 g/kg^{lwt}) was significantly greater than for Tank 2 (0.138 g/kg^{lwt}) or Tank 3 (0.132 g/kg^{lwt}), which were not significantly different from each other. Mean COD loadings to the PPW stream from Tank 1 to Tank 2 were reduced by 69% and by 83% in Tank 3. Finally, TSS values in Tank 2 and Tank 3 were reduced by 64% as compared to Tank 1.

Experiment 3

The mean pre-slaughter live body weight for all 120 commercially obtained broilers in experiment 3 was 2.101 kg. There were no significant interactions between scalding/bleed protocol and successive scalding tanks for COD (P=0.7773), TS (P=0.5869), or TSS (P=0.5077), thus the main effects were analyzed independently. The mean COD, TS and TSS loading values for the scalding/bleed protocols are included in Table 2.6 and are graphically represented in Figure 2.3. Comparing the HB vs. not HNB protocols, there was no significant differences for mean COD (0.411 and 0.346 g/kg^{lwt}; P=0.5897), TS (1.131 and 1.142 g/kg^{lwt}; P=0.9743), or TSS (0.151 and 0.095 g/kg^{lwt}; P=0.3391) PPW loadings.

The mean COD, TS and TSS loading values for the sequential scalding tanks are included in Table 2.6 and graphically represented in Figure 2.4. As seen in the previous two experiments, PPW loadings were significantly higher in Tank 1 for COD (0.702 g/kg^{lwt}; P=<0.0001), TS (1.830 g/kg^{lwt}; P=0.0012), and TSS (0.252 g/kg^{lwt}; P= 0.0010) as compared to

Tanks 2 and 3. Also, as seen in the previous two experiments, the mean loading values in Tank 2 for COD (0.246 g/kg^{lwt}), TS (0.917 g/kg^{lwt}), and TSS (0.058 g/kg^{lwt}) were not significantly different from the Tank 3 for COD (0.188 g/kg^{lwt}), TS (0.662 g/kg^{lwt}), and TSS (0.058 g/kg^{lwt}). Mean COD loadings to the PPW stream from Tank 1 to Tank 2 were reduced by 65% and by 73% in Tank 3. Likewise, mean TS values were reduced by 50% in Tank 2 compared to Tank 1 and 64% in Tank 3. Finally, TSS values in Tank 2 and Tank 3 were reduced by 77% as compared to Tank 1. For the not bled (HNB) protocol, it was also observed that the carcass skin was not red when leaving the third scalder tank but became red in color during defeathering and was noticeably apparent after exiting the picker.

DISCUSSION

Although the concentrations of poultry wastewater contaminants have been previously documented (Singh et al., 1973; Hamm, 1972; Ralph, 1950), there have been few other studies to calculate the PPW loading in g/kg^{lwt} (Plumber et al., 2012). In Plumber et al. (2012), no significant differences were observed between hard vs. soft scalding in PPW loading; although for that individual study the single scalder containers (volume of 16 L or 20 L) utilizing either 1 or 5 broiler carcasses were used rather than actual successive scalder tanks. These results were similar to the current study where no significance in PPW loading between hard vs. soft scalding protocols were observed. The PPW analytics loading values for both hard and soft scalding in the current study tended to be lower (i.e. COD hard scald- 0.739 vs. 1.86 g/kg^{lwt}; TS hard scald- 1.509 vs. 1.69 g/kg^{lwt}; TSS hard scald 0.266 vs. 0.49 g/kg^{lwt}) than the values determined in Plumber et al. (2012). This could be due to several factors including: natural variation on cleanliness of broilers and to the fact that more clean water allows for a more vigorous, robust movement of pollutants from the carcass to the adjacent water. This contrasts with a small

volume of water perhaps becoming “saturated” with pollutants which would subsequently reduce the load. Northcutt et al. (2006) compared paired carcass halves after immersion in either 2.1 L/kg (low) or 16.8 L/kg (high) chilling water volumes and the results show that using additional water during immersion chilling of inoculated broilers did remove more bacteria from the carcass surfaces, but numbers of bacteria per milliliter in the chiller water remained constant. They concluded that the bacteriological impact of using more water during commercial immersion chilling may not be enough to offset economic costs.

Previous publications have assumed that hard scalding would logically produce higher PPW loadings because the cuticle and skin lipids are being removed during scalding and defeathering at the higher water temperatures. This concept was proposed by both Russell (2007) and again by Nunes (2011) without obtaining or providing scalding water analysis data. While hard scalding does result in lower defeathered hot carcass post-evisceration yield by 1%, Buhr et al. (2014) determined that was due to the retention of the skin protein content for pre-scald and soft-scalded samples vs hard-scalded defeathered skin samples. Results from the current study do not support the concept that the higher PPW loading occurs at higher temperatures, but in fact higher PPW loadings correspond more closely to longer scalding immersion time. For both experiments 1 and 2, the protocols with the longer immersion time (120 s) had higher loading for all 3 analytics when compared to the shorter immersion time protocols (60 s). There were also no significant interactions between immersion time and temperature in experiment 2, further indicating that these factors affect loading separately. Based on the current study, it appears that immersion time had a larger impact on PPW loading compared to the scalding temperature.

A consistent result that was observed from this study was that with the use of successive scalding tanks, typically Tank 1 had significantly higher loading for COD, TS, and TSS than

tanks 2 and 3. In a commercial plant using 3, counter-current scalding tanks, Cason et al. (1999) found that TSS concentration in tank 3 was significantly lower than tanks 1 and 2. Although the Cason et al. (1999) and the current study had different methodologies, these studies support the importance of having successive scald tanks in the commercial processing plants in order to remove carcass surface debris prior to defeathering.

From this study, it was found that residual carcass blood following a 120 s bleed-out time did not significantly impact PPW loading. However, a significant difference may be observed with increased numbers of birds or on a commercial scale. Previous research of the impact of residual blood in scald tanks evaluated long (120s) vs. a short bleed (60s) time and how that effects PPW loading (Plumber et al., 2012). Results from their study indicate that the longer bleed time significantly decreases PPW loading. Finally, during experiment 3, the not bled carcasses exiting the scalder did not have red skin color, only after the birds exited the picker and the feathers removed was the change in skin color observed. This observation is supported by Griffiths (1985) where electrocuted carcasses that were not bled did not produce red carcasses after scalding at 58°C for 4 min.

CONCLUSIONS AND APPLICATIONS

1. The ability to calculate actual pollutant loading (i.e. mass) in poultry processing wastewater is an important tool to evaluate areas of the plant that contribute higher loads to the wastewater stream that will require removal prior to discharge for the processing plant into municipal sewer systems.
2. Contrary to prior beliefs, the current study indicates that soft scalding may result in higher PPW loading due to the longer immersion time. Longer immersion time (120s) protocols had the tendency to be higher in loading compared to shorter immersion time (90s) protocols.

3. All 3 experiments indicate that with the use of successive scald tanks, Tank 1 has significantly higher loading compared to Tanks 2 and 3 or all 3 tanks were significantly different from one another. This further verifies the importance of multiple-tank systems in order to clean carcasses prior to picking.
4. When comparing bled vs. not-bled protocols, residual blood after 120 s bleed-out times did not significantly impact PPW loading, although a difference might be detected on a commercial scale.

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Table 2.1. Poultry processing wastewater (PPW) mean chemical oxygen demand (COD) loading (g/kg^{lwt})[1] for commercially obtained broilers scalded using hard or soft scalding protocols in three sequential scald tanks (740L each) in 20 carcass batches; Experiment 1.

	Scalding Protocol[2]	
Tank	Hard Scald	Soft Scald
1	1.510^{B}	1.834^{A}
2	0.488^{CD}	0.673^{C}
3	0.220^{DE}	0.152^{E}

[1]grams per kilogram of pre-slaughter live weight.

[2]Hard= 60°C for 90 s; Soft= 53°C for 120 s.

$^{A, B, C, D, E}$ –different superscripts within table indicate statistically significant differences ($P \leq 0.05$).

Table 2.2. Poultry processing wastewater (PPW) probability table for chemical oxygen demand (COD) loading (g/kg^{lwt})[1] for 120 commercially obtained broilers scalded using hard or soft scalding protocols in three sequential scald tanks (740 L each) in 20 carcass batches; Experiment 1.

Protocol[2], Scald Tank	Hard, Tank 1	Hard, Tank 2	Hard, Tank 3	Soft, Tank 1	Soft, Tank 2	Soft, Tank 3
Hard, Tank 1	-	<0.0001*	<0.0001*	0.0409*	<0.0001*	<0.0001*
Hard, Tank 2	<0.0001*	-	0.1105	<0.0001*	0.4024	0.0331*
Hard, Tank 3	<0.0001*	0.1105	-	<0.0001*	0.0040*	0.9747
Soft, Tank 1	0.0409*	<0.0001*	<0.0001*	-	<0.0001*	<0.0001*
Soft, Tank 2	<0.0001*	0.4024	0.0040*	<0.0001*	-	0.0013*
Soft, Tank 3	<0.0001*	0.0331*	0.9747	<0.0001*	0.0013*	-

[1]grams per kilogram of pre-slaughter live weight.

[2]Hard= 60°C for 90 s; Soft= 53°C for 120 s.

*Indicates statistically significant differences ($P \leq 0.05$).

Table 2.3. Mean poultry processing wastewater (PPW) total solids (TS) and total suspended solids (TSS) loadings (g/kg^{lwt})[1] for 120 commercially obtained broilers scalded using soft or hard scalding protocols in three sequential scald tanks (740L each) in 20 carcass batches; Experiment 1.

		Mean Loading (g/kg^{lwt})[2]		
Scald Protocol[3]	Tank	TS	TSS	
Hard		1.509	0.266	
Soft		1.753	0.324	
		0.5291	*0.4976*	*P-value*
		±0.268	*±0.058*	*SEM*
	Tank 1	2.634[A]	0.517 [A]	
	Tank 2	1.261 [B]	0.206 [B]	
	Tank 3	0.999 [B]	0.162 [B]	
		<0.0001	*<0.0001*	*P-value*
		±0.124	*±0.024*	*SEM*

[1]grams per kilogram of pre-slaughter live weight.

[2]TS= Total Solids; TSS= Total Suspended Solids.

[3]Hard= 60°C for 90 s; Soft= 53°C for 120 s.

[A, B] –different superscripts within a column between protocols or among tanks indicate statistically significant differences ($P \leq 0.05$).

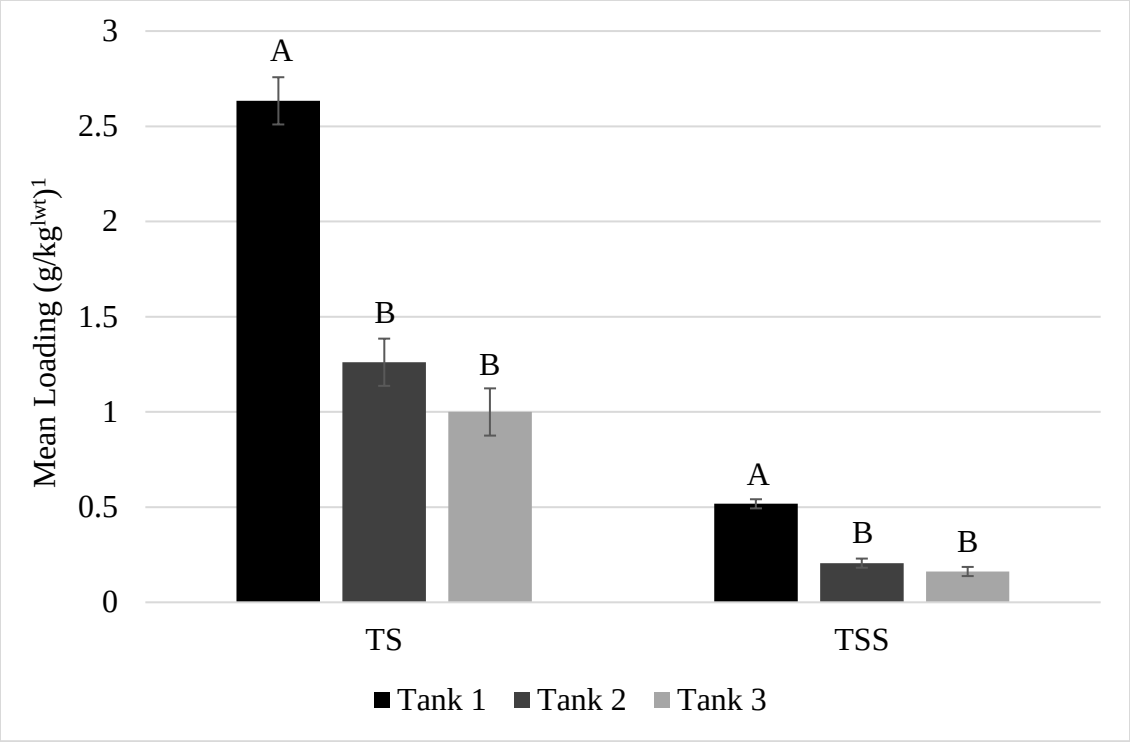

Figure 2.1. Poultry processing wastewater (PPW) total solids (TS) and total suspended solids (TSS) loadings (g/kg^{lwt}) [1, 2] for 120 commercially obtained broilers scalded in three sequential scald tanks (740L each) in 20 carcass batches; Experiment 1.

[1]grams per kilogram of pre-slaughter live weight.

[2]TS= Total Solids; TSS= Total Suspended Solids.

[A, B] different superscripts within a column indicate statistically significant differences among tanks ($P \leq 0.05$).

Table 2.4. Poultry processing wastewater (PPW) mean TS loadings (g/kg^{lwt}) [1] for 320 commercially obtained broilers scalded two scalding temperatures and two scalding durations in three sequential scald tanks (740 L each) in 20 carcass batches; Experiment 2.

Scald Temperature[3]	Immersion Time[4] x Tank	PPW Loading (g/kg^{lwt})[2] TS	
Hard		1.290	
Soft		1.246	
		0.8530	*P-value*
		0.167	*SEM*
	Long, Tank 1	2.514^{A}	
	Short, Tank 1	1.857^{B}	
	Short, Tank 2	1.060^{C}	
	Long, Tank 3	0.859^{C}	
	Long, Tank 2	0.772^{C}	
	Short, Tank 3	0.545^{C}	
		<0.0001	*P-Value*
		0.153	*SEM*

[1]grams per kilogram of pre-slaughter live weight.

[2]TS= Total Solids [3]Hard=60°C; Soft=53°C [4]Short= 90 s; Long= 120 s

$^{A, B, C}$ –different superscripts within a column indicate statistically significant differences among tanks ($P \leq 0.05$).

Table 2.5. Poultry processing wastewater (PPW) mean COD and TSS loadings (g/kg^{lwt}) [1] for 320 commercially obtained broilers scalded using different two scalding temperatures and two scalding immersion durations in three sequential scald tanks (740 L each) in 20 carcass batches; Experiment 2.

			PPW Loading (g/kg^{lwt})[2]		
Scald Temperature[3]	Immersion Time[4]	Tank	COD	TSS	
Hard			0.483	0.212	
Soft			0.465	0.216	
			0.8840	*0.9280*	*P-value*
			0.087	*0.030*	*SEM*
	Long		0.513	0.222	
	Short		0.436	0.205	
			0.5330	*0.7010*	*P-Value*
			0.087	*0.030*	*SEM*
		1	0.958^{A}	0.371^{A}	
		2	0.297^{B}	0.138^{B}	
		3	0.167^{B}	0.132^{B}	
			<0.0001	*<0.0001*	*P-value*
			0.060	*0.024*	*SEM*

[1]grams per kilogram of pre-slaughter live weight.

[2]COD= Chemical Oxygen Demand; TSS= Total Suspended Solids.

[3]Hard=60°C; Soft=53°C [4]Short= 90 s; Long= 120 s

$^{A, B}$ –different superscripts within a column indicate statistically significant differences among tanks ($P \leq 0.05$).

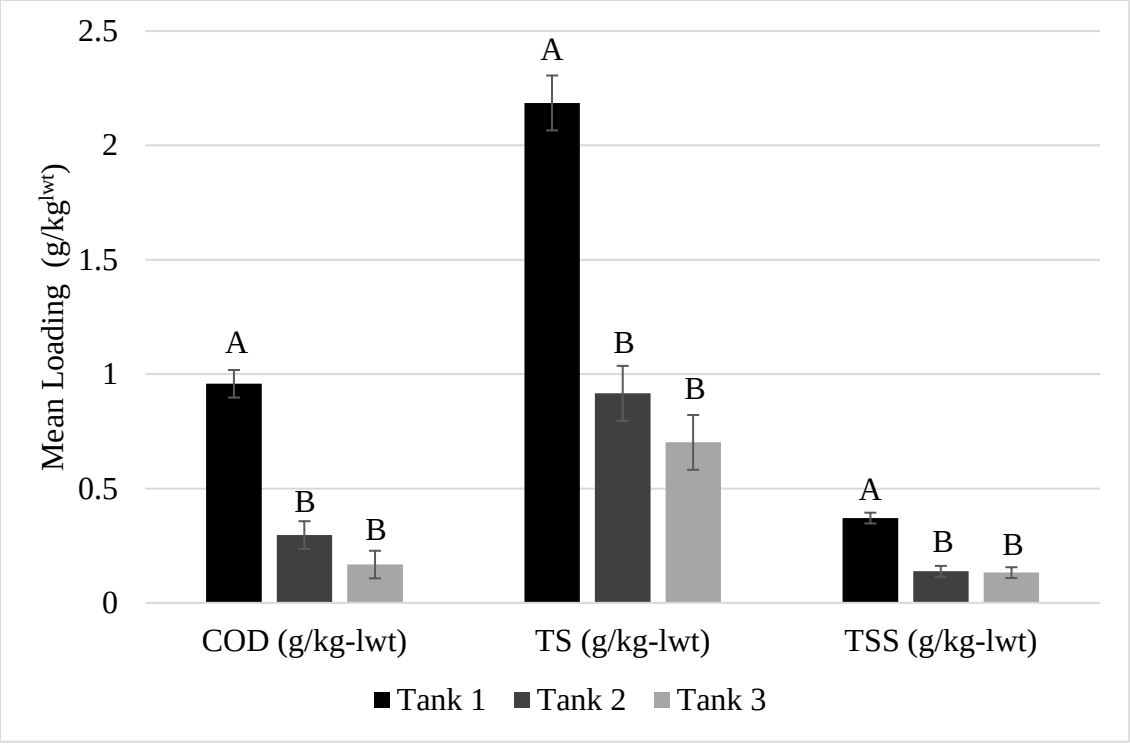

Figure 2.2. Poultry processing wastewater (PPW) mean COD and TSS loadings (g/kg^{lwt}) [1, 2] for commercially obtained broilers (20 per batch) scalded in sequential scald tanks; Experiment 2.

[1]grams per kilogram of pre-slaughter live weight.

[2]COD= Chemical Oxygen Demand; TSS= Total Suspended Solids.

[A, B] different superscripts within a column indicate statistically significant differences among tanks ($P \leq 0.05$).

Table 2.6. Poultry processing wastewater (PPW) mean COD, TS, and TSS loadings (g/kg^{lwt}) [1] for 60 bled and 60 not-bled commercially obtained broilers hard scalded[2] in three successive scald tanks (740L each) in 20 carcass batches; Experiment 3.

		PPW Loading (g/kg^{lwt}) [3]			
Scald Protocol	Tank	COD	TS	TSS	
Bled		0.411	1.131	0.151	
Not-Bled		0.346	1.142	0.095	
		0.*5897*	*0.9743*	*0.3391*	*P-Value*
		±0.084	*±0.231*	*±0.040*	*SEM*
	1	0.702^{A}	1.830^{A}	0.252^{A}	
	2	0.246^{B}	0.917^{B}	0.058^{B}	
	3	0.188^{B}	0.662^{B}	0.058^{B}	
		<0.0001	*0.0012*	*0.0010*	*P-Value*
		±0.031	*±0.124*	*±0.033*	*SEM*

[1]grams per kilogram of pre-slaughter live weight.

[2]60°C for 90 s.

[3]COD= Chemical Oxygen Demand; TS= Total Solids; TSS= Total Suspended Solids.

$^{A, B}$ –different superscripts within a column indicate statistically significant differences among tanks ($P \leq 0.05$).

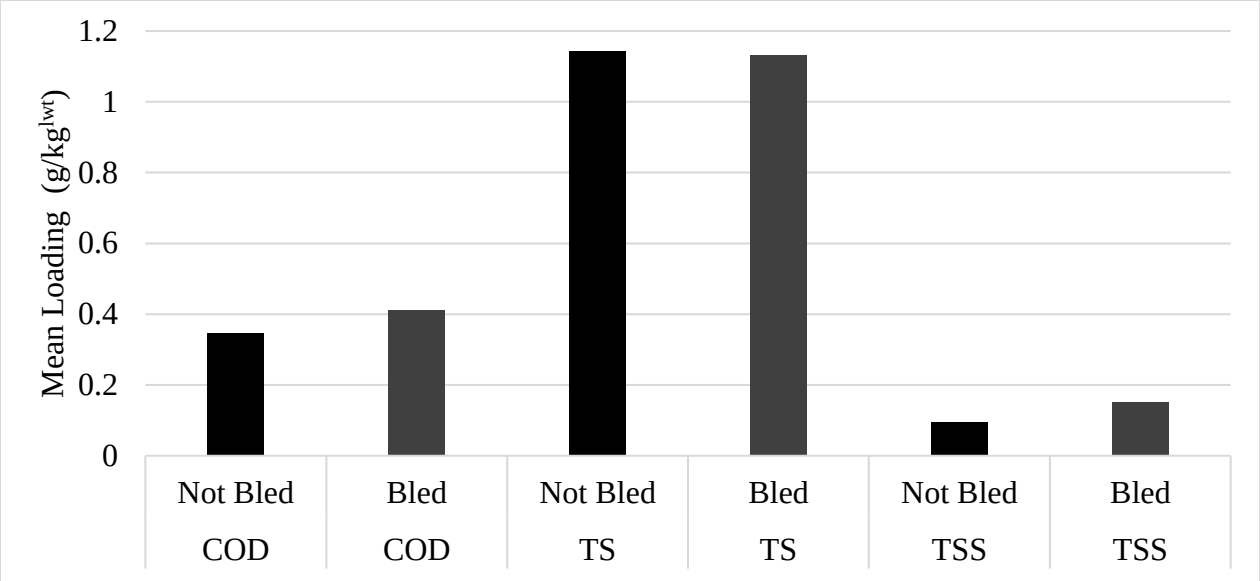

Figure 2.3 Poultry processing wastewater (PPW) mean COD, TS, and TSS loadings (g/kg^{lwt}) [1, 2] for 60 bled and 60 not-bled commercially obtained broilers hard scalded[3] in three successive scald tanks (740L each) in 20 carcass batches; Experiment 3.

[1]grams per kilogram of pre-slaughter live weight.

[2]COD= Chemical Oxygen Demand; TS= Total Solids; TSS= Total Suspended Solids.

[3]60°C for 90 s.

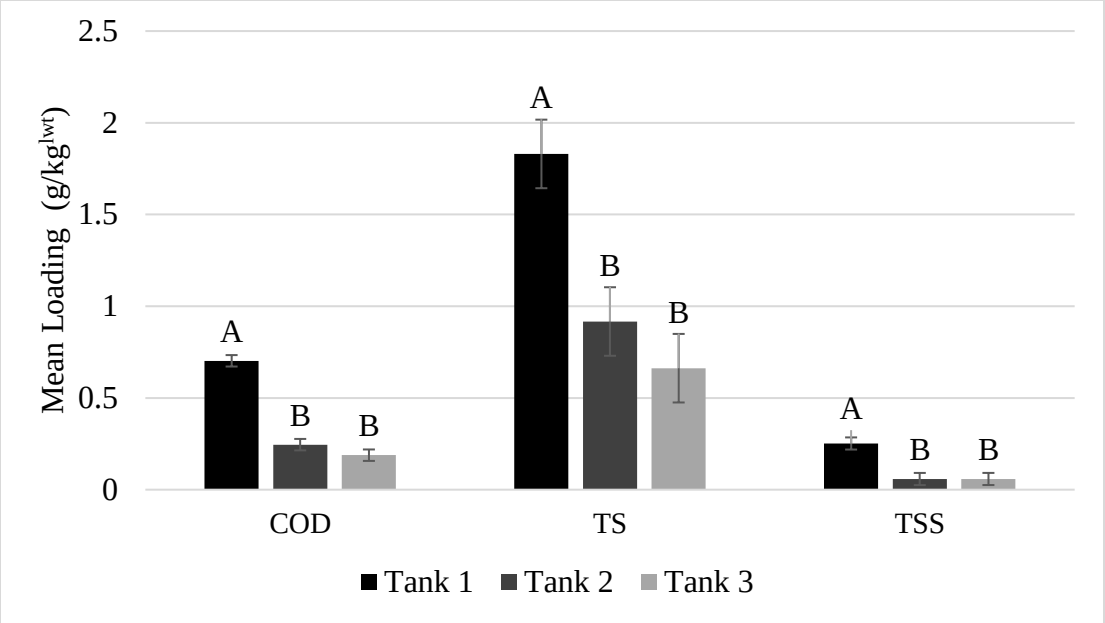

Figure 2.4. Poultry processing wastewater (PPW) mean COD, TS, and TSS loadings (g/kg^{lwt}) [1, 2] for 120 commercially obtained broilers hard scalded[3] in three successive scald tanks (740L each) in 20 carcass batches, Experiment 3.

[1]grams per kilogram of pre-slaughter live weight.

[2]COD= Chemical Oxygen Demand; TS= Total Solids; TSS= Total Suspended Solids.

[3]60°C for 90 s.

[A, B] –different superscripts within a column indicate statistically significant differences among tanks ($P<0.05$).

CHAPTER 3

EVALUATION OF DRINKING WATER ANTIMICROBIAL INTERVENTIONS ON WATER USAGE, FEED CONSUMPTION, AND *SALMONELLA* RETENTION IN BROILERS FOLLOWING FEED AND WATER[2]

[2]C. E. Harris, L. N. Bartenfeld, D. V. Bourassa, B. D. Fairchild R. J. Buhr, and B. H. Kiepper.

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ABSTRACT

A series of experiments were conducted to measure the effects of adding cetylpyridinium chloride (CPC), hydrogen peroxide (HP), and/or sodium bisulfate (SB) to commercial broiler water drinker lines on water usage, feed consumption and *Salmonella* retention in the crops and ceca of birds during multiple feed and water withdrawal schedules. Five experiments were performed that evaluated: *Salmonella* retention with the addition of CPC (Experiment 1), water and feed consumption with the addition of CPC at varying concentrations (Experiment 2), water and feed consumption with the addition of CPC and HP at varying concentrations and in combination (Experiment 3), *Salmonella* retention with the addition of HP at varying pH levels (Experiment 4), and *Salmonella* retention with the addition of HB and SB at varying pH levels (Experiment 5). For Experiment 1, drinking water usage in the control pens (0.331 L/bird/day) was significantly higher than in the 500 ppm CPC water treatment pens (0.046 L/bird/day), which had an effect on *Salmonella* retention. For Experiment 2, the water usage of broilers provided 100, 250, and 500 ppm CPC decreased 38, 62 and 72%, respectively, while feed consumption for the 250 and 500 ppm CPC treatments reduced 57 and 71%, respectively, compared to the controls. For Experiment 3, the drinking water usage for the 100 ppm CPC and 500 ppm CPC + 1% HP were significantly reduced, 48 and 96% respectively, compared to the controls. For Experiment 4, the number of *Salmonella* positive enriched crop samples was significantly lower for the 50 ppm HP + CA (pH=5.0) pens compared to the control. For Experiment 5, water treatments did not differ significantly in *Salmonella* recovery from the control for both crops (65% positive) and ceca (79% positive). From these experiments, CPC nor SB were determined to be effective *Salmonella* interventions when added to drinking water during feed and water withdrawal. 50 ppm HP + CA (pH=5.0) may be an effective *Salmonella*

intervention in bird crops when birds remain on the water treatment. Finally, the HP and SB did not significantly impact water usage and feed consumption of birds.

DESCRIPTION OF PROBLEM

Salmonella in the Poultry Industry

In the US, nontyphoidal *Salmonella* is estimated to cause 1.0 million foodborne illnesses per year and is the second leading cause of foodborne illnesses behind norovirus. Although it is the second leading cause of foodborne illness, *Salmonella* is estimated to be the leading cause of both foodborne hospitalizations (19,336/yr.) and deaths (378/yr.) in the US (Scallan et al., 2011). There is a wide variety of foods that can lead to human salmonellosis, and raw poultry products have been determined to be the most important transmission route (Mughini-Gras et al., 2014; Kimura et al., 2004). The focus on pathogen control in the poultry industry has primarily focused on the use of antimicrobials in processing plants, to minimize cross-contamination of *Salmonella* between carcasses and flocks by direct contact or contact with contaminated processing equipment (Rasschaert et al., 2008; USDA, 1996). Although raw poultry product pathogen reduction has traditionally been thought of as a role for processing plants, integrating pathogen reduction methods prior to harvesting broilers on the farm could be beneficial in decreasing the amount of pathogen contamination entering processing plants with the broilers (Arsenault et al., 2007; Gast, 2007). Recently, there has been more interest in expanding food safety measures to include poultry live production (i.e. grow-out) as well. The goal of preharvest pathogen reduction focuses on controlling the introduction, persistence, and transmission of pathogens, and common intervention methods include: vaccination of breeder flocks, litter management/treatment,

increased biosecurity, feed/heat treatment, and drinking water treatment (McKee, 2012; Gast, 2007).

Research has indicated that both the crop and ceca are important segments of the alimentary tract of broilers for *Salmonella* colonization (Hargis et al., 1995; Snoeyenbos et al., 1982). Previous research has also demonstrated that the level of *Salmonella* contamination in commercial chicken crops increases after birds were subjected to feed withdrawal for both laying hens and broilers (Corrier, D. E., 1999; Ramirez et al., 1997; Humphrey et al., 1993).

Feed and Water Withdrawal

Feed withdrawal is the total time that commercial broilers have available feed physically removed prior to slaughter (typically 8 to 12 hours). The primary purpose of feed withdrawal is to empty the alimentary tract of ingesta and digestive tract of fecal material, which then results in reduced fecal contamination in the processing plant (Smith, 2014; Northcutt and Buhr, 2010; Wabeck, 1972). The increase in *Salmonella* contamination during feed withdrawal, seen in previous research studies, is believed to the result of broilers' behavior to peck at contaminated floor litter after the feeders are raised (Corrier et al., 1999). Although, Buhr et al. (2018) reported significant increases in crop *Salmonella* recovery following a 12-hour feed withdrawal period for broilers that remained on pen litter, as well as broilers who were places into transport coops (which eliminated the potential for litter consumption). As well as pecking behavior, *Salmonella* contamination can increase because approximately 6 hours after feed withdrawal begins the crops are empty, and the lactic acid-producing bacteria population decreases in the crop because there are no longer fermentable carbohydrates that the bacteria require (Hinton et al., 2000a). Because lactic acid production decreases, the pH in the crops will increase, and as a result, the number of salmonellae and Enterobacteriaceae increases (Hinton et al., 2000a). Research

projects focused on preventing the increase of *Salmonella* colonization in the crop and ceca of broilers during feed withdrawal, and treatments have been conducted. Hinton et al. (2000b) determined that providing a glucose-based liquid cocktail during feed withdrawal will help maintain the lactic acid-producing bacteria population, therefore inhibiting the growth of *Salmonella*. Byrd et al. (2001) showed that the addition of 0.5% lactic acid to broiler drinking water during feed withdrawal significantly reduced S. enteritidis contamination in bird crops and on pre-chilled carcasses. However, extending the suppression of *Salmonella* in the alimentary tract of broilers after catching and transport to the processing plant (i.e., when not exposed directly to treatments) has not been realized.

Because there has been previous experimental success with drinking water treatments, the objective of this study was to evaluate the impact of adding antimicrobials to broiler drinking water on the retention of *Salmonella* typhimurium in the crops and ceca of broilers full fed, broilers subjected to 6 h off feed but remaining on water, or broilers 12 h off feed and 6 h off water.

MATERIALS AND METHODS

A series of 5 experiments were performed evaluating the effect of chemical interventions in drinking water on: 1. *Salmonella* retention in commercial broiler crops and ceca (Experiments 1, 4 and 5), and 2. broiler water usage and feed consumption rates (Experiments 2 and 3). Experiment 1 involved the use of CPC at 500 ppm with *Salmonella* challenged commercial broilers under varied water and feed withdrawal protocols. Experiment 2 involved monitoring commercial broiler water and feed consumption using CPC at multiple concentrations. Experiment 3 involved monitoring commercial broiler water and feed consumption using CPC and HP at multiple concentrations and in combination. Experiment 4 involved the use of HP at

50 ppm with multiple pH adjustments with *Salmonella* challenged commercial broilers under varied water and feed withdrawal protocols. Experiment 5 involved the use of SB and HP at 50 ppm with multiple pH adjustments with *Salmonella* challenged commercial broilers under varied water and feed withdrawal protocols.

Husbandry

For Experiment 1, 72 35-day-old, Cobb 500 broiler males were randomly placed into 6 pens (12 birds/pen). On day 1 of experiment, all broilers were orally inoculated with S. typhimurium and 3 days post-inoculation, water lines were disconnected from the house water supply and connected to 19 L carboys containing either drinking water (3 pens, control) or drinking water containing 500 ppm CPC (3 pens). The CPC concentration was confirmed via titration. After 2 days, the 6 pens were divided into 3 pairs, with each pair consisting of a control and CPC pen. Each pair of pens was subjected to one of the following withdrawal protocols: full fed (0 h off water; 0 h off feed), partial withdrawal (0 h off water; 6 h off feed), and full withdrawal (6 h off water; 12 h off feed). Carboy weights were measured both before and after the experiment to determine water usage. Prior to being subjected water/feed withdrawal schedules, broilers were provided feed and water ad libitum, with 24L:0D photoperiod.

For Experiment 2, 80 46-day-old, Cobb 500 broiler males were randomly placed into 8 pens (10 birds/pen). On day of placement, pens were connected to 19 L carboys to provide drinking water. One of four water treatments were provided to each group of 2 pens (reps): no chemical intervention (control), 100, 250, or 500 ppm of CPC. CPC concentrations were confirmed via titration. Broilers remained on water treatments for 96 h. Feeders and drinking water carboys were weighed at both the beginning and the end of the 4-day experiment to

determine water usage and feed consumption. For the entirety of the experiment, broilers were provided feed and water *ad libitum*.

In Experiment 3, 180 42-day-old, Cobb 500 broiler males were randomly placed into 15 pens (12 birds/pen). On day 1, pens were connected to 19 L carboys to provide drinking water. One of five water treatments were provided to each group of 3 pens (reps): no chemical intervention (control), 100 ppm CPC, 500 ppm CPC + 1% HP, 50 ppm HP, and 50 ppm HP adjusted to 5.0 pH with citric acid (CA) (HP + CA, pH=5.0). CPC concentrations were confirmed via titration. Broilers remained on water treatments for 96 h. Feeders and carboys were weighed at both the beginning and end of the 4-day experiment to determine water usage and feed consumption. For the entirety of the experiment, broilers were provided feed and water *ad libitum*. For Experiment 3, the flow rate (mL/min) was also measured for 1 nipple for 30 sec for each drinker line at the beginning of the experiment to determine the flow rates for lines connected to carboys rather than the house water supply.

For Experiment 4, 144 42-day-old, Cobb 500 broiler males were randomly placed in 12 pens (12 broiler/pen). On the day of placement, broilers were orally inoculated with S. typhimurium and 3 days post-inoculation, water lines were disconnected from the house water supply and connected to 19 L carboys, each containing one of four drinking water treatments with 3 reps: no chemical intervention (control), 50 ppm HP, 50 ppm HP adjusted to 5.0 pH with CA (HP + CA, pH=5.0), or 50 ppm HP adjusted to 6.2 pH with CA (HP + CA, pH=6.2). Similar to Experiment 1, after 2 days on the drinking water treatment, each of the four pens in each replication were subjected to one of three withdrawal schedules: full fed (0 h off water; 0 h off feed), partial withdrawal (0 h off water; 6 h off feed), and full withdrawal (6 h off water; 12 h off feed). Carboy and feeder weights were measure both before and after experiment to determine

water usage and feed consumption. Prior to being subjected water/feed withdrawal schedules, broilers were provided feed and water *ad libitum*.

For Experiment 5, 144 42-day-old, Cobb 500 broiler males were randomly placed in 12 pens (12 broiler/pen). Broilers were orally inoculated with S. typhimurium and 3 days post-inoculation, water lines were disconnected from the house water supply and connected to 19 L carboys, each containing one of four drinking water treatments with 3 reps: no chemical intervention (control), sodium bisulfate at an averaged 3.2 pH (SB), 50 ppm HP adjusted to 5.0 pH with CA (HP + CA, pH=5.0), or 50 ppm HP adjusted to 6.2 pH with CA (HP + CA, pH=6.2). Similar to Experient 4, after 2 days on the drinking water treatment, each of the four pens in each replication were subjected to one of three withdrawal schedules: full fed (0 h off water; 0 h off feed), partial withdrawal (0 h off water; 6 h off feed), and full withdrawal (6 h off water; 12 h off feed). Carboy and feeder weights were measure both before and after experiment to determine water usage and feed consumption. Prior to being subjected water/feed withdrawal schedules, broilers were provided feed and water *ad libitum*.

For all experiments the room temperature and ventilation were maintained according to the primary breeder guidelines. Broilers were fed with a standard grow-out diet. All pens were 20 ft2 to give a stocking density of 1.6ft2/bird (except Experiment 2, which had a stocking density of 2.0ft2/bird). Water lines used were also maintained according to the manufacturer's guidelines. The protocols followed for these experiments were approved by the University of Georgia Animal and Use Committees (A2015 04-029-Y3-A2).

Salmonella Challenge

For experiments 1, 4, and 5, all broilers were challenged with an oral inoculum consisting of 1.0 mL 10^8 nalidixic acid resistant S. typhimurium. For all challenges (i.e. Experiments 1, 4,

and 5), feeders were removed from pens for 4 hours both before and after the challenge to ensure colonization of *Salmonella* strain.

Sample Collection and Analysis

Crop and ceca samples were collected for Experiments 1, 4, and 5. After the withdrawal period, all broilers were euthanized individually by electrocution. For experiment 1, all broilers in each pen were sampled, while 6 broilers per pen were sampled for Experiments 4 and 5. After euthanasia, the feathers on each carcass were sprayed with 70% ethanol, the skin overlaying the crop at the base of the neck was cut and reflected, the esophagus clamped at the entrance and exit of the crop, the esophagus cut and the crop removed aseptically, and transferred to a sterile sample bag. For ceca removal, the skin covering the abdomen was reflected and the abdominal wall muscles on the right side of the carcass were incised to expose the duodenal loop. Beneath the duodenum, the ceca are located lying along the ileum and externalized through the abdominal opening and transferred to a sterile sample bag. All samples were placed on ice and transported to the laboratory for analysis.

Once transported to the lab, a random set of 5 samples of crops and ceca were weighed within plastic bags and the average of crops and ceca weights were calculated. Crops and ceca were first macerated with a rubber mallet within the sample bags to ensure that the luminal contents were exposed. To each sample bag, 1% buffered peptone water (BPW) was added at 3 times the g weight of the average crop or ceca. Crops and ceca with BPW were mixed using a stomacher for 1 min and then rinsate was streaked onto brilliant green sulfur agar plates with 200 µg/mL nalidixic acid added. All plates and samples were incubated at 37°C for 24 h.

If direct plating was negative for *Salmonella,* plates were restreaked from the enriched samples which had been incubated for 24 h and the plates were incubated again at 37°C for 24 h.

To confirm the presence of the marker *Salmonella* strain, representative suspect colonies were subjected to an agglutination test for the serogroup B. Samples that were positive from direct plating were estimated to have > 10^2 cells/mL and samples that were positive only following enrichment, were estimated to have < 10^2 cells/mL in the initial sample rinsate.

Statistical Analysis

All data were subjected to statistical analysis using SAS JMP Pro 13. For all *Salmonella* plate data, Fischer's Exact test or Chi Square test was used (Experiments 1, 4, and 5). Fisher's Exact test was used if sample size was less than 5, and Chi Square was used is sample size was greater than 5. For *Salmonella* data, the number of *Salmonella* positive plates for direct crop, enriched crop, direct ceca, and enriched ceca were compared between the control and antimicrobial water treatments using either Fischer's Exact test or Chi Square test (2x2 contingency table). Chi Square test was also used to compare the number of *Salmonella* positive plates for direct crop, enriched crop, direct ceca, and enriched ceca between withdrawal schedules (3x2 contingency table).

For all water usage and feed consumption data, data were converted to L/bird/day or kg/bird/day respectively. Data were also adjusted to account for the time off feed or water, depending on withdrawal schedule. For the water usage, feed consumption, and flow rate data, one-way ANOVA was used, and Tukey's HSD was used for means separation. For all experiments, differences in means were considered significant at $P \leq 0.05$.

RESULTS

Experiment 1

Salmonella recovery data for Experiment 1 are presented in Table 3.1, Table 3.2, and Table 3.3. Table 3.1 shows the direct and enriched *Salmonella* recovered for the crop and ceca

samples for birds not subjected to feed withdrawal. Comparing the number of *Salmonella* positive samples for the controls vs. the 500ppm CPC water treatments, there were no significant differences. The control and CPC treatments had the same number of positive samples for direct crops (75%), enriched crops (92%), direct ceca (33%), and enriched ceca (92%). Table 3.2 and Table 3.3 shows the *Salmonella* recovery from direct and enriched crop and ceca samples for partial withdrawal (0 h off water/6 off feed) and full withdrawal (6 h off water/ 12 h off feed) birds. There were no significant differences for partial withdrawal birds between the control and 500 ppm CPC treatment for enriched crop (100 vs. 100%), direct ceca (25 vs. 58%), and enriched ceca (92 vs. 92%). However, there was a significant difference for partial withdrawal between the number of *Salmonella* positive direct crops for the control (33%) vs. the 500 ppm CPC (83%) treatment (P=0.0104). There were also no significant differences for full withdrawal birds between the control and CPC treatment for direct crop (64 vs. 58%), enriched crop (92 vs. 91%), direct ceca (45 vs. 17%), and enriched ceca (100 vs. 75%). Comparing the withdrawal schedules, there were no significant differences comparing the no, partial, and full withdrawal protocols for *Salmonella* positive direct crops (61 to 75%), enriched crops (91 to 100%), direct ceca (31 to 41.5%), and enriched ceca (87.5 to 92%).

The water usage data for Experiment 1 is presented in Figure 3.1. The average water usage for the control pens (0.331 L/bird/day) was significantly higher than the water usage for the pens provided with the 500 ppm CPC water treatment (0.046 L/bird/day; P=0.0142). The 500 ppm CPC treatment pens water usage had an 86% reduction compared to the control pens.

Experiment 2

Average water usage data for Experiment 2 is presented in Figure 3.2. The average water usage for the control pens (0.299 L/bird/day) was significantly higher than the 100 ppm CPC

(0.184 L/bird/day), 250 ppm CPC (0.113 L/bird/day), and 500 ppm CPC (0.084 L/bird/day) pens (P=0.0052). The 100 ppm CPC, 250 ppm CPC, and 500 ppm CPC treatment pens water usage had a 39%, 62%, and 72% reduction compared to the control pens, respectively.

Average feed consumption data for Experiment 2 is presented in Figure 3.3. The average feed consumption for the control pens (0.201 kg/bird/day) was significantly higher than for the 250 ppm CPC (0.087 kg/bird/day) and 500 ppm CPC (0.059 kg/bird/day) pens (P=0.0121). However, the 100 ppm CPC (0.129 kg/bird/day) average feed consumption was not significantly different from the control. The 250 ppm CPC and 500 ppm CPC treatment pens feed consumption had a 57% and 71% reduction compared to the control pens, respectively.

Experiment 3

Average water usage data for Experiment 3 is presented in Figure 3.4. The average water usage for the control (0.360 L/bird/day), 50 ppm HP (0.319 L/bird/day), and 50 ppm HP + CA pens (pH=5.0; 0.359 L/bird/day) were significantly higher than the 100 ppm CPC (0.186 L/bird/day) and 500 ppm CPC + 1% HP (0.014 L/bird/day) pens, which were also significantly different from one-another (P<0.0001). The 100 ppm CPC and 500 ppm CPC + 1% HP treatment pens water usage had a 48% and 96% reduction compared to the control pens, respectively.

Average feed consumption data for Experiment 3 is presented in Figure 3.5. The average feed consumption for the control (0.194 kg/bird/day), 100 ppm CPC (0.152 kg/bird/day), 500 ppm CPC +1% HP (0.102 kg/bird/day), 50 ppm HP (0.203 kg/bird/day), and 50 ppm HP + CA pens (pH=5.0; 0.0.181 kg/bird/day) were not significantly different from one another.

Average drinker nipple flow rates (mL/min) for each treatment are presented in Figure 3.6. The average flow rates for the control (21.67 mL/min), 100 ppm CPC (23 mL/min), 500 ppm CPC + 1% HP (20 mL/min), 50 ppm HP (23 mL/min), and 50 ppm HP + CA (Ph=5.0;

26.67 mL/min) pen drinking water lines were not significantly different from one another. This indicated that flow rate from the carboys was not a significant factor.

Experiment 4

Salmonella recovery data for Experiment 4 are presented in Table 3.4, Table 3.5, and Table 3.6. Table 3.4 shows the direct and enricher *Salmonella* recovered for the crop and ceca samples for birds not subjected to feed withdrawal. Comparing the number of *Salmonella* positive samples for the control vs. 50 ppm HP, 50 ppm HP + CA (pH=5.0), or 50 ppm HP + CA (pH=6.2) water treatments, there were no significant differences for direct crop (0 to 33%), enriched crop (50 to 83%), direct ceca (0 to 67%), or enriched ceca (50 to 100%). Table 3.5 and Table 3.6 shows the *Salmonella* recovery from direct and enriched crop and ceca samples for partial withdrawal (0 h off water/6 off feed) and full withdrawal (6 h off water/ 12 h off feed) birds. There were no significant differences for partial withdrawal pens between the control, 50 ppm HP, 50 ppm HP + CA (pH=5.0), or 50 ppm HP + CA (pH=6.2) water treatments for direct crop (0 to 17%), direct ceca (0 to 17%), and enriched ceca (67 to 83%) samples. However, there was a significant difference for partial withdrawal between the number of *Salmonella* positive enriched crop samples for the control (100%) vs. 50 ppm HP + CA (pH=5.0) water treatment (17%; P=0.0033). Comparing the number of *Salmonella* positive plates for full withdrawal birds between the control vs. 50 ppm HP, 50 ppm HP + CA (pH=5.0), or 50 ppm HP + CA (pH=6.2) water treatments, there were no significant differences for direct crop (17 to 50%), enriched crop (50 to 100%), direct ceca (0 to 50%), or enriched ceca (50 to 100%). Comparing the withdrawal schedules, there were no significant differences comparing the no, partial, and full withdrawal times for *Salmonella* positive direct crops (8 to 33%), enriched crops (63 to 79%), direct ceca (4 to 29%), and enriched ceca (71 to 83%).

The drinking water usage data for Experiment 4 is presented in Figure 3.7. The average water usage for the control (0.394 L/bird/day), 50 ppm HP (0.468 L/bird/day), 50 ppm HP + CA (pH=5.0; 0.431 L/bird/day), and 50 ppm HP + CA (pH=6.2; 0.406 L/bird/day) pens were not significantly different from one another.

Average feed consumption data for Experiment 3 is presented in Figure 3.8. The feed consumption for the control (0.206 kg/bird/day), 50 ppm HP (0.217 L/bird/day), 50 ppm HP + CA (pH=5.0; 0.205 L/bird/day), and 50 ppm HP + CA (pH=6.2; 0.210 L/bird/day) pens were not significantly different from one another.

Experiment 5

Salmonella recovery data for Experiment 5 are presented in Table 3.7, Table 3.8, and Table 3.9. Table 3.7 shows the direct and enriched *Salmonella* recovered for the crop and ceca samples for birds not subjected to feed withdrawal. Comparing the number of *Salmonella* positive samples for control vs. SB, 50 ppm HP + CA (pH=5.0), or 50 ppm HP + CA (pH=6.2) water treatments, there were no significant differences for direct crop (0 to 50%), enriched crop (17 to 50%), direct ceca (50 to 83%), or enriched ceca (83 to 100%). Table 3.8 and Table 3.9 shows the *Salmonella* recovery from direct and enriched crop and ceca samples for partial withdrawal (0 h off water/6 off feed) and full withdrawal (6 h off water/ 12 h off feed) birds. There were no significant differences for partial withdrawal birds between the control vs. SB, 50 ppm HP + CA (pH=5.0), or 50 ppm HP + CA (pH=6.2) water treatments for direct crop (17 to 50%), enriched crop (67 to 100%), direct ceca (0 to 83%), or enriched ceca (67 to 100%) samples. Comparing the number of *Salmonella* positive plates for full withdrawal pens between the control vs. SB, 50 ppm HP + CA (pH=5.0), or 50 ppm HP + CA (pH=6.2) water treatments, there were no significant differences for direct crop (17 to 50%), enriched crop (67 to 100%),

direct ceca (33 to 67%), or enriched ceca (50 to 100%) samples. Comparing the withdrawal schedules, there were no significant differences comparing the no, partial, and full withdrawal times for *Salmonella* positive direct crops (21 to 33%), direct ceca (42 to 67%), and enriched ceca (75 to 83%). Compared to the full fed birds (33%), the number of *Salmonella* positive enriched crop plates was significantly higher for both the partial withdrawal (83%) pens and the full withdrawal pens (79%; P=0.0003).

The water usage data for Experiment 5 is presented in Figure 3.9. The average water usage for the control (0.313 L/bird/day), sodium bisulfate (0.334 L/bird/day), 50 ppm HP + CA (pH=5.0; 0.312 L/bird/day), and 50 ppm HP + CA (pH=6.2; 0.362 L/bird/day) pens were not significantly different from one another.

Average feed consumption data for Experiment 5 is presented in Figure 3.10. The feed consumption for the control (0.179 kg/bird/day), sodium bisulfate (0.173 L/bird/day), 50 ppm HP + CA (pH=5.0; 0.172 L/bird/day), and 50 ppm HP + CA (pH=6.2; 0.190 L/bird/day) pens were not significantly different from one another.

DISCUSSION

For Experiment 1, a significant difference between the control and 500 ppm CPC water treatment was only observed for the direct crop samples after the partial withdrawal schedule (0 h off water, 6 h off feed). In that case, the number of *Salmonella* positive crops was higher for the 500 ppm CPC treatment compared to the control. This may be due to a combination of the antimicrobial treatment effect on water usage, as well as the environment in the crop during the withdrawal. Although feed consumption was not measured for Experiment 1, Experiment 2 results and previous research indicates that there is a close relationship between feed and water consumption. Therefore, an assumption can be made that the birds in the 500 ppm CPC pens

likely had depressed feed consumption in combination with the decreased water usage (Dozier et al., 2002) due to the possible negative effect on drinking water palatability. The 500 ppm CPC treatment also may not have been effective in reducing *Salmonella* because the crops emptied more quickly due to reduced feed consumption, resulting in the lactic acid-producing bacteria population decreasing and improving the environment for pathogenic bacteria like *Salmonella* (Hinton et al., 2000a). In Experiment 4, there was also a significant reduction of *Salmonella* positive enriched crop samples for the 50 ppm HP + CA (pH=5.0). This significant difference occurred with pens that were subjected to the partial withdrawal schedule (0 h off water, 6 h off feed), so the birds in these pens would have had access to the water treatment until being euthanized for sample collection. Because this water treatment had a pH of 5.0, the 50 ppm HP + CA (pH=5.0) treatment may have reduced the impact of the changing crop environment during feed withdrawal (Hinton et al., 2000a; Hinton et al., 1991). Therefore, when having access to the 500 ppm HP + CA (pH=5.0), this antimicrobial treatment may be effective for *Salmonella* reduction in crops.

Results from Experiment 5 indicated that the number of *Salmonella* positive crop and ceca samples was higher in pens that were subjected to partial or full feed withdrawal compared to no feed withdrawal. Previous research has also determined that post-feed withdrawal had increased incidence of *Salmonella*-positive crop samples, although the feed withdrawal protocols varied between studies (Corrier et al., 1999; Ramirez et al., 1997). As previously mentioned, the increase in *Salmonella* prevalence may be caused by the changing crop environment, as well as the increased pecking behavior of contaminated litter by broilers during the time that feed is removed from grow-out houses (Hinton et al., 2000a; Corrier et al., 1999).

Finally, no significant differences were found for both the direct or enriched ceca samples for any of the antimicrobial water treatments. Prior research has also indicated that although the ceca are important area of the alimentary tract for *Salmonella* colonization, there are inconsistent results on the impact of feed withdrawal on *Salmonella* prevalence in the ceca (Corrier et al., 1999; Ramirez et al., 1997; Snoeyenbos et al., 1982). As compared to the 6 hours feed withdrawal period it takes for the crop to empty of feed, research has shown that the lower alimentary tract does not fully empty even after a 16-hour feed withdrawal period (Hinton et al., 1999; Summers and Leeson, 1979). Hinton et al. (2000a) showed that cecal weights did not significantly decrease during feed withdrawal periods lasting up to 24 hours. Hinton et al. (2000a) also suggested that feed withdrawal does not have as large of an impact on the ceca of birds compared to the crops. Therefore, it may be unlikely that antimicrobial water treatments will have an impact on the ceca and impact *Salmonella* reduction

CONCLUSIONS AND APPLICATIONS

1. CPC, at any concentration, was not an effective antimicrobial water treatment during feed and water withdrawal. This may be due to decreased drinking water usage due to decreased palatability.
2. HP in combination with CA (pH=5.0) may be effective as an antimicrobial water treatment for crop *Salmonella* reduction. Although this effect was only seen with a partial withdrawal schedule (0 h off water, 6 h off feed).
3. A sodium bisulfate drinking water treatment also did not have an impact on *Salmonella* prevalence in the crops or ceca.
4. HP and SB water treatments did not have a significant impact on the water usage or feed consumption.

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Table 3.1. Number of *Salmonella* positive crops or ceca in market-age commercial Cobb 500 male broilers (n=24) provided control or 500 ppm CPC[1] drinking water treatments for 48 hours and not subjected to feed or water withdrawal, Experiment 1.

Treatment		Birds sampled	Direct + crop	Enrich + crop	Direct + ceca	Enrich + ceca
Control	**Total:**	12	9/12	11/12	4/12	11/12
	% +		75%	92%	33%	92%
CPC	**Total:**	12	9/12	11/12	4/12	11/12
	% +		75%	92%	33%	92%
Mean	**% +**		**75.0%**	**92.0%**	**33.0%**	**92.0%**

[1]CPC= cetylpyridinium chloride.

Table 3.2. Number of *Salmonella* positive crops or ceca in market-age commercial Cobb 500 male broilers (n=23) provided control or 500 ppm CPC[1] drinking water treatments for 42 hours and subjected to a 12 h feed withdrawal/6 h water withdrawal Experiment 1.

Treatment		Birds sampled	Direct + crop	Enrich + crop	Direct + ceca	Enrich + ceca
Control	**Total:**	11	7/11	10/11	5/11	11/11
	% +		64%	91%	45%	100%
CPC	**Total:**	12	7/12	11/12	2/12	9/12
	% +		58%	92%	17%	75%
Mean	**% +**		**61.0%**	**91.5%**	**31.0%**	**87.5%**

[1]CPC= cetylpyridinium chloride.

Table 3.3. Number of *Salmonella* positive crops or ceca in market-age commercial Cobb 500 male broilers (n=24) provided control or 500 ppm CPC[1] drinking water treatments for 48 hours and subjected to a 6 h feed, Experiment 1.

Treatment		Birds sampled	Direct + crop	Enrich + crop	Direct + ceca	Enrich + ceca
Control	**Total:**	12	4/12[B]	12/12	3/12	11/12
	% +		33%	100%	25%	92%
CPC	**Total:**	12	10/12[A]	12/12	7/12	11/12
	% +		83%	100%	58%	92%
Mean	**% +**			**100.0%**	**41.5%**	**92.0%**

[1]CPC= cetylpyridinium chloride.

[A, B] –different superscripts within a column indicate statistically significant differences between treatments ($P<0.05$).

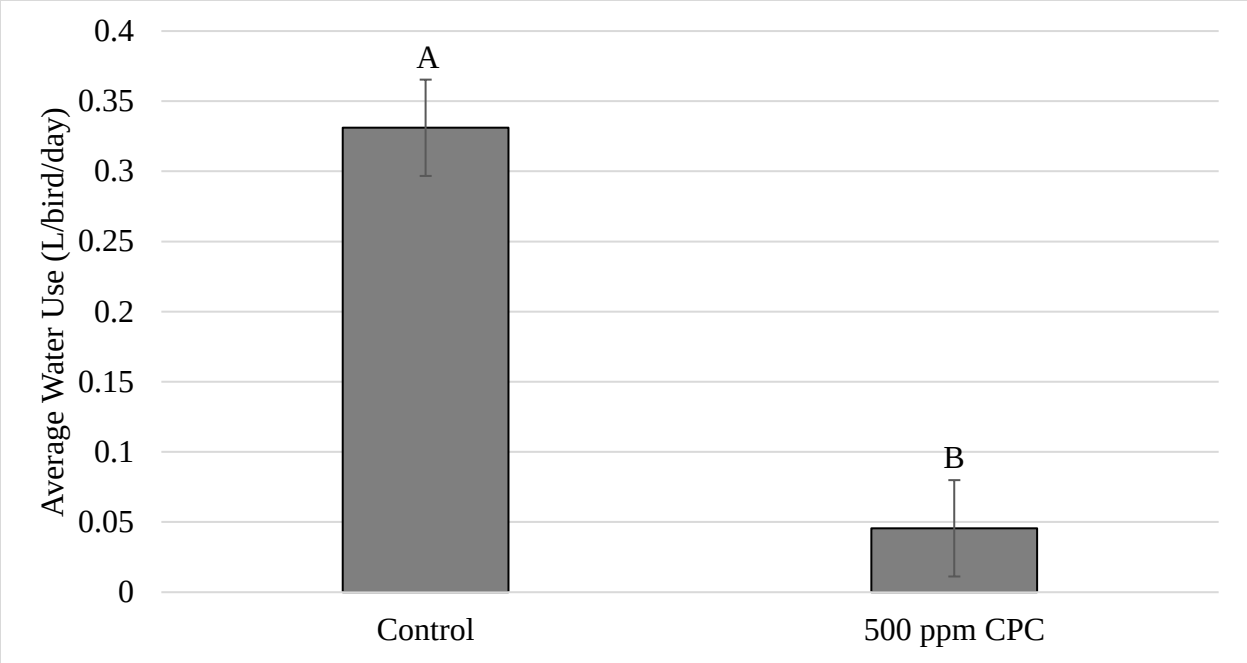

Figure 3.1. Average water use (L/bird/day) of market-age commercial Cobb 500 male broilers (n=72) in a 4-day period between pens provided control and 500 ppm CPC[1] drinking water treatments in 19 L carboys (12 birds/pen), Experiment 1.

[1]CPC= cetylpyridinium chloride.

[A, B] –different superscripts within a column indicate statistically significant differences between treatments (*P<0.05*).

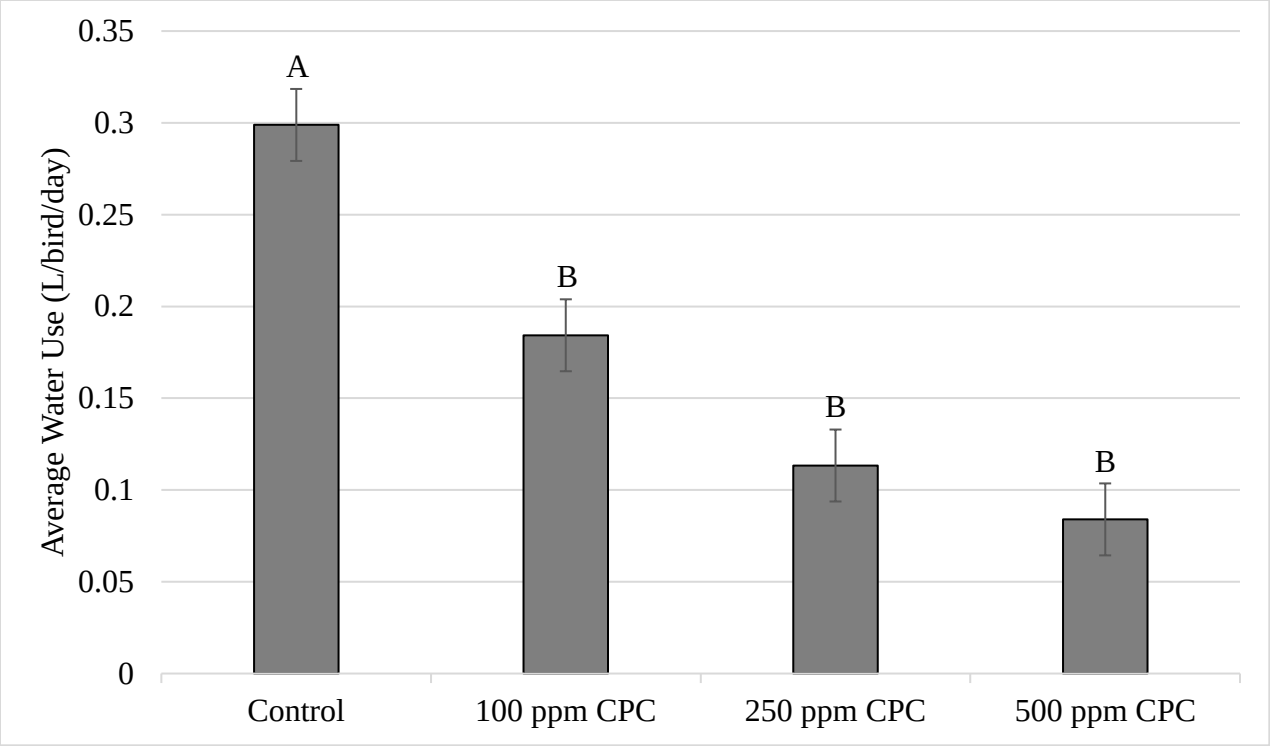

Figure 3.2. Average water use (L/bird/day) of market-age commercial Cobb 500 male broilers (n=80) in a 4-day period between pens provided control, 100 ppm, 250 ppm, or 500 ppm CPC[1] drinking water treatments in 19 L carboys (10 birds/pen), Experiment 2.

[1]CPC= cetylpyridinium chloride.

[A, B] –different superscripts within a column indicate statistically significant differences between treatments (*P<0.05*).

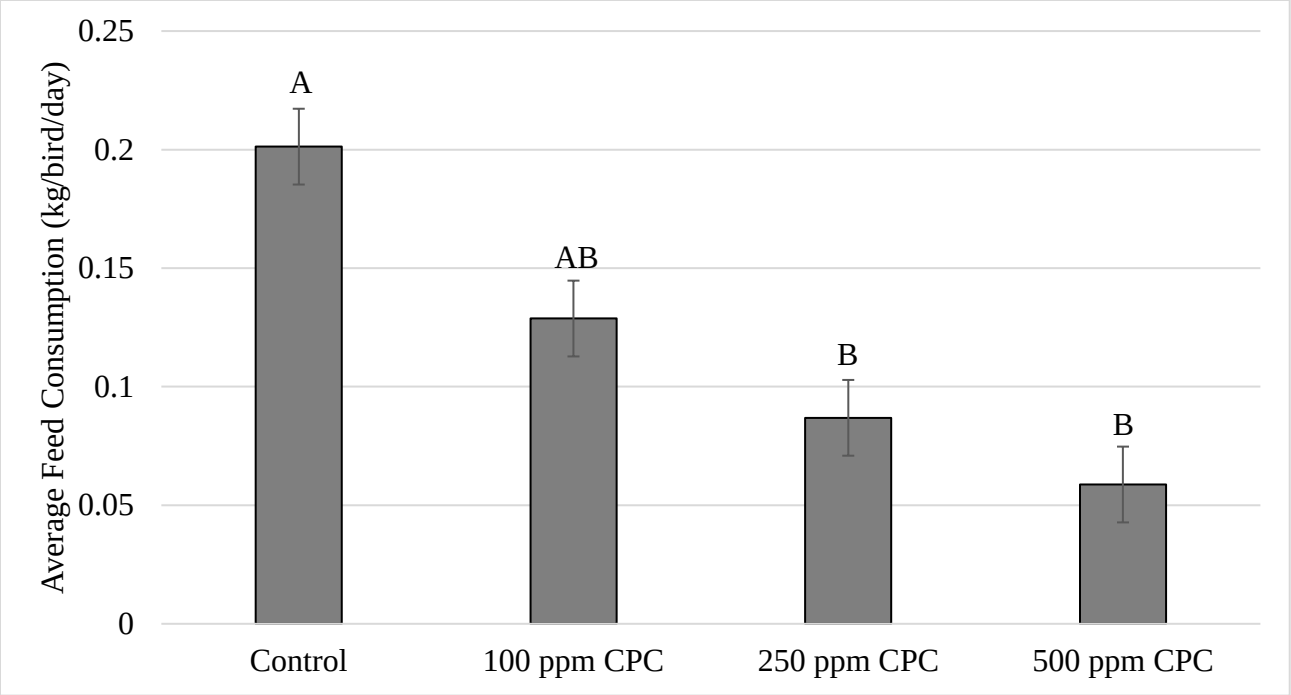

Figure 3.3. Average feed consumption (kg/bird/day) of market-age commercial Cobb 500 male broilers (n=80) in a 4-day period between pens provided control, 100 ppm, 250 ppm, or 500 ppm CPC[1] drinking water treatments in 19 L carboys (10 birds/pen), Experiment 2.

[1]CPC= cetylpyridinium chloride.

[A, B] –different superscripts within a column indicate statistically significant differences between treatments ($P<0.05$).

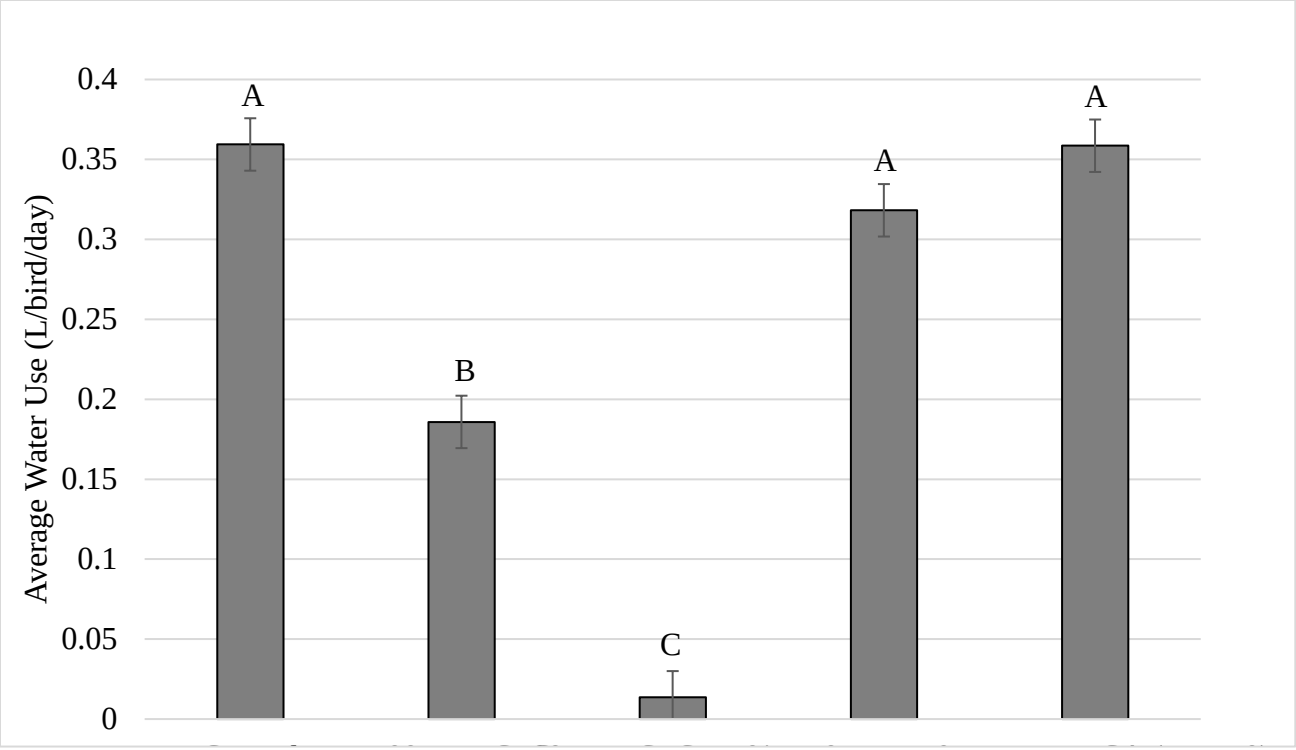

Figure 3.4. Average water use (L/bird/day) of market-age commercial Cobb 500 male broilers (n=180) in a 4-day period between pens provided control, 100 ppm CPC[1], 500 ppm CPC + 1% HP[2], 50 ppm HP, or 50 ppm HP + CP (pH=5.0) drinking water treatments in 19 L carboys (12 birds/pen), Experiment 3.

[1]CPC= cetylpyridinium chloride.

[2]HP= hydrogen peroxide.

[3]CA= citric acid.

[A, B] –different superscripts within a column indicate statistically significant differences between treatments (*P<0.05*).

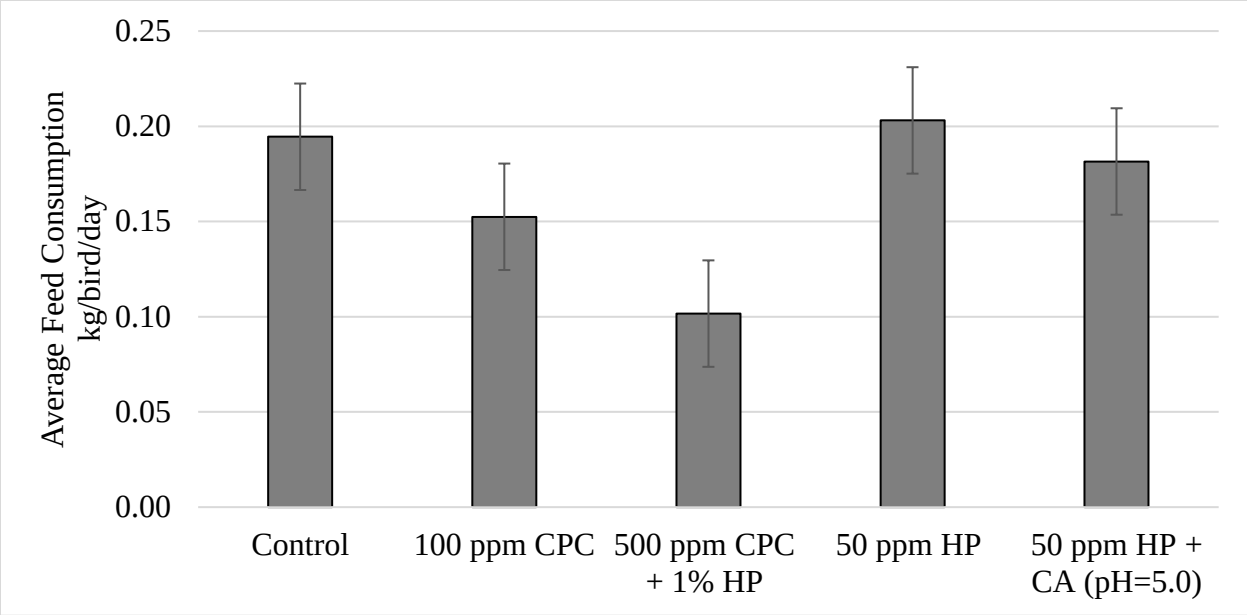

Figure 3.5. Average feed consumption (kg/bird/day) of market-age commercial Cobb 500 male broilers (n=180) in a 4-day period between pens provided control, 100 ppm CPC[1], 500 ppm CPC + 1% HP[2], 50 ppm HP, or 50 ppm HP + CP (pH=5.0) drinking water treatments in 19 L carboys (12 birds/pen), Experiment 3.

[1]CPC= cetylpyridinium chloride.

[2]HP= hydrogen peroxide.

[3]CA= citric acid.

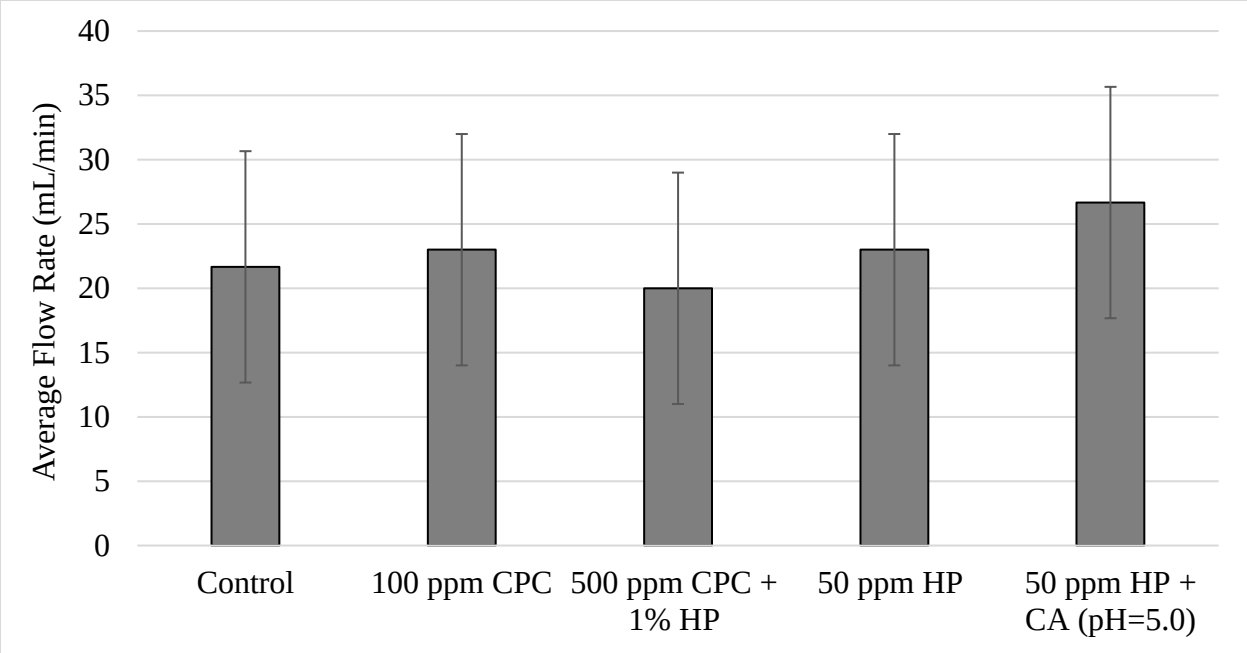

Figure 3.6. Average flow rate (mL/min) of nipple drinker water lines in 20ft^2 pens provided control, 100 ppm CPC[1], 500 ppm CPC + 1% HP, 50 ppm HP[2], or 50 ppm HP + CA[3] (pH=5.0) drinking water treatments in 19 L carboys, Experiment 3.

[1]CPC= cetylpyridinium chloride.

[2]HP= hydrogen peroxide.

[3]CA= citric acid.

Table 3.4. Number of *Salmonella* positive crops and ceca in market-age commercial Cobb 500 male broilers (n=24) provided control, 50 ppm HP[1], 50 ppm HP + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments for 48 hours and subjected to no feed/water withdrawal, Experiment 4.

Treatment		Birds sampled	Direct + crop	Enrich + crop	Direct + ceca	Enrich + ceca
Control	**Total:**	6	2/6	5/6	1/6	5/6
	% +		33%	83%	17%	83%
50 ppm HP	**Total:**	6	1/6	4/6	4/6	6/6
	% +		17%	67%	67%	100%
50 ppm HP + CA (pH=5.0)	**Total:**	6	0/6	3/6	0/6	3/6
	% +		0%	50%	0%	50%
50 ppm HP + CA (pH=6.2)	**Total:**	6	0/6	3/6	1/6	3/6
	% +		0%	50%	17%	50%
Mean	**% +**		**12.5%**	**62.5%**	**25.0%**	**71.0%**

[1]HP= hydrogen peroxide.

[2]CA= citric acid.

Table 3.5. Number of *Salmonella* positive crops and ceca in market-age commercial Cobb 500 male broilers (n=24) provided control, 50 ppm HP[1], 50 ppm HP + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments for 42 hours following 12-hour feed withdrawal/6-hour water withdrawal, Experiment 4.

Treatment		Birds sampled	Direct + crop	Enrich + crop	Direct + ceca	Enrich + ceca
Control	**Total:**	6	3/6	6/6	5/6	6/6
	% +		50%	100%	83%	100%
50 ppm HP	**Total:**	6	3/6	5/6	0/6	5/6
	% +		50%	83%	0%	83%
50 ppm HP + CA (pH=5.0)	**Total:**	6	1/6	5/6	2/6	4/6
	% +		17%	83.3%	33%	67%
50 ppm HP + CA (pH=6.2)	**Total:**	6	1/6	3/6	0/6	5/6
	% +		17%	50%	0%	83%
Mean	**% +**		**33.3%**	**79.2%**	**29.2%**	**83.3%**

[1]HP= hydrogen peroxide.

[2]CA= citric acid.

Table 3.6. Number of *Salmonella* positive crops and ceca in market-age commercial Cobb 500 male broilers (n=24) provided control, 50 ppm HP[1], 50 ppm HP + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments for 48 hours following 6-hour feed withdrawal, Experiment 4.

Treatment		Birds sampled	Direct + crop	Enrich + crop	Direct + ceca	Enrich + ceca
Control	**Total:**	6	1/6	6/6[A]	0/6	5/6
	% +		17%	100%	0%	83%
50 ppm HP	**Total:**	6	1/6	6/6[A]	1/6	5/6
	% +		17%	100%	16.6%	83%
50 ppm HP + CA (pH=5.0)	**Total:**	6	0/6	1/6[B]	0/6	5/6
	% +		0%	17%	0%	83%
50 ppm HP + CA (pH=6.2)	**Total:**	6	0/6	4/6[AB]	0/6	4/6
	% +		0%	67%	0%	67%
Mean	**% +**		**12.5%**		**4.2%**	**79.1%**

[1]HP= hydrogen peroxide.

[2]CA= citric acid.

[A, B] –different superscripts within a column indicate statistically significant differences between treatments (*P<0.05*).

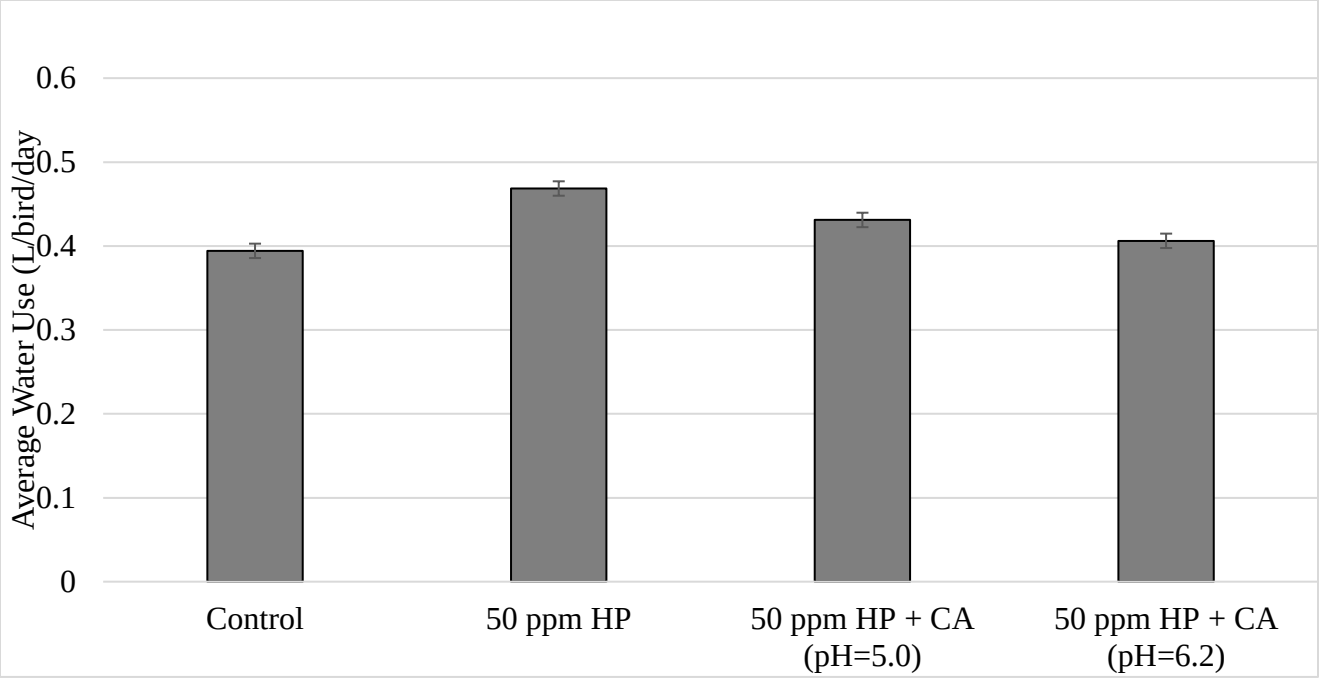

Figure 3.7. Average water use (L/bird/day) of market-age commercial Cobb 500 male broilers (n=144) in a 2-day period between pens provided control, 50 ppm HP[1], 50 ppm HP + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments in 19 L carboys (12 birds/pen), Experiment 4.

[1]HP= hydrogen peroxide.

[2]CA= citric acid.

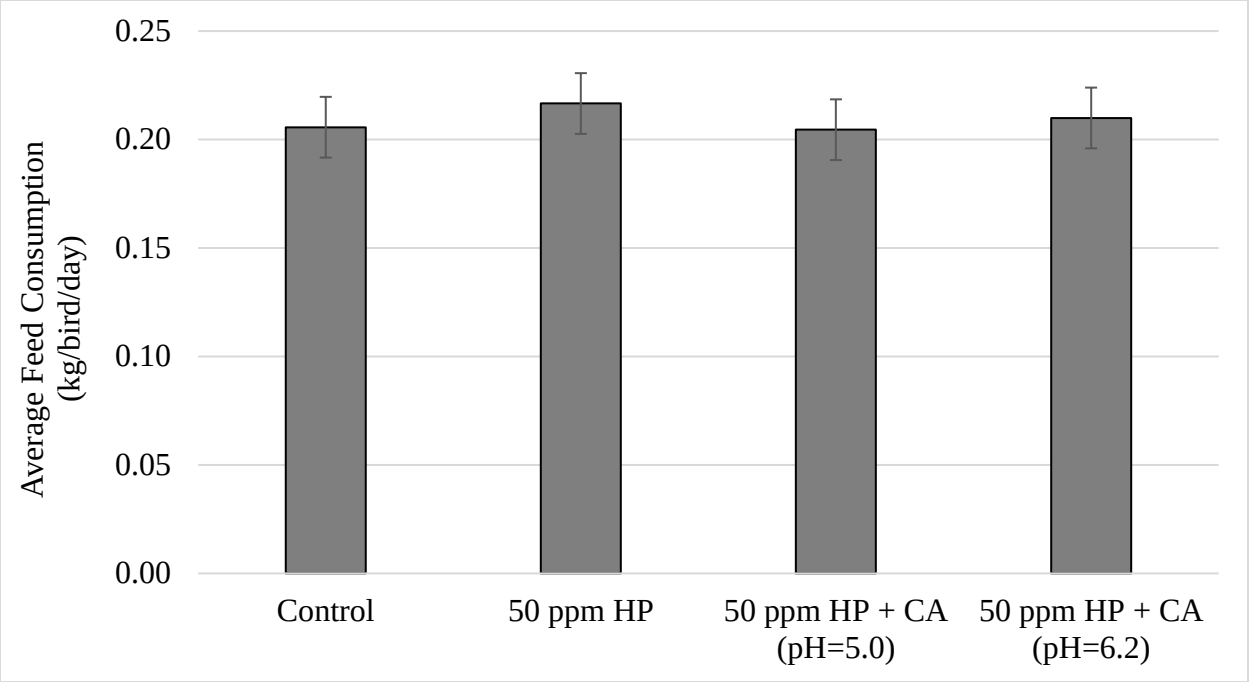

Figure 3.8. Average feed consumption (kg/bird/day) of market-age commercial Cobb 500 male broilers (n=144) in a 2-day period between pens provided control, 50 ppm HP[1], 50 ppm HP + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments in 19 L carboys (12 bird/pen), Experiment 4.

[1]HP= hydrogen peroxide.

[2]CA= citric acid.

Table 3.7. Number of Salmonella positive crops and ceca in market-age commercial Cobb 500 market age broilers (n=24) provided control, sodium bisulfate, 50 ppm HP[1] + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments for 48 hours and subjected no feed/water withdrawal, Experiment 5.

Treatment		Birds sampled	Direct + crop	Enrich + crop	Direct + ceca	Enrich + ceca
Control	**Total:**	6	0/6	1/6	5/6	6/6
	% +		0%	17%	83%	100%
Sodium Bisulfate	**Total:**	6	0/6	2/6	3/6	6/6
	% +		0%	33%	50%	100%
50 ppm HP + CA (pH=5.0)	**Total:**	6	3/6	3/6	4/6	5/6
	% +		50%	50%	67%	83%
50 ppm HP + CA (pH=6.2)	**Total:**	6	2/6	2/6	4/6	5/6
	% +		33%	33%	67%	83%
Mean	**% +**		**20.8%**	**33.3%**	**66.7%**	**91.7%**

[1]HP= hydrogen peroxide.

[2]CA= citric acid

Table 3.8. Number of Salmonella positive crops and ceca in market-age commercial Cobb 500 market age broilers (n=24) provided control, sodium bisulfate, 50 ppm HP[1] + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments for 42 hours following a 12-hour feed withdrawal/ 6-hour water withdrawal, Experiment 5.

Treatment		Birds sampled	Direct + crop	Enrich + crop	Direct + ceca	Enrich + ceca
Control	**Total:**	6	3/6	6/6	3/6	6/6
	% +		50%	100%	50%	100%
Sodium Bisulfate	**Total:**	6	1/6	4/6	3/6	4/6
	% +		17%	67%	50%	67%
50 ppm HP + CA (pH=5.0)	**Total:**	6	1/6	5/6	4/6	5/6
	% +		17%	83%	67%	83%
50 ppm HP + CA (pH=6.2)	**Total:**	6	1/6	4/6	2/6	3/6
	% +		17%	67%	33%	50%
Mean	**% +**		**25.0%**	**79.2%**	**50.0%**	**75.0%**

[1]HP= hydrogen peroxide.

[2]CA= citric acid

Table 3.9. Number of Salmonella positive crops and ceca in market-age commercial Cobb 500 male broilers (n=24) provided control, sodium bisulfate, 50 ppm HP[1] + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments for 48 hours following a 6-hour water withdrawal, Experiment 5.

Treatment		Birds sampled	Direct + crop	Enrich + crop	Direct + ceca	Enrich + ceca
Control	**Total:**	6	1/6	4/6	2/6	4/6
	% +		17%	67%	33%	67%
Sodium Bisulfate	**Total:**	6	1/6	5/6	3/6	6/6
	% +		17%	83%	50%	100%
50 ppm HP + CA (pH=5.0)	**Total:**	6	3/6	5/6	5/6	5/6
	% +		50%	83%	83%	83%
50 ppm HP + CA (pH=6.2)	**Total:**	6	3/6	6/6	0/6	4/6
	% +		50%	100%	0%	67%
Mean	**% +**		**33.3%**	**83.3%**	**41.7%**	**79.2%**

[1]HP= hydrogen peroxide.

[2]CA= citric acid

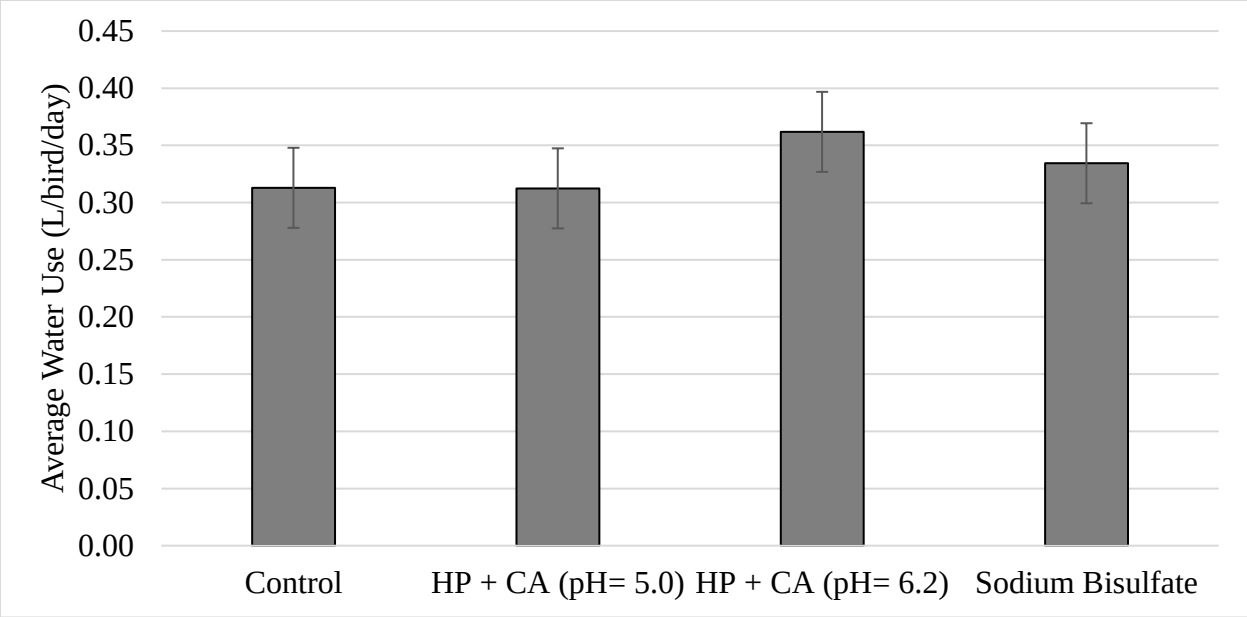

Figure 3.9. Average water use (L/bird/day) of market-age commercial Cobb 500 male broilers (n=144) in a 2-day period between pens provided control, sodium bisulfate, 50 ppm HP[1] + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments in 19 L carboys (12 bird/pen), Experiment 5.

[1]HP= hydrogen peroxide.

[2]CA= citric acid.

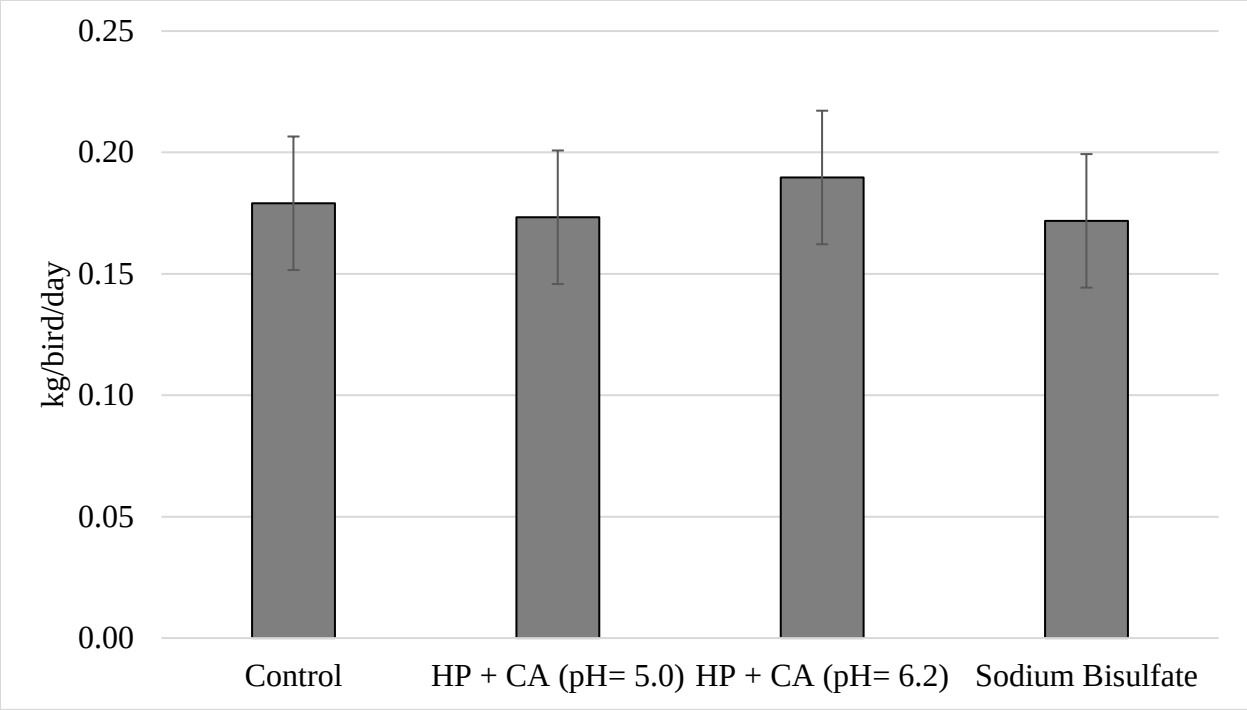

Figure 3.10. Average feed consumption (kg/bird/day) of market-age commercial Cobb 500 male broilers (n=144) in a 2-day period between pens provided control, sodium bisulfate, 50 ppm HP[1] + CA[2] (pH=5.0), or 50 ppm HP + CA (pH=6.2) drinking water treatments in 19 L carboys (12 bird/pen), Experiment 5.

[1]HP= hydrogen peroxide.

[2]CA= citric acid.

CHAPTER 4

SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

SUMMARY AND CONCLUSIONS

Chapter 2

With an average of approximately 9 billion birds processed in 2017, the annual water use in the US for poultry processing is around 234 billion L (Northcutt and Jones, 2004; USDA-ERS, 2017). The combination of potable water used and by-product waste generated from the different operation areas combines to form the poultry processing wastewater stream (PPW), and typically has relatively high concentrations of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), nitrogen, and phosphorus (USEPA, 2002). Although there is typically a pre-treatment process for the PPW prior to discharge to local wastewater treatment facilities, PPW typically still has high concentrations of these contaminants, resulting in surcharges for wastewater treatment at municipal treatment facilities (Garcia et al., 2016). The concentrations of PPW stream contaminants have been previously documented (Singh et al., 1973; Hamm, 1972; Ralph, 1950), and there have been few other studies using accurate water volumes to calculate the mass of PPW contaminants (g/kg^{lwt}; Plumber et al., 2012). Therefore, the purpose of Chapter 2 was to determine the impact of scalding procedures on load (g/kg^{lwt}) of contaminants exiting scalder tanks, rather than simply a measure of the PPW pollutant concentrations.

For this chapter, effect of traditional hard vs. soft scalding protocols (Experiment 1), scalder immersion time and scalder temperature factors analyzed independently (Experiment 2),

and the effect of residual blood post-bleeding (Experiment 3) on scalder PPW loading was evaluated. For all 3 experiments, commercial broilers were collected from a local processing facility and the same triple-pass, 3 scalder tank set-up was utilized. For all 3 experiments, the collected water samples were analyzed for concentrations of COD, total solids (TS), and TSS.

From this research 3 conclusions were made. The first being that soft scalding tends to produce PPW with higher loads on contaminants compared to hard scalding for all 3 PPW analytics (e.g., 1.753 vs. 1.509 g/kg^{lwt} TS). From Experiment 2, it was determined that although not statistically significant, the protocols utilizing the longer immersion time (120 s) tended to have higher loading values compared to the short time (90 s). Although several researchers have previously thought that hard scalding would produce significantly higher contaminated PPW (Nunes, 2011; Russell, 2007), both the current study and Plumber et al. (2012) found that soft scalding produced PPW with higher loading values. Second, in all three experiments, it was determined that Tank 1 has significantly higher loading compared to Tanks 2 and 3, or all 3 tanks were significantly different in loading values. Although it is standard to have multiple scalding tanks in a commercial processing plant, these results further showed the positive impact of having a multi-tank scalding system to clean the carcasses prior to being defeathered (Smith, 2014). Finally, the last conclusion from this study was that there was no difference in loading between the non-bled and bled protocols. Although there were no significant differences in this study, Plumber et al. (2012) determined that carcasses bled for 60 s resulted in higher scalder PPW loading compared to 120 s bleed time. Therefore, there may be a significant difference in loading when a shorter bleed time is utilized, or on a commercial scale.

Chapter 3

In the US, *Salmonella* is the second leading cause of foodborne illnesses and the main cause of foodborne hospitalizations and deaths (Scallan et al., 2011). Traditionally, foodborne pathogen control in the poultry industry has mainly been a role for processing plants, although integrating pathogen reduction methods prior to harvesting broilers on the farm could be beneficial in decreasing the amount of pathogen contamination entering the processing plants (Arsenault et al., 2007; Gast, 2007). Previous research has found that the crop and ceca of broilers are important segments for colonization of *Salmonella*, and that the process of feed withdrawing has shown to increase the amount of *Salmonella* contamination (Corrier, D. E., 1999; Ramirez et al., 1997; Hargis et al., 1995; Humphrey et al., 1993; Snoeyenbos et al., 1982).

Chapter 3 describes a study consisting of 5 experiments performed, which evaluated: *Salmonella* retention with the addition of cetylpyridinium chloride (CPC) to broiler drinking water (Experiment 1), water and feed consumption rates with the addition of CPC to broiler drinking water at varying concentrations (Experiment 2), water and feed consumption rates with the addition of CPC and hydrogen peroxide (HP) to broiler drinking water at varying concentrations and in combination (Experiment 3), *Salmonella* retention with the addition of HP to broiler drinking water at pH of 5.0 or 6.2 (Experiment 4), and *Salmonella* retention with the addition of HB and sodium bisulfate (SB) to broiler drinking water at varying pH levels (Experiment 5). For all experiments, market-age Cobb 500 broiler males were used and for Experiments 1, 4, and 5, broilers were subjected to one of three feed withdrawal schedules prior to sample collection: full withdrawal (12 hours off feed/6 hours off water), partial withdrawal (6 hours off feed/0 hours off water), or full fed (no feed/water withdrawal).

From the experiments described in Chapter 3, 4 conclusions were made. The first conclusion was that 500 ppm CPC was not effective in reducing *Salmonella* contamination either in the crop of ceca of birds. The only significant difference in Experiment 1 for partial withdrawal was between the number of *Salmonella* positive direct crops for the control (33%) vs. the 500 ppm CPC (83%) treatment (P=0.0104). In Experiment 1, 500 ppm CPC had a negative impact on water usage, decreasing from 0.331 L/bird/day to 0.046 L/bird/day compared to the control. Experiment 2 also found that all CPC concentrations tested (100, 250, and 500 ppm), significantly reduced water usage and feed consumption, therefore further indicating that CPC alone will not be an effective antimicrobial drinking water treatment. The second conclusion was that none of the HP alone, HP + CA (pH=5.0 or 6.2), or SB drinking water treatments impacted water usage and feed consumption rates as compared to the controls in Experiments 3, 4, and 5. While it was determined that these drinking water treatments did not impact water usage and feed consumption, the third conclusion of the study was that these treatments were also not effective in reducing *Salmonella* contamination. There was a significant difference in Experiment 4 between the number of *Salmonella* positive enriched crop samples for the control (100%) vs. 50 ppm HP + CA (pH=5.0) water treatment (17%; P=0.0033). Although, this significant difference was seen for the partial feed withdrawal birds, while the birds still have access to drinking water treatments. The fourth conclusion was that feed and water withdrawal impacts the *Salmonella* contamination in crops. Compared to the full fed birds (33%) in Experiment 5, the number of *Salmonella* positive crops was significantly higher for both the partial withdrawal (83%) pens and the full withdrawal pens (79%; P=0.0003). Prior research has also found that, compared to the crops, the impact of feed withdrawal on the ceca is inconsistent, likely because it takes much longer to empty the lower alimentary tract compared to the crop

(Corrier et al., 1999; Hinton et al., 1999; Ramirez et al., 1997; Snoeyenbos et al., 1982; Summers and Leeson, 1979)

FUTURE DIRECTIONS

Based on the work described in Chapter 2, there are several points of interest for future experiments. As previously stated, there have been few studies evaluating the contaminants in PPW in terms of mass loadings (g/kg^{lwt}), rather than simples concentrations (e.g., mg/L). These studies have also focused specifically on scalding operations. Therefore, further research on evaluating the PPW contamination from other operations (e.g., evisceration, chiller tanks) in mass load rather than concentration need to be performed. This will provide the opportunity to identify areas of the plant that contribute higher loads to the wastewater stream that will require removal prior to discharge for the processing plant into municipal sewer systems.

Based on the research from Chapter 3, future work will need to be performed to find an effective *Salmonella* intervention strategy for traditional commercial feed withdrawal protocols. In the study described in Chapter 3, 50 ppm HP + CA (pH=5.0) was effective in reducing *Salmonella* contamination in broiler crops. Although, this significant effect was seen when the broilers were subjected to the partial feed withdrawal schedule (6 hours off feed/0 hours off water), and when the broilers were still consuming the drinking water treatment prior to euthanasia and sample collection. As previously mentioned, the consumption of contaminated litter and the changing crop environment during feed withdrawal results in proliferation of bacteria like *Salmonella* (Hinton et al., 2000; Corrier et al., 1999). Therefore, further research in this area will need to be performed to not only find an intervention strategy that is effective when the birds are exposed to the treatment, but also has an effect once the treatment has been removed from the birds.

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