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The Use of Inorganic and Organic Acids as Short Duration Antimicrobial Dips on Mitigating Pathogens Present on Commercial Chicken During Peri-Harvest

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The Use of Inorganic and Organic Acids as Short Duration Antimicrobial Dips on Mitigating Pathogens Present on Commercial Chicken During Peri-Harvest

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by

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ABSTRACT

With poultry being the most consumed meat in the United States, poultry processors must provide consumers with safe, wholesome products. As a consequence, poultry processors are faced with the challenge of reducing the presence of foodborne pathogens such as *Salmonella* spp. and *Campylobacter jejuni* among raw poultry products. Though multi-hurdle approaches using antimicrobials are placed throughout processing to reduce these pathogens, *Salmonella* and *C. jejuni* still persist among raw poultry. Thus, it was the objective of the current dissertation to investigate various antimicrobials, organic and inorganic acids, as short duration dips and sprays as means to reduce common pathogens (*Salmonella*, *Campylobacter jejuni*, and *Escherichia coli*) among raw chicken carcasses and parts. It was hypothesized that the use of organic and inorganic acids in poultry part dips would result in a decrease pathogen load and a positive shift in the microbiota of rinsates, thus improving the safety of raw products. Therefore, three projects were devised and executed to investigate the use of inorganic and organic acids as short duration part dips and sprays. The objective of the first study was to evaluate the efficacy of TetraClean Systems aqueous ozone, O_3, in combination with PAA as an antimicrobial spray on whole chicken carcasses (**Chapter 2**). The second project aimed at determining the efficacy of varying concentrations of sodium bisulfate salt, SBS, alone or in combination with peracetic acid, PAA, in 15 s whole part dips (**Chapter 3**). The objective of the third study was to determine the influence of two antimicrobials, PAA and acidified sodium chlorite (ASC), on the microbiota of chicken thighs inoculated with *Salmonella* and *Campylobacter* (**Chapter 4**). Overall, the data presented in the current dissertation demonstrated the potential use of novel antimicrobials as short duration dips and sprays at mitigating foodborne pathogens present on raw poultry.

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DEDICATION

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INTRODUCTION

INTRODUCTION

Over the past 25 years, numerous steps have been taken to mitigate the contamination of raw poultry and products. With the implementation of the Hazzard Analysis Critical Control Point (HACCP) in 1996 (Hulebak, 2002) and the Food Safety Modernization Act (FSMA) in 2011 (FDA, 2011), rules and guidelines were established to reduce the intensity and frequency of foodborne illnesses. Although the strategies from HAACP and FSMA have had a positive impact on the incidence of foodborne illness, they are not entirely sufficient alone. As a consequence, further mitigation strategies are necessary. In fact, the U.S. Department of Health and Human Services Office of Disease Prevention and Health Promotion has created a multi-agency task force Healthy People 2030, aimed at reducing multiple foodborne illnesses (OPDHP, 2020). By the year 2030, this multi-agency effort is tasked at reducing *Campylobacter* prevalence from 15.8 to 10.6 per 100,000 population (FS-01) and reduce *Salmonella* prevalence from 14.8 to 11.1 per 100,000 population (FS-03; OPDHP, 2020). Specific to poultry, the Healthy People initiative aims at reducing outbreaks of Shiga toxin-producing *Escherichia coli*, *Campylobacter*, *Listeria*, and *Salmonella* infections linked to poultry (FS-D05; OPDHP, 2020). Thus, further intervention strategies must be integrated into the poultry industry to meet these goals.

Currently, one of the most commonly used methods to decontaminate poultry meat in poultry processing facilities across the United States (US) are antimicrobial washes and sprays at various locations throughout first and second processing (McKee, 2012). Traditionally, chlorine and peracetic acid (PAA) are the antimicrobials of choice in the chiller, post chiller, spray cabinets, and part dips in poultry processing facilities (McKee, 2012). More recently, antimicrobials such as organic acids, phosphates, chlorine derivatives, and hydrogen peroxide solutions have emerged for industrial applications (Dincer and Baysal, 2004; Loretz et al., 2010).

Antimicrobials that lower the pH of the surrounding environment are promising. However, Gram-negative species, such as *Salmonella*, are capable of developing resistance to organic acids due to the presence of lipopolysaccharide (LPS) in their cell wall (Shue and Freeze, 1973). In addition, bacteria such as *Salmonella* can generate a tolerance response to stressful conditions such as an acidic environment (Ricke, 2003). One other concern over the use of organic acids is the potential for such pathogens as *Campylobacter jejuni* to use certain organic acids as substrates in their energy metabolism and biosynthesis activities (Kaseem et al., 2013; Hofreuter, 2014). It has been demonstrated that *C. jejuni* is capable of using organic acids as intermediate substrates in their tricarboxylic acid (TCA) cycle (Line et al., 2010). Indeed, there are considerations to be made when using organic acids as the sole antimicrobial in processing.

Inorganic acids also have the potential to induce the resistance of pathogens, such as *Salmonella*, to a low environmental pH (Foster and Hall, 1991); however, it is less likely to occur. *Salmonella* can reduce their cytoplasmic pH to maintain a neutral state when there is a mild decrease in the extracellular pH. Still, this response is incredibly arduous for *Salmonella* and can lead to cell death (Hill et al., 1995). Therefore, to overcome the potential for resistance to develop against common processing antimicrobials, novel antimicrobials, and/or a combination of antimicrobials need to be evaluated and developed. In addition, these antimicrobials should be implemented at multiple locations within processing to further reduce pathogen prevalence among raw poultry.

Thus, the objective of the current dissertation was to investigate various antimicrobials, such as organic and inorganic acids, as short duration dips and sprays as means to reduce common pathogens (*Salmonella*, *Campylobacter jejuni*, and *E. coli*) among raw chicken carcasses and parts (**Figure 1**). The central hypothesis of the dissertation was that the use of

organic and inorganic acids in poultry part dips and sprays would potentially reduce foodborne pathogens. Thus, three working hypotheses were developed:

1. The use of TetraClean Systems aqueous ozone, O_3, in combination with PAA as an antimicrobial spray on whole chicken carcasses has the potential to reduce ambient PAA while maintaining the efficacy of PAA on *Salmonella* Typhimurium, *Campylobacter jejuni*, and non-pathogenic *E. coli*. (**Chapter 2**).
2. Sodium bisulfate salt, SBS, alone or in combination with peracetic acid, PAA, in 15 s part dips has the potential to reduce a nalidixic resistant strain of *Salmonella* Enteritidis (**Chapter 3**).
3. PAA and acidified sodium chlorite (ASC) have the potential to reduce *Salmonella* Enteritidis, Typhimurium, Infantis, Kentucky, Heidelberg, and *Campylobacter jejuni* and alter the microbiota of chicken thighs (**Chapter 4**).

Overall, the data presented in the current dissertation demonstrated the potential use of novel antimicrobials as short duration dips and sprays at mitigating foodborne pathogens associated with raw poultry products. With this information, poultry processors will be better equipped at providing safe products to consumers.

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TABLE AND FIGURES

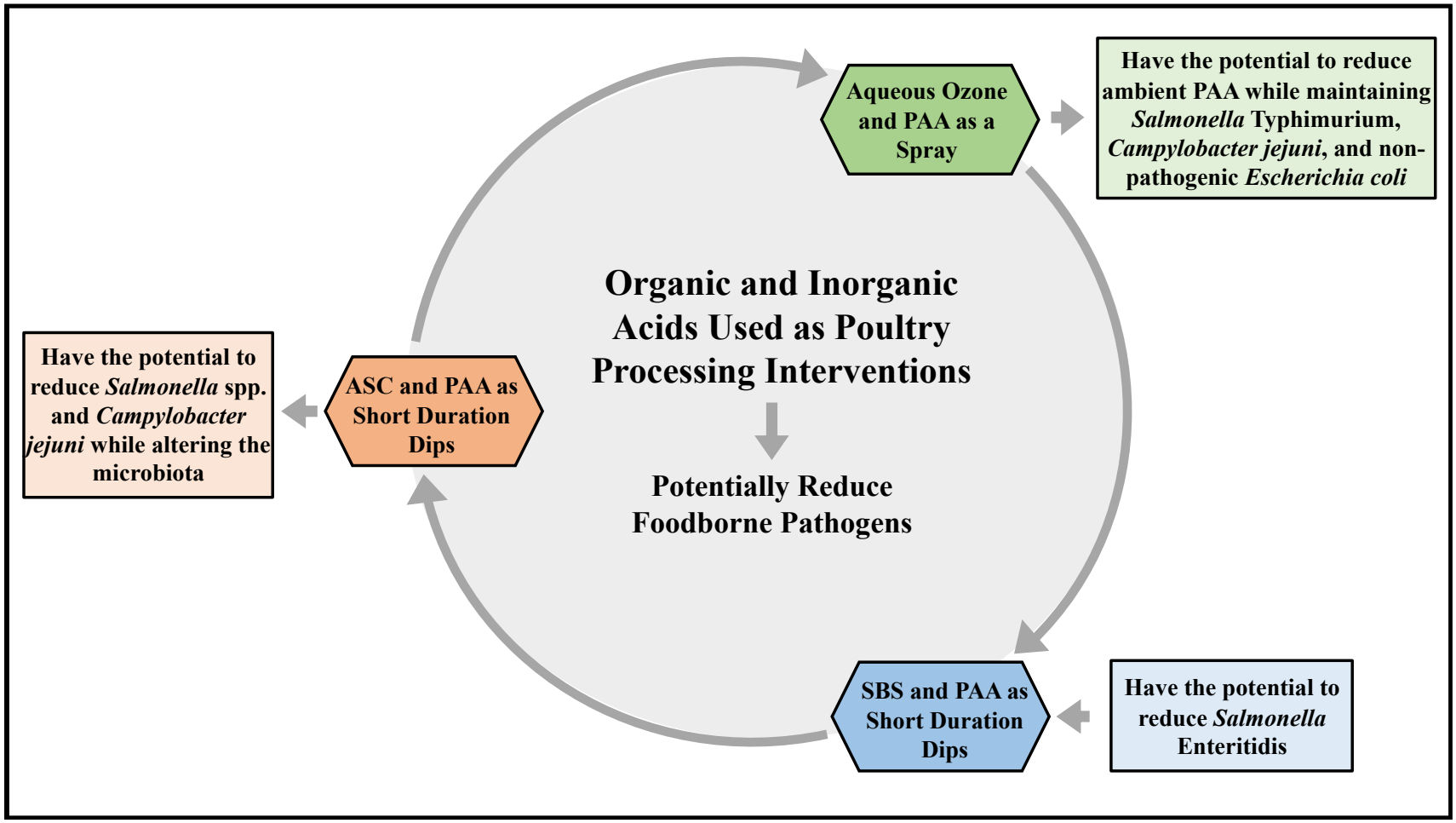

Figure 1. Mind map of the central hypothesis and working hypotheses contained within the current dissertation

CHAPTER 1

LITERATURE REVIEW

Current Antimicrobial Strategies and Interventions to Reduce Contamination of Raw Poultry Products and Their Impact on the Microbiota of Poultry Carcasses

ABSTRACT

As *Campylobacter* and *Salmonella* remain the top foodborne pathogens among poultry and poultry products, it is critical that the commercial poultry industry provide safe and wholesome products to consumers void of contaminants and pathogenic bacteria. As such, there have been tremendous strides to make commercial poultry processing a clean and efficient process. The current review describes and elaborates on the current commercial processing conditions in the United States, common foodborne pathogens, and mitigation strategies of poultry producers. Also, this review will discuss the effect of current and novel antimicrobial interventions used in poultry processing. Lastly, future considerations for the use of next-generation sequencing will be proposed.

Keywords: poultry, processing, microbiota, antimicrobials

INTRODUCTION

In the United States, poultry is the most consumed meat per capita (NCC, 2020). Not only is poultry a substantial proportion of the average diet, but it is a major part of the U.S. economy. The U.S. broiler industry is the largest in the world, exporting 16% of the total production out of the country (NCC, 2020). In 2019 alone, over 9.2 billion broilers were reared in the United States (NCC, 2020). With poultry being a signifiant part of the U.S. lifestyle and economy, it is essential to ensure the safety of these products. Therefore, the poultry industry, along with federal agencies, are tasked with reducing pathogens among poultry and poultry products.

Over the past twenty years, several interventions have been implemented, whether chemical or physical, during poultry processing, to reduce the microbial load prior to second processing (Ricke et al., 2005). Because it is well known that there are multiple critical control points where the microbial burden could be increased, numerous attempts to reduce the microbial load are employed throughout evisceration and first processing of poultry (Stopforth et al., 2007; Zweifel and Stephan, 2012; Sofos et al., 2013). These attempts are also recognized as "multi-hurdle" approaches and can reduce the risk of recontamination during harvest, which is a significant concern for processors (Schuler and Badenhop, 1972; Mead, 1974; 2004; Mead et al., 1994; Stopforth et al., 2007; Owens et al., 2010). "Multi-hurdle" interventions during processing are employed in the scalder, picker, inside-outside bird wash (IOBW), and the chiller systems, all of which have been studied extensively (Schuler and Badenhop, 1972; Mead, 2004; Stopforth et al., 2007; McKee et al., 2008; Zweifel and Stephan, 2012; Nagel et al., 2013).

Although there are interventions in place during first processing, there is still a risk of cross-contamination occurring during second processing, but there are limited hurdles

implemented at this stage. Therefore, it is the objective of the current review to discuss the more recent U.S. poultry processing procedures and the need for short antimicrobial part dips as a means to further mitigate pathogens and the microbial load during second processing. In the current review, common foodborne pathogens, the most recent operational conditions of commercial processing in the United States, and mitigation strategies of poultry producers will be described and elaborated. In addition, this review will discuss the effect of current and novel acidifiers either used currently or potentially available for use in poultry processing. Lastly, future considerations for the use of next-generation sequencing will be proposed.

FOODBORNE PATHOGENS IN POULTRY PROCESSING

Across the world, *Salmonella*, *Campylobacter*, and pathogenic *E. coli* remain among the top foodborne pathogens, causing gastroenteritis and more severe conditions in compromised individuals (World Health Organization, 2015). *Campylobacte*r remains the most frequent cause of gastroenteritis-related diarrhea, and non-typhoidal *Salmonella enterica* is one of the leading causes of foodborne related deaths (World Health Organization, 2015). In 2015, *Salmonella*, *Campylobacter*, and *E. coli* incidence were 15.74, 12.82, and 2.6% of the total U.S. foodborne infections (CDC, 2017). Also, *Salmonella*, *Campylobacter*, and *E. coli* were responsible for 411, 19, 61 foodborne outbreak-related infections in 2015 (CDC, 2017). Therefore, these pathogens continue to remain a significant source of risk to the public.

From 1998 to 2008, 20.6% reported cases of foodborne related illnesses were directly linked to contaminated poultry products (Painter et al., 2013). From 1998 to 2012, 25% of reported outbreaks (279/1114) were directly related to contaminated poultry, accounting for the highest number of outbreaks, illnesses, and hospitalizations, and the second-highest number of

deaths (Chai et al., 2017). Contributing factors included food-handling errors (64%) and inadequate cooking (53%) (Chai et al., 2017). It is evident that poultry processing is unable to eliminate all pathogens among poultry products, and thus processing controls and interventions are critical to ensure the safety of poultry products.

Salmonella, a Gram-negative, facultative aerobic bacteria, flourishes at 37 °C, and depending on the serovar, can cause typhoidal fever, enteric fevers, gastroenteritis, and septicemia (Holt et al., 2000). Most foodborne illness cases are related to contamination of food sources through the gastrointestinal tract (GIT) of livestock (Crump et al., 2015; Barrow et al., 2012; Cosby et al., 2015). Worldwide, non-typhoidal *Salmonella* causes over 938 million foodborne illness cases and 155,000 deaths each year (Majowicz et al., 2010). In the United States alone, over 1.2 million cases of non-typhoidal salmonellosis occur each year, resulting in approximately 450 deaths (Scallan et al., 2011). In humans, the infectious dose can range from 10^6 to 10^8 CFU but can be lower depending on the host immune status, with the elderly and children being more susceptible (Chen et al., 2013). In 2017, *Salmonella* accounted for the majority of these incidences of foodborne illness in the U.S., with Enteritidis, Newport, Typhimurium, and Javiana as the top serotypes (Incidence Rates > 1) (CDC, 2017). Consequently, incidences of *Salmonella*, especially Enteritidis, can be directly linked to poultry and eggs (CDC, 2014a). The high incidence of *Salmonella* in poultry is in part due to the ability of *Salmonella* to colonize poultry without detectable symptoms (subclinical infection) and proliferate and cross-contaminate via vertical and horizontal transmission (Barrow et al., 2012; Cosby et al., 2015).

Another major contributor to foodborne illness in the U.S. is *Campylobacter*, a Gram-negative, microaerophilic bacteria responsible for campylobacteriosis, an infectious GIT disease,

and Guillain-Barré Syndrome, a condition resulting in paralysis (Holt et al., 2000). *Campylobacter jejuni* and *C. coli* are the two most common strains associated with foodborne illness (CDC, 2017) and both prefer a microaerophilic environment around 42 °C, the internal temperature of poultry (41 to 42 °C) (CDC, 2014b; Holt et al., 2000). Like *Salmonella*, *Campylobacter* is present in the oral cavity, GIT, and reproductive organs of humans and animals (Holt et al., 2000). Until more recently, *Campylobacter* was considered a commensal in the GIT of poultry (Humphrey et al., 2014). However, as more is revealed, it appears *Campylobacter* does not always behave as a commensal in poultry (Lamb-Rosteski et al., 2008; Van Deun et al., 2008; Awad et al., 2014, 2015, 2016). Campylobacteriosis affects more than 1.3 million people and 76 deaths per year (CDC, 2006; CDC, 2014b). Of *Campylobacter* related infections, poultry products are estimated to be responsible for roughly half of foodborne related infections (Harris et al., 1986). Therefore, it is imperative to mitigate and control this pathogen.

Although not as prominent in the poultry industry due to extensive mitigation efforts, *E. coli* is a facultative-anaerobic, Gram-negative bacteria that colonize the lower part of the GIT of animals (Holt et al., 2000). Particular *E. coli* strains can produce enterotoxins and colonization, resulting in gastroenteritis and systemic infection in the host (Holt et al., 2000). From 2003 to 2012, 390 pathogenic *E. coli* (O157) related outbreaks occurred, resulting in 4,292 illnesses, 1,272 hospitalizations, and 33 death (Hieman et al., 2015). Approximately 65% of the reported outbreaks during those years were transmitted through food (Hieman et al., 2015). In poultry and processed poultry products, enteropathogenic *E. coli* (ETEC) is more common than Shiga-toxin producing *E. coli* (STEC). However, both are a concern for the poultry industry (Alonso et al., 2012).

Ultimately, the CDC and other federal agencies have been tasked to confront foodborne illness in the U.S. (ODPHP, 2020). The Healthy People 2030 Initiatives aim to reduce foodborne illness and its burden on the U.S. population (ODPHP, 2020). By 2030, the Healthy People Initiative aims at reducing *Salmonella* (FS-04), *Campylobacter* (FS-01), and *E. coli* (STEC; FS-02) from the baseline of 14.8, 15.8, and 4.0 to 11.1, 10.6, and 3.2 laboratory diagnosed infections per 100,000 in the U.S. (ODPHP, 2020). Consequently, the poultry industry is focused on reducing pathogen contamination within poultry. Due to these pathogens commonly being present in the GIT of livestock, specifically poultry, resulting in contaminated animals entering the processing plant, processing controls at the plant are more important than ever.

COMMERCIAL POULTRY PROCESSING IN THE UNITED STATES

Currently, in the U.S., there are over 160 poultry processing plants, 355,000 people directly employed, 1.2 million indirectly employed, and 25,500 family farms (NCC, 2020). The poultry industry consists of approximately 30 federally inspected companies that are vertically integrated, owning, and operating poultry production from start to finish (NCC, 2020). The U.S. is not only the largest poultry producer globally, but the U.S. population consumes more chicken than anyone else in the world, consuming 44.45 kg per capita in 2019 (NCC, 2020). The top 5 broiler producing states are Georgia, Alabama, Arkansas, North Carolina, and Mississippi (NCC, 2020).

These poultry companies generate varying broiler sizes depending on the carcass's final destination, such as further processing or whole carcass. The three main broiler sizes are Cornish hens (907 to 1,814 g), mid-weight broilers (1,814 to 2,722 g), and large broilers (2,722

to 3, 629 g; Wages et al., 2019). Although these birds may be of the same genetic line, their sizes are primarily determined based upon age. All broilers in the U.S. undergo a standardized processing procedure where poultry is converted to meat and processed meat products (**Figure 1**).

Live Haul and Live Hang

Before the birds reach the processing plant, they are caught by a crew of employees who typically utilize commercial mechanical chicken catching systems (Ramasamy et al., 2004). These catching systems are more efficient than manual catching with effective harvesting rates of 4,200 to 5,000 birds per hour (Ramasamy et al., 2004). Also, these commercial systems are believed to be more economically and welfare friendly and, on average, can reduce leg bruising by 9.5% compared to manual catching (Lacy and Czarich, 1998). The reduction of bruising translates to less rendered meat and properly handled birds (Lacy and Czarich, 1998).

After birds are caught on the farm, poultry are transported in crates via tractor-trailers to the processing facility. Traditionally, these modules (crates) are open to the outside environment and do not have any coverings. However, during the winter, external coverings are provided and placed around the perimeter of the crates on the trailer. In situations where 1-hour transportation time is impossible, temperature control is vital to mitigate dead upon arrivals (DOA, Mitchell and Kettlewell, 1998). Typically, the summer months are of the most concern as there are limited methods to ensure the birds are maintained at the proper temperature (Ritz et al., 2005). Many plants are equipped with loading areas where large box fans equipped with or without pressurized spray nozzles surround the trailer upon reaching the processing plants. These modified areas are where the birds will be held until they can be loaded off onto the live hang area at the back of the processing facility.

Due to the utilization of modules rather than coop systems in commercial processing in the U.S., off-loading the broilers at the processing facility is relatively free of manual labor. The module or coopless system has reduced manual labor by 30% on the back dock of processing facilities (Shackelford et al., 1981). Once broilers are delivered to the back dock, the module is typically released over a large circular conveyer belt. The birds are then picked up manually, with hocks being placed into shackles held overhead in the dark. Because poultry are photosensitive, blue light over white light has been shown to reduce stress and the development of pale, soft, and exudative (PSE) breast meat (Barbasa et al., 2013).

One of the main concerns during live haul and live hang is the amount of stress on the birds and how that affects the yield and hygiene of the bird. Many efforts have been made to reduce stressors on the farm and during the transportation of the birds. Although efforts have been made to mitigate these stressors by altering transportation methods or by supplementing diets with beneficial organisms or substrates, there is still much to improve upon (Mitchel and Ketttlewell, 1998; Ghareeb et al., 2008). Because of the stress these birds are put under during transportation and live hang, enteric foodborne pathogens are a concern during this process (Whyte et al., 2001). The amount of stress correlates to the increased production of diarrhea from the bird (Linton and Hinton, 1986). Of the enteric pathogens found within the feces of poultry, those of the genus *Salmonella* or *Campylobacter* are of concern to humans as they are the primary causative agents of foodborne illness (CDC, 2020). Therefore, cleaning transportation equipment and reducing stress among poultry during transportation and live hang is critical in reducing pathogens further down the chain (Ramesh et al. 2002; Berrang and Northcutt 2005).

Slaughter

After live hang, the broilers are hung upside down in shackles are moved using a conveyor belt system. The broiler's heads are dipped into a saline solution containing an electrical current that results in unconsciousness and cardiac arrest, also termed electrical stunning (Savenije et al., 2000; Lambooij and Hindle, 2018). In general, electrical stunning induces epileptiform insult of the brain as a function of brain damage (Savenije et al., 2000). Upon electrical stunning, birds are euthanized via exsanguination and allowed to bleed out briefly prior to entering the scalding tank (Savenije et al., 2000).

Scaling tanks consist of counterflow current, triple-pass system (3 separate tanks), and full carcass immersion. Scalding of the carcass is done to release the feather follicles from the bird's skin and prepare the bird to remove their feathers via a picker. Scalding conditions vary depending on the processing facility but generally occur as a soft scald or hard scald depending on duration and temperature of scald (90 s at 50 °C versus 45s at 56.6 °C; McKee et al., 2008). A soft scald will result in the retained waxy stratum corneum layer or the cuticle of the carcass's skin (Sams et al., 2001).

Due to the high organic load on the feathers, originating from the feces and surrounding environment, bacterial loads have been recorded as high as 6 to 9 Log_{10} CFU/mL, 10^6 CFU/cm^2, or 4×10^8 to 4×10^{11} CFU/carcass (Wilkerson et al., 1961; Lillard 1989, 1990; Kotula and Pandaya, 1995) and up to 10^8 CFU/g on the feathers alone (Barnes, 1975). In addition, Berrang et al. (2003) determined the load of *Campylobacter*, total coliforms, *E. coli*, and total aerobic bacteria increased in the respiratory tracts of broilers sampled pre and post scald (0.7 and 1.0, 1.2 and 3.0, 1.2 and 2.7, and 2.3 and 4.2 Log_{10} CFU/mL of rinse). Buhr et al. (2005) demonstrated similar results when sampling the respiratory tract of broilers pre and post scald with or without

the respiratory tract tied off above the crop. Coliforms, *E. coli*, and total aerobic bacteria were increased after exiting the scald tank when the respiratory tract was not tied off (Buhr et al., 2005). However, there were no differences in respiratory tract bacterial load when tied off (2.6, 5.0, and 2.3 Log_{10} CFU/mL, 2.4, 4.6, and 2.0 Log_{10} CFU/mL, and 3.2, 5.4, and 2.7 Log_{10} CFU/mL).

For those reasons, chemicals have been added to scalding tanks to reduce the total bacterial load on birds passing through (Humphrey et al., 1981; McKee et al., 2008). Limited research exists on the efficacy of scald additives; however, scald additives are currently provided on the market. McKee et al. (2008) investigated the addition of an alkaline commercial scald additive, RP scald (Duchem Industries, Newnan, GA). They demonstrated that the 1% addition of RP scald in scalding tanks effectively reduced *Salmonella* Typhimurium on broiler carcasses, primarily when a hard scald was utilized. Another methodology of reducing bacteria on broilers during or post scald is the use of pre-scald brushes that remove fecal matter from the carcass's surface (Pacholewicz et al., 2016). Pacholewicz et al. (2016) developed a pre-scald brushing system and demonstrated significantly reduced populations of *E. coli* and *Enterobacteriaceae*; however, these reductions were not biologically relevant (0.3 Log_{10} CFU/mL reduction).

After exiting the scalder, carcasses enter the "picker" where defeathering occurs. The mechanical picker consists of hundreds of rubber finger-like projections that vibrate or move at a high velocity and remove the already loosened feather follicles. These rubber fingers are typically ribbed and are associated with biofilms and bacterial cross-contamination (Lillard, 1986; Arnold and Silvers, 2000; Hinton et al., 2004; Arnold, 2007; Arnold and Yates, 2009). Because the scalder loosens the feather follicles and can damage intact skin, bacteria may take residence below the skin's surface and proliferate (Thomas and McMeekin, 1980; Kim et al.,

1993; Allen et al., 2003). Arnold (2007) demonstrated that the fingers of the picker might become damaged and be a reservoir for biofilm formation, making it arduous to remove and eliminate bacterial populations and reduce cross-contamination. Overall, Arnold (2007) has suggested that an early intervention step must occur in the picking process to improve sanitation procedures and pathogen control.

There is considerable evidence that scalding and feather removal are among the first steps in poultry processing to contribute to the contamination of poultry carcasses (Dickens and Whittemore, 1997; Berrang et al., 2001; Allen et al., 2003a,b; Bunic and Sofos, 2012). Wages et al. (2019) determined that not only does the size of the bird at the processing plant influence the microbiota composition of poultry rinses at post scald and post pick but also affects the total aerobic bacteria and *Enterobacteriaceae* of the rinsates. In general, Wages et al. (2019) observed less diversity and fewer observed taxonomical units (OTUs) within the carcass rinsates sampled at post pick then at post scald (Chao1 and Observed OTUs), regardless of broiler size (Cornish hens: 907 to 1,814 g; mid-weight broilers: 1,814 to 2,722 g; large broilers: 2,722 to 3,629 g). The microbiota of rinsates at post scald and post pick were significantly different, as determined by Bray Curtis and Weighted Unifrac. Post scald and post pick also affected the microbiota's overall composition, with *Proteobacteria* being greater and *Firmicutes* being less in the rinsates collected at the post-pick location (Wages et al., 2019). In summation, there is a need for the continued development of microbial interventions during and after slaughter.

1st Processing: Evisceration, Chilling, and Cut-up

Upon exiting the picker, broilers have their hocks removed and are rehung on the evisceration floor, which is separated from the kill floor by a complete wall. The first event to occur is typically the removal of the preen gland. The vent of birds is removed ("buttonhole"),

and the abdominal cavity is opened and widened. The eviscerate is subsequently removed from the cavity and displayed to the U.S. Department of Agriculture- Food Safety Inspection Service (USDA-FSIS) inspector on-site on a matching shackle as the carcass. If the eviscerate and carcass passes FSIS inspection, the carcass proceeds to have the neck, crop, and lungs removed.

At this step of the process, the digesta contained within the GIT and eviscerate must not contaminate the carcass or equipment. Because poultry digesta can commonly harbor foodborne pathogens of concern to human health, evisceration is a highly controlled process. Specifically, *Salmonella* and *Campylobacter* reside within the GIT of broilers and contaminate the carcass meat through fecal and digesta material (Hue et al., 2011; Pacholewicz al., 2016). A link has been established between the level of *Campylobacter* in the ceca and on the skin of the carcass, with 8.05 Log_{10} CFU/g of ceca correlating to 2.39 Log_{10} CFU/g of the carcass (Hue et al., 2011).

Because of the link between the bacterial load of the digesta and that of the carcass, inside-out bird washers (IOBW) are implemented to reduce fecal contamination of the skin (Jackson and Curtis, 1998; USDA-FSIS, 1998). According to FSIS standards, there is to be no visible fecal contamination of carcasses prior to entering the pre-chiller. Thus, cabinet washers, IOBW, carcass sprays, and brush washers are implemented as intervention means (Jackson and Curtis, 1998). Although the use of these IOBW reduces visible fecal matter, its use alone is not sufficient to mitigate pathogens. Previously, Northcutt et al. (2003) investigated the use of IOBW on reducing total coliforms, *E. coli*, and total aerobic bacteria. They concluded that tap water, alone, does not sufficiently remove coliforms and *E. coli* from the carcass and that there is a need to add antimicrobials to this process (Northcutt et al., 2003).

In the U.S., immersion chilling, the most widely employed chilling method, is accomplished through the use of three chiller tanks, pre-chiller, main chiller, and post-chiller.

These chillers were implemented in order to reduce the temperature of carcasses to 4.4 °C within a few hours (USDA-FSIS, 2014a, 2014b). Therefore, immediately after the IOBW spray cabinet, carcasses are loaded off their shackles and into the pre-chiller. The pre-chiller's main purpose is to reduce the organic load on the surfaces of carcasses by lowering the carcass temperature before they enter the main chiller (Barbut, 2002; Sams and McKee, 2010; James et al., 2006). Blevins et al. (2020) determined that operating the pre-chiller at temperatures at 18.3 °C was more effective at reducing pathogens such as *Campylobacter* than maintaining the pre-chiller at temperatures above 21.1 °C. When the pre-chiller is controlled at 18.3 °C, rinsates collected from carcasses entering and exiting the pre-chiller had 3.342 and 2.982, 0.002 and 0.116, and -0.613 and -0.065 Log_{10} CFU/mL of total aerobes, *Campylobacter*, and *Salmonella*, respectively (Blevins et al., 2020). Ultimately, maintaining the pre-chiller at lower temperatures improves the quality of the pre-chiller water and reduces the microbial burden present on carcasses (Blevins et al., 2020).

The main chiller consists of a large vat of chilled water where the birds are transported from one end to the other in a corkscrew motion. The chiller water has been reported to comprise the following phyla: *Proteobacteria, Firmicutes, Actinobacteria, Bacteriodetes* (Rothrock et al., 2016). Historically, chlorine has been used in the chillers to reduce the microbial load, but due to poultry's high organic load, free chlorine is quickly consumed (Tsai et al., 1992). More recently, peroxyacetic acid (PAA) has been used in the chiller system to reduce the microbial burden and has been noted to handle the organic load better than chlorine (Bauermeister et al., 2008a). Because carcasses are held in the main chiller for more extended periods (> 1 hour), antimicrobials such as PAA are added in smaller amounts, 200 ppm (Bauermeister et al., 2008a). Ultimately, carcasses leave the chiller and enter the post-chiller,

where the carcasses are maintained for a shorter duration. In the post-chiller, PAA is applied at higher concentrations, 600 to 1,000 ppm, to reduce the microbial load (Nagel et al., 2013). Nagel et al. (2013) demonstrated the use of 400 and 1,000 ppm PAA in a post-chiller reduced *Campylobacter* and *Salmonella* Typhimurium by 2 Log_{10} CFU/mL compared to 40 ppm chlorine, which decreased both pathogens to less than 1 Log_{10} CFU/mL. The reductions seen at the post-chiller are critical as it is possibly the last opportunity to reduce pathogens with antimicrobial solutions before packaging.

When examining the changes in the carcasses' overall microbial load and microbiota during first processing, few studies capture a microbial map of the entire process. Recently, Handley et al. (2018) sampled multiple abattoirs at rehang, pre-chill, and post-chill to bio-map first processing. They observed that as the carcasses moved from rehang to pre-chill, and from pre-chill to post-chill, a reduction in the total aerobic load and *Enterobacteriaceae* with 4.63, 3.15, and 0.81 and 2.99, 1.79, and 0.12 Log_{10} CFU/mL occurred at rehang, pre-chill, and post-chill, respectively (Handley et al., 2018). Also, the corresponding rinsates' microbiota composition shifted as birds exited the post-chiller (Handley et al., 2018). Although the rinsates' microbial load was almost zero by the post-chiller in the study by Handley et al. (2018), it must be remembered that processing is not complete until the carcasses are packaged. Thus, there is still room for microbial loads to increase due to cross-contamination from equipment.

2nd Processing: Deboning and Packaging

After carcasses exit the pre-chiller, they are loaded off onto a ramp where employees re-hang the carcasses on shackles. The carcass may be packaged as a whole roaster or further divided up into parts. In tray pack facilities, the carcass is divided into half below the breast but above the thighs on the back of the carcass. Humans primarily debone the front half of the

carcass, but there are facilities with automated machinery. The use of automated machinery varies depending on the machinery's effectiveness at deboning without losing substantial yield. Deboning of the front half begins when the frame is placed on a deboning cone, and a cut at the shoulder joint is made. From there, a cut is made down either underside of the breast against the ribcage. The breast is then pulled down, exposing the keel bone, and the wings are cut off the frame. The breast is pulled off the frame, and a cut is made "criss-crossing" down the sides of the keel bone, also known as the "butterfly" cut. Lastly, the tenders are pulled off. This process is done as the cones are moving and involves a team of employees, each doing a portion of the process to create efficiency and optimize yield. The hind half of the carcass is typically cut into separate parts by automated machinery and either directly enter a tray pack, are deboned, or processed further. The thighs are deboned by hand with assistance from machinery in most cases.

There is a concern of cross-contamination from the equipment to the skin-on and skinless parts during second processing. Pathogens such as *Salmonella* can form biofilms on the surface of processing equipment and can contaminate poultry meat with ease (Clayborn et al., 2015). Although processing facilities require constant cleaning and hire outside sanitization companies to sanitize equipment, biofilms remain an issue within the processing environment (Clayborn et al., 2015). Shi et al. (2019a) investigated the biofilm-forming genes, *bscA,* and *csgD,* of multiple *Salmonella* strains that were isolated from poultry processing facilities in the U.S. and discovered all serovars examined in the study had high biofilm-forming capabilities. There has been extensive research on the biofilm formation among *Salmonella* strains and the proper way to limit their formation (Obe et al., 2018; Dhakal et al., 2018; Nannapaneni et al., 2019; Shi et al., 2019a,b), but this will not be discussed in the current review.

Due to the risk of pathogen reintroduction during second processing, it is integral for the industry to implement further mitigation strategies at this stage of processing. One potential solution is to employ short duration antimicrobial part dips. These dips could last for 10 to 30 seconds and not only have the potential to mitigate pathogens but could increase the water holding capacity of the parts (USDA-FSIS, 2015a). For those reasons, short-duration antimicrobial part dips have become a focus of academic and commercial interests and will be discussed in more detail in the following section.

MITIGATION STRATEGIES AND THEIR EFFECT ON THE MICROBIOTA OF RAW POULTRY

Processing antimicrobials

In the U.S., antimicrobial washes and sprays are among the most commonly employed methods to eliminate or mitigate contamination among poultry and poultry products within poultry processing facilities (McKee, 2012). These antimicrobial washes and sprays exist throughout first processing to ensure the bacterial load is low when entering second processing (McKee, 2012). Historically, chlorine and PAA were the most commonly utilized antimicrobials in the chiller, post chiller, spray cabinets, and part dips among poultry processing (McKee, 2012). More recently, other antimicrobials have been investigated for industrial applications such as organic acids, phosphates, chlorine derivatives, and hydrogen peroxide solutions (Dincer and Baysal, 2004; Loretz et al., 2010). Although Gram-negative species, such as *Salmonella*, are capable of developing resistance to organic acids due to the presence of lipopolysaccharide (LPS), acidifiers, antimicrobials that lower the pH of the surrounding environment are promising (Shue and Freese, 1973).

Over the last decade, chlorine has been replaced with PAA as the primary sanitizer in processing facilities. As chlorine cannot remain effective in the presence of the high organic loads present during poultry processing, its effectiveness is considered limited. This ineffectiveness was evident in previous research studies. Bauermeister et al. (2008b) demonstrated that the 30 ppm of chlorine reduced *Salmonella* and *Campylobacter* presence by 43 and 13% when used in a poultry chiller. In the same study, 85 ppm of PAA reduced *Salmonella* and *Campylobacter* by 92 and 43%, on poultry carcasses, respectively (Bauermeister et al., 2008b). The use of PAA is known to reduce populations of *Staphylococcus* spp., *Listeria* spp., and generic *Escherichia coli* more than 5-log CFU in multiple food matrices (Brinez et al., 2006). Also, the use of 200 ppm in the chiller has been reported to reduce *Salmonella* and *Campylobacter* approximately 1 Log_{10} CFU/mL more than those treated with 30 ppm chlorine (Bauermeister et al. 2008a).

PAA and its Limitations

PAA, a product of the reaction between acetic acid and hydrogen peroxide, is a colorless acid with a strong odor and currently one of the most utilized antimicrobials to decontaminate poultry carcasses at concentrations ranging from 200 to 2,000 ppm (McKee, 2012). Because PAA is a potent oxidizing agent, it is bactericidal, oxidizing the cell membrane and other cell components (Oyarzabal, 2005). However, this chemical is highly corrosive and unstable. Its use has been known to be a hazard to human health (NAS, 2010) and an irritant to the upper respiratory tract, eye, and skin (Fraser and Thorbinson, 1986; Janssen, 1989a,b; Janssen and Van Doorn 1994; Merka and Urban 1978). Although direct contact in the eye and skin can be avoided if proper personal protective equipment (PPE) is worn, limited approaches exist to protect the upper respiratory tract from PAA vapors (American Thoracic Society, 1996).

As of 2020, there is no OSHA (Occupational Safety and Health Administration) limit on the acute or long-term exposure limit of PAA for employees. Other governing bodies have established PAA exposure limits and guidelines. The American Conference Governmental Hygienists (ACGIH) determined a 15-minute Short Term Exposure Limit (STEL) threshold limit of 0.04 ppm (ACGIH, 2016). Acute exposure guideline levels established by the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGLL Committee) are 0.17, 0.51, and 1.3 ppm of PAA vapor for AEGL-1, 2, and 3 during an 8 h period (NAS, 2010). The firm limits of total exposure time for AEGL-1 and 2 limits are 0.17 and 0.51 ppm of PAA vapor (NAS, 2010). As of 2015, the National Institute for Occupational Safety and Health, NIOSH, published a draft Immediately Dangerous to Life or Health (IDLH) value of PAA vapor at 0.55 ppm, though this draft has not been accepted to date (NIOSH, 2015). Thus, less corrosive and hazardous chemicals are being investigated.

Acidifiers and Poultry Rinses, Washes, and Dips

Acidifiers are promising antimicrobials for use within poultry processing. Acidifiers used in the poultry industry are comprised of organic and inorganic acids. As poultry skin has been associated with a buffering effect, these antimicrobials' efficacy can vary (Tan et al., 2014a,b). Furthermore, bacteria reside within the feather follicles and the exposed muscle surfaces (Barnes and Impey, 1968), making it arduous for antimicrobial treatments to disinfect poultry carcasses properly. However, there has been much success with acidifiers alone or in combination with other antimicrobials as short duration rinses, washes, and dips with 1 to 2 log reduction of pathogens being possible (Bauermeister et al., 2008b). More recently, Kim et al. (2017) using 16S rDNA-based sequencing demonstrated the ability of acidifiers to potentially shift the microbiota of carcasses during first processing. However, limited to no research exists

on how these acidifiers affect the microbiota of skin-on poultry parts during second processing. Due to the potential recontamination during second processing, these applications are becoming more common within processing facilities.

Peroxyacetic Acid (PAA)

Although PAA has been shown to reduce key pathogens on poultry carcasses when applied in the chiller or post-chiller, few studies have investigated its use during second processing. As such, Nagel et al. (2013) found that the post-chill application of PAA at concentration 400 and 1,000 ppm for 20 s (end of first processing) had the potential to reduce the load of *Salmonella* Typhimurium and *Campylobacter jejuni* on artificially contaminated chicken breasts by 2 logs CFU/mL. However, when applied as a 15 s part dip during second processing, the use of 220 ppm PAA (Inspexx 100, Ecolab, St. Paul, USA) did not reduce *Enterobacteriaceae* and coliforms significantly compared to tap water on chicken legs (Del Río et al., 2007). The lack of effect demonstrated by PAA when applied during second processing could be due to an initially low microbial load as Del Río et al. (2007) did inoculate the poultry parts with pathogens. However, they did demonstrate a 0.25 Log_{10} CFU/mL reduction on legs (Del Río et al., 2007).

When split, skinless, boneless chicken breasts were inoculated with *Salmonella* and *Campylobacter* (6-log CFU/mL), the use of PAA (100, 250, 500, 1,000 ppm) as a spray (5 or 10 s) and a short duration dip (4 to 30 s) reduced both populations (Kumar et al. 2020). Specifically, the use of PAA at 1,000 ppm as a 30 s antimicrobial dip, *Salmonella* and *Campylobacter* were reduced by 1.92 and 1.87 Log_{10} CFU/mL compared to the control (Kumar et al., 2020). However, the use of 500 or 1,000 ppm of PAA for 30 s did not differ in efficacy on reducing *Salmonella* (Kumar et al., 2020). When the skin's complex matrix is involved, the

reduction of pathogens is not as significant (Tan et al., 2014a,b). Zhang et al. (2018) investigated the use of various approved antimicrobials on poultry parts when inoculated with *Salmonella* Typhimurium and *Campylobacter jejuni* (10^8 CFU/mL). They demonstrated that the immersion of breasts, thighs, wings, and drumsticks in 700 or 1,000 ppm PAA for 23 s reduced both pathogens by 1.5 Log_{10} CFU/mL regardless of the part (Zhang et al., 2018). The sensory characteristics of the skin on parts were unaffected by the use of antimicrobials; whereas, skinless breasts were the most acceptable when treated with 700 ppm PAA or 3500 ppm cetylpyridinium chloride, CPC (Zhang et al., 2018).

Although there has been success with the use of PAA as an antimicrobial on pathogens during first and second processing, there are growing concerns over its use in the poultry industry. Primarily, the concern is over its effect on machinery and employees; however, more recently, there is increasing evidence for the potential for PAA to select for specific lactic acid bacterial populations that may contribute to spoilage and reduce the shelf life of poultry (Feye et al., 2020a). Therefore, there is emerging interest in investigating other alternative acidifiers that may not have this effect.

Sodium Bisulfate Salt (SBS)

The use of inorganic acids during second processing as short duration dips and across poultry processing is of increasing interest due to their effect on the surrounding pH and microbial load (Miccicche et al., 2019; Feye et al., 2020a,b; Atchley et al., 2018; Dittoe et al., 2019). Like organic acids, inorganic acids have the potential to induce resistance among pathogens to their antimicrobial effect. For example, *Salmonella* has demonstrated the ability to become tolerant to a low environmental pH (Foster and Hall, 1991). However, for the resistance to be successful, *Salmonella* must respond with an acid tolerance response and acid-shock

proteins (Foster and Hall, 1991; Foster, 1993; Foster, 2000; Bearson et al., 1997). Sodium bisulfate (SBS), an inorganic acid with a pKa of 1.9, has demonstrated the ability to decrease the extracellular pH to 2 (Knueven, 1999). Although *Salmonella* can reduce its cytoplasmic pH to maintain a neutral state (Hill et al., 1995), this response is incredibly arduous for *Salmonella* and can lead to cell death when the pH change is drastic (Hill et al., 1995). Therefore, SBS demonstrates the potential to be an antimicrobial alternative over other organic and inorganic acids.

The original use of SBS in the poultry industry was its use as Poultry Litter Treatment (PLT®). This commercially available litter amendment acidifies the litter and reduces ammonia volatilization (Payne et al., 2002). SBS's use in the litter has proven to be potent in mitigating *Salmonella* in the litter while reducing ammonia volatilization (Payne et al., 2002). When applied as a dietary amendment in poultry diets, SBS has decreased the shedding of *Salmonella* into the litter (Ruiz-Feria et al., 2011). Pertinent to poultry processing, the Environmental Protection Agency has declared SBS a safer choice antimicrobial and processing aid (EPA, 2018). Although there is limited research investigating the use of SBS as a processing aid, it can be an efficacious alternative or addition to current practices.

In 2018, Atchley et al. (2018) demonstrated SBS's potential as a processing aid during second processing. They utilized 1, 2, and 3% SBS alone or in combination with 200 ppm PAA as 15 s short antimicrobial dips on whole chicken wings and demonstrated that there was no difference among treatments in the treatments ability to reduce *Salmonella* Typhimurium on inoculated wings (Atchley et al., 2018). They did exhibit a continuous reduction of *Salmonella* Typhimurium over a 3-d refrigeration period (Atchley et al., 2018). When Dittoe et al. (2019) applied 1, 2, and 3% SBS alone or in combination with 200 ppm PAA as 15 s short antimicrobial

dips on chicken drumsticks, all treatments reduced inoculated *Salmonella* Enteritidis. Specifically, Dittoe and colleagues (2019) demonstrated the reduction of *S.* Enteritidis was greatest on drumsticks treated with 3% SBS alone or in combination with 200 ppm PAA. Lastly, Feye et al. (2020a) investigated the effect of tap water, 3% SBS, 500 ppm PAA, and the combination of 3% SBS with 500 ppm PAA on the microbial load and microbiota of poultry wings over a 21 d period. The use of 3% SBS and the combination of 3% SBS and 500 ppm PAA not only reduced total aerobic bacteria and lactic acid bacteria over a 21 d period compared to other treatments but also induced a unique microbiota that may potentially improve shelf life (Feye et al., 2020a).

Acidified Sodium Chlorite (ASC)

The most utilized USDA approved antimicrobials in poultry processing are PAA, CPC, sodium hypochlorite, and weak organic acids (Moore et al., 2017). However, there is increasing interest in utilizing acidifiers or weak acids to improve currently approved antimicrobials' efficacy. In the past 20 years, acidified sodium chlorite (ASC) has been proven to effectively reduce pathogens when utilized as a dip, spray, or post-chill intervention step on broiler carcasses (Kemp et al., 2000, 2001, 2002).

Kemp et al. (2000) investigated the effect of treating carcasses with 500, 850, and 1,200 ppm of ASC as a spray or 5 s dip with pH being lowered with the use of phosphoric acid or citric acid on the reduction of microbial load. The reduction of total aerobic bacteria, *E. coli*, and total coliforms was more significant when the pH of ASC was reduced with citric acid with an 82.9 to 90.7%, 99.4 to 99.6%, and 86.1 to 98.5% reduction being demonstrated (Kemp et al., 2000). However, the application of ASC as a dip was more efficacious at reducing the microbial load on carcasses than when applied as a spray (Kemp et al. 2000), the use of ASC in an IOBW

demonstrated the reduction of *Salmonella* and *Campylobacter* contaminated carcasses compared to offline reprocessing (Kemp 2001). *Salmonella* and *Campylobacter* presence was reduced by 21.6 and 24.1% among carcasses treated with ASC (Kemp et al., 2001). More recently, Zhang et al. (2018) investigated the effect of multiple acidifiers as 23 s rinses on artificially contaminated chicken breasts, thighs, wings, and drumsticks. Zhang et al. (2018) demonstrated less than a 1 Log_{10} CFU/mL reduction of *Salmonella* and *Campylobacter* on chicken parts rinsed in 700 ppm ASC. The reduction of *Salmonella* and *Campylobacter* by ASC was not different from the effect of rinsing parts in tap water or 30 ppm chlorine.

Organic Acid Blends

Although less commonly utilized in processing plants, organic acids and organic acid blends are alternatives to PAA. Organic acids such as lactic acid and acetic acid (1 to 2 %) when used as whole carcass sprays have been effective in reducing bacterial load present on carcasses. However, caution should be taken when using these products as higher concentration can bleach the carcass's skin (Sohaib et al., 2016; Milillo and Ricke, 2010). Specifically, at 37 °C, the minimum inhibitory concentration (MIC) of sodium citrate and sodium lactate in chicken meat was 1.25 and 2.5% to inhibit *Salmonella* Typhimurium (Milillo and Ricke, 2010). In addition, citric, malic, and tartaric acid at 150.0 mM reduced *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium by more than 5, 2, and 4 Log_{10} CFU/g on chicken breast pieces (Over et al., 2009).

Chicxide, a commercially available antimicrobial product comprised of lactic and citric acid, was utilized as an intervention on artificially contaminated broiler carcasses (*Salmonella* 10^4 CFU/100 cm^2) (Laury et al., 2009). When applied as a 5 s spray or 5, 10, or 20 s dip, the use of Chixcide reduced *Salmonella* by 1.3 Log_{10} CFU/mL when applied as a spray and by 2.3 Log_{10}

CFU/mL when applied as a dip, regardless of time (Laury et al., 2009). More recently, Ramirez-Hernandez and colleagues (2018) investigated the use of lactic acid, lactic acid–acetic acid blends, and PAA as poultry part antimicrobial sprays to reduce artificially inoculated *Salmonella* (10^6 Log_{10} CFU/mL; Enteritidis ATCC 13076, Typhimurium ATCC 14028, Typhimurium ATCC 13311, Heidelberg ATCC 3347-1, and wild-type *Salmonella* isolated from chicken thighs). Using a rising conveyor belt, the efficacy of these antimicrobials was tested on skin-on, and skinless thighs at three temperatures, and the effect of lactic acid and PAA on *Salmonella* reduction on skinless chicken breasts were investigated (Ramirez-Hernandez et al., 2018). Ramirez-Hernandez et al. (2018) demonstrated that the use of lactic acid and buffered lactic acid were most effective at reducing *Salmonella* on thighs regardless of temperature and that both lactic acid and PAA were effective at reducing *Salmonella* on skinless chicken breasts.

CURRENT MICROBIAL TESTING WITHIN POULTRY PROCESSING

As *Salmonella* and *Campylobacter* are an ongoing concern among raw poultry products, the FSIS continues to monitor their presence among raw chicken carcasses and parts. The monitoring of these pathogens is a common practice for both FSIS and processing personnel and is primarily comprised of rinsing the raw poultry in 400 mL of a sampling broth. These methods are the means to which the FSIS monitors and recalls poultry products and establishes performance standards.

Sampling Procedures

As of February of 2016, FSIS proposed new performance standards in order to further mitigate *Salmonella* and *Campylobacter* among raw poultry (USDA-FSIS, 2016a). Although the new performance standards would cost $17.96, $21.41, and $24.88 million if 30, 40 and 50% of

establishments were not in compliance, these standards could reduce the prevalence of foodborne illness among those who consume poultry products (USDA-FSIS, 2016a). In addition, the stricter performance standards for *Salmonella* and *Campylobacter* established by FSIS could provide $32.9, $58.3, and $84.2 million in health benefits even when 30, 40, and 50% of processors are not in compliance (USDA-FSIS, 2016a). The new standards will be set as 9.8, 7.1, 25.0, 13.5, 15.4 for the maximum acceptable percent possible for *Salmonella* and 15.7, 5.4, 1.9, 1.9, 7.7 % (maximum acceptable percent possible) for *Campylobacter* among raw broiler carcasses, turkey carcasses, comminated chicken (325 g), and comminated turkey (325 g) (USDA-FSIS, 2016a).

Whole bird carcass rinse. Generally, whole bird carcass rinses (WBCR) are to be collected randomly and transferred to a sterile Stomacher 3500 bag or equivalent after draining the excess fluid off of the carcass, also called a rest (1 to 2 min) (USDA-FSIS, 2015b). Chicken and turkey carcasses will have 400 and 600 mL of BPW or equivalent diluent (Buffered Phosphate Diluent) poured over and inside the interior cavity of the bird (USDA-FSIS, 2015b). The bag is closed by twisting the top of the bag and rinsed by grabbing the carcass in the bag with one hand and the twisted top with the other (USDA-FSIS, 2015b). The carcass is rinsed for 1 min approximately 35 times by rocking the carcass in a one-foot arc, rinsing all surfaces of the carcass (USDA-FSIS, 2015b). The carcass is removed aseptically and placed back into production. The rinsate is poured off into a specimen container and used for downstream analyses. These rinsates are to be used for quantifying the bacteria in foods that serve as sanitary indicators (USDA-FSIS, 2015b). For *Salmonella* and *Campylobacter* sampling, the steps are quite similar for young chickens, but not for young turkeys where sampling involves sponges and a 50 cm^2.

Sampling young chicken for *Salmonella* and *Campylobacter*. FSIS personnel are to collect carcass samples for *Salmonella* and *Campylobacter* testing. The carcasses to be sampled

are to be randomly selected and representative of all shifts, rails, chillers, coolers, etc. (USDA-FSIS, 2013). Regardless of the poultry carcass being sampled, personnel are to wash and sanitize hands, sanitize work surfaces, gather supplies, label appropriate collection containers, wash and sanitize hands again, transport supplies to the sampling location, sanitize new work surface, lay out supplies, and aseptically put sterile gloves on prior to collection (USDA-FSIS, 2013).

The current performance standards for young chickens is 7.5% and 10.4% for *Salmonella* and *Campylobacter* among 51 samples (USDA-FSIS, 2013). In order to select randomly without bias, the personnel are to select a carcass and then count forward or backward by 5 and select the next carcass for sampling (USDA-FSIS, 2013). If the carcass that is the sixth is not whole, repeat the selection process again by counting back or ahead an additional 5 carcasses (USDA-FSIS, 2013). This is to be repeated until a whole bird is selected. The carcasses are to be selected as they exit the post-chiller or where the last chemical intervention is applied before the birds are to be cut-up (USDA-FSIS, 2013).

Young chickens are to be rinsed in Buffered Peptone Water (BPW) as it is the standard FSIS procedure, although there are scenarios where neutralizing Buffered Peptone Water (nBPW) is advised (USDA-FSIS, 2013). Personnel are to open a 15” x 20” sterile rinse bag and place on sterile gloves, select the carcass, and allow the selected carcass to drip for 1 minute (USDA-FSIS, 2013). Once the carcass has had time to rest, the carcass in placed in the bag, neck first (USDA-FSIS, 2013). The bag with the carcass is placed flat on the sanitized surface and the 400 mL of pre-chilled BPW is to be opened and poured directly into the carcass cavity in the bag (USDA-FSIS, 2013). In order to prevent the bag from ripping, the loose neck skin is to be manipulated over the neck bones through the bag (USDA-FSIS, 2013). The excess air is

expelled, twisted closed, and folded over. The bag is then to mixed for 1 minute by manually agitating the carcass in 10 ° arcing motion for at least 30 times (USDA-FSIS, 2013). Aseptically remove the carcass from the bag by working the bag down in order to grab a leg without touching the inside of the bag (USDA-FSIS, 2013). The bird is not to be discarded but placed back on the conveyor or table without rinsing in potable water. Collect approximately 100 mL of the rinsate into a 120 mL sterile specimen jar without touching the inside of the jar or lid by using the "V" formed by the bag as a pouring spout (USDA-FSIS, 2013). Samples are to be sealed into a sealed bag and refrigerated within 5 minutes until shipment to the laboratory.

Sampling young turkey for *Salmonella* and *Campylobacter*. The current performance standards for young turkey carcasses are 1.7 and 0.79% for *Salmonella* and *Campylobacter* (USDA-FSIS, 2013). Although the preparation for the sampling and carcass is similar to chicken carcasses, turkey carcasses are sampled by using a cellulose sponge hydrated with BPW (USDA-FSIS, 2013). Personnel are to carefully place an absorbent pad or clean paper towel on the sanitized work surface in order to prevent the turkey from sliding off of the work surface (USDA-FSIS, 2013). Once the random turkey carcass is selected, the turkey is to be removed from the post-chill location or where the last chemical intervention was applied before cut-up by grabbing the turkey by the leg without touching the breast, sides, or back (USDA-FSIS, 2013). The turkey is placed on the absorbent pad or paper towel and gloves are aseptically removed. The bag containing the sponge is torn open and the bag aseptically opened. The cap from the 10 mL pre-chilled BPW designated for *Salmonella* testing is removed and poured directly over the sponge (USDA-FSIS, 2013). The bag is subsequently closed, and hand pressure is applied on the outside of the bag to massage the BPW into the sponge until the BPW is fully absorbed or the sponge is fully moistened (USDA-FSIS, 2013). The excess diluent out of the sponge is squeezed

while manually pushing the sponge to the upper part of the bag from the outside of the bag (USDA-FSIS, 2013). The bag is placed to the side and kept open with use of the wire closure (USDA-FSIS, 2013). Finally, the bag containing the template is carefully opened and a fresh pair of gloves are put on by the FSIS personnel (USDA-FSIS, 2013).

Personnel are to sample locations in order from least contaminated to most to avoid spreading the contamination from one location to another. The first sampling location for *Salmonella* is the left side of the vertebral column on the back, the second is the left thigh (USDA-FSIS, 2013). The sampling area of the template is 50 cm^2 (USDA-FSIS, 2013). The sponge is to be wiped vertically 10 times and then horizontally 10 times using only one side of the sponge for the first location, left side of the vertebral column (USDA-FSIS, 2013). The sponge is flipped and then used to sample the left thigh using the same methodology and placed back in the sampling bag with the air expelled (USDA-FSIS, 2013). This process is to be repeated for the *Campylobacter* testing; however, instead of 10 mL, 25 mL of BPW is to be used and the right side of the turkey carcass is to be sampled (USDA-FSIS, 2013). Once sampling is complete the carcass is to be returned to where it was collected from.

Raw part sampling. In addition to carcass sampling, the FSIS samples raw poultry parts for both *Salmonella* and *Campylobacter* (USDA-FSIS, 2016b). According to the FSIS practices, 1.8 kg ± 10% (4 lb ± 10%, 3 lb, 10 oz to 4 lb 6 oz) of raw poultry parts, legs, breasts, wings, are to be one type of part and collected from one specific shift, location, line in order to be used for sampling. First, the employee would open the collection cup or jar and remove the lid (USDA-FSIS, 2016b). It is imperative that the sample collection lid be placed aside without the inside of the lid touching anything. The employee would proceed to open the 15” x 20” sterile rinse bag and place sterile gloves on aseptically. Parts collected, totaling 1.8 kg ± 10%, would be covered

with 400 mL of sterile sampling broth that is to be poured directly over the parts (USDA-FSIS, 2016b). The collection bag, twisted and folded twice, should be inverted 30 times for approximately 1 min assuring the sampling broth covers the parts completely. Carefully opening the bag, the employee is to pour approximately 120 mL of the rinsate into a sterile sampling jar without allowing the bag to touch the inside of the specimen jar or lid. The remaining broth is to be discarded and the parts are to be placed back in production (USDA-FSIS, 2016b).

It is highly recommended that samples are collected more than once so that there is coverage over multiple shifts, conveyor belts or lines, and after interventions are used whether before packaging or in consumer packaging (USDA-FSIS, 2016b). It is also recommended that the processor have their own collection process that parallels the FSIS collection for their own record keeping and HAACP plan (USDA-FSIS, 2016b).

Microbial Testing

Once samples are collected by the FSIS or poultry processor personnel, the samples are shipped on an ice block to the main laboratory for microbial testing to occur (USDA-FSIS, 2016b, 2013). Once at the laboratory, multiple tests occur, including the quantification and identification of sanitary indicators, *Salmonella*, *Campylobacter*, and other poultry-related pathogens (USDA-FSIS, 2015b, 2019, 2016c, 2016d, 2020). In addition to microbial testing, polymerase chain reaction (PCR) and whole genome sequencing (WGS) is utilized to determine risk of the poultry products (USDA-FSIS, 2015b, 2019, 2016c, 2016d, 2020).

Microbial analyses of sanitary indicators. Among the sanitary indicators of interest to the poultry industry, aerobic plate counts (APC), *E. coli* and coliforms, *Enterobacteriaceae*, lactic acid bacteria (LAB) are the most widely referred to in reference to raw poultry products though more are of interest (USDA-FSIS, 2015b). Currently, 3M™ Petrifilms™, agar plates,

and TEMPO®, an automated system to enumerate quality and indicator organisms by Biomériex, are used to quantitate the sanitary indicators (USDA-FSIS, 2015b). In most cases, WBCR are utilized for raw poultry when testing for sanitary indicators. For WBCR, 10 mL of the rinsate is to be serially diluted (1:10) in 90 mL of a dilution blank to 10^{-6} or higher depending on the microbial load (USDA-FSIS, 2015b). Dilutions are to be shaken 25 times in a one-foot arc or vortexed thoroughly. This procedure should be completed within 20 minutes of collection (USDA-FSIS, 2015b).

Regardless of the organism type tested for, appropriate controls must be used such as a positive control and negative or sterility control. In most cases, the positive control is a stock culture, and the negative control is the diluent used in sampling (USDA-FSIS, 2015b; **Table 1**). Exactly 1 mL should be plated onto respective media for the indicator organism and then incubated for the required time (**Table 1**). For APC, the positive control is *Staphylococcus aureus* ATCC 25923 or equivalent (USDA-FSIS, 2015b). If APC Petrifilm™ is utilized, after inoculation, the plates are incubated at 35 ± 1 °C for 48 ± 3 hours in duplicate (USDA-FSIS, 2015b). Colonies on the Petrifilm™ plates that are red, regardless of their size or color intensity, are to be counted. The countable range is 25 to 250 CFU (USDA-FSIS, 2015b). If Plate Count Agar (PCA) is used, 1 mL of the diluted sample is dispensed into the bottom of duplicate petri dishes and the cooled PCA (35 ± 1 °C) is to be poured directly into the same dish (USDA-FSIS, 2015b). After gently swirling, the agar is allowed to harden and incubated for (USDA-FSIS, 2015b). All colonies are count on the agar at a countable range between 30 and 300 colonies per plate (USDA-FSIS, 2015b).

Escherichia coli and Coliforms are plated in duplicate onto *E. coli*/coliform Petrifilm™ (USDA-FSIS, 2015b). Controls for the *E. coli*/coliform Petrifilm™ are *E. coli* ATCC 25922 or equivalent (USDA-FSIS, 2015b). After inoculation, *E. coli*/coliform Petrifilms are to be incubated

at 35 ± 1 °C for 24 ± 2 hours (USDA-FSIS, 2015b). The following day, colonies are enumerated on the duplicate Petrifilms™. Blue to red-blue colonies with entrapped gas for *E. coli* and red colonies with gas for coliforms are to be counted within the countable range of 15 to 150 (USDA-FSIS, 2015b). Total Coliforms are considered the *E. coli* and coliform colonies combined (USDA-FSIS, 2015b).

To determine the concentration of *Enterobacteriaceae*, 1 mL of the sample is to be plated in duplicate on to *Enterobacteriaceae* Petrifilm™ (USDA-FSIS, 2015b). The proper positive control to be used is *Klebsiella pneumonia* ATCC 13883 or equivalent (USDA-FSIS, 2015b). After inoculation, Petrifilms™ are incubated at 35 ± 1 °C for 24 ± 2 hours and colonies that are red associated with gas bubbles or surrounded by yellow zones with or without gas are counted (USDA-FSIS, 2015b). The countable range is 15-100 CFU (USDA-FSIS, 2015b).

Other than APC, *E. coli* and coliforms, and *Enterobacteriaceae*, LAB are also quantified on raw carcasses. The proper positive control for LAB is *Lactobacillus plantarum* ATCC 14917 or equivalent (USDA-FSIS, 2015b). To enumerate LAB, the APT pour plate method is employed where 1 mL of each dilution is dispensed into duplicate petri dishes per sample (USDA-FSIS, 2015b). The APT agar is added once it has reached 42 to 25 °C and enough is added to cover the bottom of the dish (USDA-FSIS, 2015b). The dish is uniformly mixed and allowed to solidify. Once solidified, APT is incubated at 20 ± 1 °C for 4 to 5 days (USDA-FSIS, 2015b). All colonies are counted within the countable range of 30 to 300 colonies per plate (USDA-FSIS, 2015b).

***Salmonella* microbial analyses.** When quantifying *Salmonella* on whole bird and part rinses and poultry carcass and environmental sponges the sponge pre-moistened with 10 mL buffer or 30 ± 0.6 mL sample rinse fluid are utilized (USDA-FSIS, 2019; **Table 2**). For the sponge samples, 50 mL of BPW is added to the sample bag containing the sponge to bring the

total volume to 60 mL (USDA-FSIS, 2019). After the sponge and BPW are mixed well, the solution is incubated at 35 ± 2 °C for 20 to 24 h. For whole bird and part rinses, a 1:2 dilution of sample rinse fluid is prepared by using 30 ± 0.6 mL of the sample in 30 ± 0.6 mL of sterile BPW, mixed well, and incubated at 35 ± 2 °C for 20 to 24 h (USDA-FSIS, 2019). In addition to pre-enrichment, the samples could go directly to the most probable number (MPN) where the samples go through a three tube dilution series (1:10 dilutions) in enrichment broth such as Rappaport Vassiliadis broth (RV) (MLG Appendix 2.05). Based on the growth, probable number calculations are made to determine the quantity of *Salmonella* (MLG Appendix 2.05).

Following incubation, the samples are screened using the 3M™ Molecular Detection System, selective enrichment and plating, examination of colonies from plating media, and biochemical procedures (USDA-FSIS, 2019). Using the 3M™ Molecular Detection System, enriched samples are screened using the system guide (USDA-FSIS, 2019). If the screening is negative, then the samples are reported as negative, if the screen is positive then the FSIS personnel are to proceed with selective enrichment and biochemical assays on the sample.

To selectively enrich the samples that are screened positive, 0.5 ± 0.05 mL of the sample is transferred into 10 mL of tetrathionate (TT) broth and 0.1 ± 0.02 mL of the samples are transferred to 10 mL of RV broth and vortexed (USDA-FSIS, 2019). Enrichments are incubated at 42 ± 0.5 °C for 22 to 24 h or in a water bath at 42 ± 0.5 °C for 18 to 24 h (USDA-FSIS, 2019). After incubation, enriched samples are vortexed and streaked onto Brilliant Green Sulfa agar (BGS) and Double Modified Lysine Iron Agar (DMLIA) with a 10 µL loop for each plate (USDA-FSIS, 2019). Plates are to be incubated at 35 ± 2 °C for 18 to 24 h and typical colonies are to be selected (USDA-FSIS, 2019).

To select colonies off of streaked samples onto BGS and DMLIA, select colonies that are smooth, opaque, and pink with a red edge in the medium and purple colonies with (H_2S positive) or without (H_2S negative) black centers (USDA-FSIS, 2019). At least one typical colony should be isolated, but if colonies are difficult to isolate the enrichment and isolation must be repeated (USDA-FSIS, 2019). Using the selected colony, Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) slants are inoculated by stabbing the butts and streaking the slants in one motion and subsequently incubated at 35 ± 2 °C for 24 ± 2 h (USDA-FSIS, 2019). Following Table 2 of the Microbiology Laboratory Guidebook, TSI and LIA slants are screened for *Salmonella* characteristics (USDA-FSIS, 2019). According to the results, the cultures are either used further for biochemical tests with commercially available tests or automated systems (USDA-FSIS, 2019). Alternatively, follow AOAC Official Method 967.27 or "Edwards and Ewing's Identification of Enterobacteriaceae (USDA-FSIS, 2019).

***Campylobacter* microbial analyses.** Similar to *Salmonell*a analyses, *Campylobacter* quantification and presence is performed on 30 ± 0.6 mL sample rinse fluid from whole bird and part rinses and 25 mL pre-moistened poultry carcass sponges (USDA-FSIS, 2016c; **Table 3**). The rinse fluid and sponge buffer can either be directly plated for quantification or enriched for presence/absence testing (MLG 41.04). For direct plating, 250 µL of the sample is spread plated on Campy-Cefex Agar and incubated microaerophilically for 48 ± 2 hours at 42 ± 1.0 °C (MLG 41.04). Enrichment of the rinse fluid is performed by adding 30 mL 2X Blood-Free Bolton Enrichment Broth (BF-BEB) to 30 mL rinsate and incubating microaerophilically for 48 ± 2 hours at 42 ± 1.0 °C (USDA-FSIS, 2016c). After incubation, 5 µL of the enriched sample is used in a BAX® PCR assay screen test following the BAX® System User's Guide for testing (USDA-FSIS, 2016d). If the enrichment samples are negative on the BAX® system, they will

are presumed negative and testing is complete on those samples; however, if the sample is presumed positive, the sample is utilized for downstream analyses to determine if they are true positives (USDA-FSIS, 2016d).

The enriched samples that were determined positive on the BAX®, 10 μL of the enrichments are streaked for isolation on Campy-Cefex Agar and incubated microaerophilically for 48 ± 2 hours at 42 ± 1.0 °C (USDA-FSIS, 2016c). Colonies that are translucent or mucoid, glistening and pink in color, flat or slightly raised (may vary in size) are selected and utilized for confirmation analyses such as microscopy and latex agglutination immunoassays (USDA-FSIS, 2016c). As such, a colony is examined with an oil immersion microscope using phase contrast microscopy with the morphology of *Campylobacter jejuni, coli, lari* being corkscrew with darting motility (USDA-FSIS, 2016c). The agglutination assays, PanBio-Campy (jcl) or F46 Microgen *Campylobacter* assays, use the same colony that was used for microscopy and confirm presumptive colony using manufacturer's instructions (USDA-FSIS, 2016c).

16S SEQUENCING AND POULTRY PROCESSING

As previous sections of the current review have indicated, the poultry industry relies heavily on the quantification and determination of the presence of sanitary indicators and pathogens in poultry carcass and part rinses and sponges. These analyses range from the use of Petrifilms® to rapid molecular detection which can vary in completion time (USDA-FSIS, 2015b, 2019, 2016c,d). On average, these assays take at least 24 to 48 h for quantification of sanitary indicators but can take an upwards of 5 days for enrichment and confirming a presumed positive sample (USDA-FSIS, 2015b, 2019, 2016c,d). This time frame does not include the time it takes

to ship the samples to the main laboratory. Thus, the entire process is time consuming and laborious but necessary at the moment to determine the risk of the products to consumers.

More recently, it has been proposed to include more rapid molecular techniques into poultry processing monitoring in order to determine more accurately and rapidly the risk of poultry products on consumers (Feye et al., 2020; Ricke, 2020; Ricke et al., 2019). At the moment, there are two potential uses for molecular methodology and analyses within poultry processing: 1) rapid detection and quantification of foodborne pathogens and indicator organisms and 2) biomapping to evaluate multi-hurdle technology implemented throughout processing (Feye et al., 2020). Along these lines, FSIS is already making momentous strides in incorporating molecular screening technologies in the detection of pathogens such as *Salmonella* and *Campylobacter* (USDA-FSIS, 2019, 2016d).

Currently, the industry utilizes end point PCR to determine the presence of *Salmonella* and *Campylobacter* using 3M™ Molecular Detection System and the BAX® PCR System, respectively (USDA-FSIS, 2019, 2016d). In the future, it would be expected that the industry and FSIS implement more quantitative approaches of PCR beyond end point PCR. Quantitative PCR (qPCR), digital PCR (dPCR), and reverse transcriptase quantitative PCR (rt-qPCR) are all viable options to implement in poultry processing as they are quantitative and less time consuming than tradition microbiological methods (Ricke et al., 2019). In addition to end point PCR, FSIS and poultry integrators also utilize whole genome sequencing (WGS) to determine the microorganisms isolated off of poultry carcass and part rinses and carcass sponges (USDA-FSIS, 2020). The WGS of bacterial isolates in FSIS facilities is currently sequenced on Illumina MiSeq instruments (Illumina, San Diego, CA, USA) using standard Illumina practices (USDA-FSIS, 2020). The specific details regarding the library preparation can be found in chapter 42 of

the Microbiology Laboratory Guide provided by FSIS (USDA-FSIS, 2020). WGS can be used for more than just identification of the bacterial isolate but can be used to determine antimicrobial resistance patterns among the genome and epidemiological characterizations of the isolates. Specifically, this concept has been applied to *Campylobacter* isolates in several studies (Biggs et al., 2011; Revez et al., 2014; Cha et al., 2016; Clark et al., 2016; Zhao et al., 2016; Llarena et al., 2017; Joensen et al., 2018). Due to the presence of such sequencing technology and methodologies already occurring within FSIS testing, sequencing on platforms such as the Illumina MiSeq could be used for more than WGS, such as the sequencing of 16S rRNA gene of bacteria.

Sequencing the 16S rRNA gene of bacteria has become an informative tool in distinguishing the ecology of the microbiota of poultry and other food animals. Recently, Feye et al. (2020c) outlines the advantages and nuances of utilizing microbiota sequencing in poultry processing and how it's use may benefit food safety practices. In addition, there have been strides in standardizing the sequencing of poultry rinsate samples in order to utilize poultry rinses as a means to create a biomap of poultry processing (Feye and Ricke, 2019). In fact, Handley et al. (2018) utilized 16S rDNA sequencing of the (hypervariable) HV3 to HV4 region of the 16S rRNA gene on an Illumina MiSeq to develop a biomap of poultry processing facilities by utilizing carcass rinsates collected at multiple locations within poultry processing.

Microbiota data provides Alpha and Beta Diversity and compositional differences. Although these are relative measures, they can provide FSIS personnel with a more complete microbial profile than what APC or other sanitary indicators could provide (Blevins et al., 2017). Kim et al. (2017) utilized colonies from both APC and selective media to determine the microbiota selected on the Petrifilm™ media and how that differed from the microbiota of WBCR

before and after chickens were dipped in antimicrobial treatments. The study demonstrated the potential of sequencing APC Petrifilm™ and Camy-Cefex agar in determining the association of phyla and genera associated with the presence of *Salmonella* and *Campylobacter* positive rinses. This monitoring could allow FSIS and processors multiple methods of monitoring the ecological changes in the carcass rinses and subsequent plate growth in order to reduce pathogen contamination at the consumer level. Although, sequencing of the microbiota is still a relatively expensive process, the fact that Illumina MiSeq are already utilized by FSIS personnel to WGS suspected isolates is a promising note that such technologies as 16S microbiota sequencing and quantitative PCR will be utilized among FSIS and other federal agencies in the future.

CONCLUSIONS AND FURTHER CONSIDERATIONS

Although there is growing interest in the use of acidifiers to reduce common foodborne pathogens, there is limited research on how the acidifiers affect the microbiota and how the acidifiers affect the microbiota of poultry parts when inoculated with common foodborne pathogens. In the future, it will be necessary to determine not only the exact effect these antimicrobials have on the microbiota of poultry carcasses and parts and assess the impact these antimicrobials have on the microbiota when there is a high microbial or pathogen load on carcasses. Understanding these interactions will help determine the best antimicrobial to ensure a stable and healthy microbiota under a multitude of conditions.

In addition to determining these effects, future research must establish a link between the host microbiota's composition and the proceeding meat produced from the host, whether the host is poultry or other livestock. This link is necessary to better eliminate pathogens or from entering processing facilities. To determine this link, bio-mapping or mapping the microbiota

changes from the hatchery, growout, and processing will be necessary. Bio-mapping will allow integrators to determine critical control points in locations across poultry processing and determining indicator organisms.

Lastly, there is an increasing need to implement multi-hurdle technology during second processing in the form of part dips and sprays. In addition, there is a need to investigate ways to mitigate the detrimental effects that PAA has on employees and equipment without losing efficacy against pathogens and total microbial load. This may involve introducing antimicrobials that reduce PAA vapor or applying alternative acidifiers during first and second processing. Thus, the objective of the current dissertation was to investigate various antimicrobials, such as organic and inorganic acids, as short duration dips and sprays as means to reduce common pathogens (*Salmonella*, *Campylobacter jejuni*, and *E. coli*) among raw chicken carcasses and parts. Overall, the data presented in the current dissertation demonstrates the potential use of novel antimicrobials as short duration dips and sprays at mitigating foodborne pathogens associated with raw poultry products during first and second processing. With this information, poultry processors will be better equipped at providing safe products to consumers.

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TABLES AND FIGURES

Table 1. Common indicator organisms tested for on raw poultry carcasses.

Indicator or pathogen	Control Organism	Media	Incubation	Colony Appearance	Countable Range (CFU)
Aerobic Plate Count (APC)	*S. aureus* ATCC 25923 or equivalent	APC Petrifilm™	35 ± 1 °C for 48 ± 3 hours	Red regardless of size or color intensity	25 to 250
		APC Pour Plates	36 ± 1 °C for 48 ± 3 hours	All	30 to 300
Escherichia coli and Coliforms	*E. coli* ATCC 25922 or equivalent	*E. coli*/ coliform Petrifilm™	35 ± 1 °C for 24 ± 2 hours	*E. coli*: blue to red-blue with entrapped gas Coliforms: red with gas Total Coliforms: *E. coli* plus coliforms	15 to 150
Enterobacteriaceae	*Klebsiella pneumonia* ATCC 13883 or equivalent	EB Petrifilm™	35 ± 1 °C for 24 ± 2 hours	Red with gas bubbles or yellow with or without gas	15 to 100
Lactic Acid Bacteria	*Lactobacillus plantarum* ATCC 14917 or equivalent	APT Pour Plate	20 ± 1 °C for 4 to 5 days	White opaque	30 to 300

Table 2. *Salmonella* screening procedures for Whole Bird Carcass Rinses (WBCR), part rinses, and poultry carcass or environmental sponge samples.

Source	Sample	Pre-Enrichment	3M™ Molecular Detection	Enrichment	Selective Streaking	Screening	Biochemical Procedures
Whole Bird or Part Rinse	30 ± 0.6 mL rinse fluid	30 ± 0.6 mL of the sample in 30 ± 0.6 mL of sterile BPW, mixed well, and incubated at 35 ± 2 °C for 20 to 24 h	Standard protocol. If positive proceed with enrichment. If negative, report as negative.	0.5 ± 0.05 mL transferred to 10 mL TT 0.1 ± 0.02 mL transferred to 10 mL RV	Enriched samples are streaked for isolation onto BGS and DMLIA with 10 µL loop and ncubated at 35 ± 2 °C for 18 to 24 h	Select colonies and stab the butts and streak TSI and LIA slants simultaneously and incubate at 35 ± 2 °C for 24 ± 2 h	Use commercially available biochemical tests or automated systems
Poultry Carcass or Environmental Sponge	pre-moistened sponge with 10 mL buffer	50 mL of BPW added to sponge to bring total volume to 60 mL and incubated at 35 ± 2 °C for 20 to 24 h		Incubated at 42 ± 0.5 °C for 22 to 24 h or in water bath at 42 ± 0.5 °C for 18 to 24 h	Smooth, opaque, and pink colonies with a red edge (BGS) and purple colonies with or without black centers (DMLIA) selected	Use Table 2 of MLG 4.10 to determine if the reactions in TSI and LIA slants warrant biochemical tests or discard	Alternatively, follow AOAC Official Method 967.27 or "Edwards and Ewing's Identification of Enterobacteriaceae

Table 3. *Campylobacter* screening procedures for Whole Bird Carcass Rinses (WBCR), part rinses, and poultry carcass sponge samples.

Source	Whole Bird or Part Rinse	Poultry Carcass Sponge
Sample	30 ± 0.6 mL rinse fluid	pre-moistened sponge with 25 mL buffer
Direct Plating	Spread plate 250 µL of sample on Campy-Cefex Agar and incubate microaerophilically for 48 ± 2 hours at 42 ± 1.0 °C	
Enrichment	Add 30 mL 2X BF-BEB to 30 mL rinsate and incubate microaerophilically for 48 ± 2 hours at 42 ± 1.0 °C	Add 25 mL 2X BF-BEB to 30 mL rinsate and incubate microaerophilically for 48 ± 2 hours at 42 ± 1.0 °C
BAX® PCR Assay Screen Test	Using 5 µL of enriched sample, follow BAX® System User's Guide for testing. Enrichment samples that test negative will be determined negative while positive will be further examined	
Selective Streaking	Streak for isolation the enriched samples using a 10 µL loop and incubate microaerophilically for 48 ± 2 hours at 42 ± 1.0 °C	
Examination and Selection of Colonies	Select at least one colony that is translucent or mucoid, glistening and pink in color, flat or slightly raised (may vary in size)	
Confirmation Analyses	**Microscopy**: examine a colony with an oil immersion microscope using phase contrast microscopy. Morphology of *Campylobacter jejuni, coli, lari* are: • corkscrew morphology and darting motility **Latex Agglutination Immunoassay**: use same colony used for microscopy and confirm presumptive colony with PanBio-Campy (jcl) or F46 Microgen *Campylobacter* assays using manufacturer's instructions	

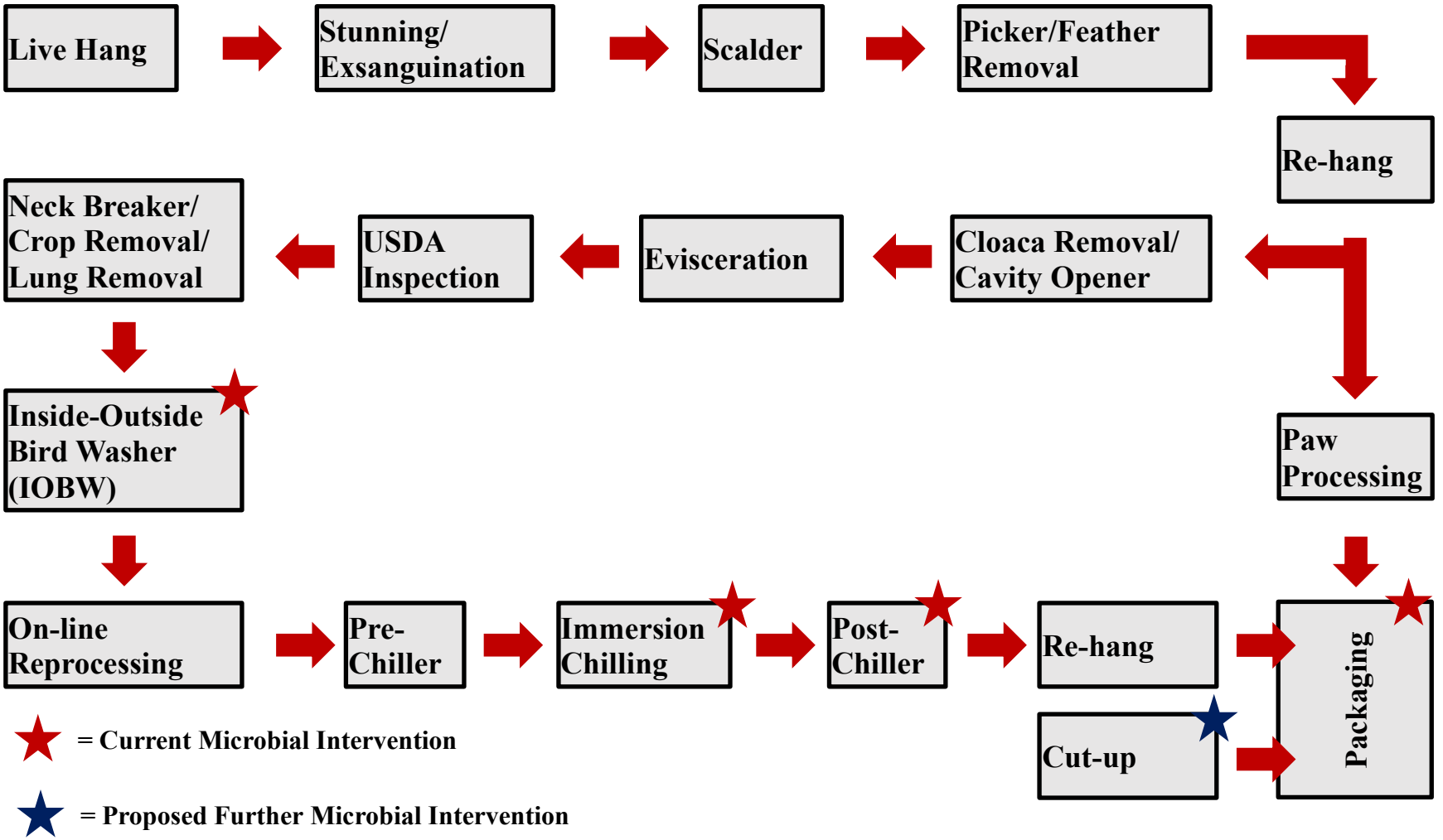

Figure 1. Typical flow of a commercial processing facility and conventional antimicrobial intervention locations.

CHAPTER 2

The Addition of Viriditec™ Aqueous Ozone to Peracetic Acid as an Antimicrobial Spray Increases Air Quality While Maintaining *Salmonella* Typhimurium, Non-Pathogenic *Escherichia Coli,* and *Campylobacter Jejuni* Reduction on Whole Carcasses

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ABSTRACT

Currently, the most utilized antimicrobial in processing facilities is peracetic acid, PAA; however, this chemical is increasingly recognized as a hazard to human health. Preliminary evidence suggests that ozone, when introduced in a specific manner, can reduce the noxious nature of PAA. Therefore, the objective of the current study was to evaluate the efficacy of TetraClean Systems aqueous ozone, O_3, in combination with PAA as an antimicrobial spray on whole chicken carcasses. This trial used 70 whole hen carcasses (7 treatments; 10 replications) that were inoculated in a 400mL cocktail containing *Salmonella*, *E.coli*, and *Campylobacter* (10^7CFU/mL) and allowed to adhere for 60min at 4 °C for a final concentration of 10^5 to 10^6CFU/g. The experimental 5sec (4x) spray treatments included: a no treatment negative control, TW; TW+O_3 (10ppm), TW+PAA (50ppm), TW+PAA (500ppm), TW+O_3+PAA (50ppm), and TW+O_3+PAA (500ppm). During treatment application, ambient PAA vapor was measured with a ChemDAQ Safecide PAA vapor sensor. After treatment, carcasses were immediately rinsed in 400mL of nBPW for 2min. Following rinsing, the dot method was utilizing for enumeration with 10µL of rinsate being serially diluted, plated on XLD and mCCDA agar, and incubated aerobically at 37˚C for 24h or microaerophilically at 42˚C for 48h. Log-transformed counts were analyzed using ANOVA in JMP 14.0. Means were separated using Tukey's HSD when $P \leq 0.05$. There was a significant treatment effect among *Salmonella*, *E. coli*, and *Campylobacter* counts, and a significant treatment effect among ambient PAA ($P<0.05$). TW+O_3+PAA (500ppm), reduced *Salmonella* significantly compared to TW (5.71 and 6.30 Log_{10} CFU/g). Furthermore, TW+PAA (500ppm), reduced the presence of *E. coli* significantly compared to TW or no treated control (5.57 and 6.18 Log_{10} CFU/g). Also, TW+PAA (50ppm), TW+PAA (500ppm), and TW+O_3+PAA (500ppm) significantly reduced *Campylobacter* compared to carcasses not treated (4.80, 4.81, and

4.86 Log_{10} CFU/g). Lastly, the addition of ozone significantly reduced the ambient PAA when O_3 was added to 500 ppm of PAA, as TW+O_3+PAA (500ppm) produced less ambient PAA than TW+PAA (500ppm) (0.052 and 0.565ppm). In conclusion, the addition of ozone to PAA may demonstrated the ability to effectively reduce ambient PAA, thus increasing employee safety.

Keywords: poultry, spray cabinet, aqueous ozone, peracetic acid, pathogenic reduction, ambient peracetic acid vapor

INTRODUCTION

Currently, the United States poultry industry utilizes peroxyacetic acid, also known as peracetic acid (PAA), to decontaminate poultry within poultry processing facilities. The disinfectant, PAA, is a product of the reaction between acetic acid and hydrogen peroxide. It is a colorless acid with a strong odor. The bactericidal effect of PAA is due to it being a strong oxidizing agent of the cell membrane and other cell components (Oyarzabal, 2005). However, this chemical is corrosive and unstable. PAA is one of the most common antimicrobials used in poultry processing facilities, as it is applied in the chillers (pre-chiller, chiller, post-chiller), part dips, spray cabinets, in and out bird washes at concentrations typically ranging from 200 to 2,000 ppm; however, it has been known to be a hazard to human health (NAS, 2010). PAA is reported to be an irritant to the upper respiratory tract, eye, and skin (Fraser and Thorbinson, 1986; Janssen, 1989a,b; Janssen and Van Doorn 1994; Merka and Urban 1978). Direct contact in the eye and skin can be avoided if the proper personal protective equipment (PPE) is worn, but there are limited approaches to protect the upper respiratory tract from the vapors emitted from PAA (American Thoracic Society, 1996).

Currently, there is no OSHA (Occupational Safety and Health Administration) limit on the acute or long-term exposure limit of PAA during shifts for employees. However, other governing bodies have set limits and guidelines for the exposure of PAA vapor. In 2014, the American Conference Governmental Hygienists (ACGIH) set a threshold limit of 0.04 ppm as the 15-minute Short Term Exposure Limit (STEL) (ACGIH, 2016). Furthermore, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGLL Committee), during an 8 h exposure time, set AEGL-1, 2, and 3 limits to 0.17, 0.51, and 1.3 ppm of PAA vapor, respectively (NAS, 2010), with firm limits of total exposure

time for AEGL-1 and 2 limits at 0.17, and 0.51 ppm (NAS, 2010). An exposure at AEGL-1 produces noticeable discomfort, and irritation, with reversible effects upon removal from exposure site. AEGL-2 exposure produces irreversible or other long-lasting serious health conditions and may impair one's ability to escape. Lastly, an AEGL-3 exposure results in life-threatening health conditions and can result in death. As of 2015, NIOSH published a draft Immediately Dangerous to Life or Health (IDLH) value for 0.64 ppm (NIOSH, 2015).

As there are no current strategies employed to reduce the ambient PAA in a processing facility, there is a significant need to develop and easily implement measures to prevent PAA vapor exposure. One novel approach is to utilize the commercial aqueous ozone product (Viriditec™, TetraClean Systems LLC., Omaha, NE) to distribute aqueous ozone directly to peracetic acid, as preliminary evidence suggests, the addition of ozone can reduce the noxious nature of PAA (data not shown).

Previously, chlorine was utilized as the primary sanitizer in processing facilities but has been replaced in the last decade with PAA. Studies have demonstrated that 85 ppm of PAA has the capability to reduce the incidence of *Salmonella* and *Campylobacter* by 92 and 43% on poultry carcasses when applied in a commercial poultry chiller (Bauermeister et al., 2008a). Whereas, 30 ppm of chlorine was only capable of reducing *Salmonella* and *Campylobacter* by 43 and 13%, when used in a poultry chiller (Bauermeister et al., 2008a). Furthermore, PAA has been shown to mitigate *Staphylococcus* spp., *Listeria* spp., and generic *Escherichia coli* more than 5- Log_{10} CFU regardless of the food source being evaluated, (Brinez et al., 2006). Bauermeister et al., (2008b) reported the reduction of *Salmonella* and *Campylobacter* to be greater in carcasses chilled in solutions containing 200 ppm of PAA compared to those chilled in 30 ppm chlorine, ≈1 Log_{10} reduction. Therefore, it is imperative to mitigate the noxious nature

of PAA without reducing the bactericidal effects of PAA in poultry processing facilities. Thus, it was the objective of the current experiment to evaluate the efficacy of a commercial aqueous ozone (O_3) alone or in combination with peracetic acid (PAA) on reducing ambient PAA and poultry pathogens when applied as an antimicrobial spray on whole chicken carcasses.

MATERIALS AND METHODS

Viriditec™ aqueous ozone generation

TetraClean's Viriditec™ aqueous ozone system has been characterized as a patented technology that utilizes Nannobubble Technology to combine water and ozone to yield aqueous ozone. In the current study the system produced ozone gas which was injected into a water stream and further infused through the systems patented configuration and mixing technology. A Q46H/64 Dissolved Ozone Monitor (Analytical Technologies Industries, Collegeville, PA, USA) was utilized to measure the specific ozone levels generated from the Viriditec™ aqueous ozone system. The result was an aqueous ozone solution that contained 10 ppm of dissolved ozone in solution.

Carcass procurement and indigenous pathogen screening

A total of 70 whole hen carcasses (7 treatments; 10 replicates) with an average weight of 1749.87 g were obtained from a free-range poultry facility immediately after processing and were void of any antimicrobial treatments prior to the onset of the current experiment. A review by the institutional animal care and use committee (IACUC) was exempted because the birds were raised in an off-campus commercial farm operation and the current study was restricted to microbiological evaluation of bird carcasses selected for study. Immediately following evisceration, on the same day all 70 carcasses were shipped on ice and upon arrival at the

University of Arkansas Center for Food Safety one carcass was screened for the background indigenous presence of *Salmonella*, *E. coli*, and *Campylobacter*. The remaining carcasses were stored at 4 °C refrigeration until the onset of the study which began within 24 h post slaughter.

Inocula Preparation and Inoculation

Prior to the study, a frozen stock of *Salmonella* Typhimurium (UK-1), *E. coli* (J53), and *Campylobacter jejuni* were streaked to isolation on respective mediums and incubated either aerobically at 37˚C for 24 h or microaerophilically at 42˚C for 48 h. Subsequently, one isolated colony from the incubated plates were streaked onto fresh medium and incubated under the previously mentioned conditions. Simultaneously, an isolated colony was streaked onto Xylose Lysine Deoxycholate (XLD; HiMedia, West Chester, Pennsylvania, USA) and modified Charcoal-Cefoperazone-Deoxycholate agar (mCCDA; HiMedia, West Chester, Pennsylvania, USA) for confirmation and incubated either aerobically at 37˚C for 24 h or microaerophilically at 42˚C for 48 h. Following confirmation, isolated colonies from the incubated media were then transferred to 40 mL of fresh Luria-Bertani Broth and Mueller Hinton Broth (Hardy Diagnostics, Irving, Texas, USA) and incubated under previously mentioned conditions in a shaking incubator at 200 rpm for 12 to 16 h. The resulting cultures of 3×10^7 CFU/mL of *Salmonella* Typhimurium (UK-1), *E. coli* (J53), and *Campylobacter jejuni*, respectively.

Directly following the overnight (12 to 16 h) incubation of the cultures, the cultures were spun down at 18,000 g for 5 min, decanted, and then washed twice in 1× Phosphate Buffered Saline (PBS; 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 per 1 L, with the pH adjusted to 7.4 with HCl). After the final wash, the pellet was resuspended in 400 mL of sterile PBS.

The carcasses were inoculated in a 400 mL cocktail containing 3×10^7 CFU/mL of *Salmonella* Typhimurium (UK-1), *E. coli* (J53), and *Campylobacter jejuni*. *Salmonella*, *E. coli*, and *Campylobacter* inocula were allowed to adhere at 4 °C for 60 min for a final attachment of 10^6, 10^6 and 10^5 CFU/g. Following the attachment period, the whole carcass weights were recorded, and the treatments were administered. The carcasses were independently placed into a spray cabinet constructed from a modified refrigerator (Model No. FFTR1814LW2, Fridgaire, Miami, Florida, USA) with 4 pressure nozzles that administered 500 mL of treatments via a high-pressurized spray (15 psi). The treatment was applied 4× with 5 sec on 5 sec off for a total duration of 20 sec treatment application. The treatments utilized in the current study were: a no-treatment negative control, tap water (TW); TW + O_3 (10 ppm), TW + PAA (50 ppm), TW + PAA (500 ppm), TW + O_3 + PAA (50 ppm), and TW + O3 + PAA (500 ppm). The commercial PAA utilized in the current study was Spectrum (FMC, Philadelphia, PA, USA). To reduce cross contamination, treated carcasses were placed into individual sterile poultry rinse bags (Nasco, Fort Atkinson, WI, USA) and allowed to rest for 2 min.

Microbial analysis

After the appropriated resting period, 400 mL of neutralizing Buffered Peptone Water (nBPW; 20.0 g of buffered peptone, 7 g of refined soy lecithin or equivalent, 1.0 g of sodium thiosulfate, 12.5 g of sodium bicarbonate, per 1 L of DI water; USDA FSIS, 2016) was poured directly on top and inside the carcasses. The carcasses were then manually agitated for 2 min in an 180° arcing motion. The carcasses were aseptically removed, discarded, and the subsequent rinsate was utilized for downstream analysis.

Salmonella, E. coli, and *Campylobacter* Enumeration

Rinsates were aliquoted to 15 mL conical tubes (VWR, Radnor, PA, USA) and subsequently 20 µL of rinsate was serially diluted to 10^{-6} in 180 µL of 1 × PBS via a flat bottom 96 well plate. The dot method was utilized in the current study where 10 µL of the rinsate was plated on Xylose Lysine Deoxycholate (XLD) and modified Charcoal-Cefoperazone-Deoxycholate agar (mCCDA), allowed to dry completely, inverted, and incubated aerobically at 37˚C for 24h or microaerophilically at 42˚C for 48h, respectively. On XLD, only colonies with black centers were considered as *Salmonella* and yellow colonies with surrounding yellow color change were considered as *E. coli*. On mCCDA, colony forming units with a silver metallic sheen were considered as *Campylobacter jejuni*.

Ambient PAA

To measure the ambient PAA vapor emitted from the treatment solution application, a SafeCide ChemDAQ sensor and meter was utilized (ChemDAQ Inc., Pittsburgh, PA, USA). The sensor was located directly outside the modified spray cabinet and measurements were recorded in real-time for each treatment application (n=10; N=70).

Statistical analysis

Each carcass was randomly assigned to a treatment prior to the onset of the study. The CFU of *Salmonella*, *E. coli*, and *Campylobacter* were log transformed and reported on a CFU of bacteria per gram of chicken basis (CFU/g). The data were analyzed using One-Way ANOVA in JMP 14.0 (SAS Institute Inc., Cary, NC, USA). Means were separated using Tukey's Protected HSD with a significant level of $P \leq 0.05$.

RESULTS

Quantification of *Salmonella, E. coli, and Campylobacter* recovered from treated carcasses

In the current experiment, there was a treatment effect for *Salmonella, E. coli,* and *Campylobacter* recovered from the treated inoculated carcasses ($P<0.05$). No treatments significantly reduced the concentration of *Salmonella* Typhimurium (UK-1) on whole carcasses compared to untreated carcasses (6.10 Log_{10} CFU/g of *Salmonella*, **Figure 1,** $P=0.0476$). However, those treated with TW + 500 ppm PAA + O_3 (5.71 Log_{10} CFU/g of *Salmonella*) had significantly lower Log_{10} CFU per gram of *Salmonella* than those treated with TW alone (6.30 Log_{10} CFU/g of *Salmonella*). Carcasses treated with TW + 500 ppm PAA + O_3 (5.71 Log_{10} CFU/g of *Salmonella*) did numerically possess the lowest Log_{10} CFU/g of *Salmonella* compared to all other treatments. However, the treatment of both TW + PAA and TW + PAA + O_3 did not differ significantly in recovered *Salmonella* (6.05, 5.86, 5.96, and 5.71 Log_{10} CFU/g of *Salmonella*).

Unlike the recovered *Salmonella, E. coli* (J53) recovered from the rinsates of carcasses treated with TW did not exhibit significantly higher counts compared to any of the other treated carcasses (**Figure 2**, $P=0.0126$). Carcasses treated with TW + 500 ppm of PAA (5.57 Log_{10} CFU/g of *E. coli*) yielded a lower load of *E. coli* than those not treated (6.18 Log_{10} CFU/g of *E. coli*). Similar to *Salmonella,* the recovery of *E. coli* did not differ from carcasses treated with TW + PAA and TW + PAA + O_3, regardless of PAA concentration.

The recovered load of *C. jejuni* (Log_{10} CFU/g of *Campylobacter*) was greatest in carcasses not treated (**Figure 3**; $P=0.0006$). The *C. jejuni* recovered from carcasses not treated (5.20 Log_{10} CFU/g of *C. jejuni*) did not differ from carcasses treated with TW, TW + O_3 and TW + 50 ppm PAA + O_3 (4.97, 5.00, and 4.96 Log_{10} CFU/g of *C. jejuni*). Further, the lowest load of

C. jejuni was recovered from carcasses treated with TW + 50 ppm PAA, TW + 500 ppm PAA, and TW + 500 ppm PAA + O_3 (4.80, 4.81, and 4.86 Log_{10} CFU/g of *C. jejuni*) which were significantly different from the untreated control.

Quantification of PAA vapor from treated carcasses

From the current experiment, it was determined that there was significant treatment effect on the production of ambient PAA (ppm) (**Figure 4**; $P<0.0001$). Further, it was demonstrated that the greatest production of ambient PAA was derived from the treatment solution TW + 500 ppm PAA (0.565 ppm of Ambient PAA). The treatment solutions consisting of, NT, TW, TW + O_3, and TW + 50 ppm PPA + O_3 did not produce any ambient PAA; however, the ambient PAA produced from those treatments was not different that the ambient PAA produced off of the treatments: TW + 50 ppm PAA and TW + 500 ppm PAA + O_3 (0.011 and 0.008 ppm of ambient PAA).

DISCUSSION

Impact of sanitizer treatments on *Salmonella, E. coli, and Campylobacter* inocula

In the current study, the addition of Viriditec™ aqueous ozone to a commercial PAA was utilized to determine if the addition of aqueous ozone possessed synergistic affects in mitigating three Gram-negative bacteria, commonly associated with poultry. Previously, it has been suggested that Gram-negative bacteria may be more sensitive to ozone than Gram-positive bacteria due to the greater presence of peptidoglycan in the cell wall of Gram-positive bacteria. Rey et al (1995) demonstrated the resistance to aqueous ozone was enhanced when N-acetyl glucosamine, a constituent of the peptidoglycan of bacterial cell walls, was present (pH 3 to 7).

In addition, the utilization of ozone has been demonstrated to possess the ability to disrupt the cell membrane and induce membrane permeability in *Salmonella* and *E. coli* ssp., respectively, thus weakening the bacterial cell wall and ultimately leading to cell death (Dave, 1999; Komanapalli and Lau, 1996). However, in the current study, there was no difference on the reduction of any of the Gram-negative bacteria, *Salmonella, E. coli*, or *Campylobacter*, between the use of tap water or aqueous ozone (10 ppm) when sprayed for 5 sec (4x) on whole hen carcasses.

The lack of effect of aqueous ozone, alone, may have been in part due to the short duration of the treatment application (5 sec; 4x) utilized in the current study. Previous research has generally utilized aqueous ozone for a longer duration of exposure, when evaluating bactericidal effects of food matrices. Gertzou et al. (2016) determined the addition of gaseous ozone for 1 h at 10 ppm to fresh chicken legs extended their shelf life 4 days more than the control when packaged in polyamide/polyethylene (PA/PE) packaging. Others have seen various levels of gaseous ozone (1, 0.1, and 33 ppm) applied for 5, 20 and 9 minutes, respectively, to be effective at reducing *L. monocytogenes* in water, fish, and poultry samples (Vaz-Velho et al., 2001; Fisher et al., 2000; Muthukumar and Muthuchamy 2013). Previously, aqueous ozone (4.5 ppm) in poultry chillers has demonstrated the potential to significantly mitigate total aerobes, psychrotrophs, coliforms, fecal coliforms, and *Salmonella* (78, 37, 91, 91, and 81%, respectively) on chicken broiler carcasses that had been chilled for 45 min compared to those not chilled (Sheldon and Brown, 1986). When aqueous ozone (0.5 to 6.5 ppm) was applied to poultry meat, in a separate study, it reduced the load of *Salmonella* Enteritidis by 0.6 to 4 Log_{10} CFU (Dave, 1999).

However, ozone has demonstrated to be more effective in reducing bacteria when suspended in pure water than in food products (Khadre et al., 2001). In agreement with the current study, where aqueous ozone did not have an effect on mitigating pathogen load when utilized alone at 10 ppm, Fabrizio and colleagues (2002) determined that the spray application of distilled water, 10 ppm aqueous ozone, 10% trisodium phosphate (TSP), 2% acetic acid (AA), 20 ppm sodium hypochlorite, electrolyzed oxidizing water (pH 2.4 to 2.7, 1,150 mV ORP, 50 ppm free CL) on chicken whole carcasses did not have an effect on *Salmonella* Typhimurium load on day 0. However, Fabrizio et al. (2002) did report that the submersion of electrolyzed oxidizing water, TSP, AA, and aqueous ozone reduced *Salmonella* Typhimurium to levels of detection only after selective enrichment on whole chicken carcasses. The submersion of whole carcasses in aqueous ozone has also demonstrated the potential to reduce total aerobic bacteria on d 0 and reduce *E. coli* and total coliforms on d 7 compared to whole carcasses not treated (Fabrizio et al., 2002). Thus, the complete submersion of carcasses may prove to have a greater bactericidal effect than sprays may have, as observed in the current study.

In the current study when PAA was utilized alone, both concentrations of PAA, 50 and 500 ppm, reduced *Campylobacter jejuni* load, but only 500 ppm reduced *E. coli*, and no concentration of PAA reduced *Salmonella* compared to the control. Although PAA has demonstrated to be an effective antimicrobial in previous research, mitigating pathogens by 2 Log_{10} or greater (Bauermeister et al., 2008b), the current research did not demonstrate the same efficacy. In fact, the current study demonstrated no treatments were capable of exhibiting practical reductions of pathogen load of 1 Log_{10} or greater. Unlike the current research, Bauermeister et al. (2008b) demonstrated that when PAA is applied in the chiller at 200 ppm *Salmonella* and *Campylobacter* load are reduced roughly below 2 and 2.5 Log_{10} CFU when

artificially inoculated with 10^6 CFU/mL of *Salmonella* and *Campylobacter*, respectively. However, as with aqueous ozone, the application method may play a part in the differences in pathogen reduction.

Other short duration antimicrobial treatments of poultry meat with PAA have demonstrated little consistency. Del Río et al. (2007) demonstrated that when chicken legs were dipped in solutions containing 220 ppm PAA (Inspexx 100, Ecolab, St. Paul, USA) for 15 s, *Enterobacteriaceae* and coliforms were reduced 0.24±0.19 and 0.28±0.84, respectively, on d 0; however, the reduction of bacteria on legs dipped in PAA was not significantly different than the legs treated with water. In contrast, Nagel et al. (2013) found that the post chill application of PAA at concentration 400 and 1,000 ppm for 20 s had the potential to reduce the load of *Salmonella* Typhimurium and *Campylobacter jejuni* on artificially contaminated chicken breasts by 2 Log_{10} CFU/mL.

Although, aqueous ozone and PAA, alone, did not mitigate pathogens as previous studies have shown, the combination of aqueous and PAA demonstrated an additive effect. This additive effect may be in part due to the byproducts of PAA and O_3. As PAA is the equilibrium product of acetic acid and hydrogen peroxide, when it dissociates acetic acid and hydrogen peroxide molecules are released. Hydrogen peroxide in aqueous solution is then capable of partially dissociating to hydroperoxide anion (HO_2^-) which is very reactive to ozone (Taube and Bray, 1940). Further, as acetic acid directly affects the pH, ozone is stabilized as ozone is more stable at a low pH (Khadre et al., 2001).

Overall, the current study demonstrated that the reduction of pathogens while utilizing the modified spray cabinet, while significant, was not extensive. Previously, it has been demonstrated that bacteria reside not only on the exposed muscle surfaces, but within the feather

follicles (Barnes and Impey, 1968). Thus, creating difficulties for antimicrobial treatments to properly disinfect poultry carcasses. This may explain the small reductions seen in the current study. This was also seen in a study performed by Sheldon and Brown (1986), who demonstrated less than a 1 Log_{10} reduction of total aerobic bacteria, psychrotrophs, coliforms and fecal coliforms, and *Salmonella* when broiler carcasses were chilled in aqueous ozone for 45 min compared to those not chilled.

Decomposition of PAA vapor

In the current study, the addition of aqueous ozone to PAA reduced the ambient PAA emitted when carcasses were treated in a modified spray cabinet. There is limited research on the proposed mechanism behind the reduction of PAA vapor, however, the authors have two proposed hypotheses to describe reduction in ambient PAA vapor. First, peracetic acid (CH_3COOOH) is formed from the equilibrium of hydrogen peroxide (H_2O_2 or OH) and acetic acid (CH_3COOH). From the reaction of the acetic acid radical (Reaction 1) and ozone (O_3, Reaction 2) result in the formation of the peroxyacetic acid radical which disproportionates (Reaction 3) to produce 70% hydrogen peroxide (H_2O_2) and other products: formaldehyde, glyoxylic acid, glycolic acid, and organic peroxides (Sehested et al., 1991) as seen in the following reactions:

$$1. OH + CH_3COOH \rightarrow 'CH_2COOH + H_2O$$

$$2. 'CH_2COOH + O_3 \rightarrow 'OOCH_2COOH$$

$$3.\ 2'OOCH_2COOH \rightarrow 70\%\ H_2O_2 + other\ products$$

The second explanation for the loss of ambient PAA vapor is that the ozone is being "robbed" an oxygen from the peracetic acid to reduce it to acetic acid in the gas state, preventing the OH radical formation as seen in the following reaction:

$$CH_3OOOH + O_3 \rightarrow CH_3OOH + 2O_2$$

CONCLUSIONS

In conclusion, the combination of 10 ppm of aqueous ozone, Viriditec™, and 500 ppm of PAA has the potential to mitigate the presence of *Salmonella* Typhimurium (UK-1), *Escherichia coli* J53, and *Campylobacter jejuni*. Furthermore, the combination of 10 ppm of aqueous ozone with 500 ppm of PAA demonstrated the ability of ozone to reduce the ambient PAA vapor by 90%, when compared to 500 ppm of PAA alone. Thus, the application of TetraClean's product Viriditec™ has the ability to enhance the safety for poultry processing employees. Although the current study demonstrated the promising capabilities of aqueous ozone and PAA, in combination, future research is necessary to develop an understanding of the impact the combination of aqueous ozone and PAA has on the shelf life of processed poultry and the subsequent changes in the microbiome.

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TABLES AND FIGURES

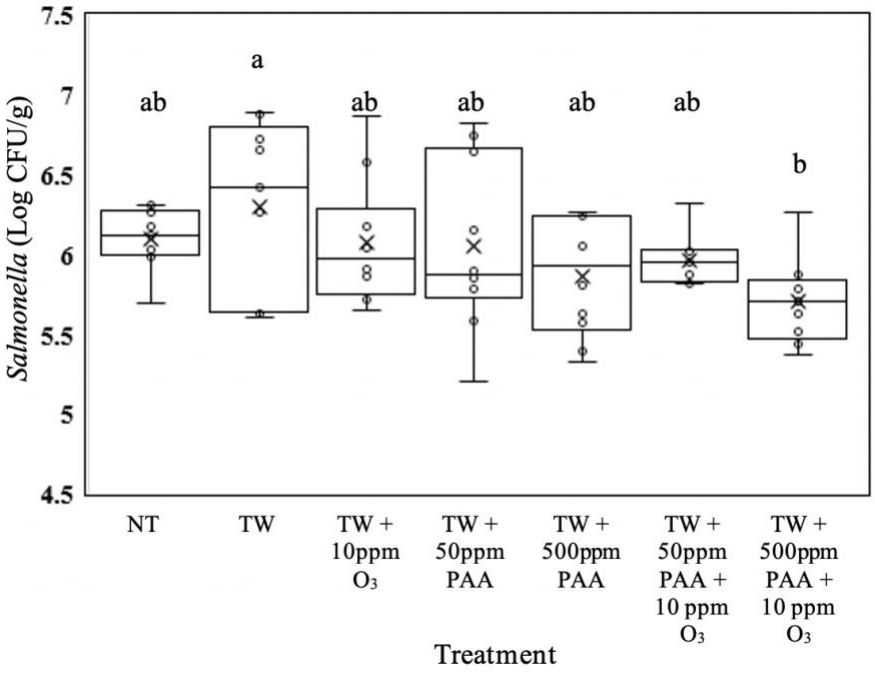

Figure 1. The effect of applying peracetic acid (PAA) alone and in combination with aqueous ozone at 10 ppm on the mean Log_{10} CFU/g of *Salmonella* Typhimurium UK-1 on whole hen carcasses[1,2]. Carcasses were inoculated with 10^7 CFU/mL of *Salmonella* for a final attachment of 10^6 CFU/g of *Salmonella* Typhimurium UK-1. Birds were then placed in a modified spray cabinet to be treated for 5 sec (4x) via a low pressurized spray. Immediately after, birds were rinsed in 400 mL of neutralizing buffered peptone water (nBPW) and subsequently plated to determine load of *Salmonella*.

[1]N=67, n=10, P=0.0476

[2]Means with different superscripts are considered significantly different (a-b)

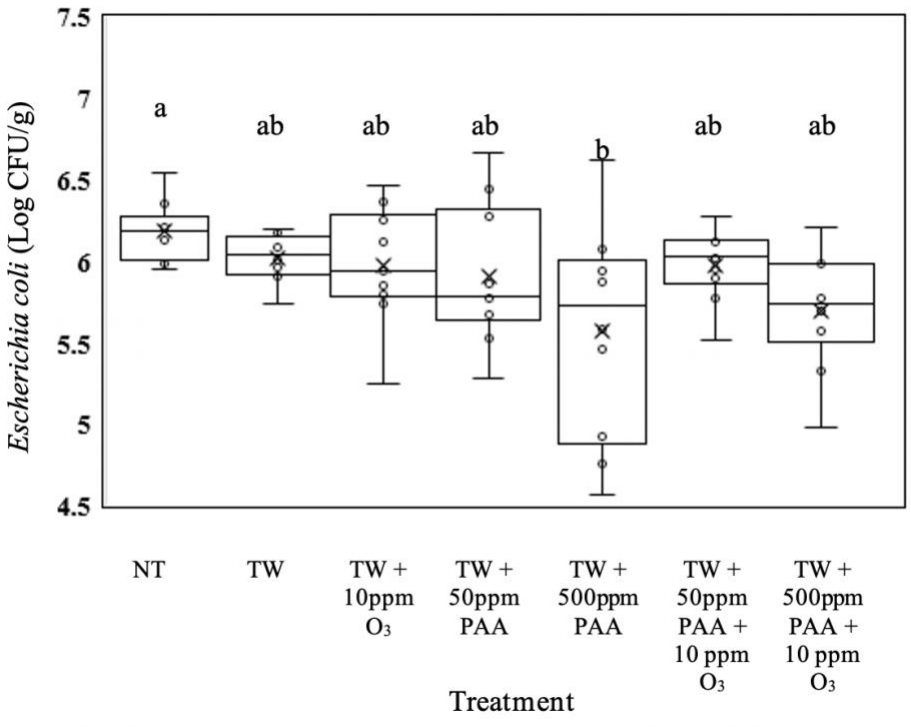

Figure 2. The effect of applying peracetic acid (PAA) alone and in combination with aqueous ozone at 10 ppm on the mean Log_{10} CFU/g of *Escherichia coli* J53 on whole hen carcasses[1,2]. Carcasses were inoculated with 10^7 CFU/mL of *E. coli* for a final attachment of 10^6 CFU/g of *Escherichia coli* J53. Birds were then placed in a modified spray cabinet to be treated for 5 sec (4x) via a low pressurized spray. Immediately after, birds were rinsed in 400 mL of neutralizing buffered peptone water (nBPW) and subsequently plated to determine load of *E. coli*.
[1]N=68, n=10, P=0.0126
[2]Means with different superscripts are considered significantly different (a-b)

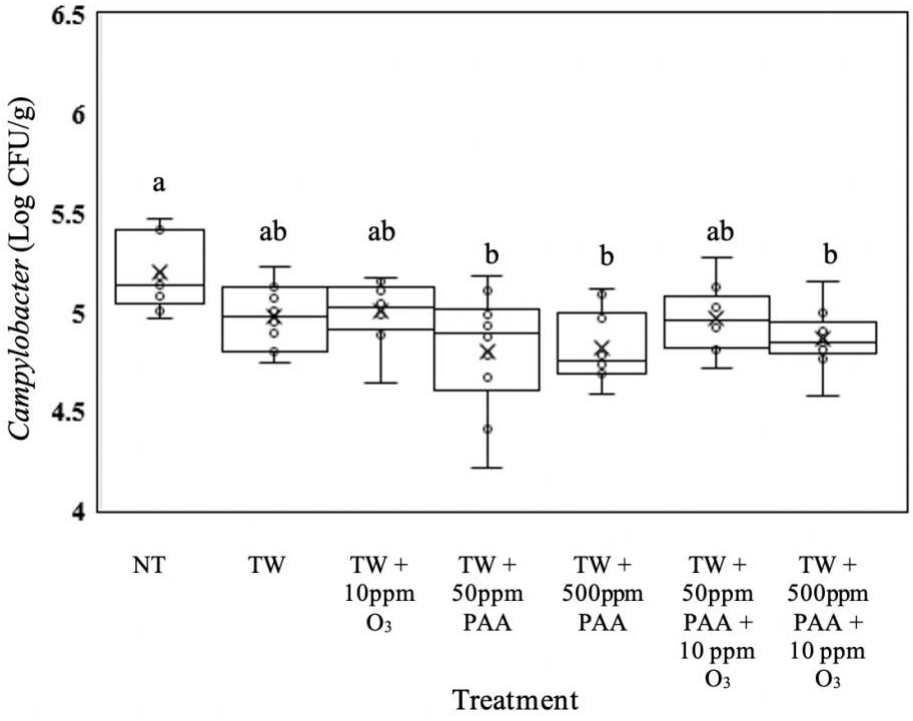

Figure 3. The effect of applying peracetic acid (PAA) alone and in combination with aqueous ozone at 10 ppm on the mean Log_{10} CFU/g of *Campylobacter jejuni* on whole hen carcasses[1,2]. Carcasses were inoculated with 10^6 CFU/mL of *C. jejuni* for a final attachment of 10^5 CFU/g of *Campylobacter jejuni*. Birds were then placed in a modified spray cabinet to be treated for 5 sec (4x) via a low pressurized spray. Immediately after, birds were rinsed in 400 mL of neutralizing buffered peptone water (nBPW) and subsequently plated to determine load of *Campylobacter*.
[1]N=69, n=10, P=0.0006
[2]Means with different superscripts are considered significantly different (a-b)

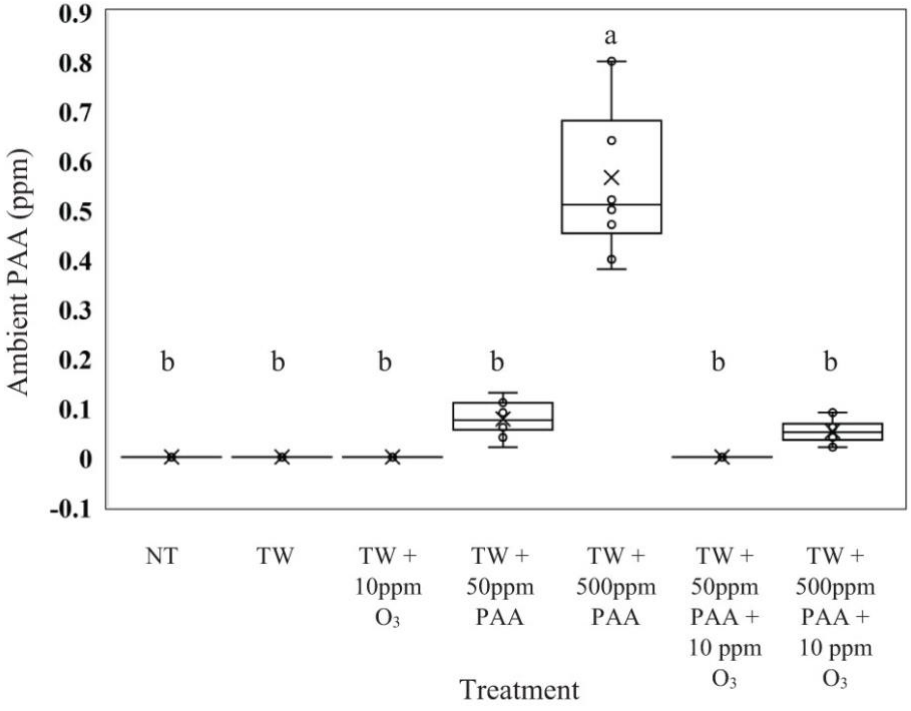

Figure 4. The effect of applying PAA alone and in combination with aqueous ozone at 10 ppm on whole hen carcasses on the mean ppm of the surrounding ambient PAA[1,2]. Carcasses were inoculated with *Salmonella* Typhimurium UK-1, *E. coli* J53, and *C. jejuni*. Birds were then placed in a modified spray cabinet to be treated for 5 s (4×) via a low pressurized spray. While the treatments were being applied, a ChemDaq SafeCide ambient PAA monitor and sensor, located outside the modified spray cabinet, was utilized to determine ppm of ambient PAA.
[1]N = 70, n = 10, $P < 0.0001$.
[2]Means with different superscripts are considered significantly different (a,b).

CHAPTER 3

The Efficacy of Sodium Bisulfate Salt (SBS) Alone and Combined with Peracetic Acid (PAA) as an Antimicrobial on Whole Chicken Drumsticks Artificially Inoculated with *Salmonella* Enteritidis

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ABSTRACT

The presence of *Salmonella* spp. on poultry products is one of the leading causes of foodborne illness in the United States. Therefore, novel antimicrobial substances are being explored as potential interventions in poultry processing facilities. The objective of the current study was to evaluate the efficacy of varying concentrations of sodium bisulfate salt, SBS, alone or in combination with peracetic acid, PAA, in 15 s whole part dips. Drumsticks (4 replications, 8 treatments, 3 days) were inoculated separately in a 400 mL solution of nalidixic resistant (NA) *Salmonella* Enteritidis (10^7 CFU/mL) and allowed to adhere for 60 to 90 min at 4 °C for a final concentration of 10^6 CFU/g. The experimental treatments included: a no treatment (control), and 15 s dips in 300 mL of tap water alone (TW) or with the addition of 1; 2; and 3% SBS; 1; 2; and 3% SBS+PAA. After treatment, drumsticks were stored at 4◦C until microbial sampling was conducted. On d 0, l, and 3, drumsticks were rinsed in 150 mL of nBPW for 1 min, 100 µL of rinsate was serially diluted, spread plated on XLT4+NA (20 µg/mL), and incubated aerobically at 37 °C for 24 h. Log-transformed counts were analyzed using a randomized complete block design (day) using One-Way ANOVA, polynomial contrasts, and pairwise comparisons with means being separated by Tukey's HSD with a significance level of $P \leq 0.05$. A treatment by day interaction ($P = 0.14071$) was not substantial. Thus, the treatment effect was investigated separately by days. Over time, a linear trend was observed in *S.* Enteritidis concentration when SBS was increased (1 < 2 < 3%). The concentration of *S.* Enteritidis was different between 1% SBS and 1% SBS+PAA on d 0. However, the level of *S.* Enteritidis was not different among drumsticks treated in 2 and 3% SBS and 2 and 3% SBS+PAA across d 0, 1, 3. The application of 3% SBS alone or in combination with 200 ppm of PAA is capable of reducing the presence of

Salmonella over a 3-d refrigeration period, potentially increasing the safety of poultry products for consumers.

Keywords: *S.* Enteritidis, sodium bisulfate salt, processing, poultry, part dips

INTRODUCTION

There is a need to enhance food safety strategies in the poultry industry. In the past 25 years, numerous steps have been taken to lessen the contamination of poultry products. The implantation of the Hazzard Analysis Critical Control Point (HACCP) in 1996 (Hulebak and Schlosser, 2002) and the Food Safety Modernization Act (FSMA) in 2011 (FDA, 2011) established rules and guidelines for the food industry to follow in order to reduce the intensity and amount of foodborne illnesses. Although these strategies have had an impact on the incidence of foodborne illness, they are not entirely sufficient alone. As a result, the Center for Disease Control and Prevention (CDC) has set limits and goals on microbial reduction in the poultry industry. One pathogen the CDC is particularly interested in is *Salmonella*, especially Enteritidis, with outbreaks linked to poultry and eggs (Finstad et al., 2012; Ricke, 2017). The CDC aims to lower *Salmonella* incidence by 5% by the year 2020 (FDA. 2004). In order to meet the CDC's goal, further intervention strategies must be integrated into the poultry industry.

In poultry processing facilities in the United States, one of the most commonly used methods to decontaminate poultry meat are antimicrobial washes and sprays at various locations during processing (McKee, 2012). Traditionally, chlorine and peracetic acid (PAA) are the antimicrobials of choice in the chiller, post chiller, spray cabinets, and part dips in poultry processing facilities (McKee, 2012). Recently, alternative antimicrobials have emerged for industrial application such as organic acids, phosphates, chlorine derivatives, and hydrogen peroxide solutions (Dincer and Baysal, 2004, Loretz et al., 2010). Antimicrobials that lower the pH of the surrounding environment are promising; however, Gram-negative species, such as *Salmonella*, are capable of developing resistance to organic acids due to the presence of LPS

(Shue and Freese, 1973). In addition, bacteria such as *Salmonella* can build a tolerance to stressful environments (Ricke, 2003).

Inorganic acids also have the potential to induce the resistance of pathogens, such as *Salmonella*, to a low environmental pH (Foster and Hall, 1991). Research conducted by Foster and Hall (1991) revealed that in order for inorganic acids to induce an acid tolerance response, new protein synthesis and the development of a pH homeostasis system is required. As a consequence, both the acid tolerance response and acid-shock proteins are required for *Salmonella* to survive acidic conditions induced by inorganic acids (Foster, 199; Foster, 200; Foster, Bearson et al., 1997). Sodium bisulfate (SBS), an inorganic acid, has demonstrated the ability to decrease the extracellular pH to around 2 with a pKa of 1.9 (Knueven, 1999). If there is a mild decrease in the extracellular pH, *Salmonella* reduce cytoplasmic pH to maintain a neutral state (Hill et al, 1995). This response is incredibly arduous for *Salmonella* and can lead to cell death (Hill et al., 1995). Because of these features, SBS demonstrates the potential to be a valid antimicrobial over other organic and inorganic acids as it has the ability to create a highly acidic environment that is not easily adapted to.

Historically, SBS has been utilized commercially as an acidifier on poultry litter. When used as a litter amendment at high doses, SBS not only reduced ammonia volatilization from poultry litter but also the presence of *Salmonella* (Payne et al., 2002). Additionally, the dietary inclusion of SBS decreased the shedding of *Salmonella* into the litter (Ruiz-Feria et al., 2011). Pertinent to poultry processing, SBS was used as an antimicrobial rinse agent on apples to reduce artificially inoculated *Listeria monocytogenes* (Kim et al., 2018). The Environmental Protection Agency has declared SBS as a safer choice as an antimicrobial and processing aid (EPA, 2018). In addition, according to the World Health Organization (WHO), the use of SBS is approved

with no restrictions on allowable daily intake in over 150 countries that recognize the WHO codex (WHO, 2007). Because of all of the preliminary data as well as the Environmental Protection Agency designation, it is evident that SBS could be a valid agent for reducing *Salmonella*, during multiple stages of poultry production, including processing. Therefore, it was the objective of the current study to investigate the potential of SBS as an antimicrobial intervention in poultry processing by determining the efficacy of SBS alone (1, 2, and 3%) or in combination with PAA (200 ppm) on mitigating the presence of a nalidixic acid (NA) resistant strains of Salmonella Enteritidis on whole chicken drumsticks.

MATERIALS AND METHODS

Whole Chicken Drumsticks and *Salmonella* Screening

A total of 96 drumsticks (8 treatments, 3 days, 4 replicates) were obtained from a local supermarket no longer than 24 h before the onset of the study and chosen based on the furthest expiration date. Prior to the start of the study, one drumstick was screened for the background unintended presence of *Salmonella*. One drumstick was rinsed in 150 mL of neutralizing Buffered Peptone Water [nBPW; (USDA-FSIS, 2016)] and manually agitated for 1 min. Subsequently, 100 µL of the rinsate was spread plated onto Xylose Lysine Tergitol 4 with the addition of 20µg/mL of NA (XLT 4 + NA) and incubated for 24 h at 37◦C.

Inocula Preparation

Before the onset of the current study, a frozen stock of *Salmonella* Enteritidis that was selected to be resistant to 20µg/mL of NA was streaked for isolation on Tryptic Soy Agar (TSA) and incubated for 24 h aerobically at 37◦C. Subsequently, one isolated colony from the incubated plate was streaked onto fresh TSA with the addition of 20µg/mL of NA (TSA + NA) and

incubated under the previously mentioned conditions. Simultaneously, an isolated colony was streaked onto XLT 4 plus 20µg/mL of NA (XLT 4 + NA) for confirmation and incubated aerobically at 37◦C for 24 h. An isolated colony from the incubated TSA + NA plate was then transferred to 40 mL of fresh Tryptic Soy Broth the addition of 20µg/mL of NA (TSB +NA) and incubated aerobically at 37 °C in a shaking incubator at 200 rpm for 12 to 16 h. The resulting cultures of *S*. Enteritidis were determined to contain 10^8 CFU/mL. Directly following the overnight (12 to 16 h) incubation of the *S*. Enteritidis cultures, the cultures were spun down at 18,000 g for 5 min, decanted, followed by washing twice in 1× Phosphate Buffered Saline (PBS). After the final wash, the pellet was resuspended in 400 mL of sterile DI water.

Inoculation

A separate inoculum of *S*. Enteritidis was utilized per replication of drumsticks (4 replications). Approximately 24 drumsticks were placed into sterile Whirl-Pak® (Nasco, Atkins, WI, USA) bags, where the inoculum was administered. The inoculated drumsticks were then massaged manually for 5 min and allotted 60 to 90 min at 4 °C to allow for attachment.

Treatment pH and Application

Following inoculation, the eight experimental antimicrobial treatments were created by combining TW with the appropriate amounts of SBS and 200 ppm of PAA (Sigma-Aldrich, St. Louis, MO, USA) to create proper concentration of the following treatments: a no treatment (control), a 15 s dip in 300 mL of tap water alone (TW), and TW with the addition of 1% SBS (TW+SBS1); 2% SBS (TW+SBS2); 3% SBS (TW+SBS3); 1% SBS + PAA (TW+SBS1+PAA); 2% SBS + PAA (TW+SBS2+PAA); and 3% SBS + PAA (TW+SBS3+PAA). Before drumsticks were treated, one replicate of each treatment was analyzed for pH with a SympHony pH meter and probe (VWR International, Radnor, PA, USA). Immediately following the attachment

period, the drumstick weights were recorded and the treatments were administered. The whole chicken drumsticks were independently dipped for 15 s into sterile Whirl-Pak bags containing the eight previously described treatments.

Microbial Analysis and *Salmonella* Enumeration

Following the 15 s dips, the drumsticks were transferred to new sterile Whirl-Pak bags and allowed to rest for 2 min. The drumsticks were evaluated immediately on d 0 or maintained at 4 °C for an additional 24 h (d 1) or 72 h (d 3) and then analyzed for *Salmonella* Enteritidis concentration. At each time point posttreatment, d 0, 1, and 3, the drumsticks were rinsed with 150 mL of sterile nBPW. The Whirl-Pak bags containing the 150 mL of nBPW and drumsticks were then manually agitated for 1 min and the resulting rinsates were collected for downstream analysis. Whole chicken drumstick rinsates were aliquoted to 1.5 mL microcentrifuge tubes and subsequently serially diluted to 10^{-6} (1:10 dilution factor). After diluting the samples, a 100 µL aliquot of each dilution was spread plated onto XLT 4 + NA (20µg/mL) agar in duplicate using sterile spreaders. The plates were then inverted and incubated aerobically for 24 h at 37 °C. Only the plated dilutions with CFU counts between 30 and 300 were enumerated and recorded. The following equation was utilized to calculate the CFU of *Salmonella* per gram of whole chicken drumstick:

$$\frac{\left(\frac{\text{Number of colonies}}{\text{0.1 mL plated}}\right)*\text{Dilution Factor}}{\frac{Drumstick\ Weight\ (g)}{Original\ Homogenate\ (mL)}} = CFU/\text{gram}\ of\ Drumstick$$

Statistical Analysis

Each drumstick was randomly assigned to a treatment and a time point prior to analyses. The CFU of *Salmonella* were Log_{10} transformed and reported on a Log_{10} CFU of *S.* Enteritidis per gram of drumstick basis (Log_{10} CFU/g). The data were analyzed as a Randomized Complete Block design with replications (n = 4) where the blocks are designated as day, d 0, 1, and 3,

using oneway ANOVA, polynomial contrasts, and pairwise comparisons. The differences were assessed statistically by using Tukey's protected HSD at 0.05 level of significance. Data analyses were performed in R version 3.3.2 (R Core Team, 2018).

RESULTS

In the current study, the overall one-way ANOVA did not produce a significant interaction between the block (day) and treatment ($P = 0.1407$). There was no main effect of day ($P = 0.0948$); however, there was a main effect of treatment ($P < 0.0001$; **Figure 1**). Overall, all treatments, TW, TW+SBS1, TW+SBS2, TW+SBS3, TW+SBS1+PAA, TW+SBS2+PAA, and TW+SBS3+PAA (6.22, 5.93, 5.76, 5.28, 5.42, 5.19, 5.27 Log_{10} CFU/g), reduced the population of *S*. Enteritidis on the drumsticks compared to the no treatment control (6.85 Log_{10} CFU/g). Also, drumsticks treated with TW+SBS3, TW+SBS2+PAA, and TW+SBS3+PAA (5.28, 5.19, and 5.27 Log_{10} CFU/g) had populations of *S*. Enteritidis 1 to 2 log CFU per g of drumstick lower than those treated with the Control, TW, and TW+SBS1 (6.85, 6.22, and 5.93 Log_{10} CFU/g).

Although there was not a significant interaction between treatment and day ($P = 0.1407$), the main effects of each treatment on each day were evaluated using polynomial contrasts and pairwise comparisons of treatments. Linear trends were investigated for the increasing concentrations of SBS within treatments: Control, TW, TW+SBS1 (SBS1 and SBS1+PAA), TW+SBS2 (SBS2 and SBS2+PAA), and TW+SBS3 (SBS3 and SBS3+PAA). By combining treatments with similar concentrations of SBS, negative linear trends of Log_{10} CFU of *Salmonella* per gram of drumstick occurred as SBS increased (1% < 2% < 3%) on d 0, 1, and 3 ($P = 0.0008$, $P < 0.0001$, $P < 0.0001$, **Figure 2**), where d 1 and 3 had distinct linear trends. On d 0, there was no detectable difference between TW+SBS 1, 2, and 3; however, on both d 1 and 3,

TW+SBS1 and TW+SBS3 had detectable differences with SBS3 yielding a lower population of *S.* Enteritidis per gram of drumstick (5.99 and 5.13 Log_{10} CFU/g on d 1 and 6.07 and 5.24 Log_{10} CFU/g on d 3).

To further evaluate the effects of the treatments, TW+SBS and TW+SBS+PAA treatments were examined separately alongside the control and TW in pairwise comparisons by day (Figures 3– 5). Although not statistically different, TW+SBS1+PAA and TW+SBS2+PAA treatments exhibited a lower presence of *S.* Enteritidis per gram of drumstick than those treated with TW+SBS1 and TW+SBS2 alone. Only on d 0 did TW+SBS1+PAA have a significantly lower population of *S.* Enteritidis than TW+SBS1 (4.80 and 5.72 Log_{10} CFU/g). Thus, both TW+SBS1+PAA and TW+SBS2+PAA treatments show a slight advantage over TW+SBS treatments. Drumsticks treated with TW+SBS3 and TW+SBS3+PAA did not yield the previously mentioned pattern. In fact, TW+SBS3+PAA (5.33, 5.28, and 5.19 Log_{10} CFU/g on d 0, 1, and 3, respectively) was not more effective at reducing the population of *S.* Enteritidis on drumsticks than TW+SBS3 (5.48, 5.13, and 5.24 Log_{10} CFU/g on d 0, 1, and 3, respectively), primarily as time continued.

Though the pH of the treatments was not statistically analyzed, due to insufficient replication, there was a clear numerical difference between TW and treatments (**Table 1**). The mean pH of the TW solution was 7.42; whereas, the mean pH of TW+SBS1, TW+SBS2, TW+SBS3, TW+SBS1+PAA, TW+SBS2+PAA, and TW+SBS3+PAA was 1.64, 1.45, 1.31, 1.51, 1.33, and 1.29, respectively. It should be noted that there was a numerical drop in the pH level of the TW+SBS1 and TW+SBS2 solutions when PAA was added. There was not a substantial drop in TW+SBS3 when PAA was added.

DISCUSSION

Although the authors did not evaluate the *Salmonella* recovered in this study for invasion or infectivity, data presented herein is promising as it demonstrates the possibility of SBS to improve food safety. Throughout the course of this study, SBS treatments reduced the concentration of *S.* Enteritidis below the typical infectious dose of ingested *Salmonella* to humans, 10^6 to 10^8 CFU, though the infectious dose of *Salmonella* can vary based on the matrix and the immune status of the affected individual (Chen et al., 2013). Despite the fact that the infectious dose has been reported to be much lower in other studies (McEntire et al., 2014), the Log_{10} reduction of 1.75 CFU/g on d 3 in the current study demonstrates the ability of SBS to effectively reduce pathogens to a potentially non-infectious dose for those who are not immunocompromised. Therefore, data presented herein warrants further investigations into whether or not treating poultry carcasses with SBS reduces salmonellosis.

The use of PAA has been shown to be an effective antimicrobial in poultry processing and its potential synergism with SBS (McEntire et al., 2014). Two commercial acidifiers, acetic acid and hydrogen peroxide, are individually effective against pathogens. In combining both acids, synergism is demonstrated and yields PAA (Brinez et al., 2006). This effect is likely driven by the acidification of the hydrogen peroxide by acetic acid (Brinez et al., 2006). It is possible that the acidification of PAA may enhance its antimicrobial properties. As SBS is a strong acidifier with a pKa of 1.9 (Knueven, 1999), it was important to evaluate the potential synergism between SBS and PAA. As demonstrated by the current study, the combination of 3% SBS and 200 ppm of PAA had a lower pH (1.29) and reduced *S.* Enteritidis more than 1.7 Log_{10} CFU of S. Enteritidis per g of drumstick. Consequently, the combination of SBS with PAA demonstrated similar trends in reducing *Salmonella* as other studies investigating the use of PAA

alone. The application of 85 ppm of PAA in a chilling tank resulted in a 1 Log_{10} reduction (91.8% reduction) of *Salmonella*-positive carcasses (Baurmeister et al., 2008). In other research investigating the effects of PAA as a post-chill dip (10 or 20 s), Nagel et al. (2013) reported a 2-Log_{10} reduction of *S.* Typhimurium among whole carcasses treated in a 20 s postchiller dip of 400 or 1000 ppm of PAA. Though the current study indicates the potential combinatorial effect of SBS and PAA, it also confirms the validity of SBS as an antimicrobial when used alone.

In previous studies, the use of SBS alone has demonstrated to be an effective antimicrobial agent. Previously, when SBS was applied as a pre-chill 90 s spray, it resulted in a 2.4 Log_{10} CFU reduction of *S.* Typhimurium (Li et al., 1997). Another study similar to the one conducted herein demonstrated a similar reduction of *Salmonella* was exhibited in research by Yang et al. (1998). Yang et al. (1998) showed that a 17 s application of 5% SBS in an inside-outside bird wash reduced *Salmonella* by 1.66 Log_{10} CFU per carcass. As demonstrated in the current and past studies, the acidification of water induced by SBS has the potential to effectively reduce foodborne pathogens, nevertheless, their use may be hindered due to the complex nature of poultry skin.

Although SBS proved to be a potent antimicrobial in the current study, there is a possible buffering effect of poultry meat and skin that may inhibit the competency of antimicrobials on poultry parts. Tan et al. (Tan et al., 2014a) demonstrated that the use of organic acids was capable of reducing *Salmonella* on chicken skin. However, the use of organic acids was only effective after the pH was reduced below 2, with acetic acid being the most efficacious (Tan et al., 2014a). This is consistent as chicken skin exhibits a stronger buffering effect than skin remnants and adipose tissue alone (Tan et al., 2014b). As a result, the efficacy of SBS may be inhibited, but the application of a surfactant in conjunction with SBS may counteract some of the

potential buffering ability of poultry skin and meat. This can potentially be overcome with the use of surfactants, which disrupt the surface topography of the skin and reduce the buffering effect. To illustrate the advantage of combining inorganic acids with surfactants, Kim and Day (Kim and Day, 2007) combined hydrogen peroxide, sodium bisulfate, and thymol, a surfactant, and evaluated the effect of the combined solutions on *E. coli* and *S.* Typhimurium. Kim and Day (2007) demonstrated the combination yielded a synergistic effect on MIC's as the combination lowered *E. coli* and *S.* Typhimurium three-fold greater than the MIC's of the individual components and reduced both pathogens by 2 Log_{10}. As a result, the incorporation of a surfactant such as thymol should be included in future studies to enhance the anti-pathogenic effects of SBS and PAA.

Another factor that may have also played a role in the inhibition of SBS was the acquisition of drumsticks from a local supermarket rather than acquiring them immediately after cut-up in a local processing plant. Therefore, the drumsticks purchased may have had antimicrobials such as PAA already applied to them. Although this may have influenced the results, the effect in the current study would be relatively small as untreated controls were utilized. Thus, any bias toward pretreatment of drumsticks should be accounted for when comparing the results. In addition, because of the small sample size (n = 4 per treatment) used in the current experiment, there is a room for future research to validate our current result with a larger sample size.

CONCLUSIONS

The current study demonstrated that there is a greater efficacy on *S.* Enteritidis reduction as SBS concentration is increased, with no visual discoloration and 3% SBS being most effective. Drumsticks treated with 3% SBS, 2% SBS with the addition of 200 ppm of PAA, and

3% SBS with the addition of 200 ppm of PAA had the most significant reductions of *S.* Enteritidis over a 3-d refrigeration period (1.7 Log_{10} CFU of *S.* Enteritidis per g of drumstick). The treatment of drumsticks with 3% SBS demonstrated the effective reduction of *S.* Enteritidis regardless of the presence of 200 ppm of PAA. Therefore, the application of 3% SBS as an antimicrobial part dip has the potential to be an advantageous tool to further reduce the contamination of poultry parts past the post-chilling stages of processing.

Further research should be conducted to determine the effects these specific concentrations of SBS have on the overall shelf life of poultry parts and on diminishing *Salmonella* when combined with a surfactant. In order to determine whether or not efficacy is consistent across all major poultry serovars, SBS needs to be tested with other *Salmonella* serovars. Lastly, studies that optimize the application of SBS to reduce *Salmonella* and determine other potentially synergistic compounds must be conducted. In doing so, investigators will continue to develop potent antimicrobials for poultry processing that will reduce the transmission of pathogens to the food supply.

AUTHOR CONTRIBUTIONS

All authors significantly contributed to the work of the current study. DD, JA, KF, and SR designed and prepared the current study with the assistance from CK. DD and JA conducted the experiment. JL analyzed the data and DD wrote the manuscript with assistance from KF, JL, CK, and SR.

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TABLES AND FIGURES

Table 1 The mean pH values of Sodium Bisulfate (SBS) salt and PAA, used alone or in combination when utilized as 15 sec dip solutions[1].

Treatment[2]	Mean pH of Part Dips[3]
Control	-
TW	7.42
TW + SBS1	1.64
TW + SBS2	1.45
TW + SBS3	1.31
TW + SBS1 + PAA[4,5]	1.51
TW + SBS2 + PAA[4,5]	1.33
TW + SBS3 + PAA[4,5]	1.29

[1]Contact time: 15 seconds based on Morris recommendation and current industry practice.
[2]There were eight antimicrobial treatments consisting of: a no treatment Control, tap water (TW), tap water with the addition of either 1, 2, or 3% sodium bisulfate (SBS) indicated as TW+SBS1, TW+SBS2, and TW+SBS3, and the combination of 1, 2, and 3% SBS with 200 ppm of peracetic acid (PAA), represented as TW+SBS1+PAA, TW+SBS2+PAA, and TW+SBS3+PAA.
[3]Mean pH of part dips was determined using a SympHony pH meter (VWR International, Radnor, PA). The mean pH of the solutions prepared for drumsticks was based on the four replicated experiments. There was no pH for the no treatment group (Control) as no solution was prepared.
[4]200 ppm concentration of PAA
[5]Peracetic acid solution; 39% PAA; Sigma-Aldrich, 3050 Spruce St., St. Louis, MO

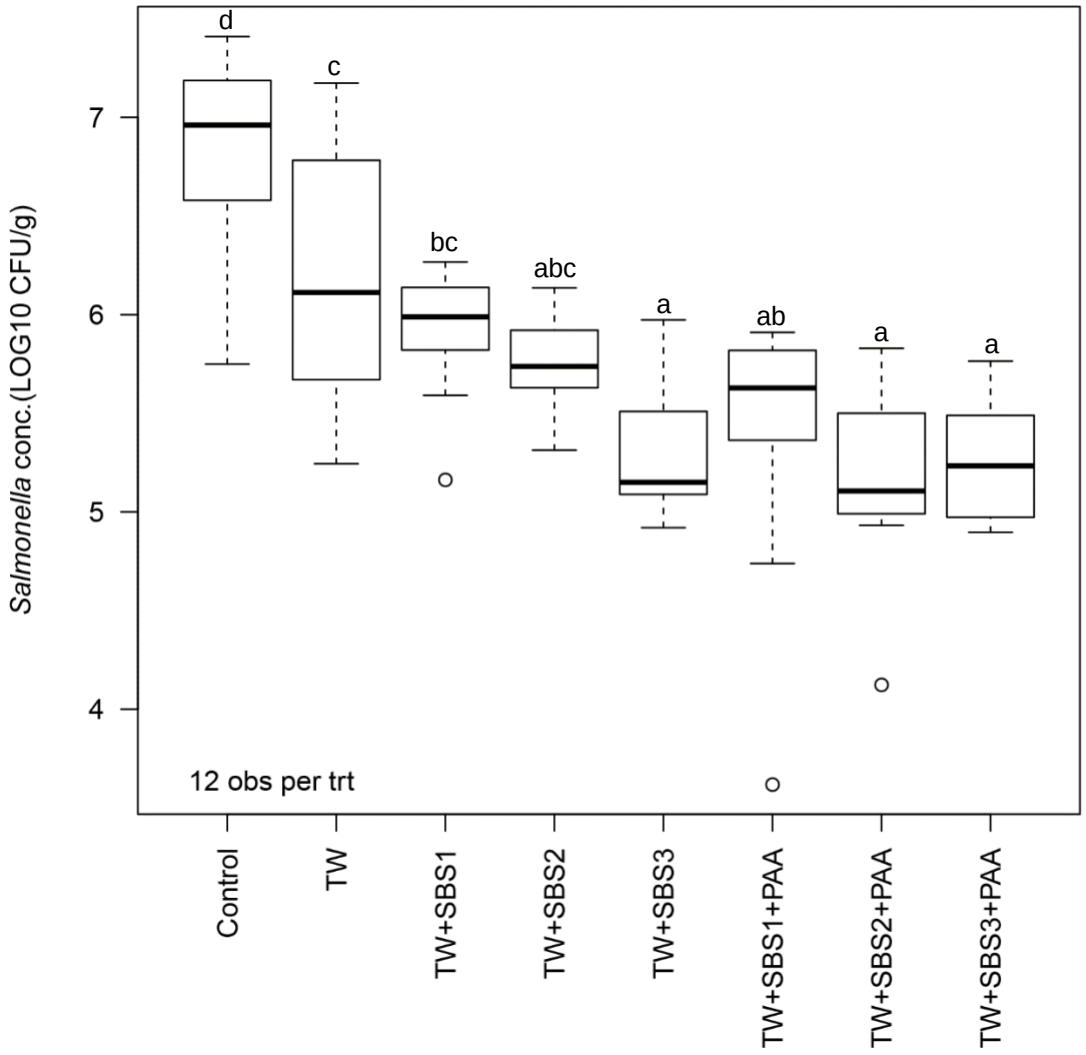

Figure 1. The effect of Sodium Bisulfate, SBS, and 200 ppm of peracetic acid, PAA, utilized alone or in combination as an antimicrobial 15 s part dip on the population of *Salmonella* Enteritidis on whole chicken drumsticks. In the current study, drumsticks were artificially inoculated with 107 CFU/g of S. Enteritidis and subsequently treated in 300 mL of antimicrobial treatments to identify the remaining population of *Salmonella*. There were eight treatments consisting of: a no treatment Control, tap water (TW), tap water with the addition of either 1, 2, or 3% SBS indicated as TW+SBS1, TW+SBS2, and TW+SBS3, and the combination of 1, 2, and 3% SBS with 200 ppm of peracetic acid (PAA), represented as TW+SBS1+PAA, TW+SBS2+PAA, and TW+SBS3+PAA. The current figure demonstrates the effect the treatments had on *Salmonella* population regardless of refrigeration (4 °C) time, d 0, 1, and 3. Individual standard error of the mean (SEM) for Control, TW, TW+SBS1, TW+SBS2, TW+SBS3, TW+SBS1+PAA, TW+SBS2+PAA, and TW+SBS3+PAA were 0.136, 0.180, 0.089, 0.066, 0.091, 0.190, 0.132, 0.087 Log_{10} CFU/g, respectively. F-test P-value < 0.0001; Pooled SEM = 0.129; N = 96; n = 12. Means with different superscripts are considered different (a–d).

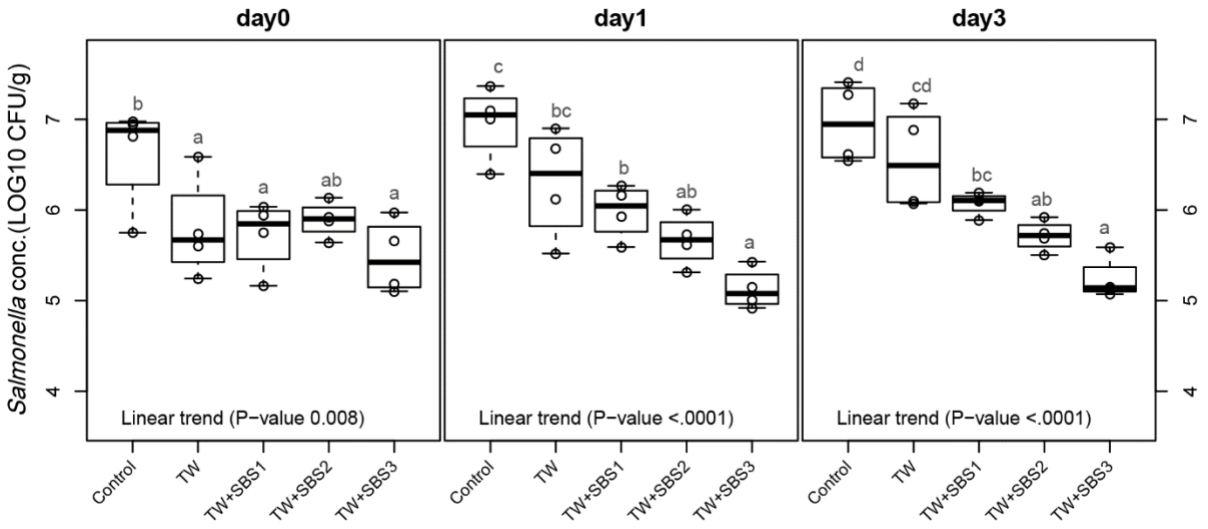

Figure 2. The linear effect of Sodium Bisulfate, SBS, as an antimicrobial 15 sec part dip on suppressing the population of *Salmonella* Enteritidis on whole chicken drumsticks on d 0, 1, and 3. Drumsticks were artificially inoculated with 10^7 CFU/g of *S.* Enteritidis and subsequently treated in 300 mL of antimicrobial treatments to identify the remaining population of *Salmonella*. There were eight antimicrobial treatments consisting of: a no treatment Control, tap water (TW), tap water with the addition of either 1, 2, or 3% SBS indicated as TW+SBS1, TW+SBS2, and TW+SBS3, and the combination of 1, 2, and 3% SBS with 200 ppm of peracetic acid (PAA), represented as TW+SBS1+PAA, TW+SBS2+PAA, and TW+SBS3+PAA. In the current figure, a linear trend was investigated for the incremental increase in SBS concentration, Control (n = 4), TW (n = 4), TW+SBS1 (TW+SBS1 and TW+SBS1+PAA, n=8), TW+SBS2 (TW+SBS2 and TW+SBS2+PAA, n=8), and TW+SBS3 (TW+SBS3 and TW+SBS3+PAA, n=8), over a 3-d refrigeration period at 4 °C. Individual SEM for Control, TW, TW+SBS1, TW+SBS2, and TW+SBS3 was 0.292, 0.284, 0.281, 0.229, 0.146 Log_{10} CFU/g for d 0, 0.205, 0.309, 0.092, 0.122, and 0.089 Log_{10} CFU/g for d 1, and 0.223, 0.278, 0.082, 0.131, and 0.067 for d 3, respectively. Pooled SEM for d 0 is 0.227, 0.196 for d 1, and 0.175 for d 3; N = 32. Means with different superscripts are considered different (a–d).

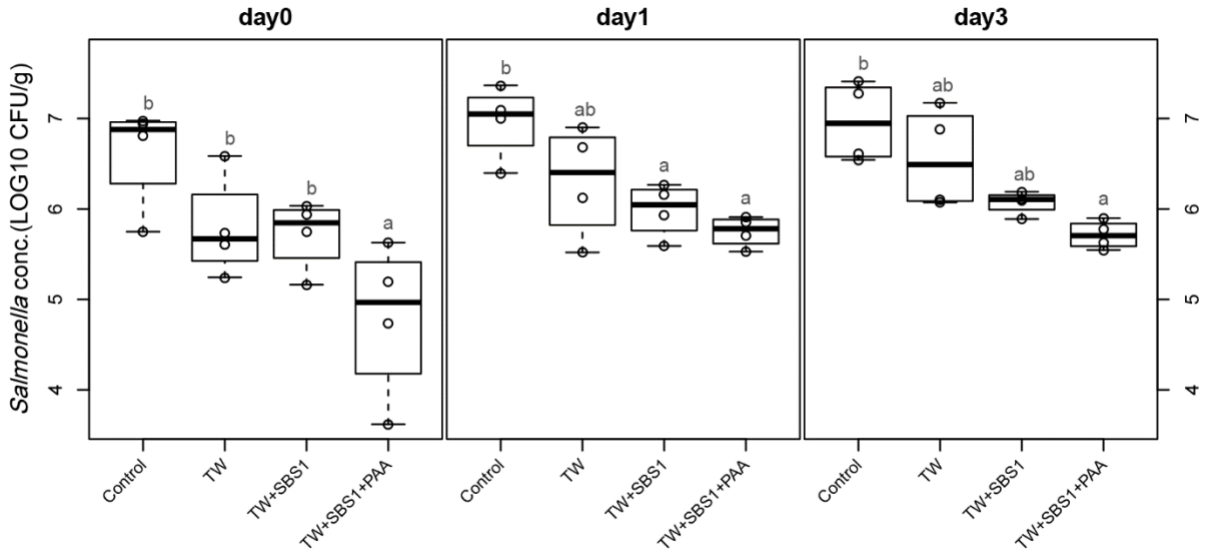

Figure 3. The comparative effect of 1% Sodium Bisulfate, SBS, and 200 ppm of peracetic acid, PAA, utilized alone or in combination as antimicrobial 15 sec part dips on the presence of *Salmonella* Enteritidis on whole chicken drumsticks on d 0, 1, and 3. In the current study, drumsticks were artificially inoculated with 10^7 CFU/g of *S.* Enteritidis and subsequently treated in 300 mL of antimicrobial treatments to identify the remaining population of *Salmonella*. In the study, there were eight treatments, consisting of: a no treatment Control, tap water (TW), tap water with the addition of either 1, 2, or 3% SBS indicated as TW+SBS1, TW+SBS2, and TW+SBS3, and the combination of 1, 2, and 3% SBS with 200 ppm of peracetic acid (PAA), represented as TW+SBS1+PAA, TW+SBS2+PAA, and TW+SBS3+PAA. However, in the current figure only the Control, TW, TW+SBS1, and TW+SBS1+PAA is represented and is separated by d 0, 1, and 3 of 4 °C incubation. Individual SEM for Control, TW, TW+SBS1, and TW+SBS1+PAA was 0.293, 0.284, 0.196, and 0.432 for d 0; 0.205, 0.309, 0.150, and 0.086 for d 1; and 0.223, 0.278, 0.064, and 0.078 for d 3, respectively. P-value for d 0 is 0.0116, 0.0067 for d 1, and 0.0024 for d 3; Pooled SEM for d 0 is 0.313, 0.204 for d 1, and 0.0024 for d 3; Per day N = 16 and n = 4. Means with different superscripts are considered different (a,b).

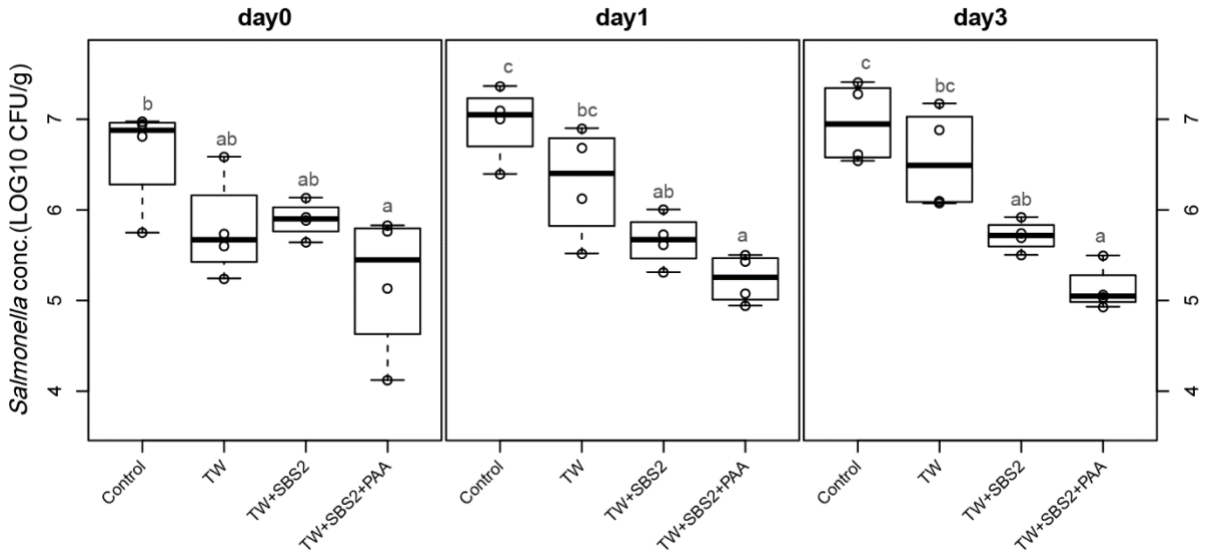

Figure 4. The comparative effect of 2% Sodium Bisulfate, SBS, and 200 ppm of peracetic acid, PAA, utilized alone or in combination as antimicrobial 15 sec part dips on the population of *Salmonella* Enteritidis on whole chicken drumsticks on d 0, 1, and 3. In the current study, drumsticks were artificially inoculated with 10^7 CFU/g of *S*. Enteritidis and subsequently treated in 300 mL of antimicrobial treatments to identify the remaining population of *Salmonella*. In the study, there were eight treatments, consisting of: a no treatment Control, tap water (TW), tap water with the addition of either 1, 2, or 3% SBS indicated as TW+SBS1, TW+SBS2, and TW+SBS3, and the combination of 1, 2, and 3% SBS with 200 ppm of peracetic acid (PAA), represented as TW+SBS1+PAA, TW+SBS2+PAA, and TW+SBS3+PAA. However, in the current figure only the Control, TW, TW+SBS2, and TW+SBS2+PAA is represented and is separated by d 0, 1, and 3 of 4 °C incubation. Individual SEM for Control, TW, TW+SBS2, and TW+SBS2+PAA was 0.293, 0.284, 0.101, and 0.396 for d 0; 0.205, 0.309, 0.143, and 0.136 for d 1; and 0.223, 0.278, 0.086, and 0.125 for d 3, respectively. P-value for d 0 is 0.0343, 0.0005 for d 1, and < 0.0001 for d 3; Pooled SEM for d 0 is 0.289, 0.210 for d 1, and 0.194 for d 3; Per day N = 16 and n = 4. Means with different superscripts are considered different (a,c).

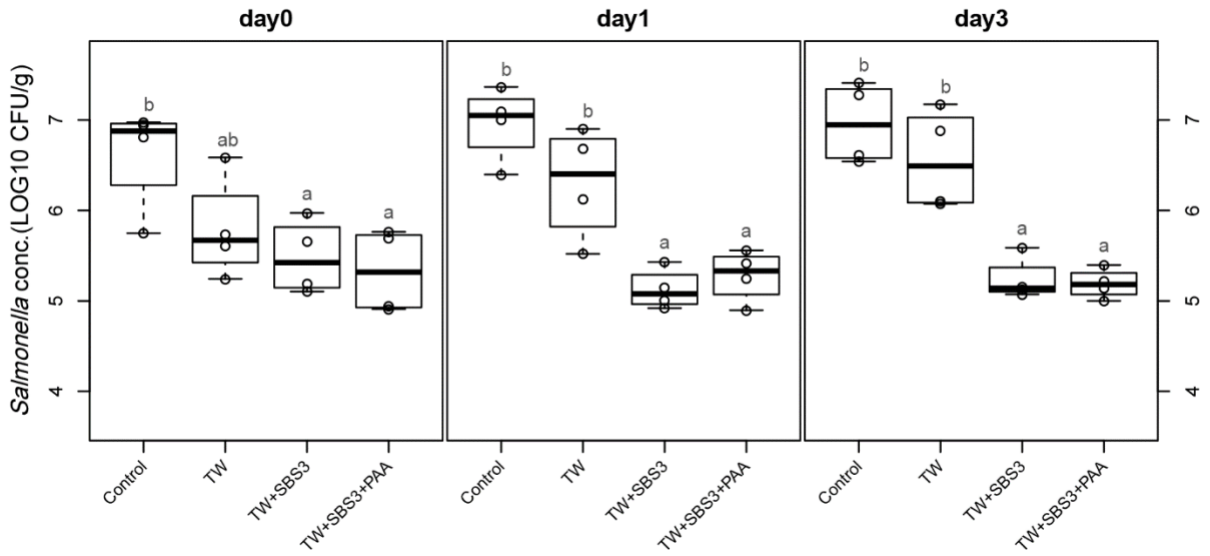

Figure 5. The comparative effect of 3% Sodium Bisulfate, SBS, and 200 ppm of peracetic acid, PAA, utilized alone or in combination as antimicrobial 15 sec part dips on the presence of *Salmonella* Enteritidis on whole chicken drumsticks on d 0, 1, and 3. In the current study, drumsticks were artificially inoculated with 10^7 CFU/g of *S*. Enteritidis and subsequently treated in 300 mL of antimicrobial treatments to identify the remaining population of *Salmonella*. In the study, there were eight treatments, consisting of: a no treatment Control, tap water (TW), tap water with the addition of either 1, 2, or 3% sodium bisulfate (SBS) indicated as TW+SBS1, TW+SBS2, and TW+SBS3, and the combination of 1, 2, and 3% SBS with 200 ppm of peracetic acid (PAA), represented as TW+SBS1+PAA, TW+SBS2+PAA, and TW+SBS3+PAA. However, in the current figure only the Control, TW, TW+SBS3, and TW+SBS3+PAA is represented and is separated by d 0, 1, and 3 of 4 °C incubation. Individual SEM for Control, TW, TW+SBS3, and TW+SBS3+PAA was 0.293, 0.284, 0.205, and 0.232 for d 0; 0.205, 0.309, 0.112, and 0.143 for d 1; and 0.223, 0.278, 0.119, and 0.083 for d 3, respectively. F test P-values are 0.0169 for d 0, 0.0001 for d 1, and < 0.0001 for d 3; Pooled SEMs are 0.256 for d 0, 0.206 for d 1, and 0.192 for d 3; Per day N = 16 and n = 4. Means with different superscripts are considered different (a,b).

CHAPTER 4

Salmonella and *Campylobacter* Influence the Microbiota Response of Skin-On, Bone-In Chicken Thighs Treated with Different Antimicrobials

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ABSTRACT

In order to provide consumers with safe poultry producers, integrators are tasked with implementing further multi-hurdle technology during second processing such as poultry part dips. However, to utilize these interventions properly, it is important to understand the effect these antimicrobials have on key pathogens and the resulting microbiota of poultry parts. Therefore, the objective of this study was to determine the influence of two antimicrobials, peracetic acid (PAA) and acidified sodium chlorite (ASC), on the microbiota of chicken thighs inoculated with *Salmonella* and *Campylobacter*. In two independent trials, chicken thighs (N = 360, n = 5, k = 9, 2 time points, 2 inocula, 2 trials) were inoculated with either a cocktail of *S.* Enteritidis, Heidelberg, Infantis, Kentucky, Typhimurium or a cocktail containing *C. jejuni* (10^8 and 10^7 CFU/mL) and incubated at 4 °C for 90 min. Inoculated thighs were independently dipped for 15 s into sterile bags containing 400 mL of the following treatments: Tap water (TW), TW + 800 or 1100 ppm of ASC (pH 2.4 and 2.8), and TW + 200, 350, 500, or 650 ppm of PAA. After treatment, samples were immediately stored at 4 °C. At 0 and 24 h, thighs were rinsed in 150 mL of nBPW and rinsates were aliquoted for pathogen detection and microbiota analyses. *Salmonella* and *Campylobacter* were enumerated using the drop plating method. Genomic DNA of rinsates was extracted, and the 16S rDNA was sequenced (Illumina MiSeq platform). Pathogen data were analyzed using 2-Way ANOVA in JMP 14, with means separated by Tukey's Protected HSD ($P \leq 0.05$). Microbiota data was filtered and aligned using the QIIME 2-2020.2 pipeline, with data considered significant at $P \leq 0.05$ for main effects and $Q \leq 0.05$ for pairwise differences. The results of the study demonstrated that there were differences between the load of inoculated pathogens during trial 1 and 2, therefore, trials were analyzed separately for both the microbiological and microbiota data. Both ASC and PAA treatments were effective at

reducing inoculated pathogens over both trials ($P<0.05$) with PAA potentially being more effective on *Salmonella* and ASC on *Campylobacter*. There were no differences among Alpha or Beta Diversity metrics during trial 1 and 2 when thighs were inoculated with either *Salmonella* or *Campylobacter*. In the *Salmonella* study, *Enterobacteriaceae* and *Bacillus* spp. were significantly different in treatments across time, regardless of trial. In the *Campylobacter* study, *Campylobacter* and *Bacillus* spp. were significantly different among treatments and time, regardless of trial. During Trial 2 of that study, there was a significant relative abundance of *Pseudomonas* and *Proteus* spp. Overall, both ASC and PAA were effective at altering the microbiota composition of chicken thighs when inoculated with *Salmonella* or *Campylobacter* with the microbiota varying across trials.

Key Words: broiler thighs, peracetic acid, acidified sodium chlorite, 16S rRNA, pathogens

INTRODUCTION

With the Food Safety Inspection Service (FSIS) increasing the *Salmonella* and *Campylobacter* performance standards for chicken and turkey products, the poultry industry must take great strides to meet the new standards (FSIS, 2015). As such, the new performance standards for broiler, turkey carcasses, and chicken parts are 9.8, 7.1, and 15.4% for *Salmonella* and 15.7, 5.4, and 7.7% for *Campylobacter*, respectively (maximum acceptable percent) (FSIS, 2015). These food safety standards directly reflect the goals set by the CDC in the Healthy People 2030 objectives. The Healthy People 2030 initiative aims at reducing *Campylobacter* prevalence from 15.8 to 10.6 per 100,000 population (FS-01) and reduce *Salmonella* prevalence from 14.8 to 11.1 per 100,000 population (FS-03; OPDHP, 2020). Therefore, the poultry industry must implement novel hurdles and strategies to meet and exceed the standards set by both consumers and regulatory agencies.

The utilization of short duration part dips after cut-up, during second processing may be one such strategy. Short duration dips with the inclusion of antimicrobials would add an extra step at further mitigating pathogens from raw poultry meat. Among the numerous products on the market currently used as food additives to reduce foodborne pathogens, the most widely used antimicrobials in poultry processing are peracetic acid (PAA), cetylpyridinium chloride (CPC), sodium chlorite, and weak organic acids (FSIS, 2019; Moore et al., 2017). Among those, sodium chlorite has become a focus of the poultry allied as they attempt to improve its efficacy against pathogens through its acidification. Acidified sodium chlorite (ASC), the acidification of sodium chlorite with weak acids, has demonstrated the ability to reduce pathogens significantly when utilized as a dip, spray, and post-chill intervention step on broiler carcasses (Kemp et al., 2000, 2001, 2002). Though one would think that the effects demonstrated on cut-up parts would

parallel the effects demonstrated on broiler carcasses, this is not necessarily true as parts have exposed muscle, different fat content, and allowed water holding capacity. Therefore, there is little known of its efficacy as a short duration dip on poultry parts and how ASC's use compares to other industry standards such as PAA during second processing.

Additionally, there is limited knowledge on how these acidifiers affect the microbiota of poultry skin and the microbiota of the parts, especially in the presence of a high bacterial load. Previous research has indicated that acidifiers potentially shift the microbiota of carcasses during first processing but limited to no research exists on how these acidifiers affect the microbiota of skin-on poultry parts during second processing (Kim et al., 2017). Using next generation sequencing (NGS), these affects and the ecology of the microbiota of the poultry parts could be characterized without the need for selective or enrichment media (Kim et al., 2017; Handley et al., 2018). The composition of the microbiota of poultry parts during second processing could provide the industry with detailed information regarding the efficacy of multi-hurdle strategies beyond that of indicator and pathogenic microorganisms involved in poultry processing (Handley et al., 2018).

Therefore, the central objective of the current study was to determine the shift in the microbiota when thighs were treated with PAA and ASC when inoculated with a cocktail of *Salmonella* Enteritidis, Heidelberg, Infantis, Kentucky, Typhimurium or *Campylobacter jejuni*. The further objectives were to evaluate the anti-*Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, Typhimurium) and the anti-*Campylobacter jejuni* effects of ASC versus PAA used as short duration part dips on artificially contaminated commercial chicken thighs.

MATERIALS AND METHODS

The following experiment was divided into two separate studies: 1) the effect of ASC and PAA on *Salmonella* Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium; and 2) the effect of ASC and PAA on *Campylobacter jejuni*. Where each study consisted of two independent trials. In total, the experiment consisted of 180 commercial chicken thighs per study that were investigated at 0 and 24 hours over 2 trials for their effects on the inoculated pathogen(s) (N = 180, k = 9, n = 5, t = 2, trial = 2).

Study 1: *Salmonella*

Inocula Preparation. In preparation for the current study, 48 h prior to the onset of study 1, frozen stocks of *Salmonella* Enteritidis (CFS Collection 38-0087), Heidelberg (ATCC 8326), Infantis (CDC H3536), Kentucky (CFS Collection 38-0056), and Typhimurium (ATCC 14028) were prepared using the following methodology. Frozen stocks of *Salmonella* species were separately streaked for isolation on Xylose Lysine Deoxycholate (XLD) agar and incubated for 24 h aerobically at 37 °C. Following, one isolated colony of each *Salmonella* species (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium) from the incubated plates were transferred to 40 mL of fresh Tryptic Soy Broth (TSB) and incubated under previously mentioned conditions overnight (12 to 16 h). The resulting cultures of *Salmonella* Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium were determined to contain 10^7 CFU/mL. Directly following incubation of the *Salmonella* Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium cultures, the individual cultures were spun down at 13,500 g for 2 min, decanted, and washed twice in 1× Phosphate Buffered Saline (PBS). After the final wash, one individual pellet of each strain was combined and resuspended in 40 mL of 1× PBS to create a *Salmonella* cocktail containing 10^8 CFU/mL.

Treatment and Sampling. A total of 180 commercial chicken skin-on, bone-in thighs (k = 9, n = 5, t = 2, trial = 2) with an average weight of 233 g were obtained from a commercial poultry facility no longer than 24-hours before the onset of each trial. Prior to each trial, one thigh was screened for the indigenous background presence of *Salmonella* by rinsing the thighs in 150 mL of neutralizing Buffered Peptone Water (nBPW), spread plating 100 μL on XLD, and incubating at 37 °C for 24 h.

The remaining thighs (N = 180, k = 9, n = 5, t = 2, trial = 2) were inoculated with a cocktail containing 2.5×10^8 CFU/mL of *S*. Enteritidis, *S*. Heidelberg, S. Infantis, *S*. Kentucky, *S*. Typhimurium (1 ml of inoculate per 25 g of chicken). Immediately after inoculation, the thighs were incubated at 4 °C for 60 to 90 min to allow for the attachment of *Salmonella.* Following the attachment period, the thigh weights were recorded, and the treatments were administered. The thighs were independently dipped into individual sterile whirl-pack bags containing 400 mL of the following experimental treatment for 10 seconds: peracetic acid (PAA) at 200, 350, 500, and 650 ppm and sodium chlorite (ASC) at 800 and 1100 ppm with the pH adjusted to both 2.4 and 2.8 (**Table 1**).

As thighs were being inoculated, new solutions of the experimental dips were being prepared (1 to 2 hours prior to 0 h). All solutions were prepared in 15 L of fresh tap water. Sodium chlorite (Crimson Chemicals, Fort Worth, TX, USA) was manually acidified with the addition of citric acid (Crimson Chemicals, Fort Worth, TX, USA) until the pH was within range of the expected pH (pH of 2.3 to 2.5 for pH of 2.4 and pH of 2.7 to 2.9 for pH of 2.8). The sodium chlorite level was verified via titration (Sodium Chlorite Test Kit, Crimson Chemicals, Fort Worth, TX, USA). The commercial PAA utilized in the current study was Xgenex™ (Xgenex™, Ponte Vedra Beach, FL, USA). The peracetic acid level was confirmed via titration

(PAA Test Kit, Crimson Chemicals, Crimson Chemicals, Fort Worth, TX, USA). Following treatment, thighs were placed into individual sterile poultry rinse bags (Nasco, Fort Atkinson, WI, USA) and allowed to rest for 2 min.

Following the two-minute rest period, the thighs were evaluated for anti-*Salmonella* effects. Regardless of trial, thighs (n = 5) were either assessed the same day (0 h) or stored for an additional 24-hours (24 h, n = 5) at 4 °C and analyzed. At each time point post-treatment, 0 and 24 h, the thighs were rinsed with 150 mL of nBPW, where the whirl-pak bags were manually agitated for 1 minute. The thighs were discarded, and the rinsates were collected for downstream analysis.

Microbiological Analysis. Rinsates were transferred to a biosafety cabinet where 25 µL of rinsate was serially diluted to 10^{-7} in 225 µL of 1 × PBS in a flat bottom 96 well plate. The dot plating method was utilized in the current study where 10 µL of the rinsate was plated on XLD and mCCDA agar, allowed to dry completely, inverted and incubated aerobically at 37 ˚C for 24 h. On XLD, only colonies with black centers were considered as *Salmonella*.

The following equation was utilized to calculate the CFU of bacteria per gram of chicken thigh:

$$\frac{\left(\frac{Number\ of colonies}{0.01\ mL\ plated}\right) * \text{Dilution Factor}}{\frac{\text{Thigh Weight (g)}}{\text{Original Hemogenate (mL)}}} = \text{CFU/ gram of Chicken Thigh}$$

DNA Extraction and Microbiota Analysis. At each time point, 0 and 24 hours, and for each trial, rinsates were collected after treatment, and 1 mL was aliquoted to 1.5 mL microcentrifuge tubes. Aliquoted rinsates for microbiota analysis were stored at -80 °C prior to extraction. Rinsates were pelleted for 10 minutes at 5,000 × g (approximately 7,500 rpm), and the supernatant was discarded. The pellet was then resuspended in 180 µL of ATL buffer, and

DNA from the pellet was extracted using the standard protocol from the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany).

The16S rDNA sequencing libraries targeting the V4 region were prepared using the extracted DNA (n = 2) with custom primers developed by Kozich et al. (2013). The libraries were amplified using a high-fidelity polymerase, Pfx, using the recommended PCR procedure (Invitrogen, Carlsbad, CA, USA). Libraries were then quantified with the KAPA Library quantification kit (Kapa Biosystems, Inc., Wilmington, MA, USA) specified for Illumina platforms and with a Qubit fluorometer using broad range dsDNA kit (Invitrogen, Carlsbad, CA, USA). Library amplicon size in base pairs (bp) was determined using an Agilent bioanalyzer (Agilent Technologies, Santa Clara, CA). After the library was thoroughly prepared, it was diluted to 20 pM with HT1 buffer and 30 % PhiX, loaded into a Miseq V2 cartridge, and sequenced on an Illumina MiSeq as per standard Illumina practices (Illumina, San Diego, CA, USA).

Study 2: *Campylobacter*

Inocula Preparation. Approximately 48 h before the onset of the experiment investigating the anti-*Campylobacter* effects, a frozen stock of *Campylobacter jujuni* (Wild Type) was streaked for isolation on modified Charcoal-Cefoperazone Deoxycholate (mCCDA) agar and incubated for 48 h microaerophilically at 42 °C. An isolated colony from the incubated mCCDA plates were transferred to 40 mL of fresh Mueller Hinton broth and incubated under previously mentioned conditions for 24 h. The resulting cultures of *Campylobacter jejuni* were determined to contain 10^7 CFU/mL. Directly following incubation of the *Campylobacter* cultures, the individual cultures were spun down at 13,500 g for 2 min, decanted, followed by

washing twice in Maximum Recovery Diluent (MRD;1 g Peptone, 8.5 g of NaCl). After the final wash, the pellet was resuspended in 40 mL of MRD.

Treatment and Sampling. A total of 180 commercial chicken skin-on, bone-in thighs (9 treatments, 5 replications, 2-time points, 0 and 24 hours, 2 independent trials) with an average weight of 293 g were obtained from a commercial poultry facility no longer than 24-hours before the onset of the study Prior to each trial during study 2, a thigh was screened for the indigenous background presence of *Campylobacter* by rinsing the thighs in 150 mL of nBPW, spread plating 100 μL on mCCDA, and incubating microaerophilically at 42 °C for 48 h.

The remaining thighs were inoculated with 2.5×10^7 CFU/mL of *Campylobacter jejuni* (1 ml of inoculate per 25 g of chicken). Immediately after inoculation, the thighs were incubated at 4 °C for 60 to 90 min to allow for the attachment of the bacteria. Following the attachment period, the thigh weights were recorded, and the treatments were administered. The thighs were independently dipped into individual sterile whirl-pack bags containing 400 mL of the aforementioned experimental treatment for 10 seconds and allowed to rest for 2 min. All solutions were prepared identically as prepared in study 1 with *Salmonella*.

Following the two-minute rest period, treatments were evaluated for their effects on the loosely attached *Campylobacter* on the skin of inoculated chicken thighs. In both trials, the thighs were either assessed the same day (0 h, n = 5) or stored for an additional 24-hours (24 h, n = 5) at 4 °C and analyzed. At each time point post-treatment, the thighs were rinsed with 150 mL of nBPW in sterile whirl-pak bags and manually shaken for 1 minute. The thighs were discarded and the rinsates were collected for downstream analysis.

Microbiological Analysis. As in study 1, rinsates were transferred to a biosafety cabinet where 25 μL of rinsate was serially diluted to 10^{-7} in 225 μL of 1 × PBS in a flat bottom 96 well

plate. Serially diluted rinsates were dot plated on mCCDA agar, allowed to dry completely, inverted and incubated microaerophilically at 42 ˚C for 48h. Only colony-forming units with a silver metallic sheen were considered as *Campylobacter*. The aforementioned equation in study 1 was utilized to calculate the CFU of bacteria per gram of chicken thigh.

DNA Extraction and Microbiota Analysis. As described in study 1, rinsates were collected, aliquoted to 1.5 mL microcentrifuge tubes in duplicate, and stored at -80 °C until genomic extractions could occur. Once thawed, rinsates were pelleted and resuspended in 180 µL of ATL buffer. The DNA was extracted using the standard protocol from the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany).

The16S rDNA sequencing libraries targeting the V4 region were prepared using the extracted DNA with custom primers developed by Kozich et al. (2013) as in study 1. The libraries were amplified, normalized, and quantified according to Illumina standards. After the library was thoroughly prepared, it was diluted to 20 pM with HT1 buffer and 30 % PhiX, loaded into a Miseq V2 cartridge, and sequenced on an Illumina MiSeq as per standard Illumina practices (Illumina, San Diego, CA, USA).

Statistical and Bioinformatic Analyses

Prior to either study and trial, each thigh was randomly assigned to a treatment and a time point prior to analyses. The CFU of *Salmonella* and *Campylobacter* were Log_{10} transformed and reported as a Log_{10} CFU of *Salmonella* or *Campylobacter* per gram chicken thigh basis (Log_{10} CFU/g). The distribution of the data per trial was investigated and data were analyzed using a General Linear Model in R (R Studio). Studies were analyzed separately, with the main effect and interactions of trial, treatment, and time being investigated. Means were separated by using Tukey's Protected HSD, with a significance level of $P \leq 0.05$.

The microbiota sequencing data were downloaded from Illumina BaseSpace, de-multiplexed, and locally uploaded into QIIME2-2020.2 (Boylen et al., 2019). Amplicon Sequence Variants (ASVs) were filtered and trimmed via DADA2 for quality, with chimeras filtered by consensus (q2-dada2; Callahan et al. 2016). The phylogenetic trees were created in mafft (q2-alignment; Katoh et al. 2002). ASVs were aligned to SILVA full OTU sequence with a confidence limit of 95% (Bokulich et al. 2018). Using metadata-based filtering in Qiime2, study, *Salmonella* or *Campylobacter*, and trial, 1 or 2, were separated out. Therefore, the remaining analytics were performed on trial 1 and 2 of both study 1 and 2.

Alpha and Beta diversity analyses were analyzed using core metrics results. Alpha diversity was determined using the Shannon diversity index and Pielou's Evenness, which included the Kruskal-Wallis tests for pairwise differences (Kruskal and Wallis, 1952). Beta-diversity metrics were analyzed with qualitative and quantitative indices, Bray Curtis, and Weighted UniFrac Distance Matrix (Lozupone et al. 2007). Pairwise differences and distances across time for treatment were investigated for Alpha and Beta Metrics using Qiime2 longitudinal analyses. Due to the small sample size ($n = 2$), there was not enough statistical power to determine statistical differences among the taxa. Therefore, the relative abundance of the taxa at the phyla and genera was generated from Qiime2 output. Pairwise differences were considered significant when $P \leq 0.05$ and $Q \leq 0.05$. Q values were used as they include the False Discovery Rate, which accounts for the occurrence of type I errors, rejecting a true null hypothesis (false positive), when conducting multiple comparisons.

RESULTS AND DISCUSSION

Quantification of *Salmonella* and *Campylobacter* recovered from treated thighs

Previous research investigating the effect of acidifiers on chicken parts has demonstrated the ability to reduce the pH of poultry skin and foodborne pathogens such as *Salmonella* (Tan et al., 2014). Previously, PAA has been shown to be an effective antimicrobial in the post or finishing chiller, reducing *Salmonella* and *Campylobacter* on chicken carcasses (Nagel et al., 2013). Also, Zhang et al. (2018) demonstrated that various acidifiers such as 30 ppm chlorine, 700 ppm ASC, 700 or 1000 ppm PAA, and 0.35 or 0.60% cetylpyridinium chloride (CPC) are capable of reducing both *Salmonella* and *Campylobacter* on poultry breasts, thighs, wings, and drumsticks. Although acidifiers are commonly used in poultry processing to reduce common foodborne pathogens, there is limited research on how the acidifiers affect the ecology of the microbiota loosely attached on the skin of poultry carcasses during second processing. Therefore, the results discussed herein may potentially provide the industry with additional information on how the ecology of the loosely attached microbiota of poultry parts are affected by acidifiers such as PAA and ASC when inoculated with high doses of a *Salmonella* cocktail and *Campylobacter jejuni*.

The results of the current study demonstrate that both PAA and ASC have the ability to reduce *Salmonella* and *Campylobacter* on broiler thighs. As there was a significant effect of trial ($P < 0.05$; data not shown), trials (1 and 2) were analyzed separately for both studies (*Salmonella* and *Campylobacter* study). There was a treatment by time (0 and 24-h post-treatment) interaction demonstrated during both trials in each study (**Figure 2-3,** $P<0.05$). Therefore, time may have played a role in the way treatments were capable of reducing *Salmonella* and *Campylobacter* effectively.

Study 1: *Salmonella*. During trial 1 (**Figure 1A**) at 0 h, all thighs treated with ASC or PAA reduced the load of *Salmonella* compared to the control, Tap Water (6.04 Log_{10} CFU/g). Over 24 h, the load of *Salmonella* of the thighs treated with Tap Water did not increase (5.86 Log_{10} CFU/g). Thighs treated with 800 ppm ASC (2.4 pH), 1100 ppm ASC (2.4 and 2.8 pH), 200, 350, 500, and 650 ppm PAA had lower loads of *Salmonella* than Tap Water at 24 h (5.28, 4.91, 4.37, 5.18, 4.85, 4.91, 4.36, and 5.86 Log_{10} CFU/g respectively). Over 24 h, the only treatment to continue to reduce the concentration of *Salmonella* was 1100 ppm ASC (2.8 pH) with thighs being treated having 4.27 Log_{10} CFU/g. Overall, the lowest load of *Salmonella* on the thighs was on those treated with 350 ppm PAA at 0 h (3.99 Log_{10} CFU/g) and was not different than those treated with 1100 ppm ASC (2.8 pH) or 650 ppm PAA at 24 h (4.37 and 4.36 Log_{10} CFU/g).

Of thighs inoculated with *Salmonella* during trial 2, those dipped in Tap Water had a *Salmonella* load of 6.21 and 6.80 Log_{10} CFU/g at 0 and 24 h. Compared to the load of *Salmonella* on thighs treated with Tap Water at 0 h, those treated with all levels of PAA (200, 350, 500, and 650 ppm) had lower loads (5.23, 5.03, 5.11, 4.32 Log_{10} CFU/g). At 24 h, thighs treated with 800 ppm ASC (2.4 pH), 800 ppm ASC (2.8 pH) at 24 h, 1100 ppm ASC (2.4), 200, 350, 500, and 650 ppm PAA had lower concentration of *Salmonella* compared to those treated with Tap Water (4.50, 5.32, 5.04, 5.46, 5.22, 5.39, 4.61, and 6.80 Log_{10} CFU/g, respectively). Over 24 h, two treatments were capable of reducing the concentration of *Salmonella*. Both the treatment of thighs with 800 ppm ASC (2.4 pH) and 1100 ppm (2.4 pH) was capable of reducing *Salmonella* over a 24 h period time on inoculated thighs (5.55 to 4.5 and 5.91 to 5.04 Log_{10} CFU/g) and had significantly lower loads than those treated with Tap Water at 0 and 24 h (6.21 and 6.80 Log_{10} CFU/g). However, the treatment of thighs with 650 ppm PAA reduced

Salmonella the most on inoculated thighs and maintained this reduction over a 24 h period (4.32 and 4.61 Log_{10} CFU/g). At 24 h, thighs treated with 650 ppm PAA were not different than those treated with 800 ppm ASC (2.4 pH) or 1100 ppm ASC (2.4 pH) at 24 h (4.50 and 5.03 Log_{10} CFU/g).

Ultimately over both trial 1 and 2, thighs treated with 650 ppm PAA reduced *Salmonella* 1.7 and 2.5 Log_{10} CFU/g compared to thighs treated with Tap Water alone. However, at 0 h during trial 1 and 2, 350 ppm PAA proved to be a sufficient concentration of PAA to limit *Salmonella* growth by 2 and 1.8 Log_{10} CFU/g compared to thighs treated with Tap Water. At 24 h, data from both trials demonstrated the potential of ASC; however, the efficacy of the concentrations and pH varied by trial.

Study 2: *Campylobacter*. The data presented herein also demonstrates the capability of all treatments to effectively reduce artificially inoculated *Campylobacter* on commercial chicken thighs compared to those treated with Tap Water during trial 1 and 2. During trial 1, thighs dipped for 15 seconds in Tap Water had *Campylobacter* levels of 3.83 Log_{10} CFU/g at 0 and was reduced to 2.29 Log_{10} CFU/g over 24 h. Regardless of time (0 and 24 h), all treatments (< 1.36 Log_{10} CFU/g) reduced *Campylobacter* on inoculated thighs compared to those treated with Tap Water alone. At 0 and 24 h h, Thighs treated with 800 ppm ASC (2.4 and 2.8 pH), 1100 ppm (2.8 pH), 350 and 500 ppm PAA had below 0.5 Log_{10} CFU of *Campylobacter* per g of thigh. At 0 and 24, thighs treated with 800 ppm ASC (2.8 pH) reduced *Campylobacter* below detection (no growth on mCCDA).

Thighs treated with Tap Water during trial had levels of *Campylobacter* at 2.94 and 3.09 Log_{10} CFU/g and were not different than 200 and 500 ppm PAA at 0 h (2.57 and 2.11 Log_{10} CFU/g), and 800 ppm ASC (2.4 and 2.8 pH), 1100 ppm ASC (2.8 pH), 200, 350 and 500 ppm

PAA at 24 h (2.68, 2.58, 2.42, 3.07, 2.81, and 2.72 Log_{10} CFU/g). From 0 to 24 h, no treatments were able to reduce *Campylobacter* compared to their own levels at initial treatment (0 h). However, at 0 h, thighs treated with 800 ppm (2.4 and 2.8 pH), 1100 ppm ASC (2.4 and 2.8 pH), 350 and 650 ppm PAA reduced the load of *Campylobacter* on inoculated thighs compared to those treated with Tap Water (1.85, 1.21, 1.52, 1.01, 1.76, and 1.47 Log_{10} CFU/g). At 24 h, the lowest numerical load of *Campylobacter* was observed on thighs treated with 1100 ppm ASC (2.4 pH) and 650 ppm PAA (1.78 and 1.58 Log_{10} CFU/g).

Overall, 1100 ppm ASC (2.8 pH) reduced *Campylobacter* by 3.8 and 2 Log_{10} CFU/g compared to those treated with Tap Water alone while 650 ppm PAA reduced *Campylobacter* by 3.1 and 1.6 Log_{10} CFU/g at 0 h during both trials. At 24 h, this was not necessarily true with the treatment of thighs with 800 ppm ASC (2.8 pH) reducing *Campylobacter* below detectable limits during trial 1 and 650 ppm PAA reducing *Campylobacter* to 1.58 Log_{10} CFU/g. This effect was also seen with the inoculation of *Salmonella* in study 1; where at 24 h, the efficacy of the concentrations and pH of ASC treatments varied by trial.

Acidified Sodium Chlorite and Peracetic Acid as Effective Antimicrobials

Previously, Zhang et al. (2018) investigated the effect of multiple acidifiers as 23-s rinses on chicken breasts, thighs, wings, and drumsticks after being inoculated with *Salmonella* and *Campylobacter*. Zhang et al. (2018) determined that the use of PAA could reduce both *Salmonella* and *Campylobacter* on parts by 1.5 Log_{10} CFU/mL. In addition, Zhang et al. (2018) demonstrated less than a 1 Log_{10} CFU/mL reduction of *Salmonella* and *Campylobacter* on chicken parts rinsed in 700 ppm ASC. The reduction of *Salmonella* and *Campylobacter* by ASC was not different from the reduction of rinsing parts in tap water or 30 ppm chlorine. Unlike Zhang et al. (2018), the current study demonstrated that PAA and ASC reduced *Salmonella* and

Campylobacter load compared to those treated with tap water treatment (TW). In addition, there was a 0.6 to 2.3 Log_{10} CFU/g reduction of *Salmonella* among ASC treatments and a 0.7 to 2.5 Log_{10} CFU/g reduction of *Salmonella* among thighs treated with PAA. The current study also demonstrated a 0.4 to 3.83 Log_{10} CFU/g reduction of *Campylobacter* among ASC treatments and a 0.02 to 3.74 Log_{10} CFU/g reduction of *Campylobacter* regardless of experimental treatment compared to TW alone. Therefore, there was a small differential effect of acidifiers based on the pathogen present, with *Salmonella* being reduced more with PAA than ASC, and ASC being more effective on *Campylobacter*. However, these differences were not significant.

The use of PAA has also been shown to reduce *Salmonella* and *Campylobacter* on chicken fillets when immersed in 4, 10, and 30-s dips of 100, 250, 500, and 1,000 ppm of PAA (Kumar et al., 2020). Specifically, the use of 500 or 1,000 ppm PAA effectively reduced *Salmonella* on breast fillets when immersed for 30 seconds (1.77 and 1.92 Log_{10} CFU/mL reduction compared to control). The use of 1,000 ppm PAA was most effective at reducing *Campylobacter* on breast fillets when dipped in the antimicrobial solution for 30 s (1.87 Log_{10} CFU/mL reduction compared to the control). When breast fillets were immersed in 500 ppm PAA for 10 s (similar conditions to the current study), *Salmonella* and *Campylobacter* were reduced (1.16 and 1.25 Log_{10} CFU/mL). The reduction of *Salmonella* and *Campylobacter* at 500 ppm for 10 s was comparable to the results of the current study despite skin being a factor in the current study. Poultry skin has been associated with a buffering effect and reducing the efficacy of acidifiers (Tan et al., 2014).

Microbiota Diversity of Inoculated Thighs

Due to the statistical differences demonstrated between *Salmonella* and *Campylobacter* reduction during trial 1 and 2 ($P < 0.05$), preliminary analyses investigating the effect of trial

were performed. Due to significant effect of study and trial on the diversity metrics demonstrated during the preliminary analyses, microbiota data were separated after DADA2 in Qiime2 by study and trial. The main effect of treatment and interaction of treatment and time were investigated for Alpha and Beta Diversity indices with differences and distances being investigated over time.

Of the Alpha Diversity metrics, the qualitative measurement of evenness, Pielou's Evenness, and a quantitative measure of richness that accounts for the abundance and the evenness of the data, Shannon Diversity, were investigated. Both Pielou's Evenness and Shannon Diversity were utilized as they complement one another with Pielou's determining the how even data are and Shannon's determining the diversity of the data by incorporating both evenness and abundance into its analyses. Beta Diversity indices such as the quantitative measurement of community dissimilarity, Bray Curtis, and the phylogenetic community dissimilarity, Weighted Unifrac, were investigated using PERMANOVA and PERMDISP. PERMANOVA is a multivariate form of ANOVA with permutations to reduce bias used to determine if the distribution and abundances of chosen treatment are different. However, this method possesses limitation in the sense that is does not account for the dispersion of the data. Therefore, PERMDISP was used additionally to determine if the significant effect of treatments was due to the dispersion of the data from the centroids driving the significance.

Study 1: *Salmonella*. There was no significant effect of treatment on the Alpha or Beta Diversity metrics in either trial. During trial 1, both Pielou's Evenness and Shannon's Diversity Index were not significant (**Supplemental Table 1**; $P > 0.05$; $Q > 0.05$). In addition, there was no interaction between treatment and hour when investigating the evenness and diversity of trial 1 using ANOVA (**Supplemental Table 2**; $P > 0.05$). The main effect of treatment for the Beta

Diversity metrics during trial 1 were not significant for either PERMANOVA or PERMDISP (**Figure 4A**; **Supplemental Table 3**; $P > 0.05$; $Q > 0.05$). There was also no interaction among treatment and time for both Beta Diversity metrics, Bray Curtis and Weighted Unifrac, during trial 1 when using ADONIS, a nonparametric multivariate analysis of variance (**Supplemental Table 4**; $P > 0.05$). The lack of significance for the main effect and interactions of treatment and time was also demonstrated in trial 2 (**Figure 4B**; **Supplemental Table 5-8**; $P > 0.05$; $Q > 0.05$). There were also no differences in the distances and differences over time for both Alpha and Beta diversity metrics over a 24 h period of time (Data not shown; $P > 0.05$).

Study 2: *Campylobacter*. As in study 1, there was no significant effect of treatment on the Alpha or Beta Diversity metrics in either trial (1 or 2). During trial 1, neither Alpha Diversity Indexes, Pielou's Evenness and Shannon Diveristy Index, were significant (**Supplemental Table 9**; $P > 0.05$; $Q > 0.05$). There was no interaction between treatment and time during trial 1 among either Alpha Diversity metrics using ANOVA (**Supplemental Table 10**; $P > 0.05$). In addition, the main effect of treatment (PERMANOVA and PERMDISP) nor the interaction of treatment and time for the Beta Diversity metrics, Bray Curtis and Weighted Unifrac, during trial 1 were not significant (**Figure 5A**; **Supplemental Table 11-12**; $P > 0.05$; $Q > 0.05$). The lack of significance for the main effect and interactions of treatment and time was also demonstrated in trial 2 (**Figure 5B; Supplemental Table 13-16**; $P > 0.05$; $Q > 0.05$). There were also no differences in the distances and differences over time for both Alpha and Beta diversity metrics over a 24 h period of time (Data not shown; $P > 0.05$).

Microbiota Composition of Inoculated Thighs

Due to the small sample size in both studies (n = 2), taxa bar plots of the microbiota were generated in order to highlight any potential shifts of the microbiota in response to treatment

during both studies. Relative abundance at the phylum and genus level was used to determine the effect of treatments during trial 1 and 2 at 0 and 24 h when inoculated with either *Salmonella* or *Campylobacter* on the microbiota composition.

Study 1: *Salmonella*. Of thighs inoculated with *Salmonella* during trial 1 and 2 at both 0 and 24 h, proportionally, Proteobacteria represented the highest proportion of the compositions of the inoculated thighs during both trials at the phylum level (**Figure 7**). Firmicutes were the second highest proportionally represented phyla among the microbiota of inoculated thighs regardless of trial. There did seem to be a pattern of the proportion of firmicutes increasing after 24 h when held at 4 °C, but this was not consistently seen across treatments. Interestingly, during trial 2 at 0h, thighs treated with 500 ppm PAA had the smallest relative abundance of Proteobacteria (67%), highest relative abundance of Firmicutes (27%) and Bacteroidetes (4%). This effect could be due to the core microbiota of the skin of the part that was utilized in the experiment. At the genus level, the patterns seen at the phyla level were also demonstrated with the highest proportions of genera being among *Enterobacteriaceae* and *Bacillus* (**Figure 8**). The genera among the microbiota of thighs treated with 500 ppm PAA during trial 2 at 0 h had the lowest relative abundance of *Enterobacteriaceae* (67%) which reflected the level of Proteobacteria among the phyla. In addition, microbiota of thighs treated with 500 ppm PAA during trial 2 at 0 h were comprised of 9% *Lachnospiraceae*, 4% *Bacillus*, 3% *Ruminococcaceae* UCG-014, 2% *Ruminococcaceae*, 2% *Bacteroides*, 1% *Alistipes*, 1% *Anaerinibacillus*, 1% *Ruminococcus* torques group, and the remaining 10% was comprised of genera < 1%.

Like the current study, Kim et al. (2017) demonstrated that *Bacillus* was among the microbiota of carcass rinsates treated with or without 750 ppm PAA 15 s post-chill dips. In general, Kim et al. (2017) demonstrated the use of 750 ppm PAA and Amplon (1.3 pH) as 15-s

dips could significantly alter the microbiota of poultry carcasses during processing. From broiler carcass rinsates collected before entering the post-chiller, Kim et al. (2017) observed that their microbiota was mainly comprised of *Paenaibacillaceae* (≈25 %), *Bacillus* (≈ 20%), *Clostridium* (≈ 10%), *and Clostridiaceae* (≈10%).

Overall, during study 1, the inoculation of chicken thighs with a cocktail of *Salmonella*, resulted in a high proportion of Proteobacteria and consequently *Enterobacteriacea*. This observation is similar to how *Salmonella* invades and dominates the microbiota of the gastrointestinal tract by altering the conditions of the environment in its favor (Khan and Chousalkar, 2020; Dieye et al., 2009; Stecher et al., 2008). Further, the microbiota observed in the current study is similar to what has been seen in previously seen. Kim et al. (2017) observed that 98.7% of the commercial chicken rinsate microbiota phyla profiles that consisted of Firmicutes, Proteobacteria, Bacteriodetes, Actinobacter, and Cynaobacter. Handley et al. (2018), who biomapped the microbiota profiles of commercial broilers through evisceration and immersion chilling, identified Proteobacteria as the primary phyla of all carcass rinsates collected regardless of sampling location within the processing facility (48%). In addition, Handley et al. (2018) determined that *Pseudomonas* and *Enterobacteriaceae* had the highest relative abundance at post-chill (83.5 and 2.2%). Unlike Handley et al. (2018), in the current study, *Enterobacteriaceae* was the highest relative genera among thigh rinsates, but *Pseudomonas* was not. This difference was most likely due to the inoculation of *Salmonella*. However, it does not lessen the fact that *Enterobacteriaceae* comprises a high proportion of post chill carcass rinsates and could be the result of the contamination of the carcass with high levels of *Salmonella* as was seen in the current study.

Study 2: *Campylobacter*. During study 2, the microbiota of thighs inoculated of thighs were comprised of the same phyla and genera; however, the composition of these were not consistent between trials 1 and 2 (**Figure 8-9**). Of thighs inoculated with *Campylobacter* during trial 1, Proteobacteria, Firmicutes, and Epsilonbacteraeota were the most represented phyla among the microbiota. During trial 1, Proteobacteria, Firmicutes, and Epsilonbacteraeota comprised 38, 55, and 5% and 41, 55, and 3% of the microbiota of thighs at 0 and 24 h (**Figure 8A-B**). The microbiota profile at the phylum level observed during trial 1 is consistent with a high microbial load of *Campylobacter*; however, this was not demonstrated in trial 2. During trial 2, Proteobacteria, Firmicutes, and Epsilonbacteraeota comprised 5, 16, and 78% and 2, 10, and 88% of the microbiota of thighs at 0 and 24 h, respectively (**Figure 8C-D**).

The microbiota composition of thighs at the genus level differed between the two trials; however, thighs during both trials had a core microbiota consisting of *Campylobacter* and *Bacillus* (**Figure 9**). During trial 1, across treatments, *Campylobacter* and *Bacillus* comprised approximately 38 and 52% of the microbiota at 0 h and 41 and 44% of the microbiota of thighs at 24 h (**Figure 9A-B**). In contrast, *Campylobacter* and *Bacillus* comprised approximately 5 and 15% of the microbiota at 0 h and 41 and 44% of the microbiota of thighs at 24 h during trial 2 (**Figure 9C-D**). In addition to *Campylobacter* and *Bacillus*, the thighs used during trial 2 had a high relative abundance of *Pseudomonas* (41 and 43%), *Psychrobacter* (33 and 41%), and *Proteus* (4 and 3%) at 0 and 24 h post treatment. Populations of *Pseudomonas*, *Psychrobacter*, *Proteus*, and *Bacillus* are not uncommon among the microbiota of raw poultry (Handley et al., 2018; Kim et al., 2017).

Campylobacter was most likely among the microbiota composition due to it being the inocula used during this study. Previously, Kim et al. (2017) sequenced the 16S rDNA of

mCCDA plates that had been inoculated with poultry rinsates. Kim et al. (2017) demonstrated the association of *Campylobacter* (47%) with *Oscillospira* (12.7%), *Actinobacter* (10%), *Enterococcus* (9.71%), *Bacillus* (7.25%), *Paenibacillus* (2.91%), *Sporanaerobater* (1.65%), *Lactobacillus* (1.60%), *Clostridium* (1.02%), and others (< 1%) among *Campylobacter* selective plates. Indicating that *Campylobacter* may thrive in a more diverse environment. As mentioned previously, *Salmonella* alters the microbiota when it invades due to its pathogenicity and ability to survive inflammation (Khan and Chousalkar, 2020; Dieye et al., 2009; Stecher et al., 2008). However, *Campylobacter* may survive with the help of other microbiota and therefore does not disturb the composition of the microbiota as *Salmonella* does (Indikova et al., 2015).

Oakley et al. (2013) investigated the microbiota of poultry during the farm to fork continuum and identified *Pseudomonas* as the key genera associated with retail poultry. In addition, Oakley et al. (2013) demonstrated that *Campylobacter* is among the core microbiota of poultry on the farm and this does translate to the microbiota of processed poultry, though *Campylobacter* abundance was significantly reduced throughout processing. Oakley et al. (2013) through network analyses determined that *Campylobacter* does not interact significantly with other taxa and this may be due to the ecological niches certain *Campylobacter* taxa prefer. Thus, making the elimination of *Campylobacter* from poultry more difficult than other foodborne pathogens.

Interestingly, *Acinetobacter* was only among the composition of thighs treated with 350 ppm PAA, but even then, *Acinetobacter* only constituted less than 1% of the total composition. Some multi-drug resistant *Acinetobacter* strains that have been isolated from the environment and food have the potential to be human pathogens. *Acinetobacter* has been associated with *Campylobacter* isolation from poultry carcass rinses using such enrichment and cultivation

media as Bolton and Preston Broth and modified charcoal-cefoperazone-deoxycholate agar (mCCDA; Kim et al., 2019; Chin et al., 2013; Kim et al., 2017). Also, Handley et al. (2018) and Kim et al. (2017) previously demonstrated the association of *Acinetobacter* among the microbiota of poultry rinsates.

CONCLUSIONS

Both peracetic acid and acidified sodium chlorite reduced the load of *Salmonella* and *Campylobacter* on inoculated commercial chicken thighs. Overall, thighs treated with PAA demonstrated a greater reduction of *Salmonella* after 24-h of refrigeration, while ASC may have been more efficacious a reducing *Campylobacter*. The main effect of treatment nor the interaction of treatment and time were significant among either Alpha or Beta Diversity metrics used in either study. Additionally, there were no differences among distances and differences across time for either Alpha or Beta Diversity metrics. Among the microbiota composition, Proteobacteria and Firmicutes at the phylum level and *Enterobacteriaceae* and *Bacillus* at the genus level comprised the highest proportions of the microbiota of thighs when commercial chicken thighs were inoculated with *Salmonella*, regardless of trial. In study 2, when thighs were inoculated with *Campylobacter*, Firmicutes, and Epsilonbacteraeota were among the highest relative proportions of the phyla and *Bacillus* and *Campylobacter* were among the highest relative genera during trial 1. However, during trial 2, the microbiota differed to trial as the composition was more comprised of *Pseudomonas*, *Psychrobacter*, *Proteus* than *Bacillus* and *Campylobacter*.

Due to the random variability between trial, as fresh chicken thighs were obtained from a commercial processor, trial and study (*Salmonella* and *Campylobacter*) were statistically

different from one another. In future studies, batches of poultry or poultry parts should be cofounded to the same source and day as this study demonstrates the variability of the microbiota from trial to trial in response to *Salmonella* or *Campylobacter* inoculation. As more studies are conducted to biomap the core microbiota of processed poultry and how it affects the stability and safety of poultry products, reducing the variability among data will be important.

Ultimately, the data presented herein demonstrated the use of NGS such as 16S rDNA sequencing to illuminate the changes in the core microbiota when poultry parts are inoculated with either a cocktail of *Salmonella* or *Campylobacter* and dipped in antimicrobial solutions. The results of the current study could potentially provide integrators with the means to select the appropriate antimicrobials for certain foodborne pathogen issues such as the high incidence of *Salmonella* or *Campylobacter*.

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TABLES AND FIGURES

Table 1. Description of the experimental treatment of thighs in both trial one and two investigating the anti-*Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium) and anti-*Campylobacter jejuni* effects of acidified sodium chlorite (ASC) and peracetic acid (PAA)

Treatment Description	Tap Water	Contact Time
Tap Water (TW)	400 mL	10 sec
200 ppm PAA[1]	400 mL	10 sec
350 ppm PAA	400 mL	10 sec
500 ppm PAA	400 mL	10 sec
650 ppm PAA	400 mL	10 sec
800 ppm ASC[2] (2.4 pH)	400 mL	10 sec
800 ppm ASC (2.8 pH)	400 mL	10 sec
1100 ppm ASC (2.4 pH)	400 mL	10 sec
1100 ppm ASC (2.8 pH)	400 mL	10 sec

[1] Xgenex™, Ponte Vedra Beach, FL, USA

[2]Sodium chlorite was manually acidified with the addition of citric acid until the pH was within range of the expected pH (pH of 2.3 to 2.5 for pH of 2.4 and pH of 2.7 to 2.9 for pH of 2.8).

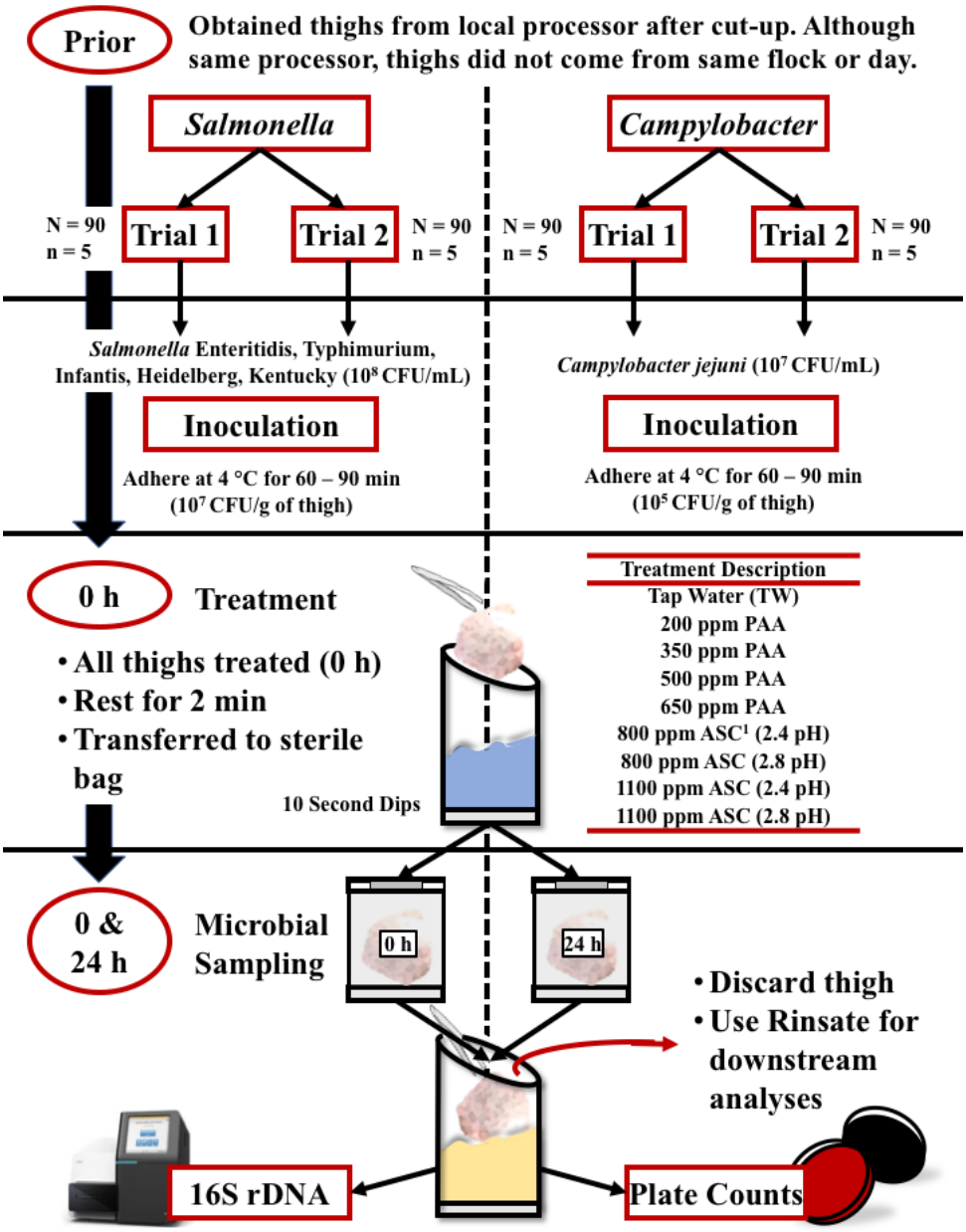

Figure 1. Schematic of the current study where thighs were either inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Kentucky, Typhimurium, and Infantis) or *Campylobacter* jejuni over two independent trials.

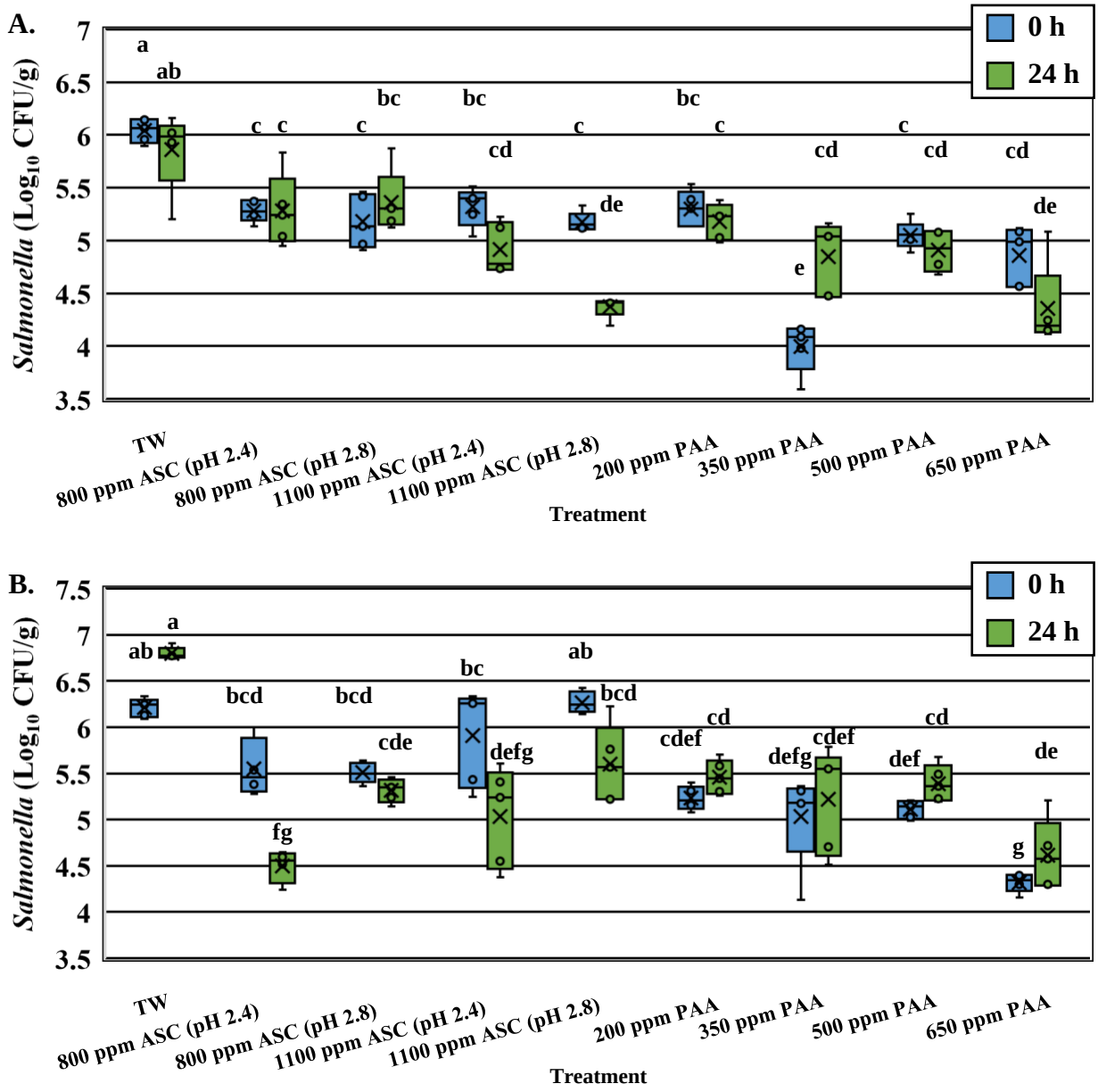

Figure 2. The interaction of treatment and time on the load of inoculated *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium) (Log_{10} CFU/g chicken thigh) on whole skin-on bone-in chicken thighs when treated with 400 mL of antimicrobial treatment dips for 10-sec in trial 1 (**A**) and 2 (**B**) ($P < 0.0001$, N = 90, n = 5 and $P = 0.0034$, N = 90, n = 5). Levels not connected by same letter are significantly different.

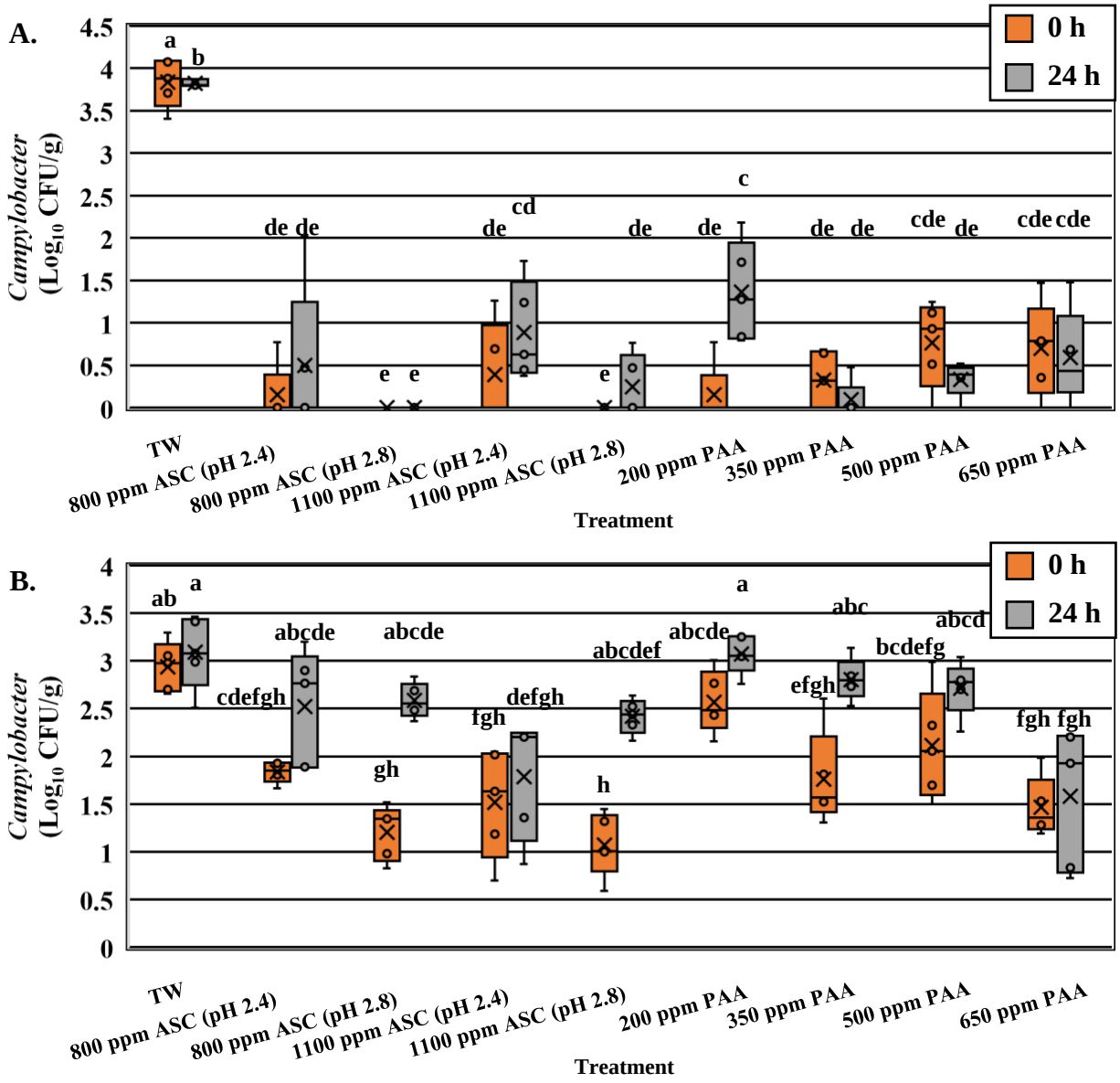

Figure 3. The interaction of treatment and time on the load of inoculated *Campylobacter jejuni* (Log_{10} CFU/g chicken thigh) on whole skin-on bone-in chicken thighs when treated with 400 mL of antimicrobial treatment dips for 10-sec in trial 1 (**A**) and 2 (**B**) (P = 0.0034, N = 90, n = 5 and P = 0.0020, N = 90, n = 5). Levels not connected by same letter are significantly different

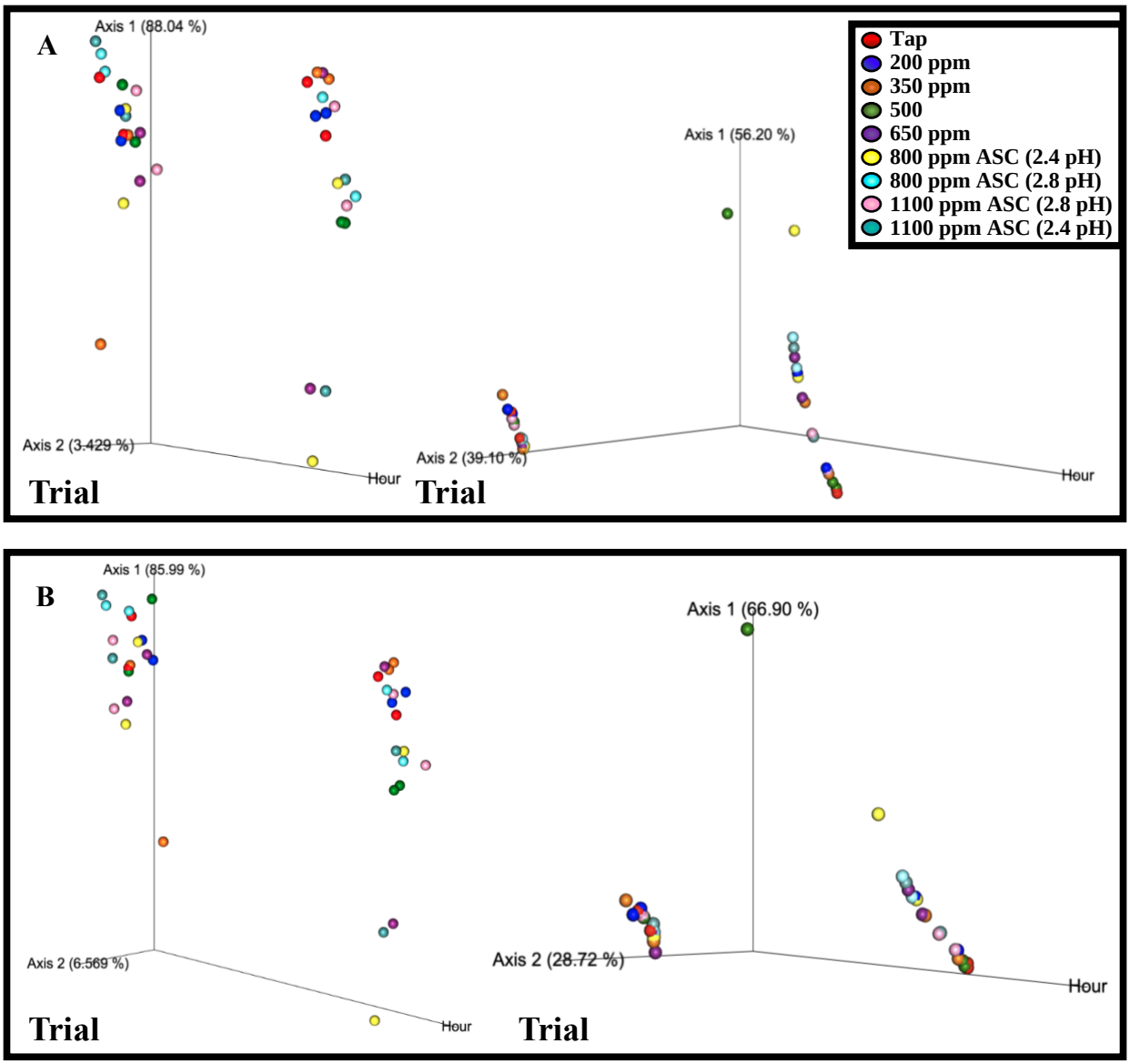

Figure 4. PcoA plots of the Bray Curtis dissimilarity (**A**) and Unweighted Unifrac (**B**) of the microbiota of the rinsate of thighs inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium).

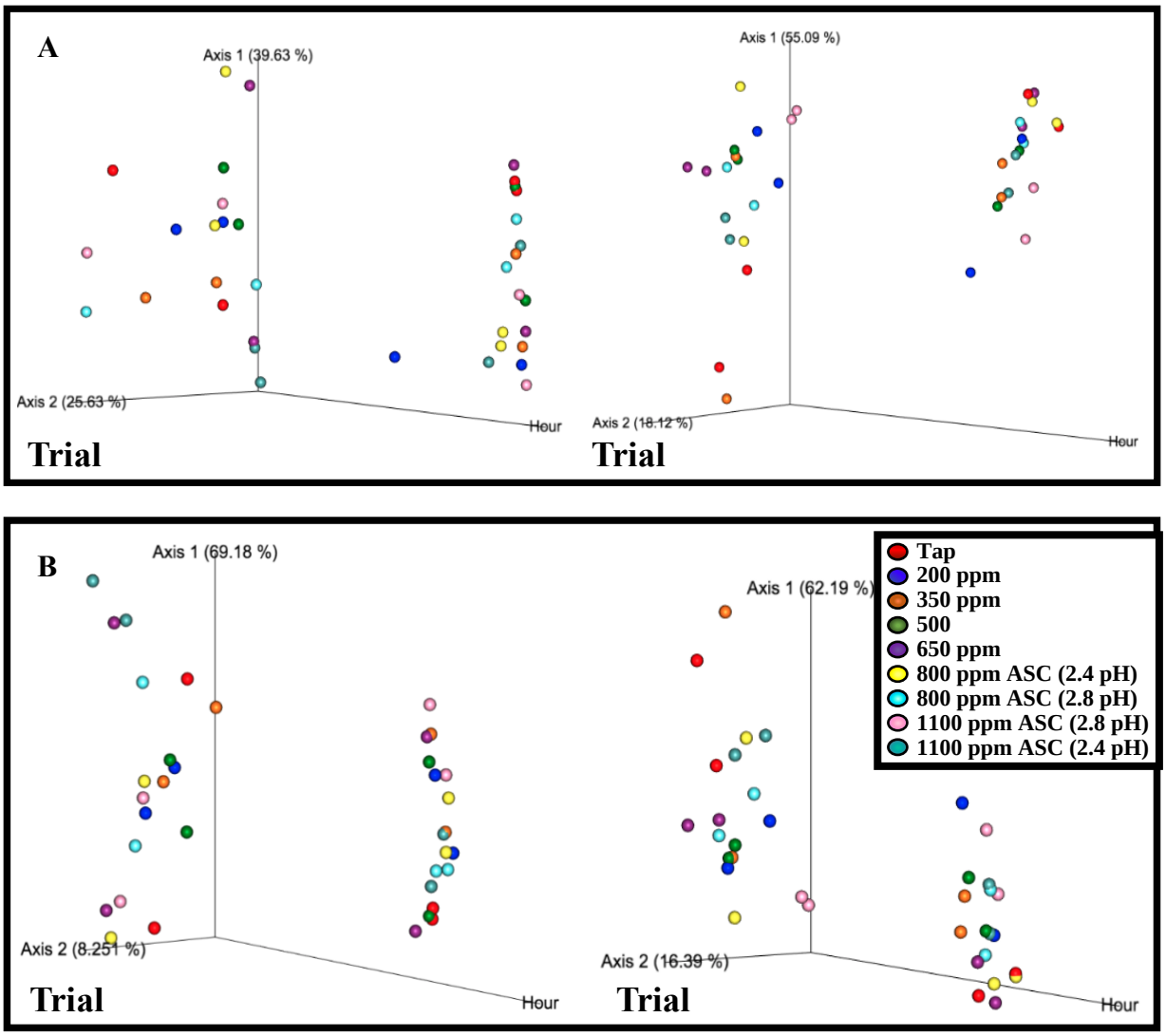

Figure 5. PcoA plots of the Bray Curtis dissimilarity (**A**) and Unweighted Unifrac (**B**) of the microbiota of the rinsate of thighs inoculated with *Campylobacter jejuni*.

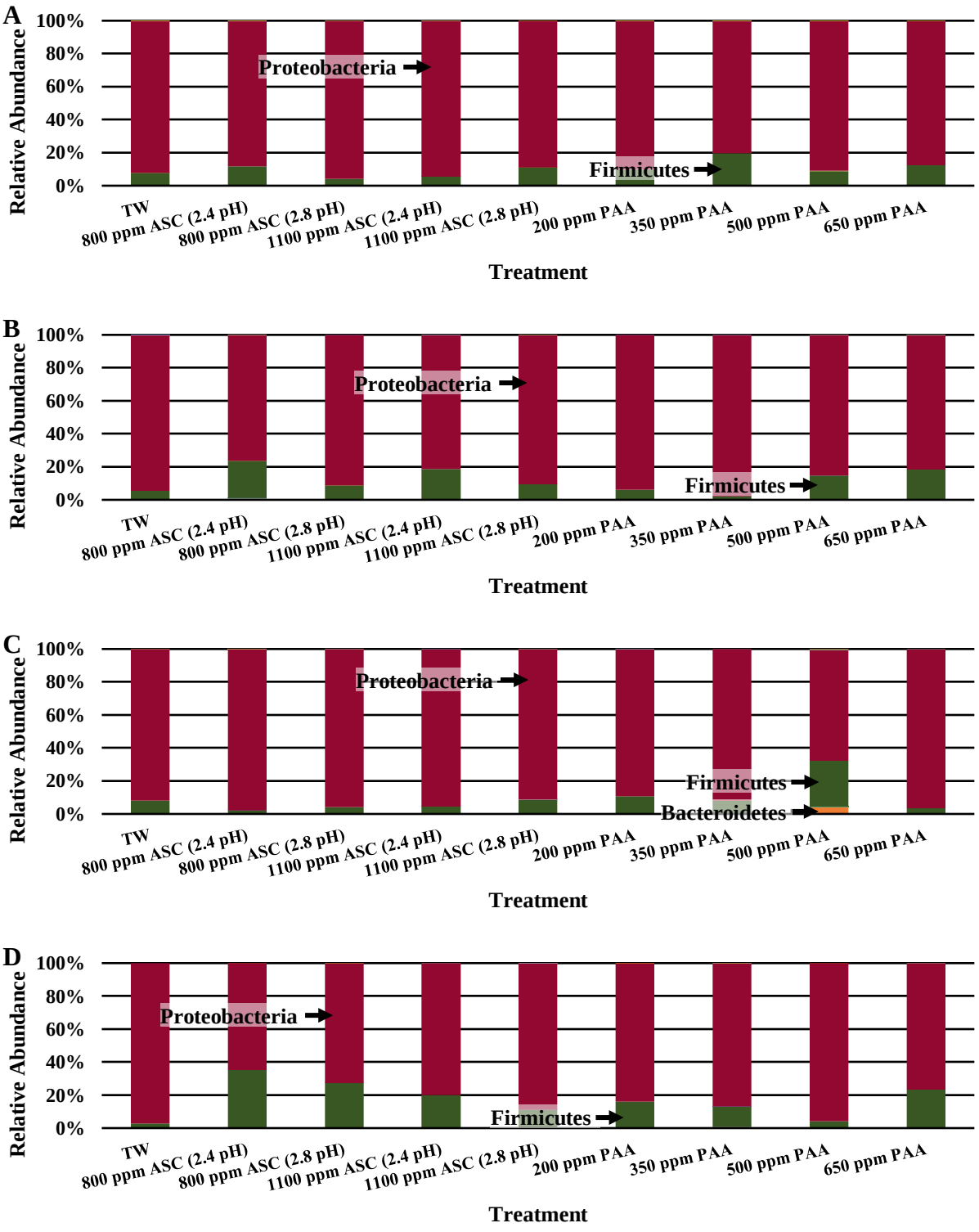

Figure 6. Microbiota composition at the phylum level of rinsates collected from chicken thighs artificially inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium) during trial 1 at 0 (**A**) and 24 h (**B**) and trial 2 at 0 (**C**) and 24 h (**D**).

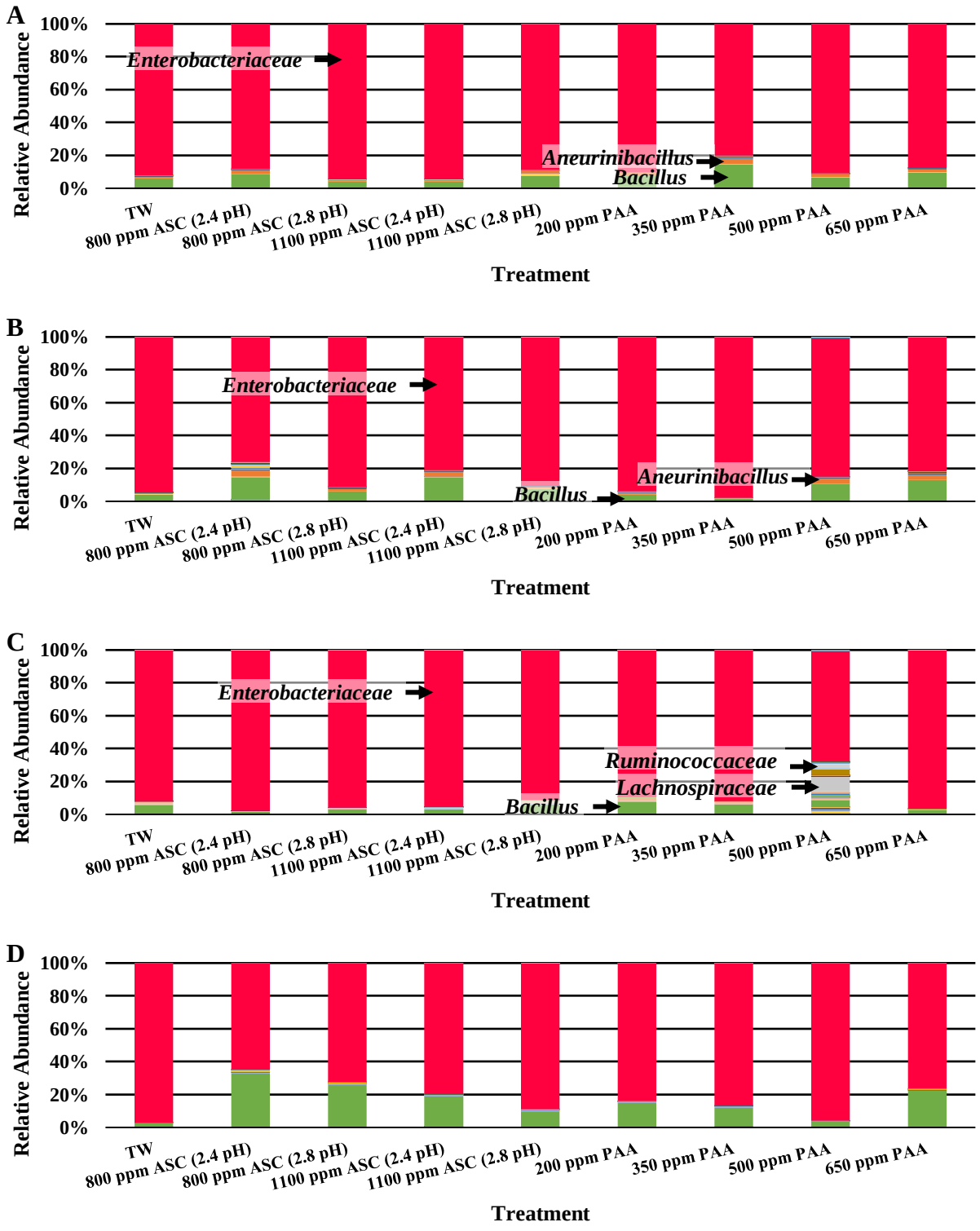

Figure 7. Microbiota composition at the genus level of rinsates collected from chicken thighs artificially inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium) during trial 1 at 0 (**A**) and 24 h (**B**) and trial 2 at 0 (**C**) and 24 h (**D**).

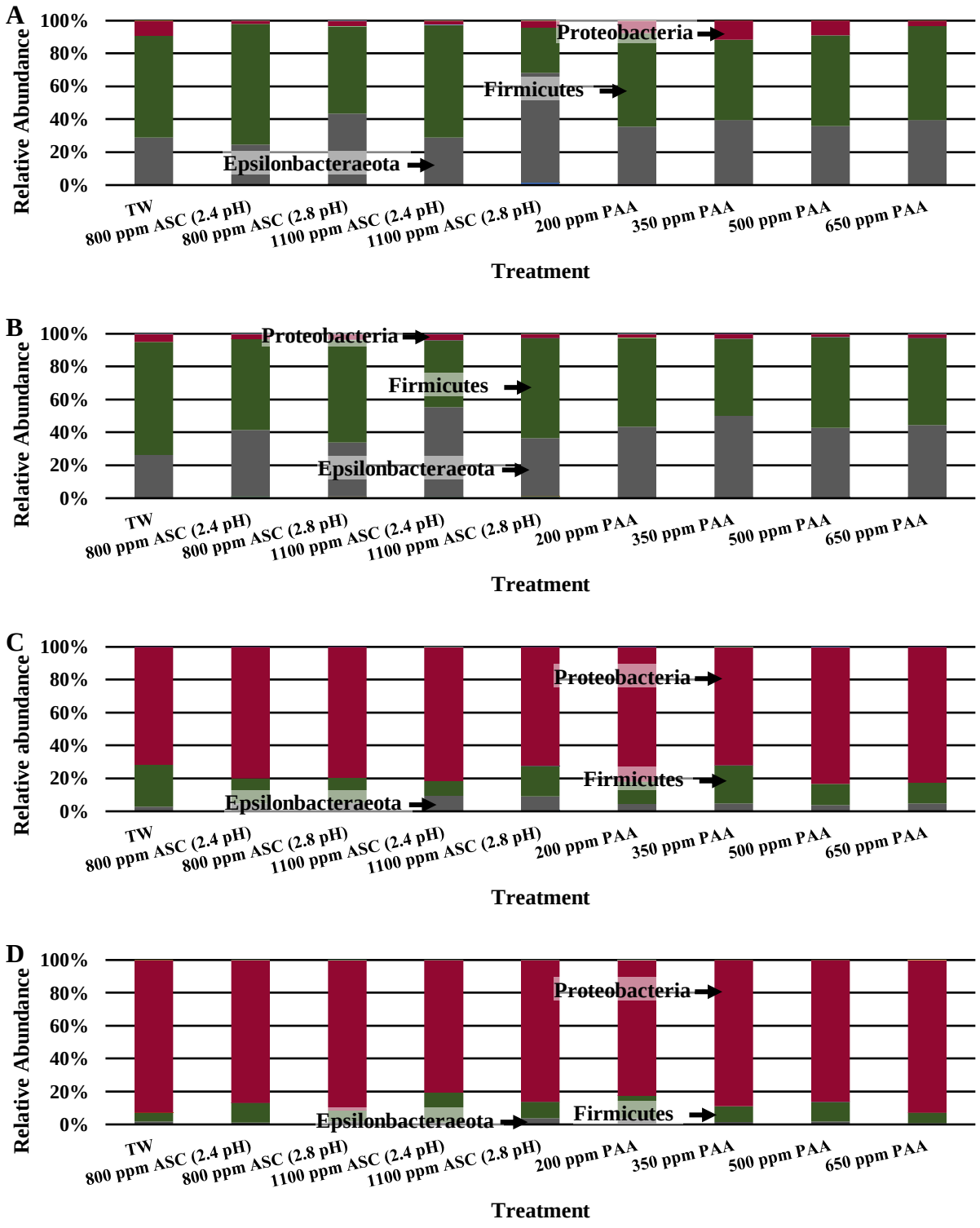

Figure 8. Microbiota composition at the phylum level of rinsates collected from chicken thighs artificially inoculated with *Campylobacter jejuni* during trial 1 at 0 (**A**) and 24 h (**B**) and trial 2 at 0 (**C**) and 24 h (**D**).

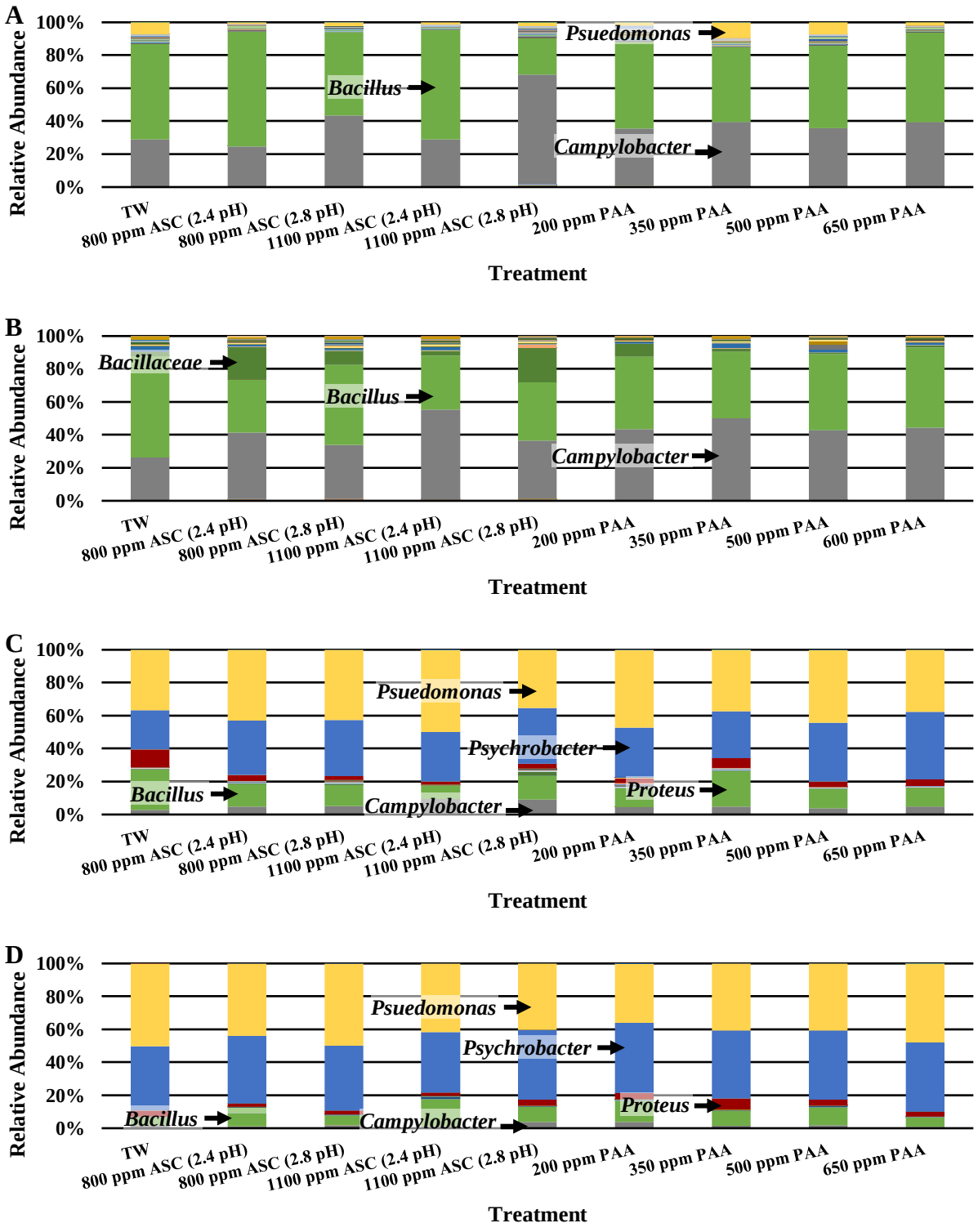

Figure 9. Microbiota composition at the genus level of rinsates collected from chicken thighs artificially inoculated with *Campylobacter jejuni* during trial 1 at 0 (**A**) and 24 h (**B**) and trial 2 at 0 (**C**) and 24 h (**D**).

SUPPLEMENTAL MATERIAL

Supplemental Table 1. Richness and evenness of the microbiota of carcass rinsate during trial 1 when inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium). Differences were determined using Kruskal-Wallis and pairwise comparisons. No differences were detected for either diversity metrics, Pielou's Evenness or Shannon's Diversity Index (P > 0.05).

		Pielou's Evenness			Shannon's Diversity		
Group 1	Group 2	H	p-value	q-value	H	p-value	q-value
Tap Water (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	1.333	0.248	0.558	0.750	0.386	0.696
Tap Water (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	2.083	0.149	0.558	2.083	0.149	0.670
Tap Water (n = 4)	200 ppm PAA (n = 4)	1.333	0.248	0.558	0.750	0.386	0.696
Tap Water (n = 4)	350 ppm PAA (n = 4)	0.333	0.564	0.700	0.000	1.000	1.000
Tap Water (n = 4)	500 ppm PAA (n = 4)	4.083	0.043	0.500	4.083	0.043	0.428
Tap Water (n = 4)	650 ppm PAA (n = 4)	2.083	0.149	0.558	1.333	0.248	0.687
Tap Water (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	4.083	0.043	0.500	4.083	0.043	0.428
Tap Water (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
1100 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.083	0.773	0.818	0.000	1.000	1.000
1100 ppm ASC (2.4 pH) (n = 4)	200 ppm PAA (n = 4)	0.333	0.564	0.700	0.750	0.386	0.696
1100 ppm ASC (2.4 pH) (n = 4)	350 ppm PAA (n = 4)	0.083	0.773	0.818	0.083	0.773	0.927
1100 ppm ASC (2.4 pH) (n = 4)	500 ppm PAA (n = 4)	0.750	0.386	0.700	0.333	0.564	0.781
1100 ppm ASC (2.4 pH) (n = 4)	650 ppm PAA (n = 4)	0.333	0.564	0.700	0.333	0.564	0.781
1100 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.750	0.386	0.700	0.750	0.386	0.696
1100 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.333	0.564	0.700	0.333	0.564	0.781
1100 ppm ASC (2.8 pH) (n = 4)	200 ppm PAA (n = 4)	0.333	0.564	0.700	1.333	0.248	0.687
1100 ppm ASC (2.8 pH) (n = 4)	350 ppm PAA (n = 4)	0.333	0.564	0.700	0.333	0.564	0.781
1100 ppm ASC (2.8 pH) (n = 4)	500 ppm PAA (n = 4)	0.083	0.773	0.818	0.083	0.773	0.927
1100 ppm ASC (2.8 pH) (n = 4)	650 ppm PAA (n = 4)	0.333	0.564	0.700	0.000	1.000	1.000
1100 ppm ASC (2.8 pH) (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.750	0.386	0.700	0.750	0.386	0.696
1100 ppm ASC (2.8 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	2.083	0.149	0.558	3.000	0.083	0.428
200 ppm PAA (n = 4)	350 ppm PAA (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
200 ppm PAA (n = 4)	500 ppm PAA (n = 4)	3.000	0.083	0.500	3.000	0.083	0.428
200 ppm PAA (n = 4)	650 ppm PAA (n = 4)	1.333	0.248	0.558	1.333	0.248	0.687
200 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	3.000	0.083	0.500	4.083	0.043	0.428
200 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.333	0.248	0.558	1.333	0.248	0.687
350 ppm PAA (n = 4)	500 ppm PAA (n = 4)	0.333	0.564	0.700	0.750	0.386	0.696
350 ppm PAA (n = 4)	650 ppm PAA (n = 4)	1.333	0.248	0.558	0.083	0.773	0.927
350 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	1.333	0.248	0.558	1.333	0.248	0.687
350 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.083	0.773	0.818	0.000	1.000	1.000
500 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.333	0.564	0.700	0.083	0.773	0.927
500 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.333	0.564	0.700	0.333	0.564	0.781
500 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	3.000	0.083	0.500	3.000	0.083	0.428
650 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.083	0.773	0.818	0.333	0.564	0.781
650 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.333	0.248	0.558	0.750	0.386	0.696
800 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	3.000	0.083	0.500	3.000	0.083	0.428

Supplemental Table 2. Main effects and interactions of treatment and time on the evenness and richness of the microbiota of carcass rinsate during trial 1 when inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium). Model main effects and interactions were determined using ANOVA in Qiime2.

		Pielou's Evenness			Shannon's Diversity Index		
	Df	Sums of Sqs	F.Model	Pr(>F)	Sums of Sqs	F.Model	Pr(>F)
Treatment	8	0.031	1.252	0.327	1.450	1.016	0.459
Hour	1	0.001	0.191	0.667	0.053	0.299	0.591
Treatment:Hour	8	0.043	1.764	0.151	1.871	1.312	0.299
Residual	18	0.055			3.210		

Supplemental Table 3. Bray Curtis dissimilarity and Unweighted Unifrac of the microbiota of the rinsate of thighs inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium) during trial 1. Differences were determined using Permanova and Permdisp. No differences were detected (P > 0.05).

		Bray Curtis						Weighted Unifrac					
		Permanova			Permdisp			Permanova			Permdisp		
Group 1	Group 2	pseudo-F	P-value	Q-value	F-value	P-value	Q-value	pseudo-F	P-value	Q-value	F-value	P-value	Q-value
Tap Water (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	1.182	0.355	0.872	1.397	0.140	0.801	1.170	0.387	0.904	1.197	0.261	0.841
Tap Water (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	2.189	0.179	0.749	2.086	0.241	0.867	1.437	0.269	0.904	0.532	0.505	0.841
Tap Water (n = 4)	200 ppm PAA (n = 4)	0.544	0.558	0.872	0.059	0.918	0.973	0.528	0.548	0.909	0.037	0.889	1.000
Tap Water (n = 4)	350 ppm PAA (n = 4)	0.486	0.732	0.909	1.476	0.176	0.801	0.481	0.708	0.910	1.440	0.128	0.841
Tap Water (n = 4)	500 ppm PAA (n = 4)	3.744	0.118	0.749	0.300	0.452	0.871	1.958	0.258	0.904	0.560	0.398	0.841
Tap Water (n = 4)	650 ppm PAA (n = 4)	1.656	0.189	0.749	2.098	0.118	0.801	1.394	0.306	0.904	2.119	0.124	0.841
Tap Water (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	3.060	0.052	0.749	1.192	0.337	0.867	2.686	0.065	0.904	1.337	0.311	0.841
Tap Water (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.451	0.610	0.872	0.036	0.885	0.973	0.093	0.941	0.968	0.000	1.000	1.000
1100 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.434	0.678	0.901	0.738	0.576	0.902	0.411	0.669	0.910	0.641	0.613	0.841
200 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.789	0.553	0.872	1.561	0.178	0.801	1.139	0.324	0.904	1.356	0.283	0.841
200 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.257	0.291	0.872	4.280	0.143	0.801	1.112	0.341	0.904	1.423	0.340	0.841
200 ppm PAA (n = 4)	350 ppm PAA (n = 4)	0.335	0.847	0.955	1.683	0.073	0.801	0.311	0.845	0.933	1.408	0.152	0.841
200 ppm PAA (n = 4)	500 ppm PAA (n = 4)	2.531	0.125	0.749	0.643	0.336	0.867	1.078	0.428	0.904	0.632	0.236	0.841
200 ppm PAA (n = 4)	650 ppm PAA (n = 4)	1.143	0.354	0.872	2.362	0.059	0.801	1.138	0.379	0.904	2.292	0.149	0.841
200 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	2.572	0.085	0.749	1.341	0.367	0.871	2.453	0.065	0.904	1.429	0.374	0.841
200 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.387	0.603	0.872	0.120	0.715	0.973	0.811	0.457	0.904	0.023	0.798	0.958
350 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.067	0.895	0.955	0.019	0.925	0.973	0.221	0.522	0.909	0.039	0.553	0.841
350 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.252	0.853	0.955	0.901	0.469	0.871	0.178	0.869	0.933	0.869	0.562	0.841
350 ppm PAA (n = 4)	500 ppm PAA (n = 4)	0.143	0.878	0.955	1.047	0.257	0.867	0.090	0.970	0.970	0.887	0.273	0.841
350 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.171	0.630	0.872	0.008	0.973	0.973	0.073	0.881	0.933	0.010	0.926	1.000
350 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.703	0.437	0.872	0.003	0.921	0.973	0.653	0.477	0.904	0.002	0.966	1.000
350 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.520	0.701	0.901	1.237	0.469	0.871	0.528	0.701	0.910	1.225	0.536	0.841
500 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.128	0.955	0.955	0.879	0.405	0.871	0.327	0.746	0.926	0.494	0.603	0.841
500 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.718	0.431	0.872	0.118	0.709	0.973	0.107	0.852	0.933	0.003	0.972	1.000
500 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.137	0.939	0.955	1.351	0.307	0.867	0.181	0.861	0.933	0.897	0.319	0.841
500 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.931	0.448	0.872	0.792	0.564	0.902	1.012	0.404	0.904	0.712	0.522	0.841
500 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	2.897	0.124	0.749	0.046	0.697	0.973	2.116	0.208	0.904	0.324	0.200	0.841
650 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.026	0.939	0.955	0.004	0.904	0.973	0.111	0.695	0.910	0.011	0.757	0.958
650 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.624	0.562	0.872	1.181	0.320	0.867	0.421	0.651	0.910	1.218	0.164	0.841
650 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.315	0.563	0.872	0.001	0.952	0.973	0.378	0.581	0.909	0.022	0.831	0.965
650 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.471	0.325	0.872	1.506	0.106	0.801	1.406	0.294	0.904	1.734	0.054	0.841
800 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.396	0.540	0.872	0.006	0.847	0.973	0.438	0.564	0.909	0.058	0.783	0.958
800 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.665	0.208	0.749	0.676	0.766	0.973	1.305	0.290	0.904	0.799	0.625	0.841
800 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	3.000	0.100	0.749	0.939	0.508	0.871	2.768	0.102	0.904	1.187	0.387	0.841
800 ppm ASC (2.8 pH) (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	1.065	0.390	0.872	1.032	0.493	0.871	1.255	0.357	0.904	1.060	0.593	0.841

Supplemental Table 4. Main effects and interactions of treatment and time on the dissimilarity and phylogenetic diversity of the microbiota of carcass rinsate during trial 1 when inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium). Model main effects and interactions were determined using ADONIS in Qiime2.

		Bray Curtis					Weighted Unifrac				
	Df	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)
Treatment	8	0.056	0.007	0.950	0.205	0.499	0.007	0.001	0.933	0.199	0.498
Hour	1	0.005	0.005	0.716	0.019	0.420	0.001	0.001	1.441	0.038	0.240
Treatment:Hour	8	0.080	0.010	1.354	0.292	0.264	0.009	0.001	1.322	0.282	0.255
Residuals	18	0.133	0.007	NA	0.485	NA	0.016	0.001	NA	0.480	NA
Total	35	0.274	NA	NA	1.000	NA	0.033	NA	NA	1.000	NA

Supplemental Table 5. Richness and evenness of the microbiota of carcass rinsate during trial 2 when inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium). Differences were determined using Kruskal-Wallis and pairwise comparisons. No differences were detected for either diversity metrics, Pielou's Evenness or Shannon's Diversity Index (P > 0.05).

		Pielou's Evenness			Shannon's Diversity		
Group 1	Group 2	H	p-value	q-value	H	p-value	q-value
Tap Water (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	2.083	0.149	1.000	2.083	0.149	1.000
Tap Water (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	2.083	0.149	1.000	4.083	0.043	0.780
Tap Water (n = 4)	200 ppm PAA (n = 4)	2.083	0.149	1.000	4.083	0.043	0.780
Tap Water (n = 4)	350 ppm PAA (n = 4)	1.333	0.248	1.000	0.750	0.386	1.000
Tap Water (n = 4)	500 ppm PAA (n = 4)	1.333	0.248	1.000	1.333	0.248	1.000
Tap Water (n = 4)	650 ppm PAA (n = 4)	2.083	0.149	1.000	3.000	0.083	0.999
Tap Water (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.750	0.386	1.000	0.000	1.000	1.000
Tap Water (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	2.083	0.149	1.000	1.333	0.248	1.000
1100 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.083	0.773	1.000	0.083	0.773	1.000
1100 ppm ASC (2.4 pH) (n = 4)	200 ppm PAA (n = 4)	0.083	0.773	1.000	0.083	0.773	1.000
1100 ppm ASC (2.4 pH) (n = 4)	350 ppm PAA (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
1100 ppm ASC (2.4 pH) (n = 4)	500 ppm PAA (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
1100 ppm ASC (2.4 pH) (n = 4)	650 ppm PAA (n = 4)	0.083	0.773	1.000	0.083	0.773	1.000
1100 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.083	0.773	1.000	0.083	0.773	1.000
1100 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.083	0.773	1.000	0.083	0.773	1.000
1100 ppm ASC (2.8 pH) (n = 4)	200 ppm PAA (n = 4)	0.333	0.564	1.000	0.000	1.000	1.000
1100 ppm ASC (2.8 pH) (n = 4)	350 ppm PAA (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
1100 ppm ASC (2.8 pH) (n = 4)	500 ppm PAA (n = 4)	0.333	0.564	1.000	1.333	0.248	1.000
1100 ppm ASC (2.8 pH) (n = 4)	650 ppm PAA (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
1100 ppm ASC (2.8 pH) (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
1100 ppm ASC (2.8 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
200 ppm PAA (n = 4)	350 ppm PAA (n = 4)	0.083	0.773	1.000	0.333	0.564	1.000
200 ppm PAA (n = 4)	500 ppm PAA (n = 4)	0.750	0.386	1.000	1.333	0.248	1.000
200 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.083	0.773	1.000	0.083	0.773	1.000
200 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.000	1.000	1.000	0.083	0.773	1.000
200 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
350 ppm PAA (n = 4)	500 ppm PAA (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
350 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.083	0.773	1.000	0.333	0.564	1.000
350 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.083	0.773	1.000	0.000	1.000	1.000
350 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.333	0.564	1.000	0.333	0.564	1.000
500 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.083	0.773	1.000	0.000	1.000	1.000
500 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.333	0.564	1.000	0.333	0.564	1.000
500 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.083	0.773	1.000	0.000	1.000	1.000
650 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.083	0.773	1.000	0.083	0.773	1.000
650 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.750	0.386	1.000	0.000	1.000	1.000
800 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.333	0.564	1.000	0.333	0.564	1.000

Supplemental Table 6. Main effects and interactions of treatment and time on the evenness and richness of the microbiota of carcass rinsate during trial 2 when inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium). Model main effects and interactions were determined using ANOVA in Qiime2.

		Pielou's Evenness			Shannon's Diversity Index		
	Df	Sums of Sqs	F.Model	Pr(>F)	Sums of Sqs	F.Model	Pr(>F)
Treatment	8	0.038	0.624	0.747	3.286	0.709	0.681
Hour	1	0.022	2.868	0.108	0.165	0.285	0.600
Treatment:Hour	8	0.172	2.820	0.032	9.334	2.015	0.104
Residual	18	0.137			10.423		

Supplemental Table 7. Bray Curtis dissimilarity and Unweighted Unifrac of the microbiota of the rinsate of thighs inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium) during trial 2. Differences were determined using Permanova and Permdisp. No differences were detected (P > 0.05).

		Bray Curtis						Weighted Unifrac					
		Permanova			Permdisp			Permanova			Permdisp		
Group 1	Group 2	pseudo-F	P-value	Q-value	F-value	P-value	Q-value	pseudo-F	P-value	Q-value	F-value	P-value	Q-value
Tap Water (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	1.450	0.257	0.911	1.587	0.410	0.873	1.472	0.213	0.978	1.619	0.417	0.790
Tap Water (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	2.812	0.111	0.911	0.011	0.964	0.964	3.330	0.104	0.978	0.284	0.495	0.813
Tap Water (n = 4)	200 ppm PAA (n = 4)	2.787	0.077	0.911	0.621	0.695	0.896	2.910	0.083	0.978	0.454	0.815	0.923
Tap Water (n = 4)	350 ppm PAA (n = 4)	1.392	0.292	0.911	2.378	0.166	0.664	1.462	0.244	0.978	2.183	0.363	0.790
Tap Water (n = 4)	500 ppm PAA (n = 4)	0.968	0.626	0.911	0.910	0.747	0.896	0.982	0.529	0.978	0.885	0.732	0.923
Tap Water (n = 4)	650 ppm PAA (n = 4)	1.709	0.386	0.911	7.108	0.446	0.873	1.221	0.368	0.978	10.287	0.091	0.410
Tap Water (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	1.688	0.445	0.911	3.484	0.383	0.873	1.590	0.453	0.978	2.308	0.410	0.790
Tap Water (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	2.054	0.329	0.911	10.367	0.361	0.873	1.925	0.404	0.978	12.165	0.356	0.790
1100 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.460	0.723	0.911	1.671	0.221	0.723	0.475	0.705	0.978	3.041	0.029	0.281
200 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.047	0.970	1.000	0.286	0.465	0.873	0.147	0.732	0.978	0.290	0.497	0.813
200 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.757	0.520	0.911	0.576	0.711	0.896	0.645	0.540	0.978	1.097	0.464	0.813
200 ppm PAA (n = 4)	350 ppm PAA (n = 4)	0.263	0.715	0.911	0.082	0.694	0.896	0.328	0.672	0.978	0.080	0.746	0.923
200 ppm PAA (n = 4)	500 ppm PAA (n = 4)	1.039	0.358	0.911	0.516	0.691	0.896	0.784	0.877	0.989	0.607	0.649	0.899
200 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.116	0.889	0.976	1.370	0.109	0.664	0.120	0.879	0.989	1.837	0.076	0.410
200 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.392	0.735	0.911	2.228	0.126	0.664	0.368	0.691	0.978	1.510	0.395	0.790
200 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.249	0.758	0.911	2.407	0.026	0.468	0.246	0.778	0.978	3.175	0.039	0.281
350 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.146	0.895	0.976	0.135	0.795	0.923	0.327	0.608	0.978	0.182	0.774	0.923
350 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.306	0.759	0.911	2.469	0.089	0.664	0.300	0.604	0.978	4.009	0.033	0.281
350 ppm PAA (n = 4)	500 ppm PAA (n = 4)	0.965	0.499	0.911	0.419	0.944	0.964	0.875	0.690	0.978	0.527	0.906	0.981
350 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.242	0.719	0.911	1.509	0.130	0.664	0.133	0.772	0.978	2.455	0.091	0.410
350 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.658	0.533	0.911	1.578	0.342	0.873	0.625	0.464	0.978	1.492	0.299	0.790
350 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.484	0.582	0.911	3.667	0.115	0.664	0.497	0.556	0.978	5.024	0.121	0.484
500 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.905	0.695	0.911	0.247	0.860	0.964	0.717	0.953	1.000	0.379	0.820	0.923
500 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.032	0.154	0.911	0.886	0.593	0.896	0.839	1.000	1.000	1.024	0.331	0.790
500 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.942	0.544	0.911	0.161	0.961	0.964	0.802	0.708	0.978	0.243	0.954	0.981
500 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.830	0.750	0.911	0.002	0.732	0.896	0.623	0.688	0.978	0.025	1.000	1.000
500 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.961	0.436	0.911	0.068	0.889	0.964	0.715	1.000	1.000	0.180	0.940	0.981
650 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.005	1.000	1.000	0.129	0.632	0.896	0.082	0.854	0.989	0.409	0.547	0.821
650 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.640	0.484	0.911	9.982	0.025	0.468	0.335	0.625	0.978	18.914	0.024	0.281
650 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.197	0.730	0.911	0.821	0.485	0.873	0.246	0.663	0.978	0.436	0.632	0.899
650 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.069	0.727	0.911	0.317	0.373	0.873	0.120	0.665	0.978	0.242	0.308	0.790
800 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.295	0.711	0.911	1.012	0.541	0.896	0.280	0.733	0.978	0.728	0.531	0.821
800 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.953	0.417	0.911	3.863	0.213	0.723	0.753	0.520	0.978	2.791	0.258	0.790
800 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.078	1.000	1.000	0.391	0.602	0.896	0.055	1.000	1.000	0.242	0.752	0.923
800 ppm ASC (2.8 pH) (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.134	0.787	0.914	0.647	0.425	0.873	0.101	0.788	0.978	1.018	0.236	0.790
800 ppm ASC (2.8 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.979	0.414	0.911	11.478	0.151	0.664	0.749	0.426	0.978	31.369	0.032	0.281

Supplemental Table 8. Main effects and interactions of treatment and time on the dissimilarity and phylogenetic diversity of the microbiota of carcass rinsate during trial 2 when inoculated with a cocktail of *Salmonella* (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium). Model main effects and interactions were determined using ADONIS in Qiime2.

		Bray Curtis					Weighted Unifrac				
	Df	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)
Treatment	8	0.134	0.017	1.227	0.183	0.221	0.016	0.002	1.002	0.168	0.442
Hour	1	0.130	0.130	9.560	0.178	0.001	0.011	0.011	5.402	0.113	0.004
Treatment:Hour	8	0.223	0.028	2.052	0.305	0.009	0.032	0.004	2.044	0.342	0.017
Residuals	18	0.245	0.014	NA	0.335	NA	0.035	0.002	NA	0.377	NA
Total	35	0.732	NA	NA	1.000	NA	0.094	NA	NA	1.000	NA

Supplemental Table 9. Richness and evenness of the microbiota of carcass rinsate during trial 1 when inoculated with *Campylobacter jejuni*. Differences were determined using Kruskal-Wallis and pairwise comparisons. No differences were detected for either diversity metrics, Pielou's Evenness or Shannon's Diversity Index (P > 0.05).

		Pielou's Evenness			Shannon's Diversity		
Group 1	Group 2	H	P-value	Q-value	H	P-value	Q-value
Tap Water (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	5.333	0.021	0.188	2.083	0.149	0.745
Tap Water (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	5.333	0.021	0.188	0.750	0.386	0.773
Tap Water (n = 4)	200 ppm PAA (n = 4)	0.750	0.386	0.535	0.000	1.000	1.000
Tap Water (n = 4)	350 ppm PAA (n = 4)	0.750	0.386	0.535	1.333	0.248	0.745
Tap Water (n = 4)	500 ppm PAA (n = 4)	0.083	0.773	0.818	0.083	0.773	0.897
Tap Water (n = 4)	650 ppm PAA (n = 4)	5.333	0.021	0.188	4.083	0.043	0.745
Tap Water (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	4.083	0.043	0.260	0.333	0.564	0.812
Tap Water (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	3.000	0.083	0.273	1.333	0.248	0.745
1100 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.750	0.386	0.535	0.333	0.564	0.812
1100 ppm ASC (2.4 pH) (n = 4)	200 ppm PAA (n = 4)	1.333	0.248	0.426	0.750	0.386	0.773
1100 ppm ASC (2.4 pH) (n = 4)	350 ppm PAA (n = 4)	1.333	0.248	0.426	0.083	0.773	0.897
1100 ppm ASC (2.4 pH) (n = 4)	500 ppm PAA (n = 4)	1.333	0.248	0.426	1.333	0.248	0.745
1100 ppm ASC (2.4 pH) (n = 4)	650 ppm PAA (n = 4)	0.750	0.386	0.535	2.083	0.149	0.745
1100 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	4.083	0.043	0.260	1.333	0.248	0.745
1100 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.333	0.564	0.655	0.333	0.564	0.812
1100 ppm ASC (2.8 pH) (n = 4)	200 ppm PAA (n = 4)	3.000	0.083	0.273	0.333	0.564	0.812
1100 ppm ASC (2.8 pH) (n = 4)	350 ppm PAA (n = 4)	3.000	0.083	0.273	0.000	1.000	1.000
1100 ppm ASC (2.8 pH) (n = 4)	500 ppm PAA (n = 4)	2.083	0.149	0.357	0.750	0.386	0.773
1100 ppm ASC (2.8 pH) (n = 4)	650 ppm PAA (n = 4)	0.333	0.564	0.655	0.750	0.386	0.773
1100 ppm ASC (2.8 pH) (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	2.083	0.149	0.357	0.083	0.773	0.897
1100 ppm ASC (2.8 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.750	0.386	0.535	0.000	1.000	1.000
200 ppm PAA (n = 4)	350 ppm PAA (n = 4)	0.000	1.000	1.000	0.333	0.564	0.812
200 ppm PAA (n = 4)	500 ppm PAA (n = 4)	0.333	0.564	0.655	0.000	1.000	1.000
200 ppm PAA (n = 4)	650 ppm PAA (n = 4)	3.000	0.083	0.273	3.000	0.083	0.745
200 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
200 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.333	0.248	0.426	0.750	0.386	0.773
350 ppm PAA (n = 4)	500 ppm PAA (n = 4)	0.333	0.564	0.655	1.333	0.248	0.745
350 ppm PAA (n = 4)	650 ppm PAA (n = 4)	3.000	0.083	0.273	1.333	0.248	0.745
350 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.083	0.773	0.818	0.333	0.564	0.812
350 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.333	0.248	0.426	0.083	0.773	0.897
500 ppm PAA (n = 4)	650 ppm PAA (n = 4)	2.083	0.149	0.357	3.000	0.083	0.745
500 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.333	0.564	0.655	0.083	0.773	0.897
500 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	2.083	0.149	0.357	0.750	0.386	0.773
650 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	5.333	0.021	0.188	3.000	0.083	0.745
650 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.083	0.773	0.818	0.083	0.773	0.897
800 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.333	0.248	0.426	0.333	0.564	0.812

Supplemental Table 10. Main effects and interactions of treatment and time on the evenness and richness of the microbiota of carcass rinsate during trial 1 when inoculated with *Campylobacter jejuni.* Model main effects and interactions were determined using ANOVA in Qiime2.

	Pielou's Evenness				Shannon's Diversity Index		
	Df	Sums of Sqs	F.Model	Pr(>F)	Sums of Sqs	F.Model	Pr(>F)
Treatment	8	0.037	3.931	0.008	0.794	0.937	0.511
Hour	1	0.000	0.120	0.733	0.090	0.853	0.368
Treatment:Hour	8	0.028	2.958	0.027	1.797	2.121	0.088
Residual	18	0.021			1.906		

Supplemental Table 11. Bray Curtis dissimilarity and Unweighted Unifrac of the microbiota of the rinsate of thighs inoculated with *Campylobacter jejuni* during trial 1. Differences were determined using Permanova and Permdisp. No differences were detected (P > 0.05).

		Bray Curtis						Weighted Unifrac					
		Permanova			Permdisp			Permanova			Permdisp		
Group 1	Group 2	pseudo-F	P-value	Q-value	F-value	P-value	Q-value	pseudo-F	P-value	Q-value	F-value	P-value	Q-value
Tap Water (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.539	0.679	0.877	0.012	0.938	1.000	0.812	0.456	0.789	0.110	0.704	0.981
Tap Water (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	2.451	0.074	0.843	0.050	0.763	0.979	2.833	0.141	0.789	0.427	0.518	0.927
Tap Water (n = 4)	200 ppm PAA (n = 4)	1.172	0.341	0.843	0.041	0.717	0.979	1.631	0.213	0.789	0.234	0.541	0.927
Tap Water (n = 4)	350 ppm PAA (n = 4)	1.261	0.387	0.843	0.268	0.569	0.979	2.177	0.195	0.789	0.135	0.616	0.981
Tap Water (n = 4)	500 ppm PAA (n = 4)	0.443	0.889	0.970	0.493	0.481	0.979	0.549	0.598	0.789	0.012	0.973	0.981
Tap Water (n = 4)	650 ppm PAA (n = 4)	0.646	0.618	0.877	0.077	0.696	0.979	0.739	0.360	0.789	1.064	0.386	0.782
Tap Water (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.566	0.682	0.877	0.001	1.000	1.000	0.583	0.532	0.789	0.022	0.866	0.981
Tap Water (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.595	0.644	0.877	0.005	0.973	1.000	0.829	0.503	0.789	0.076	0.848	0.981
1100 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.958	0.429	0.843	0.026	0.733	0.979	0.673	0.482	0.789	0.178	0.484	0.917
200 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.137	1.000	1.000	0.154	0.648	0.979	0.360	0.722	0.789	1.913	0.105	0.540
200 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.185	0.336	0.843	0.309	0.621	0.979	1.610	0.207	0.789	7.115	0.190	0.693
200 ppm PAA (n = 4)	350 ppm PAA (n = 4)	0.476	0.817	0.970	0.392	0.297	0.979	1.332	0.251	0.789	0.051	0.816	0.981
200 ppm PAA (n = 4)	500 ppm PAA (n = 4)	2.406	0.121	0.843	1.048	0.321	0.979	1.050	0.411	0.789	0.613	0.229	0.693
200 ppm PAA (n = 4)	650 ppm PAA (n = 4)	1.666	0.260	0.843	0.935	0.216	0.979	0.778	0.442	0.789	8.874	0.035	0.432
200 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.808	0.557	0.843	0.106	0.728	0.979	0.842	0.506	0.789	0.137	0.959	0.981
200 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.226	0.904	0.970	0.117	0.704	0.979	0.550	0.665	0.789	0.069	0.889	0.981
350 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.249	0.943	0.970	0.754	0.504	0.979	0.444	0.726	0.789	1.117	0.227	0.693
350 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.256	0.201	0.843	1.012	0.357	0.979	0.881	0.374	0.789	3.680	0.104	0.540
350 ppm PAA (n = 4)	500 ppm PAA (n = 4)	2.417	0.090	0.843	0.475	0.517	0.979	1.357	0.296	0.789	0.168	0.699	0.981
350 ppm PAA (n = 4)	650 ppm PAA (n = 4)	1.504	0.261	0.843	5.646	0.055	0.979	0.818	0.431	0.789	7.532	0.050	0.450
350 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.995	0.415	0.843	0.698	0.518	0.979	2.037	0.159	0.789	0.041	0.786	0.981
350 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.732	0.554	0.843	0.666	0.549	0.979	1.344	0.286	0.789	0.007	0.882	0.981
500 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	1.296	0.275	0.843	1.314	0.240	0.979	0.493	0.640	0.789	0.556	0.323	0.775
500 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	2.623	0.086	0.843	1.602	0.228	0.979	1.799	0.207	0.789	2.396	0.231	0.693
500 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.594	0.562	0.843	7.051	0.030	0.979	0.422	0.601	0.789	4.061	0.036	0.432
500 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	1.048	0.373	0.843	1.280	0.223	0.979	0.580	0.593	0.789	0.006	0.981	0.981
500 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.171	0.278	0.843	1.194	0.238	0.979	0.394	0.705	0.789	0.041	0.778	0.981
650 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.733	0.518	0.843	0.054	0.789	0.979	0.124	0.895	0.895	0.857	0.210	0.693
650 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.459	0.223	0.843	0.000	1.000	1.000	0.649	0.479	0.789	0.434	0.391	0.782
650 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.710	0.490	0.843	0.101	0.602	0.979	0.502	0.554	0.789	2.034	0.094	0.540
650 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.895	0.407	0.843	0.068	0.776	0.979	0.460	0.683	0.789	3.484	0.024	0.432
800 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.594	0.776	0.963	0.006	0.911	1.000	0.534	0.544	0.789	0.369	0.668	0.981
800 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.399	0.328	0.843	0.055	0.720	0.979	2.222	0.255	0.789	1.066	0.288	0.775
800 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.551	0.838	0.970	0.004	0.912	1.000	0.551	0.745	0.789	0.009	0.931	0.981
800 ppm ASC (2.8 pH) (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.147	0.923	0.970	0.001	1.000	1.000	0.209	0.820	0.843	0.640	0.360	0.782
800 ppm ASC (2.8 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.910	0.473	0.843	0.027	0.766	0.979	1.462	0.229	0.789	1.811	0.320	0.775

Supplemental Table 12. Main effects and interactions of treatment and time on the dissimilarity and phylogenetic diversity of the microbiota of carcass rinsate during trial 1 when inoculated with *Campylobacter jejuni*. Model main effects and interactions were determined using ADONIS in Qiime2.

		Bray Curtis					Weighted Unifrac				
	Df	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)
Treatment	8	0.461	0.058	1.149	0.226	0.323	0.461	0.058	1.149	0.226	0.323
Hour	1	0.163	0.163	3.257	0.080	0.029	0.163	0.163	3.257	0.080	0.029
Treatment:Hour	8	0.511	0.064	1.274	0.251	0.189	0.511	0.064	1.274	0.251	0.189
Residuals	18	0.903	0.050	NA	0.443	NA	0.903	0.050	NA	0.443	NA
Total	35	2.039	NA	NA	1.000	NA	2.039	NA	NA	1.000	NA

Supplemental Table 13. Richness and evenness of the microbiota of carcass rinsate during trial 2 when inoculated with *Campylobacter jejuni*. Differences were determined using Kruskal-Wallis and pairwise comparisons. No differences were detected for either diversity metrics, Pielou's Evenness or Shannon's Diversity Index ($P > 0.05$).

		Pielou's Evenness			Shannon's Diversity		
Group 1	Group 2	H	P-value	Q-value	H	P-value	Q-value
Tap Water (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.333	0.564	0.846	0.000	1.000	1.000
Tap Water (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.000	1.000	1.000	0.750	0.386	0.773
Tap Water (n = 4)	200 ppm PAA (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
Tap Water (n = 4)	350 ppm PAA (n = 4)	0.333	0.564	0.846	0.333	0.564	0.882
Tap Water (n = 4)	500 ppm PAA (n = 4)	0.000	1.000	1.000	0.083	0.773	0.897
Tap Water (n = 4)	650 ppm PAA (n = 4)	0.000	1.000	1.000	0.750	0.386	0.773
Tap Water (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.000	1.000	1.000	0.083	0.773	0.897
Tap Water (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.333	0.564	0.846	0.083	0.773	0.897
1100 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.333	0.564	0.846	0.333	0.564	0.882
1100 ppm ASC (2.4 pH) (n = 4)	200 ppm PAA (n = 4)	0.333	0.564	0.846	1.333	0.248	0.687
1100 ppm ASC (2.4 pH) (n = 4)	350 ppm PAA (n = 4)	0.750	0.386	0.846	0.083	0.773	0.897
1100 ppm ASC (2.4 pH) (n = 4)	500 ppm PAA (n = 4)	0.333	0.564	0.846	0.750	0.386	0.773
1100 ppm ASC (2.4 pH) (n = 4)	650 ppm PAA (n = 4)	0.000	1.000	1.000	0.750	0.386	0.773
1100 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
1100 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.083	0.773	1.000	1.333	0.248	0.687
1100 ppm ASC (2.8 pH) (n = 4)	200 ppm PAA (n = 4)	0.000	1.000	1.000	0.333	0.564	0.882
1100 ppm ASC (2.8 pH) (n = 4)	350 ppm PAA (n = 4)	1.333	0.248	0.846	0.083	0.773	0.897
1100 ppm ASC (2.8 pH) (n = 4)	500 ppm PAA (n = 4)	0.750	0.386	0.846	1.333	0.248	0.687
1100 ppm ASC (2.8 pH) (n = 4)	650 ppm PAA (n = 4)	0.750	0.386	0.846	2.083	0.149	0.687
1100 ppm ASC (2.8 pH) (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.750	0.386	0.846	1.333	0.248	0.687
1100 ppm ASC (2.8 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.333	0.248	0.846	2.083	0.149	0.687
200 ppm PAA (n = 4)	350 ppm PAA (n = 4)	1.333	0.248	0.846	0.083	0.773	0.897
200 ppm PAA (n = 4)	500 ppm PAA (n = 4)	0.750	0.386	0.846	1.333	0.248	0.687
200 ppm PAA (n = 4)	650 ppm PAA (n = 4)	1.333	0.248	0.846	3.000	0.083	0.687
200 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.333	0.564	0.846	0.750	0.386	0.773
200 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.750	0.386	0.846	1.333	0.248	0.687
350 ppm PAA (n = 4)	500 ppm PAA (n = 4)	4.083	0.043	0.749	0.000	1.000	1.000
350 ppm PAA (n = 4)	650 ppm PAA (n = 4)	3.000	0.083	0.749	0.333	0.564	0.882
350 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	3.000	0.083	0.749	0.083	0.773	0.897
350 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	3.000	0.083	0.749	2.083	0.149	0.687
500 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.083	0.773	1.000	0.083	0.773	0.897
500 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.333	0.564	0.846	0.333	0.564	0.882
500 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.083	0.773	1.000	1.333	0.248	0.687
650 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.333	0.564	0.846	1.333	0.248	0.687
650 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.000	1.000	1.000	0.000	1.000	1.000
800 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.333	0.564	0.846	1.333	0.248	0.687

Supplemental Table 14. Main effects and interactions of treatment and time on the evenness and richness of the microbiota of carcass rinsate during trial 2 when inoculated with *Campylobacter jejuni.* Model main effects and interactions were determined using ANOVA in Qiime2.

	Pielou's Evenness				Shannon's Diversity Index		
	Df	Sums of Sqs	F.Model	Pr(>F)	Sums of Sqs	F.Model	Pr(>F)
Treatment	8	0.011	1.220	0.342	0.444	0.934	0.513
Hour	1	0.015	12.938	0.002	1.060	17.841	0.001
Treatment:Hour	8	0.021	2.335	0.064	0.451	0.950	0.502
Residual	18	0.021			1.069		

Supplemental Table 15. Bray Curtis dissimilarity and Unweighted Unifrac of the microbiota of the rinsate of thighs inoculated with Campylobacter jejuni during trial 1. Differences were determined using Permanova and Permdisp. No differences were detected (P > 0.05).

		Bray Curtis						Weighted Unifrac					
		Permanova			Permdisp			Permanova			Permdisp		
Group 1	Group 2	pseudo-F	P-value	Q-value	F-value	P-value	Q-value	pseudo-F	P-value	Q-value	F-value	P-value	Q-value
Tap Water (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.801	0.495	0.875	2.794	0.217	0.651	0.683	0.498	0.886	1.251	0.134	0.439
Tap Water (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.404	0.311	0.806	5.533	0.063	0.552	1.034	0.363	0.886	3.222	0.021	0.303
Tap Water (n = 4)	200 ppm PAA (n = 4)	0.455	0.729	0.875	1.342	0.259	0.666	0.193	0.862	0.970	2.004	0.168	0.504
Tap Water (n = 4)	350 ppm PAA (n = 4)	0.218	0.766	0.890	0.546	0.514	0.946	0.236	0.670	0.886	0.248	0.542	0.848
Tap Water (n = 4)	500 ppm PAA (n = 4)	0.829	0.450	0.853	14.345	0.109	0.561	0.505	0.576	0.886	11.653	0.029	0.303
Tap Water (n = 4)	650 ppm PAA (n = 4)	1.619	0.343	0.806	9.271	0.186	0.651	0.955	0.467	0.886	5.526	0.052	0.303
Tap Water (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	1.054	0.395	0.806	6.031	0.147	0.651	0.416	0.646	0.886	8.372	0.038	0.303
Tap Water (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.238	0.252	0.806	2.512	0.235	0.651	0.700	0.483	0.886	1.327	0.330	0.697
1100 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.587	0.287	0.806	0.112	0.611	0.946	1.379	0.321	0.886	0.429	0.368	0.697
200 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.539	0.641	0.875	0.160	0.725	0.946	0.636	0.568	0.886	0.109	0.731	0.919
200 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.456	0.722	0.875	0.461	0.333	0.799	0.718	0.543	0.886	0.079	0.669	0.919
200 ppm PAA (n = 4)	350 ppm PAA (n = 4)	0.453	0.695	0.875	0.000	1.000	1.000	0.273	0.778	0.913	0.045	1.000	1.000
200 ppm PAA (n = 4)	500 ppm PAA (n = 4)	0.078	1.000	1.000	5.674	0.050	0.552	0.303	0.931	0.986	4.288	0.051	0.303
200 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.543	0.696	0.875	3.508	0.075	0.552	0.661	0.664	0.886	0.666	0.315	0.697
200 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.184	1.000	1.000	1.363	0.186	0.651	0.129	1.000	1.000	2.149	0.092	0.382
200 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.154	0.940	1.000	0.398	0.477	0.946	0.379	0.657	0.886	0.087	0.464	0.835
350 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	1.382	0.298	0.806	0.054	0.914	0.968	1.223	0.327	0.886	0.003	0.979	1.000
350 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	0.894	0.382	0.806	0.155	0.808	0.946	0.572	0.714	0.886	0.125	0.771	0.925
350 ppm PAA (n = 4)	500 ppm PAA (n = 4)	1.043	0.403	0.806	0.875	0.590	0.946	0.843	0.642	0.886	0.967	0.275	0.697
350 ppm PAA (n = 4)	650 ppm PAA (n = 4)	1.654	0.298	0.806	0.486	0.832	0.946	1.180	0.369	0.886	0.254	0.974	1.000
350 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	1.264	0.394	0.806	0.251	0.826	0.946	0.744	0.620	0.886	0.592	0.740	0.919
350 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	1.618	0.304	0.806	0.182	0.865	0.946	1.228	0.409	0.886	0.125	0.946	1.000
500 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	1.474	0.258	0.806	2.630	0.088	0.552	1.086	0.365	0.886	4.462	0.059	0.303
500 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.224	0.310	0.806	1.449	0.441	0.934	1.625	0.242	0.886	1.943	0.359	0.697
500 ppm PAA (n = 4)	650 ppm PAA (n = 4)	0.664	0.552	0.875	0.913	0.083	0.552	0.333	0.786	0.913	3.726	0.050	0.303
500 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	0.240	1.000	1.000	1.154	0.211	0.651	0.329	0.893	0.974	0.394	0.536	0.848
500 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.604	0.590	0.875	0.444	0.686	0.946	0.412	0.689	0.886	0.506	0.683	0.919
650 ppm PAA (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	2.018	0.142	0.806	1.327	0.092	0.552	1.183	0.391	0.886	1.157	0.106	0.382
650 ppm PAA (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	2.362	0.186	0.806	0.310	0.691	0.946	2.418	0.163	0.886	0.128	0.717	0.919
650 ppm PAA (n = 4)	800 ppm ASC (2.4 pH) (n = 4)	1.227	0.335	0.806	0.220	0.572	0.946	1.215	0.253	0.886	0.875	0.271	0.697
650 ppm PAA (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.745	0.519	0.875	0.064	0.859	0.946	0.367	0.683	0.886	0.002	0.978	1.000
800 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	0.566	0.568	0.875	0.504	0.377	0.848	0.674	0.454	0.886	2.662	0.101	0.382
800 ppm ASC (2.4 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.380	0.268	0.806	0.112	0.699	0.946	1.644	0.218	0.886	1.256	0.309	0.697
800 ppm ASC (2.4 pH) (n = 4)	800 ppm ASC (2.8 pH) (n = 4)	0.444	0.620	0.875	0.000	1.000	1.000	0.548	0.475	0.886	0.174	0.720	0.919
800 ppm ASC (2.8 pH) (n = 4)	1100 ppm ASC (2.4 pH) (n = 4)	-0.112	1.000	1.000	0.161	0.538	0.946	0.045	0.969	0.997	0.209	0.498	0.848
800 ppm ASC (2.8 pH) (n = 4)	1100 ppm ASC (2.8 pH) (n = 4)	1.793	0.181	0.806	0.026	0.867	0.946	1.818	0.213	0.886	0.023	0.874	1.000

Supplemental Table 16. Main effects and interactions of treatment and time on the dissimilarity and phylogenetic diversity of the microbiota of carcass rinsate during trial 2 when inoculated with *Campylobacter jejuni*. Model main effects and interactions were determined using ADONIS in Qiime2.

		Bray Curtis					Weighted Unifrac				
	Df	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)
Treatment	8	0.096	0.012	1.981	0.216	0.041	0.009	0.001	1.443	0.184	0.155
Hour	1	0.106	0.106	17.450	0.238	0.001	0.010	0.010	13.077	0.209	0.001
Treatment:Hour	8	0.133	0.017	2.754	0.300	0.002	0.016	0.002	2.497	0.319	0.013
Residuals	18	0.109	0.006	NA	0.245	NA	0.014	0.001	NA	0.288	NA
Total	35	0.444	NA	NA	1.000	NA	0.050	NA	NA	1.000	NA

CONCLUSIONS

CONCLUSIONS

Due to the increasing need for multi-hurdle technology during second processing and the need for suitable alternatives to PAA, the objective of the current dissertation was to investigate various antimicrobials, such as organic and inorganic acids, as short duration dips and sprays as means to reduce common pathogens (*Salmonella*, *Campylobacter jejuni*, and *Escherichia coli*) among raw chicken carcasses and parts. Overall, the data presented in the current dissertation demonstrates the potential use of novel antimicrobials as short duration dips and sprays at mitigating foodborne pathogens associated with raw poultry products during first and second processing. With this information, poultry processors will be better equipped at providing safe products to consumers.

Chapter 2

Sodium bisulfate salt, SBS, alone or in combination with peracetic acid, PAA, in 15 s whole part dips demonstrated the potential to reduce a nalidixic resistant strain of *Salmonella* Enteritidis. It was hypothesized that SBS in combination with PAA would more effectively than SBS alone. However, results demonstrated that the application of 3% SBS alone or in combination with 200 ppm of PAA was capable of reducing the presence of *Salmonella* over a 3-d refrigeration period.

The results demonstrated a greater efficacy on *S.* Enteritidis reduction as SBS concentration is increased, with no visual discoloration and 3% SBS being most effective. Drumsticks treated with 3% SBS, 2% SBS with the addition of 200 ppm of PAA, and 3% SBS with the addition of 200 ppm of PAA had the most significant reductions of *S.* Enteritidis over a 3-d refrigeration period (1.7 log CFU of *S.* Enteritidis per g of drumstick). The treatment of

drumsticks with 3% SBS demonstrated the effective reduction of *S.* Enteritidis regardless of the presence of 200 ppm of PAA. Therefore, the application of 3% SBS as an antimicrobial part dip has the potential to be an advantageous tool to further reduce the contamination of poultry parts during second processing.

Further research should be conducted to determine the effects these specific concentrations of SBS have on the overall shelf life of poultry parts and on diminishing *Salmonella* when combined with a surfactant. In order to determine whether or not efficacy is consistent across all major poultry serovars, SBS needs to be tested with other *Salmonella* serovars. Lastly, studies that optimize the application of SBS to reduce *Salmonella* and determine other potentially synergistic compounds must be conducted. In doing so, investigators will continue to develop potent antimicrobials for poultry processing that will reduce the transmission of pathogens to the food supply.

Chapter 3

The objective of the second study was to evaluate the efficacy of TetraClean Systems aqueous ozone, O_3, in combination with PAA as an antimicrobial spray on whole chicken carcasses. It was hypothesized that the combination of ozone and PAA would reduce ambient PAA while mainting the efficacy of PAA on *Salmonella* Typhimurium, *Campylobacter jejuni,* and non pathogenic *Escherichia coli.* Results indicated that the addition of ozone to PAA demonstrated the ability to effectively reduce ambient PAA, thus increasing employee safety.

Specifically, the combination of 10 ppm of aqueous ozone, Viriditec™, and 500 ppm of PAA reduced the presence of *Salmonella* Typhimurium (UK-1), *Escherichia coli* J53, and *Campylobacter jejuni*. The combination of 10 ppm of aqueous ozone with 500 ppm of PAA

reduced ambient PAA vapor by 90%, when compared to the vapors emitted off of 500 ppm of PAA. Although the current study demonstrated the promising capabilities of aqueous ozone and PAA, in combination, future research is necessary to develop an understanding of the impact the combination of aqueous ozone and PAA has on the shelf life of processed poultry and the subsequent changes in the microbiome.

Chapter 4

Although there is growing interest in the use of acidifiers to reduce common foodborne pathogens, there is limited research on how the acidifiers affect the microbiota and how the acidifiers affect the microbiota of poultry parts when inoculated with common foodborne pathogens. Thus, the objective of the third study was to determine the influence of two antimicrobials, PAA and acidified sodium chlorite (ASC), on the microbiota of chicken thighs inoculated with *Salmonella* and *Campylobacter*. It was hypothesized that the acidification of sodium chlorite would be as effective at reducing *Salmonella* Enteritidis, Typhimurium, Infantis, Kentucky, Heidelberg and *Campylobacter jejuni* and altering the microbiota of chicken thighs as PAA.

Due to the variation between trial, as fresh chicken thighs were obtained from a commercial processor, trial and study (*Salmonella* and *Campylobacter*) were investigated separately from one another. Regardless of trial, results demonstrated that both peracetic acid and acidified sodium chlorite reduced the load of inoculated *Salmonella* and *Campylobacter* on commercial chicken thighs during trial 1 and 2. Both ASC and PAA treatments were effective at reducing inoculated pathogens over both trials with PAA potentially being more effective on *Salmonella* and ASC on *Campylobacter*. Overall, there were no differences among Alpha or

Beta Diversity metrics during trial 1 and 2 when thighs were inoculated with either *Salmonella* or *Campylobacter*. In the *Salmonella* study, the core microbiota of thighs was comprised of *Enterobacteriaceae* and *Bacillus* spp. across time irrespective of trial. In the *Campylobacter* study, the core microbiota of thighs was comprised of *Campylobacter* and *Bacillus* spp. across time during trial 1 and 2. In addition to the core microbiota being comprised of *Campylobacter* and *Bacillus* spp., during trial 2, the relative abundance of *Psychrobacter*, *Pseudomonas* and *Proteus* spp constituted a major portion of the microbiota compostion. Thus, there microbiota of the thighs was not only different between inocula, but during study 2, trial 1 and 2 did not show a similar microbiota composition. These differences between trial demonstrated during study 2 could be due to the initial microbial populations of the thighs during each study of potentially the differences in the invasion mechanisms between *Salmonella* and *Campylobacter,* but further studies are necessary to validate this hypothesis.

Ultimately, the data presented herein demonstrated the use of NGS such as 16S rDNA sequencing to illuminate the changes in the core microbiota when poultry parts are inoculated with either a cocktail of *Salmonella* or *Campylobacter* and dipped in antimicrobial solutions. The results of the current study could potentially provide integrators with the means to select the appropriate antimicrobials for certain foodborne pathogen issues such as the high incidence of *Salmonella* or *Campylobacter*. In the future, integrating 16S rDNA sequencing and quantitative PCR of the bacteria isolated from media utilized during these studies will be integral in monitoring shifts in the microbiota. In addition, it should be determined which *Salmonella* serovar within the *Salmonella* cocktail is out competing the other serovars on poultry parts using molecular techniques such as quantitative PCR.

APPENDIX

Appendix 1. Approved Protocol 18035, "Effect of antimicrobial substances as poultry processing interventions" by the Institutional Biosafety Committee at the University of Arkansas.

UNIVERSITY OF ARKANSAS

Office of Research Compliance

April 20, 2018

MEMORANDUM

TO:	Dr. Steven Ricke
FROM:	Bob Beitle, Acting Biosafety Committee Chair
RE:	New Protocol
PROTOCOL #:	18035
PROTOCOL TITLE:	Effect of antimicrobial substances as poultry processing interventions
APPROVED PROJECT PERIOD:	**Start Date** April 19, 2018 **Expiration Date** April 18, 2021

The Institutional Biosafety Committee (IBC) has approved Protocol 18035, "Effect of antimicrobial substances as poultry processing interventions". You may begin your study.

If modifications are made to the protocol during the study, please submit a written request to the IBC for review and approval before initiating any changes.

The IBC appreciates your assistance and cooperation in complying with University and Federal guidelines for research involving hazardous biological materials.

1424 W. Martin Luther King, Jr. • Fayetteville, AR 72701
Voice (479) 575-4572 • Fax (479) 575-6527

University of Arkansas

Institutional Biosafety Committee

Registration for Research Projects
Form 1: GENERAL INFORMATION

IBC Number: 18035

For Committee Use Only.

Principal Investigator Name: First Steven M.I. C Last Ricke

Please check the boxes for any of the forms below that are applicable to the research project you are registering. *The General Information Form (Form 1) MUST be completed on all submitted project* registrations, regardless of the type of research.

- [x] General information (*must be completed*) **(FORM 1)**
- [ ] Recombinant DNA (***EVEN IF IT IS EXEMPT*** *from the NIH Guidelines.*) **(FORM 2)**
- [x] Pathogens (*human/animal/plant*) **(FORM 3)**
- [ ] Biotoxins **(FORM 4)**
- [ ] Human materials/nonhuman primate materials **(FORM 5)**
- [ ] Animals or animal tissues and any of the above catagories; transgenic animals, plant tissues; wild vertebrates or tissues. **(FORM 6)**
- [ ] Plants, plant tissues, or seed and any of the above categories; transgenic plants, plant tissues, or seeds **(FORM 7)**
- [ ] CDC regulated select agents **(FORM 8)**
- [x] Notice to Pat Walker Health Center **(FORM 9)**

1. To initiate the review process, you must attach and send all completed registration forms via email to: ibc@uark.edu . **ALL REGISTRATION FORMS MUST BE SUBMITTED ELECTRONICALLY.**

2. To complete your registration, you must print out page 1 of this form, sign, date (by the Principal Investigator), and mail to: Compliance Coordinator-IBC, 1 Admin. Building, Fayetteville, AR 72701. You may also FAX it to 479-575-3846.

As Principal Investigator:

- [x] I attest that the information in the registration is accurate and complete and I will submit changes to the Institutional Biosafety Committee in a timely manner.
- [x] I am familiar with and agree to abide by the current, applicable guidelines and regulations governing my research, including, but not limited to, the NIH *Guidelines for Research Involving Recombinant DNA Molecules* and the *Biosafety in Microbiological and Biomedical Laboratories*.
- [x] I agree to accept responsibility for training all laboratory and animal care personnel involved in this research on potential biohazards, relevant biosafety practices, techniques, and emergency procedures.
- [x] If applicable, I have carefully reviewed the NIH Guidelines and accept the responsibilities described therein for principal investigators (Section IV-B-7).
- [x] I will submit a written report to the Institutional Biosafety Committee and the Office of Recombinant DNA Activities at NIH (if applicable) concerning: any research related accident, exposure incident, or release of rDNA materials to the environment; problems pertaining to the implementation of biological and physical containment procedures; or violations of the NIH Guidelines.
- [x] I agree that no work will be initiated prior to project approval by the Institutional Biosafety Committee.
- [x] I will submit in a timely fashion my annual progress report to the IBC.

Signature (PI): _____ **Date:** _____

CONTACT INFORMATION

Principal Investigator:

Name: Dr. Steven Ricke | Title: Professor

Department: Food Science | Campus Address: FDSC E3

Phone#: A/C [ ] Phone 575-4678 | E-Mail sricke@uark.edu

Fax#: A/C [ ] Phone 575-6936

After hours phone number (required if research is at Biosafety Level 2 or higher):

Phone#: A/C [ ] Phone 1-479-387-4433

Co-Principal Investigator:

Name: Dr. Kristina Feye | Title: Postdoctoral Associate

Department: FDSC | Campus Address: BIOR 134

Phone#: A/C [ ] Phone 281-705-2948 | E-Mail feye@uark.edu

Fax#: A/C [ ] Phone 575-6936

After hours phone number (required if research is at Biosafety Level 2 or higher):

Phone#: A/C [ ] Phone 281-705-2948

PROJECT INFORMATION

Have you registered ANY project previously with the Institutional Bio Safety Committee? (*Check one*) [ ] Yes [ ] No

Are you registering a new project or renewing a previous project registration?

[✓] New project [ ] Renewal (*Mandatory after 3 years*)

Project Title: Effect of antimicrobial substances as poultry processing interventions

Project Duration: Start Date 5/25/2018 End Date 5/25/2020

Indicate what containment conditions you propose to use (check all that apply):

Biosafety Level 1 *(2,3)*	Biosafety Level 2 *(2,3)*	Biosafety Level 3 *(2,3)*
Animal Biosafety Level 1 *(2,3a,3b)*	Animal Biosafety Level 2 *(2,3a,3b)*	Animal Biosafety Level 3 *(2,3a,3b)*
Plant Biosafety Level 1 *(3)*	Plant Biosafety Level 2 *(3)*	Plant Biosafety Level 3 *(3)*

NOTE: Hyperlinks for references throughout this form are available on the last page of this form.

References for Biosafety criteria:

(1) University of Arkansas Biological Safety Manual - http://ehs.uark.edu/DocumentPages/BiosafetyManual04.pdf

(2) Biosafety in Microbiological and Biomedical Laboratories (BMBL) - 4th edition. CDC - Dept. of Health & Human Services - http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

(3) NIH Guidelines for work involving recombinant DNA molecules; "http://www4.od.nih.gov/oba/ibc/ibcindexpg.htm"

If working at BL-2, has your laboratory received an onsite inspection by the Biosafety Officer or a member of the IBC?

[✓] Yes Date (if known) 05/30/2013 [ ] No (*If No* - schedule the inspection with the BSO)

Please provide the following information on the research project **(Please DO NOT attach or insert entire grant proposals unless it is a Research Support & Sponsored Programs proposal)** -

Project Abstract:

Salmonella and Campylobacter are major concerns to the poultry industry as they are two of the leading causes of foodborne illness in the United States. Currently, the industry standard is to utilize various chemical and antimicrobial interventions, such as paracetic acid (PAA), in the chiller, post-chiller, spray cabinets, and part dips to reduce pathogen loads on poultry carcasses and their products within a poultry processing facility. However, the foodborne pathogens such as Salmonella and Campylobacter. Thus, there is further need to manage and reduces these pathogens within a processing environment. Therefor, the current study was developed to specifically evaluate the inclusion of novel antimicrobial compounds (surfactant, acidifier, etc.) in various locations (chiller, post-chiller, spray, dip) within a processing facility environment as either an alternative or additive to current antimicrobial practices employed by food safety and quality personnel.

Specific Aims:

Objective 1: A. To identify the optimal applications (chiller, post-chiller, spray, dip) and concentrations of various antimicrobial compounds (surfactant, acidifier, etc.) in processing environments. B. To determine the efficacy of various antimicrobial compounds (surfactant, acidifier, etc.) on mitigating the presence of inoculated pathogens such as Salmonella, Campylobacter, and etc.

Objective 2: To determine the impact various antimicrobial compounds (surfactant, acidifier, etc.) have on the microbiome of poultry products.

Relevant *Materials and Methods*: *(this information should be specific to the research project)*

Objective 1: Preparation of Salmonella and Campylobacter Cultures: A cryogenic vial containing a bacterial culture in glycerol will be removed from the freezer and placed in a biological safety cabinet. A loop full of the bacterial culture will be inoculated into the appropriate media and allowed to grow in the incubator under either aerobic or anaerobic conditions at either 37*C or 42*C(Salmonella: aerobic incubation at 37*C for 24 h; Campylobacter: anaerobic incubation at 42*C for 48 h). After 24 to 48 hours, the cultures will be washed by centrifugation and resuspended in fresh broth or saline solution and stored under refrigeration (4*C. Three days before each experiment, the bacteria will be transferred to 10 ml of tryptic soy broth (TSB) and incubated at 37*C or 42*C for 24 h, followed on the second day by a 1:10 transfer into fresh 9 ml of TSB with incubation again at 37*C or 42*C for 24 h. The third day a final culture will be inoculated 1:10 into 9 ml of fresh TSB with incubation at 37*C or 42*C for 12 h. The day before the inoculation of the chicken wings the 12 h culture will be dispensed into sterile centrifuge tubes (40 ml) and harvested by centrifugation at 5,000 xg for 10 min at room temperature. Each pellet then will be re-suspended in sterile 1x phosphate buffered saline (PBS) to a final concentration of approximately 10^8 CFU/ml.

Inoculation of Salmonella and Campylobacter on Chicken Wings: Fresh, never-frozen chicken wings will either be obtained from collaborating companies or local retailers no greater than 24 h prior to the onset of the study. The study will be replicated ten times and at the onset of each trial the absence of Salmonella and Campylobacter will be confirmed by microbiological and molecular techniques. One mL of the prepared inoculum (either Salmonella or Campylobacter) will be used to inoculate 25 g of chicken wings and allowed to attach for 60-90 minutes in 4*C refrigeration. After attachment, wings will be removed from inoculum and placed into sterile Whirl-pak bags. The inoculation of Salmonella and Campylobacter on chicken wing will occur separately, meaning their inoculation will occur on separate wings and on days, thus reducing cross contamination

Treatment of Inoculated Wings: Treatments (surfactant, acidifier, etc.) will be reconstituted in 350 mL of tap water in sterile Whirl-pak bags (22 oz). Inoculated wings will be placed into respective treatments, separately, for desired time to mimic conditions of various processing steps, chiller, post-chiller, spray cabinet, parts dip. Each wing will be retrieved with flame sterilized forceps and placed into a sterile Whirl-pak bag (22 oz.). Each wing will be allowed to rest for 5 min before treatment effects are neutralized with the addition of 150 mL of neutralizing buffer in the Whirl-pak and shaken for 1 minute. The wing will be aseptically removed with flame sterilized forceps, discarded in autoclave bags, and immediately autoclaved.

Bacterial Enumeration: One hundred microliters (µ) of the chicken wing rinsates will be transferred to 900 µ of 1x PBS and serially diluted up to 10-6. Following, 100 µ of the serial dilutions will be plated on XLD (with the addition of 20 **g**/mL of nalidixic acid) and Blood Free Campylobacter Media plates and incubated aerobically for 24 h at 37*C and anaerobically for 48 h at 42*C. Only colonies between 30 and 300 will be counted. All inoculated media used in this experiment will be immediately autoclaved and disposed following data collection.

Objective 2: The non-enriched neat dilution (or undiluted) of the rinsates will be subject to DNA extraction and microbiome analysis.

Biohazards: Possible exposure to pathogens via aerosols. To protect researchers from this risk, scientists will wear nitrile gloves, a face shield, goggles, a lab coat, and observe standard aseptic

** This information can be attached as an electronic document (Word or Word Perfect formats) or entered in the space provided (If you add an attachment, to your e-mail or FAX. Please indicate "see attached" and list the file name in the space provided):*

PERSONNEL QUALIFICATIONS & FACILITY INFORMATION:

List all personnel (including PI and Co-PI) to be involved in this project:

Name: (first and last) **- POSITION** (Title, academic degrees, certifications, and material field ofexpertise)	**QUALIFICATIONS/TRAINING/RELEVANT EXPERIENCE** Describe previous work or training with biohazardous and/or recombinant DNA and include Biosafety Levels)
Example: Bob Biohazard - Associate Professor, PhD Microbiology	14 yrs working with E. coli at BL1, Salmonella enterica at BL2, 8 yrs working with transgenic mice.
Steven C. Ricke, PhD Bacteriology/Anin	20 years experience with anaerobic bacteria and foodbo
Peter Rubinelli, PhD Molecular Cell Biol	20 years experience with pathogens
Kristina Feye, PhD Biomedical Sciences	5 years experience with pathogens, 3 years experience
Laura Meyer, MS, Animal Science, Prog	2 years experience with BSL2 pathogens
Jennifer Wages, MS in Operational Man	15 years experience with pathogens
Andrew Miccichi, grad student	5 years experience with pathogens
Dana Dittoe, MS Poultry Science	4 years experience working with pathogens
Zhaohao Shi, grad student	3 years experience working with pathogens
Aaron Bodie, grad student	3 years experience working with pathogens
Julie Atchley	4 years experience working with pathogens

Additional Personnel Information (if needed):

Conner Sherman, undergraduate, 0 years of experience with pathogens
Madison Looper, undergraduate, 0 years experiene with pathogens

Form 1: GENERAL INFORMATION, contd.

List all the laboratories/facilities where research is to be conducted (specify building, room number and category for each **(e.g. laboratories, cold/warm rooms, animal care facilities or farms, growth chambers and greenhouses, biological material storage areas, tissue culture rooms.)**

	Building	Room Number	Category	New Biohazard Door Sign?
1.	Biomass	102	Laboratory	☑ Yes
2.	Biomass	132	Laboratory	☑ Yes
3.	Biomass	101	Autoclave/Biosto	☑ Yes
4.				☐ Yes
5.				☐ Yes
6.				☐ Yes
7.				☐ Yes
8.				☐ Yes
9.				☐ Yes
10.				☐ Yes
11.				☐ Yes
12.				☐ Yes

Additional Laboratory/Facility Information (if needed):

*** Biohazard signs are required for entrances to Biosafety Level 2 areas (including Animal Biosafety Level 2 areas). The Office of Environmental Health & Safety will supply these signs.**

If an updated biohazard sign is required, please indicate the location and what agents/organisms/hazards should be listed on the sign in addition to what is being registered. Describe below:

SAFETY PROCEDURES:

Please indicate which of the following personal protective equipment (PPE) will be used to minimize the exposure of laboratory personnel during all procedures requiring handling or manipulation of the registered biological materials.

GLOVES:

☐ Latex ☐ Vinyl ☑ Nitrile ☐ Leather ☐ Other (specify)

FACE & EYE PROTECTION:

☑ Face Shield ☑ Safety Goggles ☐ Safety Glasses ☐ Other (specify)

CLOTHING PROTECTION:

☐ Disposable clothing protection
☑ Re-usable Lab Coat
☐ Re-usable Coverall
☐ Other (specify)

How will protective clothing be cleaned once dirty or contaminated? (Check all that apply)

☑ Autoclaved prior to laundering or disposal
☐ Laundered with qualified commercial service
☐ Laundered in on-site facilities with bleach
☐ Other (specify)

Outline procedures for routine decontamination of work surfaces, instruments, equipment, glassware and liquid containing infectious materials (Autoclaving or fresh 10% bleach as a chemical disinfectant are preferred treatments; please specify and justify exceptions):

Work surfaces will be decontaminated with a freshly prepared 10% bleach solution before and after working. Exception is biosafety cabinets which will be disinfected before and after use with LysolÆ No Rinse Sanitizer in order to avoid the corrosiveness of the bleach on the metal of the biosafety cabinets. Instruments and equipment will be decontaminated by wiping down with 10% bleach. Paper towels used for these purposes will be discarded in biohazard bags. Glassware, waste, and disposable tubes will be autoclaved under standard conditions (15 psi, 121 C, 20 min). Disposable items (pipette tips, pipets, etc) will be discarded into a biohazard bag for autoclaving before disposal.

Describe waste disposal methods employed for all biological and recombinant materials used. (Please include methods involving the following types of waste)**

Sharps:	Placed into 10% bleach solution for decontamination followed by discarding into sharps waste container
Cultures, stocks, and disposable labware:	Liquid cultures in tubes will be autoclaved prior to disposal down a drain. Cultures in petri dishes are disposed of in biohazard bags which are autoclaved
Pathological Waste:	Not Applicable
Other:	Rinsates will be disposed of in biohazard bags which are autoclaved and disposed of in the trash.

**** For more information, please reference the Biological Safety Manual *at*** http://ehs.uark.edu/DocumentPages/BiosafetyManual04.pdf

Indicate autoclave location(s) used for waste disposal and describe autoclave validation procedures:

Autoclaves are located in Biomass 101. The autoclaves are checked monthly using SteriGage test strips (3M) and SporAmpule vials to ensure autoclaves completely sterilize all bacterial life forms including spores.

Will you be using a biological safety cabinet? *(choose one)* ☑ Yes ☐ No

If yes, please provide the following information:

SERIAL NUMBER	CERTIFICATION EXPIRATION DATE	LOCATION OF UNIT (Bldg./room)	MAKE/MODEL
170238664B	06/2018	Biomass 132	Labconco Logic +
12118-128	10/2018	Biomass 132	Biosafety Cabine

Indicate if any of the following aerosol-producing procedures will occur:

Centrifuging	Grinding	Blending	Vigorous shaking or mixing	Sonic disruption
Pipetting	Dissection	Stomacher	Inoculating animals intranasally	

Other (please describe):

Describe the procedures/equipment that will be used to prevent personnel exposure during aerosol-producing procedures:

Centrifugation and Stomacher: Centrifugation and Stomacher use will occur outside of a biosafety hood. Researchers will observe proper PPE and proceedures which will include: a laboratory coat buttoned over street clothes, two pairs of gloves, goggles and a face shield.

Pipetting, Vortexing (Vigorous Shaking): Mechanical pipettors will always be used. This will occur in the biosafety cabinate at all times. Researchers will observe proper PPE and proceedures which will include: a laboratory coat buttoned over street clothes, two pairs of gloves, goggles and a face shield.

EMERGENCY PROCEDURES

In the event of **personnel** exposure (e.g. mucous membrane exposure or parenteral inoculation), describe what steps will be taken including treatment, notification of proper supervisory and administrative officials, and medical follow up evaluation or treatment.

In the event of accidental exposure of personnel the person exposed should notify the laboratory supervisor immediately. Treatable exposures will be treated by use of the first aid kit. Mucous membrane exposure or puncture with contaminated material will result in the person being taken to the Health Center for evaluation.

In the event of **environmental** contamination, describe what steps will be taken including a spill response plan incorporating necessary personal protective equipment (PPE) and decontamination procedures.

In case of a large environmental spill, persons interacting with spilled area should protect themselves with personal protective equipment such as gloves, eye mask, coat, etc. The area will be secured to prevent spreading. Appropriate disinfectant dependent upon spill will be selected. Freshly prepared 10% bleach as a chemical disinfectant is a preferred treatment. All spill-related material will be autoclaved. All individuals exposed to the environmental contamination will be sent to receive medical attention. The incident will be reported to biological safety officer and principal investigator

TRANSPORTATION/SHIPMENT OF BIOLOGICAL MATERIALS

As per the Department of Transportation **49 CFR Parts 171-173** (5), some biological materials are regulated as hazardous materials and require special training of all personnel involved in shipping. ***Will you be transporting or shipping any of the following off campus?*** *(Yes* ***or*** *No)*

☐ Yes ☑ No

If yes, check all that apply.

☐ Cultures of human or animal pathogens

☐ Environmental samples known or suspected to contain a human or animal pathogen

☐ Human or animal material (including excreta, secreta, blood and its components, tissue or tissue fluids, and cell lines) containing or suspected or containing a human or animal pathogen

Have you or anyone in your lab involved in packaging, labeling, or completing/signing paper work received training to ship infectious substances or diagnostic specimens within the past 3 years? *(Choose Yes **or** No.)*

☐ Yes

☑ No

If yes, please provide the following information.

Name	Date Trained	Certified Shipping Trainer

REFERENCES

1. Research and Sponsored programs Research Compliance: http://avcf.uark.edu/

2. Biosafety in Microbiological and Biomedical Laboratories (BMBL) - 4th ed. CDC - Dept. of Health and Human Services: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

3. NIH Guidelines for work involving recombinant DNA molecules: http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html

4. University of Arkansas Office of Environmental Health & Safety: http://ehs.uark.edu/

5. Department of Transportation - Hazardous Materials: Standards for Infectious Substances: **49 CFR Parts 171-173**

Appendix 2. *Curriculum Vitae*

EDUCATION:

Doctor of Philosophy in Cell and Molecular Biology: Concentration in Food Safety, December 2020
University of Arkansas, Fayetteville, Arkansas

Dissertation title: "*The use of inorganic and organic acids as short duration antimicrobial dips on mitigating pathogens present on commercial chicken during peri-harvest*"

- Cumulative GPA 3.78/4.0

Master of Science in Agriculture: Concentration in Poultry Science, 2017
Mississippi State University, Starkville, Mississippi

Thesis title: "*In vitro and in vivo effects of an encapsulated butyric acid and a lactic acid producing bacteria used alone or in combination*"

- Cumulative GPA: 3.98/4.0

Bachelor of Science in Poultry Science: Concentration in Business Management, 2016
Mississippi State University, Starkville, Mississippi

- Cumulative GPA: 3.74/4.0

EQUIPMENT AND LABORATORY SKILLS:

16S Microbiome Sequencing

Library Preparation, Normalization, Illumina Miseq Operation

Bioinformatics

Qiime 2-2020.8 (Intermediate Understanding), R software (Basic Understanding)

PCR amplification:

DNA Extraction, Thermocycler, Gel Imaging Systems, Gel Electrophoresis, qPCR, Reverse Transcriptase qPCR

Microbiology:

Anaerobic and Aerobic assays, Media Preparation, Pipetting, Serial Dilution, Inoculation, Streaking, Anoxamat, Most Probable Number, Incubators, Gram Staining, Spot Plating

Other:

Particle Size, pH, Water Bath, Scales and Balances, Hazardous Waste Treatment, *In Ovo* Vaccination, Necropsy, Poultry Processing, Feed Formulation (basic knowledge), and Animal Husbandry

Training:

Hazardous Waste, Bloodborne Pathogens, Biosafety: Principles and Practices, Food Protection, Animal Handling

RESEARCH AND ACADEMIC EXPERIENCE:

Graduate Research Assistant, Spring 2018-Present
Pre and Post-Harvest Food Safety
Under the direction of Dr. Steve Ricke, University of Arkansas

Investigate various antimicrobials, organic and inorganic acids, as short duration dips and sprays as means to reduce common pathogens (*Salmonella*, *Campylobacter jejuni*, and *Escherichia coli*) among raw chicken carcasses and parts.

Graduate Research Assistant, Fall 2016-Fall 2017
Evaluating Dietary Alternatives, lactic acid producing bacteria, butyric acid, and their combination, in vitro and in vivo.
Under the direction of Dr. Aaron Kiess, Mississippi State University

Evaluated lactic acid producing bacteria and encapsulated butyric acid on *Salmonella* prevalence, *in vitro* over an 8h period. Also evaluated the effect of lactic acid producing bacteria and encapsulated butyric acid, and their combination in commercial broiler diets from day 0-57 on performance, processing, intestinal and immune tissues, A1-AGP, and intestinal histology.

Recipient of CALS' Undergraduate Research Scholars Program, Fall 2014-Spring 2016
Windrowing Poultry Litter after a Broiler House is Sprinkled with Water
Under the direction of Dr. Aaron Kiess, Mississippi State University

Litter was analyzed for moisture, pH, particle size, N, P, K, and NH_3.
Manuscript published in Journal of Applied Poultry Research in 2018*
*Among the top ten most read articles in JAPR in 2018

PROFESSIONAL EXPERIENCES:

Residence Advisor, July 2013- May 2016
Mississippi State University, Starkville, Mississippi

- Created community among residence, supervised and cared for residents, enforced rules and ensured safety of residents, and coordinated staff development.

Poultry Production and Processing Internship, May 2014-July 2014
Tyson Foods, Temperanceville, Virginia

- Received experience in poultry processing, laboratory methods and sampling procedures, hatchery operations, broiler farm maintenance and scheduled pre-slaughter testing, pullet vaccination procedures, pullet farm operations, and breeder facility operations.

PUBLICATIONS (IN REVIEW):

Deng, W., **D.K. Dittoe**, H. O. Pavlidis, W. E. Chaney, Y. Yang, and S. C. Ricke. 2020. Current perspectives and potential of probiotics to limit foodborne *Campylobacter* in poultry. **Submitted to** Frontiers in Microbiology.

Olson, E. G., **D. K. Dittoe**, A. C. Micciche, and S. C. Ricke. 2020. Using next generation sequencing to discover potential hygienic microorganisms within the microbiota of commercial poultry feed. **Submitted to** Journal of Environmental Science and Health, Part B

PUBLICATIONS:

Ricke, S.C., **D.K. Dittoe,** and K. Richardson. 2020. Formic acid as an antimicrobial for poultry production: a review. Frontiers in Veterinary Science 7: 563. doi: 10.3389/fvets.2020.00563

Castañeda, C. D., **D. K. Dittoe**, K. G. S. Wamsley, C. D. McDaniel, A. Blanch, D. Sandvang, and A. S. Kiess. 2020. *In ovo* inoculation of an Enterococcus faecium–based product to enhance broiler hatchability, live performance, and intestinal morphology. Poultry Science. doi: 10.1016/j.psj.2020.08.002.

Olson, E. G., L. A. Wythe, **D. K. Dittoe**, K. M. Feye, S. C. Ricke. 2020. Application of Amplon™ in combination with peroxyacetic acid for the reduction of Nalidixic Acid Resistant *Salmonella* Typhimurium and *Salmonella* Reading on skin-on tom turkey drumsticks. Poultry Science. doi: 10.1016/j.psj.2020.08.078.

Dittoe, **D.K.,** R.D. Barabote, M.J. Rothrock, S.C. Ricke. 2020. Assessment of a potential role of *Dickeya dadantii* DSM 18020 as a pectinase producer for utilization in poultry diets based on *in silico* analyses. Frontiers in Nutrition. Frontiers in Microbiology 11: 751. doi:10.3389/fmicb.2020.00751

Blevins, R.E., K.M. Feye, **D.K. Dittoe**, L. Bench, B.J. Bench, and S.C. Ricke. 2020. *Campylobacter* and *Salmonella* Levels and Chemical Composition of Commercial Whole Bird Carcass Pre-Chiller Rinses. Journal of Agriculture and Food Research 2:100041. doi:10.1016/j.jafr.2020.100041.

Micciche, A.C., R.D. Barabote, **D.K. Dittoe**, S.C. Ricke. 2020. *In silico* genome analysis of an acid mine drainage species, *Acidiphilium multivorum,* for potential commercial acetic acid production and biomining. Journal of Environmental Science and Health, Part B. 15: 1-8. Doi: 10.1080/03601234.2019.1710985

Blevins, R.E., K.M. Feye, **D.K. Dittoe**, L. Bench, B.J. Bench, and S.C. Ricke. 2019. Aerobic plate count, *Salmonella* and *Campylobacter* loads of whole bird carcass rinses from pre-chillers with different water management strategies in a commercial poultry processing plant. Journal of Environmental Science and Health, Part B. 55(2): 155-165. Doi:10.1080/03601234.2019.1670522.

Feye, K.M., **D.K. Dittoe**, Z. Shi, J. Woitte, C.M. Owens, M.H. Kogut, and S.C. Ricke. 2019. The Reduction of Pathogen Load on Ross 708 Broilers when Using Different Sources of Commercial Peracetic Acid Sanitizers in a Pilot Processing Plant. Microorganisms, 7(11): 503. Doi: 10.3390/microorganisms7110503

Shi, Z., **D.K. Dittoe**, K.M. Feye, M. Kogut, S.C. Ricke. 2019. Short Communication: Preliminary Differences Identified in Genes Responsible for Biofilm Formation in Poultry Isolates of *Salmonella enterica* Heidelberg, Enteritidis, and Kentucky. Microorganisms, 7(7), 196. Doi: 10.3390/microorganisms7070196

Shi, Z., **D.K. Dittoe**, and S.C. Ricke. 2019. Non-molecular characterization of pellicle formation by poultry *Salmonella* Kentucky strains and other poultry-associated *Salmonella* serovars in Luria Bertani broth. Journal of Environmental Science and Health, Part B. 54. 1-7. Doi:10.1080/03601234.2019.1661210.

PUBLICATIONS:

Ricke, S.C., K. Richardson and **D.K. Dittoe**. 2019. Formaldehydes in Feed and Their Potential Interaction With the Poultry Gastrointestinal Tract Microbial Community–A Review. Frontiers in Veterinary Science. 6. Doi: 10.3389/fvets.2019.00188.

Bodie, A.R. **D.K. Dittoe**, K.M. Feye, C.J. Knueven, and S.C. Ricke. 2019. Application of an Alternative Inorganic Acid Antimicrobial for Controlling Listeria monocytogenes in Frankfurters. Frontiers in Sustainable Food Systems. 3. 34. Doi: 10.3389/fsufs.2019.00034.

Dittoe, D.K., J.A. Atchley, K.M. Feye, J. Lee, C.J. Knueven, and S.C. Ricke. 2019 The Efficacy of Sodium Bisulfate Salt (SBS) Alone and Combined With Peracetic Acid (PAA) as an Antimicrobial on Whole Chicken Drumsticks Artificially Inoculated With *Salmonella* Enteritidis. Frontiers in Veterinary Science. 6. Doi: 10.3389/fvets.2019.00006.

Dittoe, D.K., K.M. Feye, B. Peyton, D. Worlie, M.J. Draper, and S.C. Ricke. 2019. The Addition of Viriditec™ Aqueous Ozone to Peracetic Acid as an Antimicrobial Spray Increases Air Quality While Maintaining *Salmonella* Typhimurium, Non-pathogenic *Escherichia coli*, and *Campylobacter jejuni* Reduction on Whole Carcasses. Front. Microbiolology. 9:3180. Doi: 10.3389/fmicb.2018.03180

Dittoe, D.K., S.C. Ricke, and A.S. Kiess. 2018. Organic Acids and Potential for Modifying the Avian Gastrointestinal Tract and Reducing Pathogens and Disease. Frontiers in Veterinary Science. 5. 216. Doi:10.3389/fvets.2018.00216.

Dittoe, D.K., C.D. McDaniel, and A.S. Kiess. 2018. Windrowing Poultry Litter after a Broiler House is Sprinkled with Water. Journal of Applied Poultry Research 27(1):1-15. ***Top Ten Most Read JAPR Articles in 2018 (https://academic.oup.com/japr/pages/highly_read_articles)**

CONFERENCE PRESENTATIONS:

D. K. Dittoe, K. M. Feye, and S. C. Ricke. 2020. *Salmonella* and *Campylobacter* influence the microbiota response of skin-on, bone-in chicken thighs treated with different antimicrobials. Poultry Science Association Virtual Annual Meeting, July 2020.

Olson, E. G., L. A. Wythe, **D. K. Dittoe**, K. M. Feye, S. C. Ricke. 2020. Application of cetylpyridinium chloride (CPC) on poultry skin for the reduction of Nalidixic resistant *Salmonella* Typhimurium and *S.* Infantis on chicken skin. Poultry Science Association Virtual Annual Meeting, July 2020.

Olson, E. G., L. A. Wythe, **D. K. Dittoe**, K. M. Feye, S. C. Ricke. 2020. Application of Amplon® in combination with peroxyacetic acid for the reduction of *Salmonella* Typhimurium and *S.* Reading on skin-on, bone-in tom turkey drumsticks. Poultry Science Association Virtual Annual Meeting, July 2020.

Wythe, L. A., E. G. Olson, **D. K. Dittoe**, K. M. Feye, and S. C. Ricke. 2020. The effects of cetylpyridinium chloride for the reduction of *Salmonella* Infantis onSkin- on, bone-in chicken thighs. Poultry Science Association Virtual Annual Meeting, July 2020.

CONFERENCE PRESENTATIONS:

Wythe, L. A., K. M. Feye, **D. K. Dittoe**, and S. C. Ricke. 2020. The effects of yeast fermentate prebiotic feed additive on the lactic acid producing bacteria in an in vitro microaerophilic cecal culture model. Poultry Science Association Virtual Annual Meeting, July 2020.

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Dittoe, D. K., K. M. Feye, C. Ovall, C. J. Knueven, and S. C. Ricke. 2020. Influence of organic and inorganic acids as used for poultry part dips on the emergence of spoilage organisms during a shelf-life study. International Poultry Scientific Forum, Atlanta, GA, January 2020.

Dittoe, D. K., K. M. Feye, Z. Shi, J. Woitte, and S. C. Ricke. 2019. Determining the efficacy of acidified sodium chlorite for the reduction of inoculated Salmonella and Campylobacter on skin-on, bone-in chicken thighs. Poultry Science Association Annual Meeting, Montreal, Canada, July 2019.

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Dittoe, D.K., J.A. Atchley, K.M. Feye, L.R. Meyer, C.J. Knueven, and S.C. Ricke. 2018. Effect of Sodium Bisulfate Salt on mitigating the presence of an antibiotic resistant strain of *Salmonella* Enteritidis on chicken drumsticks. Poultry Science Association Annual Meeting, San Antonio, TX, July 2018.

Atchley, J.A, **D.K. Dittoe**, K.M. Feye, L.R. Meyer, C.J. Knueven, and S.C. Ricke. 2018. Effect of Sodium Bisulfate Salt (SBS) on reducing the presence of an antibiotic resistant *Salmonella* Typhimurium on whole chicken wing parts. Poultry Science Association Annual Meeting, San Antonio, TX, July 2018.

Bodie, A.R., S.A. Kim, **D.K. Dittoe**, L.R. Meyer, C.J. Knueven, and S.C. Ricke. 2018. Potential antimicrobial combinations for controlling *Listeria monocytogenes* in hotdogs. International Association of Food Protection Annual Meeting, Salt Lake City, UT, July, 2018.

Dittoe, D.K., K.G.S. Wamsley, C.D. McDaniel, A. Blanch, D. Sandvang, and A.S. Kiess. 2018. Changes to the gastrointestinal tract and yolk sac of broilers previously in ovo injected with different concentrations of GALLIPRO® Hatch. International Poultry Scientific Forum, Atlanta, GA, January 2018.

Dittoe, D.K., C.D. McDaniel, K.G. Wamsley, W. Zhai and A.S. Kiess. 2017. Effects of ButiPEARL™ and PrimaLac® used alone or in combination on 57 d broiler intestinal morphology and immune tissues. Poultry Science Association Annual Meeting, Orlando, FL, July 2017.

CONFERENCE PRESENTATIONS:

Dittoe, D.K., C.D. McDaniel, K.G. Wamsley, W. Zhai and A.S. Kiess. 2017. Effects of ButiPEARL™ and PrimaLac® used alone or in combination on 57 d broiler performance and processing. Poultry Science Association Annual Meeting, Orlando, FL, July 2017.

Dittoe, D.K., C.D. McDaniel, and A.S. Kiess. 2017. Effects of lactic acid bacteria and organic acids on the concentration of an antibiotic resistant strain of *Salmonella* Heidelberg, *in vitro.* International Poultry Scientific Forum, Atlanta, GA, January, 2017.

Dittoe, D.K., C.D. McDaniel, and A.S. Kiess. 2015. Windrowing Poultry Litter after a Broiler House is Sprinkled with Water. Poultry Science Association Annual Meeting, Louisville, KY, July 2015. -Award Winner

HONORS AND AWARDS:

Doctoral Academy Fellowship, University of Arkansas	2018-Present
Poster Session Winner, 3rd place, Category 1: Food Safety Including Fundamental Understanding of Pathogens (Graduate), Arkansas Association for Food Protection	2019
Poster Session Winner, 3rd place, Category 2: Interventions, Pre and Post Harvest (Graduate), Arkansas Association for Food Protection	2019
Poster Session Winner, 3rd place, Category 4: Other and Miscellaneous (Graduate), Arkansas Association for Food Protection	2019

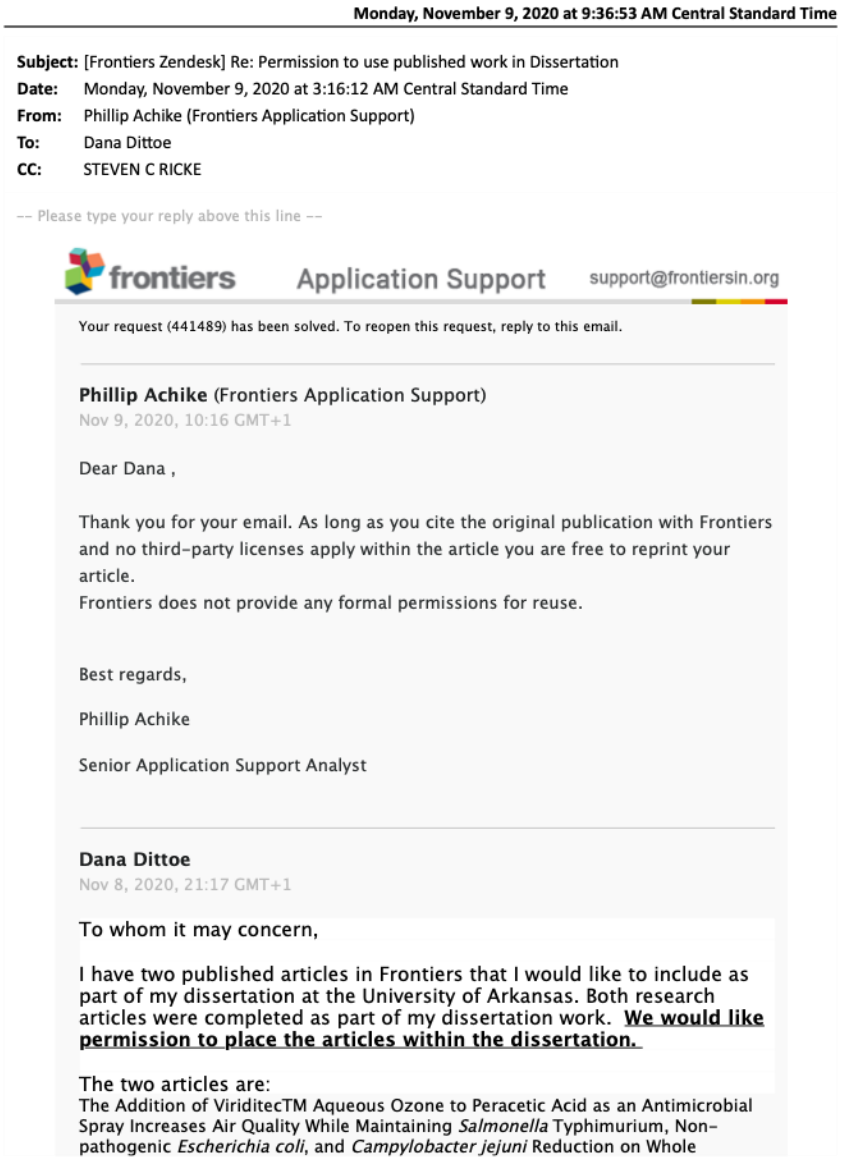
HONORS AND AWARDS:

Doctoral Student Travel Grant, University of Arkansas	2019
Poultry Science Graduate Association, 2018 PSA Practice Session Winner, University of Arkansas	2018
Doctoral Student Travel Grant, University of Arkansas	2018
DPI Emerson Morgan Graduate Scholarship	2017
Oliver J. Hubbard Memorial Scholarship	2016
H.F. McCarty, Jr. Family Scholarship	2012 – 2016
Robert H. Dunlap Loyalty Scholarship	2012 – 2016
Edward Ellis Gandy Endowed Scholarship	2015
Delmarva Poultry Industry Scholarship	2012

PROFESSIONAL MEMBERSHIPS:

Arkansas Association for Food Protection
Gamma Sigma Delta, Honor Society for Agriculture
International Association for Food Protection
Poultry Science Association
Southern Poultry Science Society
The National Society of Collegiate Scholars
World Poultry Science Association

Appendix 3: Permission by Frontier's editorial office to use Chapters 2 and 3 in the current dissertation.



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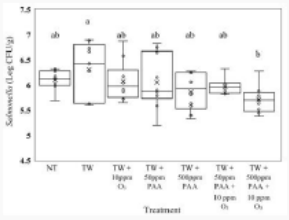
To whom it may concern,

I have two published articles in Frontiers that I would like to include as part of my dissertation at the University of Arkansas. Both research articles were completed as part of my dissertation work. **We would like permission to place the articles within the dissertation.**

The two articles are:
The Addition of ViriditecTM Aqueous Ozone to Peracetic Acid as an Antimicrobial Spray Increases Air Quality While Maintaining *Salmonella* Typhimurium, Non-pathogenic *Escherichia coli*, and *Campylobacter jejuni* Reduction on Whole

Carcasses
Front. Microbiol., 08 January 2019 |
https://doi.org/10.3389/fmicb.2018.03180



The Addition of ViriditecTM Aqueous Ozone to Peracetic Acid as an Antimicrobial Spray Increases Air Quality While Maintaining Salmonella Typhimurium, Non-pathogenic Escherichia coli, and Campylobacter jejuni Reduction on Whole Carcasses

Currently, the most utilized antimicrobial in processing facilities is peracetic acid, PAA; however, this chemical is increasingly recognized as a hazard to human health. Preliminary evidence suggests that

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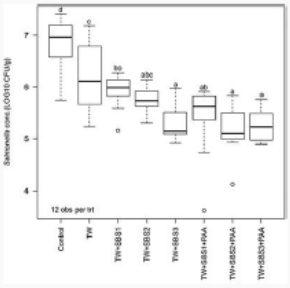
The Efficacy of Sodium Bisulfate Salt (SBS) Alone and Combined With Peracetic Acid (PAA) as an Antimicrobial on Whole Chicken Drumsticks Artificially Inoculated With Salmonella Enteritidis
Front. Vet. Sci., 30 January 2019 |
https://doi.org/10.3389/fvets.2019.00006



The Efficacy of Sodium Bisulfate Salt (SBS) Alone and Combined With Peracetic Acid (PAA) as an Antimicrobial on Whole Chicken Drumsticks Artificially Inoculated With Salmonella Enteritidis

The presence of Salmonella spp. on poultry products is one of the leading causes of foodborne illness in the United States. Therefore, novel antimicrobial substances are being explored as potential

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Please reach out to me at dkdittoe@uark.edu if you have any questions.

Thank you,

Dana Dittoe