THESIS

EFFECTS OF ANTIMICROBIAL INTERVENTIONS ON FOOD SAFETY AND AN ASSESSMENT OF THE COLORADO PORK SUPPLY

Submitted by

Brianna C. Britton

Department of Animal Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2018

Master's Committee:

Advisor: Dale R. Woerner
Co-Advisor: Keith E. Belk

Ifigenia Geornaras
Jessica E. Prenni

ABSTRACT

EFFECTS OF ANTIMICROBIAL INTERVENTIONS ON FOOD SAFETY
AND AN ASSESSMENT OF THE COLORADO PORK SUPPLY

Three experiments (six total studies) were conducted to complete this thesis. The first two experiments explored the efficacy of antimicrobial interventions against inoculated bacterial populations on beef and poultry products, while the final experiment was conducted to characterize the pork processing industry in the state of Colorado. In the first experiment, three studies were conducted to determine whether addition of a surfactant to various chemical solutions enhanced antimicrobial effects against inoculated bacterial populations on beef trimmings (part A – study 1), chicken wings (part A – study 2), and prerigor beef carcass surface tissue (part B). In part A – study 1, beef trimmings were inoculated (6-7 log CFU/g) with a five-strain mixture of nonpathogenic *Escherichia coli* biotype I surrogates for *E. coli* O157:H7, non-O157 Shiga toxin-producing *E. coli*, and *Salmonella* using a sanitized paint brush. The trimmings (n = 10) were spray treated (10 s, 20 psi) with peroxyacetic acid (PAA; 400ppm), a sulfuric acid and sodium sulfate blend (SSS; pH 1.1), or PAA (400ppm) acidified with SSS (pH 1.1; aPAA), with and without addition of a proprietary alkyl polyglycoside surfactant (AP; 0.4%) to the solution. In part A – study 2, whole, skin-on chicken wings were spot inoculated (6-7 log CFU/ml of sample rinsate) with a five-strain mixture of pathogenic *Salmonella* resistant to novobiocin and naladixic acid. Chicken wings (n = 10) were immersed (15 s) in PAA (500 ppm), SSS (pH 1.2) or aPAA (500 ppm PAA acidified with SSS, pH 1.2) solutions that were or were not supplemented with AP (0.4%). In part B, prerigor beef carcass surface tissue pieces (10 x 10

cm) were inoculated on the external adipose side with 200 µl of the aforementioned nonpathogenic *E. coli* biotype I surrogates for pathogens. The prerigor surface tissue pieces (n = 10) were spray treated (10 s, 15 psi) with PAA (400 ppm), SSS (pH 1.2), or aPAA (400 ppm PAA acidified with SSS, pH 1.2), with and without the addition of a different proprietary alkyl polyglycoside (DB; 0.5%) from the studies in part A. All samples in part A were analyzed 5 min following treatment to determine surviving bacterial populations. Data were analyzed using the Mixed Models Procedures of SAS and with a model that included independent variables of antimicrobial treatment and surfactant addition, along with respective interactions. Samples in part B were analyzed 5 min after treatment as well as after 24 h at 4°C for surviving *Enterobacteriaceae* populations. Data were analyzed using the Mixed Models Procedures of SAS and the model included independent variables of antimicrobial treatment, surfactant addition, and sampling time, as well as the respective interactions. Least squares means for all studies were separated using a significance level of $\alpha = 0.05$. In part A, the addition of surfactant did not affect ($P \geq 0.05$) the efficacy of any of the tested antimicrobial treatments during application in either study 1 or 2. All PAA-containing treatments effectively ($P < 0.05$) reduced inoculated populations on beef trimmings (6.5 log CFU/g) and chicken wings (6.0 log CFU/ml) by 0.5 to 0.6 log CFU/g and 1.7 to 1.8 log CFU/ml, respectively. In part B, an interaction was detected between antimicrobial treatment and addition of DB ($P < 0.05$); however, after treatment with PAA and aPAA with DB added, surviving bacterial populations were 0.3 log-units greater than the same treatments without surfactant addition. All spray treatments reduced ($P < 0.05$) *E. coli* populations, though PAA-containing treatments were most effective, reducing ($P < 0.05$) initial bacterial populations by 1.4 to 1.7 log CFU/cm^2.

In the second experiment, two studies were conducted to evaluate the antimicrobial effects of blends of PAA acidified with various acids against inoculated populations of the same nonpathogenic *E. coli* inoculum used in the previous studies on warm, prerigor brisket tissue to simulate applications to warm carcasses. In the first study, prerigor beef carcass surface brisket tissue pieces (10 × 10 cm) were inoculated (6-7 log CFU/cm^2) with a five-strain mixture of nonpathogenic *E. coli* biotype I surrogates. Samples were either left untreated (control) or were immersed for 10 s in PAA (400 ppm) acidified with lactic acid (3.5%), PAA (400 ppm) acidified with acetic acid (2%), PAA (400 ppm) acidified with citric acid (1%), PAA (400 ppm) acidified with a sulfuric acid and sodium sulfate blend (SSS; pH 1.2 and pH 1.8), and PAA (300 ppm) acidified with SSS (pH 1.2). In study 2, 10 × 10 cm pieces (n = 10) of prerigor beef tissue inoculated (6 to 7 log CFU/cm^2) with the same five-strain mixture of nonpathogenic *E. coli* surrogates were either left untreated or were spray-treated (10 s) with water, PAA (350 ppm), PAA (400 ppm), PAA (400 ppm) acidified with acetic acid (2%), PAA (400 ppm) acidified with SSS (pH 1.2), or PAA (350 ppm) acidified with SSS (pH 1.2). All samples in both studies were analyzed 5 min post-treatment for surviving *Enterobacteriaceae* populations and data were analyzed using the lsmeans package in R using antimicrobial treatment as the independent variable. Least-squares means were separated using a significance level of $\alpha = 0.05$. All immersion treatments evaluated in study 1 effectively ($P < 0.05$) reduced inoculated *E. coli* populations (6.2 log CFU/cm^2) on the prerigor beef carcass surface tissue by at least 2.3 log CFU/cm^2. The 400 ppm PAA treatments acidified with lactic acid, SSS (pH 1.2), or acetic acid were the most ($P < 0.05$) effective, reducing inoculated bacterial counts from 6.2 log CFU/cm^2 to 3.4, 3.4, and 3.7 log CFU/cm^2, respectively. In study 2, all of the tested antimicrobial spray treatments lowered ($P < 0.05$) initial inoculated *E. coli* counts (6.4 log CFU/cm^2) by 1.7 to 1.9

log CFU/cm^2. No ($P \geq 0.05$) differences in efficacy were observed between the five antimicrobial treatments in the second study.

In the third experiment, a survey was administered to the small and very small processors in Colorado to determine how many processors harvest pigs, what their desired traits for live pigs were, and to determine the processor's views of the pork industry in Colorado. This survey was the first of its type to be conducted in Colorado. An online survey was designed to establish definitions for various quality factors, determine the likelihood that a company would select a factor as a "must have", and assess image, strength, weakness, and potential threats (i.e. SWAT analysis) to the Colorado pork industry from a small processor's perspective. Those making purchasing decisions or are knowledgeable in the daily activities of each company, were asked to complete the survey. Initial contact was made via telephone in February 2018, and surveys were decimated and completed during the following three month period (February to April 2018). A dynamic routing survey was designed utilizing the Qualtrics platform, and routed questions asked of processors based on their initial response to a question of whether they did or did not harvest pigs. If they did not harvest pigs, further questions were asked to determine the primary causes for processors to not harvest them. If the processor did harvest pigs, they were routed to questions pertaining specifically to their business. Definitions for predetermined quality factors of 1) how and where the pigs are raised, 2) weight and size, 3) conformation, 4) food safety, and 5) quality were recorded and analyzed to assist in determining perceived meaning for each quality factor and interpret the importance of these factors to the processors. Financial considerations was the most common reason for small processors to not harvest pigs; the input costs of updating their facilities, purchasing additional equipment to harvest or further process, and the costs of updating their HACCP plans or abiding by regulatory standards outweighed the

minimal profit margins they would receive from harvesting pigs and processing pork products. Custom exempt meat processors were the most common type of facility and most harvested less than five head per week. How and where the pigs are raised was most likely (42.7%) to be selected as a must have, followed by quality (35.5%). The respondents believed that a strength of the industry is the number of small and local producers, though, they are concerned about the lack of supply and the low quality of the livestock that are harvested. Responses suggested that there is positive image of the pork industry in Colorado by the processors surveyed. Overall, the results of the study were able to characterize the size and scope of the small pork processors in Colorado and provide initial information to further improve the pork industry.

ACKNOWLEDGEMENTS

Words cannot express my gratitude for all of those who have encouraged me, helped me, and inspired me to get to this point in life. First and foremost, I must thank my fellow graduate students for all of the blood, sweat, tears, late nights, and early mornings. I would never have completed these projects without your constant assistance and willingness to sacrifice your time and energy to collect samples at the plant, struggle with the spray cabinet, sweat it out in the micro lab, and jump to help at the last second. I'd specifically like to thank KatieRose McCullough for pushing me (literally) into my first project and training me in all of the tasks that are necessary to complete a food safety study. I truly do not think that I would have been successful in the early stages of my degree without your encouragement and guidance.

To my committee, thank you for teaching me how to conduct research, enhancing my writing and communication skills, and for supporting and guiding me during my degree program. Dr. Dale Woerner, I could never truly express the gratitude I have for you. Not only did you give me a chance when it felt like no one else would, but you have constantly supported and encouraged me through the ups and downs and have given me every opportunity that I needed to discover the path that I would take in this field. Dr. Keith Belk, thank you for constantly believing in my abilities and pushing me to do more, even when I didn't think I could. Thank you for your guidance, patience, and encouragement to become a scientist over the past two years. To Dr. Jessica Prenni, thank you for your desire to be more involved in our program and serving as my committee member. I truly appreciate you diving in and supporting me along the way! Dr. Ifigenia Geornaras, thank you for being my mentor, teacher, and friend. If it weren't for

you I would not be a “microbiologist.” You have truly trained me from the ground up and there are no words that could possibly explain all that you have done not only for me, but for all of us.

Finally, I must thank my family for their unconditional love and support through all of the challenges and changes these past few years. To my roommate, Erin, thank you for putting up with my crazy. You have been a constant support system for me throughout grad school and I really don’t know what I will do without you next year! To my beautiful mother, Tessa, for instilling in me the strength and boldness to fight for what I want. Mama, thank you for constantly pushing me and motivating me to do more. To my pops, Preston, thank you for your continued support in all of my endeavors. Regardless of if you actually understand what I do or not, you are always excited to learn more and I couldn’t express how much I appreciate that. Lastly, to my baby sister, Katie, you are my rock! I could not have made it to this point without your listening ear and constant pep talks; I am truly grateful that my sister is also my best friend and biggest cheerleader! Thank you for letting me be the “favorite child!”

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CHAPTER 1

Review of Literature – Part 1

1.1 Meat Safety

Knowledge of meat safety began in the 1880's when meat inspection processes were incorporated into processing facilities around the globe (*40*). Ante- and postmortem inspection of livestock was implemented in processing facilities in the U.S. after congress gave the U.S. Department of Agriculture (USDA) the responsibility of ensuring that beef was safe to export to European countries, and for interstate transport within the U.S. (*40*, *62*). Following the release of Upton Sinclair's *The Jungle* in 1906, President Theodore Roosevelt signed the Federal Meat Inspection Act (FIMA) mandating that all meat intended for human consumption be inspected by the USDA (*114*).

In 1926, rising consumer concerns about the safety of poultry products influenced the USDA to offer voluntary inspection of poultry through the Federal Poultry Inspection Act (*105*, *141*). Three decades later, there was an increased demand for ready-to-eat poultry products following WWII, leading congress to pass the Poultry Products Inspection Act in 1957 to ensure that the supply of these products was safe to consume (*141*). Similar to FIMA, this act mandated the ante- and postmortem inspection of all poultry products intended for human consumption (*105*). These traditional inspection methods were predominately organoleptic, relying heavily on the senses such as sight, touch, and smell (*40*, *141*).

In 1993, an outbreak of *Escherichia coli* O157:H7 from undercooked ground beef hamburger patties in the Pacific Northwest hospitalized over 400 people and killed 4 children (*6*, *62*, *149*). This incident reintroduced the public health concern for consuming beef and

revolutionized meat safety, influencing reform for intervention protocols and increased outbreak monitoring (*149*). In response, the USDA Food Safety and Inspection Service (USDA-FSIS) implemented the "zero tolerance" policy, requiring the removal of all udder contents, fecal matter, and ingesta to be trimmed from the carcass before washing (*6*). Additionally, in 1994, FSIS declared *E. coli* O157:H7 an adulterant in raw ground beef products (*6*, *149*). In 1996, FSIS delivered the Pathogen Reduction/Hazard Analysis Critical Control Point (PR/HACCP) system final rule, which would utilize a science-based approach to reduce the risk of foodborne illness in meat and poultry products by identifying where in the processing system unacceptable food safety risk could occur and implementing a monitoring system for these critical control points (*135*). The PR/HACCP final rule also shifted control from USDA-FSIS to each individual processing facility, and required that all red meat production facilities implement a HACCP system, sanitation standard operating procedures (SSOPs), and microbial testing (*137*).

With this ruling, inspection protocols shifted from visual inspection procedures to microbiological testing, with the focus being on the top four pathogens: Shiga toxin-producing *E. coli*, *Salmonella*, *Campylobacter* spp., and *Listeria monocytogenes* (*62*, *137*, *141*). Since 1996, there has been a significant reduction in foodborne illness outbreaks due to these pathogens, particularly with *E. coli* contamination. It has been recognized by the Centers for Disease Control and Prevention (CDC) that this reduction can be attributed to proper implementation and monitoring of HACCP protocols throughout the meat industry (*141*).

1.2 *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli*

E. coli is a rod-shaped, gram-negative, facultative anaerobe, first described by Theodor Escherich in 1885 (*83*). There are over 700 known serotypes of *E. coli*, most of which are harmless to humans and have been found to be a natural inhabitant of the gut in both humans and

animals (*83*, *127*). However, there are some that have been found to be highly pathogenic to humans after consuming contaminated food or water. There are four basic classes of pathogenic *E. coli*: enterotoxigenic, enteropathogenic, enteroinvasive, and enterohemorrhagic (*127*).

The STECs (also called verotoxin-producing *E. coli*) are part of the enterohemorrhagic classification with clinical symptoms that include hemorrhagic colitis and, in serious cases, hemolytic uremic syndrome (HUS) and death (*83*). These pathogens are classified in the *Enterobacteriaceae* family and are named according to their somatic (O) and flagellar (H) antigens (*49*, *130*). The STECs were first described in 1982 after two outbreaks of hemorrhagic colitis in Oregon and Michigan, where patients complained of abdominal cramps and bloody diarrhea, but had no fever (*65*). The pathogens that are of the greatest concern in this category include *E. coli* O157:H7 and six non-O157 STEC serogroups, also referred to as the "Big 6" non-O157 STECs (O26, O45, O103, O111, O121, and O145), among many others (*49*). In the United States, STECs cause approximately 100,000 illnesses each year and nearly 90 deaths; it has been estimated that *E. coli* O157:H7 is responsible for 73,000 of these and all STEC-related fatalities (*16*, *49*).

The STECs were differentiated from other pathogenic *E. coli* after discovery of the Shiga-like toxin in the 1970's (*98*). The toxin was described as being similar in structure and function to the Shiga toxin (Stx) that is produced by *Shigella dysenteriae* type I, but some could be neutralized by antibodies against Stx (anti-Stx), resulting in the "Shiga-like toxin" nomenclature (*49*, *98*). There are two different Shiga toxins that have been identified in STECs: Stx1 can be neutralized by anti-Stx and Stx2 is non-neutralizable (*49*, *98*). STECs can also be characterized by presence of the *eae* gene, which encodes the membrane for the protein intimin, and allows for the bacteria to attach to the lining of the host's gastrointestinal tract (*65*).

Infections with STEC can occur with as few as 4 to 10 cells and are characterized by gastroenteritis, enterocolitis, bloody diarrhea, and weight loss; symptoms can also include hemolytic uremic syndrome in severe cases, leading to renal failure and death (*49*, *127*).

E. coli *O157:H7*

Before 1982, *E. coli* O157:H7 was considered a rare foodborne pathogen, but soon became the new headache of the meat and food industry. *E. coli* O157:H7 became widely recognized as a threatening pathogen in 1993 after a devastating multi-state outbreak linked to undercooked hamburger patties from a fast-food restaurant chain (*49*, *107*). This outbreak resulted in more than 700 illnesses and four deaths of children (*4*), which spread significant awareness of the effects of *E. coli* O157:H7 and challenged the government's approach to food safety. This initiated immediate reform policies from the U.S. government, causing FSIS to implement their zero tolerance policy and declare *E. coli* O157:H7 an adulterant in raw ground beef in 1994; later, in 1999, *E. coli* O157:H7 was deemed an adulterant in non-intact beef products (*137*, *149*).

E. coli O157:H7 outbreaks are most commonly associated with ground beef products, but in recent years, have also been associated with non-meat sources, such as unpasteurized milk, apple juice, and various vegetables (*89*). Cattle, and other ruminant animals, have been identified as natural reservoirs for *E. coli* O157:H7 and are typically asymptomatic when infected (*49*, *83*, *101*). Infection of cattle with *E. coli* O157:H7 is more likely to occur in warmer climates; therefore shedding of the pathogen occurs more often in the summer months, causing an increased rate of infection in humans at this time (*89*). *E. coli* O157:H7 organisms are shed in the feces, and subsequently can contaminate the hide and carcass during the slaughter process (*41*,

89). Due to the virulent nature of *E. coli* O157:H7, and its relevance in beef production, the cattle industry has spent more than $2 billion on combating this pathogen (*127*).

Non-O157 STECs

Symptoms associated with non-O157 STEC infections range from mild diarrhea to HUS, and even death, and are indistinguishable from those resulting from an O157:H7 infection (*61*). This poses a concern to clinicians because they are unable to determine which of the STECs are the causative agent of an infection. Since infections of non-O157 STECs are difficult to differentiate from *E. coli* O157:H7 infections, diagnosis of these pathogens went nearly undetected until 2000 (*16*, *49*). FoodNet conducted a surveillance survey for non-O157 STEC infections from 2000 to 2010; by 2010, it was determined that the number of non-O157 STEC infections equaled that of O157:H7 (*49*). In 2012, FSIS declared that all raw, non-intact beef products contaminated with the "big 6" STECs be considered adulterated and now enforces routine agency testing for non-O157 STECs in addition to *E. coli* O157:H7 (*134*). Routine sampling for non-O157 STECs has determined that these pathogens are present in ground beef products, though outbreaks associated with non-O157 STECs have not been linked to ground beef, but – rather – to milk, salad bars, lake water, flour, and human contact, among other unknown sources in recent years (*16*, *27*).

1.3 *Salmonella enterica*

The genus *Salmonella* was named in 1885 by Daniel E. Salmon (*150*) and are described as gram-negative, rod-shaped, non-spore forming, facultative anaerobes, which are classified in the *Enterobacteriaceae* family (*51*). The two species of *Salmonella* that are recognized today are *S. bongori* and *S. enterica*, of which *Salmonella enterica* is most commonly associated with human foodborne disease (*150*). *Salmonella enterica* is further divided into six subspecies: *S.*

enterica (I), *S. salamae* (II), *S. arizonae* (IIIa), *S. diarizonae* (IIIb), *S. houtenae* (IV), and *S. indica* (VI; *6*, *40*). There are more than 2,500 identified *Salmonella enterica* subspecies *enterica* serovars and more than 99% of the serovars that have been found to cause disease in humans and animals fall under this subspecies (*28*, *34*). Additionally, *Salmonella enterica* subspecies *enterica* are further classified based on their surface lipopolysaccharide and flagellar antigenic properties into individual serotypes and are commonly referred to by their serotype names, such as *S.* Typhimurium, *S.* Enteritidis, and *S.* Heidelberg (*42*, *150*).

As a method of entry, invasive *Salmonella* species, such as *S.* Typhimurium, will initiate cytoskeletal changes (membrane "ruffles") at the membrane surface of the host's epithelial cell lining in the small intestine which, in turn induces an inflammatory response from the host's immune system (*33*, *34*). This inflammation is partially due to the presence of the flagella and chemotaxis, but requires presence of the *Salmonella* enterotoxin gene (*stn*) and various virulence factors (*145*). After emerging from the cell membrane, the *Salmonella* will be engulfed by the macrophages and delivered to the mesenteric lymph nodes, where they will multiply and disperse throughout the body (*33*, *145*). Virulence genes and Stn that are responsible for the pathogenesis of *Salmonella* are carried on what scientists call *Salmonella* pathogenicity islands (SPI), which give each bacteria a specific virulence phenotype (*145*). The SPI-1 is important to the virulence of *Salmonella enterica* subspecies I strains because it carries a type III secretion system apparatus that injects bacterial proteins into the host membrane, directly influencing the cell physiology (*34*). Two types of illness can result from infection with *Salmonella* spp. within subspecies I: nontyphoidal salmonellosis and typhoid fever (*34*, *37*).

Infection with *Salmonella enterica* subspecies I serovars most commonly results in nontyphoidal salmonellosis, but infection with *S. typhi* or *S. paratyphi* can cause typhoid fever

(*37*). Nontyphoidal salmonellosis is a self-limiting pathogen that requires a relatively high infective dose of about 50,000 cells to become symptomatic, in healthy hosts (*28*, *34*). It is characterized by enterocolitis after an 8 to 72 h incubation period and symptoms typically include abdominal pain, nausea, and diarrhea (*34*, *37*). Though, small children, the elderly, and immunocompromised individuals are more susceptible to illness and often progress to a more serious condition including bacteremia and sometimes death (*37*, *42*). Salmonellosis can be contracted from contact with contaminated surfaces and consuming contaminated food and water (*42*). It is estimated that *Salmonella* infection is the cause of nearly 1.4 million illnesses each year in the U.S., along with 16,000 hospitalizations and approximately 600 deaths; reported cases are most commonly associated with *S.* Typhimurium and *S.* Enteritidis (*80*).

Salmonella is the leading bacterial cause of foodborne illness in the United States and is considered a significant public health concern (*3*, *147*). Livestock are the primary reservoirs for *Salmonella* bacteria, and meat products are a common route for human infection (*3*). Poultry products have been identified as the primary cause of human salmonellosis, though ground beef is the fourth leading cause (*78*). In an attempt to reduce the prevalence of *Salmonella* in meat and poultry products, FSIS issued a pathogen performance reduction standard in the PR/HACCP system final rule in 1996 (*135*). The verification system was designed to ensure that processing facilities were appropriately following the reduction standards for *Salmonella* in regards to the most recent national microbiological baseline data for each major species (*135*). In recent years, *Salmonella* has been identified as a possible adulterant in beef products, but according to court ruling in the American Public Health Association vs. Earl Butz case, it cannot be considered an adulterant because proper handling and cooking procedures by the consumer is expected to kill

any pathogens (*78*, *133*). Regardless, salmonellosis outbreaks continue to occur and *Salmonella* infections are still the leading cause of death by foodborne illness (*118*).

1.4 Microbial Contamination of Beef Carcasses

Skeletal muscle of beef carcasses is inherently sterile, however, it can become contaminated when the carcass comes into contact with extrinsic sources, such as the fecal material, the viscera, and the hide (*11*, *60*). Cattle are considered natural reservoirs for STEC and *Salmonella,* and these bacteria are typically shed in the fecal matter of infected animals (*8*, *11*, *41*). In 1994, the USDA-FSIS implemented a "zero tolerance" policy for any visible carcass contamination with fecal, milk, or ingesta material in response to the 1993 *E. coli* O157:H7 outbreak linked to undercooked ground beef (*135*). This has led the industry to implement multiple hurdle systems consisting of washes, steam vacuuming, and chemical decontamination steps to reduce the presence of pathogens, such as STEC and *Salmonella*, on the carcass throughout the harvest process (*5*, *104*). Regardless of the industry and government's attempts to eliminate bacterial pathogens in processing systems, some pathogens are able to evade even the multiple hurdle intervention systems, leading to the contamination of subprimals and trim during fabrication of the carcass (*60*).

The hide has been identified as a significant source of carcass contamination during the harvest process due to pre-harvest contamination by fecal matter during feeding and transportation (*5*, *50*, *149*). Transfer of bacterial populations from the hide to the carcasses is nearly unavoidable during hide removal both due to direct contact with the carcass and indirect contact from workers knives and gloves (*87*). Hides sometimes undergo a spray washing step before removal to reduce the pathogen contamination (*5*). Unfortunately, there is concern that this hide washing step is actually transferring bacteria from highly contaminated regions to all

areas of the hide (*7*). The greatest concern for bacterial transfer from the hides onto the carcass results from contamination at the pattern mark (the midline)where the initial openings are made (*7*). These initial cut locations along the hides are likely where STEC and *Salmonella* contaminate the carcass.

Lymph nodes of cattle can be a source of contamination, especially in ground beef (*50*, *55*, *82*). Research has shown that there is minimal (less than 1.0%) *Salmonella* recovered at the end of the slaughter process from the carcass, yet there are still recalls for ground beef products (*50*, *82*). Studies have found a *Salmonella* prevalence level of greater than 2.0% in U.S. beef samples after fabrication and grinding (*55*, *82*), leading to a general consensus that *Salmonella* is harbored in the lymph nodes; a dilemma becuase current decontamination methods are ineffective against lymphatic contamination (*50*).

1.5 Microbial Contamination of Poultry Carcasses

Poultry meat is considered a major reservoir for transmission of *Salmonella* spp. and *Campylobacter jejuni* into the human food chain (*39*, *67*, *95*). Poultry are typically contaminated with pathogens during pre-harvest stages, including in the hatching environment and during broiler housing (*39*, *67*, *88*). This occurs by transmission from infected breeding stock, contaminated feed, and exposure via handling (*88*). Unfortunately, studies have determined that it is challenging and uneconomical to completely eliminate *Salmonella* (*67*). Thus, high standards of handling hygiene are essential to controlling pathogen contamination pre-harvest (*88*).

Poultry processing facilities have procedures in place that are designed to decontaminate carcasses, but research has shown that these same processing steps may be a source of cross-contamination within a facility (*10*, *14*, *59*). Often, these steps include scalding, defeathering,

evisceration, and chilling; broilers are placed into the same piece of equipment or water where contaminated birds have been processed and contamination can be transferred (*59*). Scalding and defeathering can open new surfaces of the carcass for bacteria to colonize because it removes the epidermis of the skin (*59*), while evisceration introduces the intestinal tract to the processing environment and this can lead to contamination if the bird is infected (*102*). In an attempt to improve contamination on raw poultry products, pre- and post-harvest interventions have been utilized by the industry and HACCP protocols have been implemented in processing facilities (*10*, *14*, *59*, *88*).

Contamination of poultry products with *Salmonella* spp. and/or *Campylobacter* spp. is considered a significant biological hazard due to the increased risk of foodborne illness in consumers who unintentionally undercook poultry (*17*). Therefore, USDA-FSIS has implemented pathogen testing programs in poultry processing facilities and the prevalence of *Salmonella* and *Campylobacter* are reported quarterly (*139*). *Salmonella* was selected as a target bacteria because it is naturally occurring in the intestinal tract of poultry and performance standards have been determined for the allowable positives in a processing facility (*94*). The allowable percent positives for *Salmonella* in broiler chickens, comminuted chicken chicken, and chicken parts is 9.8%, 25.0% and 15.4%, respectively (Insert new performance standards, *91*). These pathogen testing programs and HACCP protocols are investments made by the government and industry to minimize foodborne pathogen contamination.

1.6 Chemical Decontamination of Beef Carcasses

Since *E. coli* O157:H7 was deemed an adulterant in ground beef in 1994, a considerable amount of effort has been expended to control pathogens in beef products (*121*). Many physical and chemical interventions have been developed and implemented to aid in these control

processes (*47*). Research has shown that a multiple hurdle intervention process in beef processing facilities can successfully reduce the risk of pathogens on carcasses during harvest (*6*, *47*, *152*). These multiple hurdle systems typically consist of two or more physical and chemical decontamination methods, including steam pasteurization, hot water washes, and chemical treatments (*6*, *121*). Additionally, chemical interventions have been extensively studied for reducing pathogen loads on beef carcasses and are utilized heavily within the industry as either a spray treatment in a wash cabinet or as an immersion treatment (*22*, *48*, *77*, *91*, *121*, *152*).

Organic acids are commonly utilized as chemical treatments in meat processing facilities and are generally recognized as safe (GRAS) by the FDA (*142*). The mode of action for most organic acids is to permeate across the cell membrane to a higher pH level in a non-dissociated form (*85*). Once inside the cell, the alkaline environment favors the dissociation of the acid, acidifying the cytoplasm of the cell and inhibiting it's functionality, ultimately causing cell death (*85*).

Lactic Acid

Research has shown that lactic acid is an effective antimicrobial intervention on beef and is the most widely used organic acid in the industry (*77*). USDA-FSIS (*142*) has approved lactic acid as a processing aid on beef products with the following parameters: i) on carcasses prior to fabrication, variety meats, and offal up to 5.0% in solution, ii) on subprimals and trimmings at a concentration of 2.0% to 5.0% and up to 55°C, and iii) on heads and tongues at 2.0% to 2.8% in solution. Studies show that lactic acid can effectively reduce contamination of *E. coli* O157:H7, STECs, and *Salmonella* spp. in beef products (*24*, *111*, *121*, *152*).

Bosilevac et al. (*15*) determined that a 2.0% lactic acid spray (42°C) applied to hot beef carcasses post-evisceration successfully reduced aerobic plate counts (APC) by 1.6 log CFU/cm^2

and reduced the prevelance of *E. coli* O157:H7 by 35%. Castillo et al. (*24*) reported reductions in aerobic plate counts, coliforms, and *E. coli* populations recovered from post-chilled beef carcasses after treatment with a 4.0% lactic acid solution (55°C). Additionally, Hardin et al. (*56*) determined that a 2.0% lactic acid treatment reduced *E. coli* O157:H7 and *Salmonella* Typhimurium contamination levels to below detection limit more often than other decontamination methods, including acetic acid, trimming, and a hot water wash. The efficacy of lactic acid is variable depending on many factors, including application method, pressure and time, solution concentration and temperature, and product type.

Acetic Acid

Acetic acid is one of the oldest known organic acids and, along with lactic acid, is one of the most widely accepted carcass interventions in the beef industry (*85*, *129*). Acetic acid is a primary ingredient in vinegar and has a strong odor; therefore, it may negatively affect the flavor and sensory attributes of meat products (*129*). The USDA-FSIS (*142*) approved the use of a 4.0% acetic acid solution in dried and fermented sausages and in prosciutto.

Acetic acid has been observed to have varying antimicrobial effects with different application parameters. Berry and Cutter (*13*) spray treated inoculated beef carcass tissue with a 2.0% acetic acid solution and reported initial reductions of *E. coli* O157:H7 of > 1.0 log CFU/cm^2. In another study (*70*), fresh beef samples were immersed in 3.0% and 5.0% solutions of lactic acid, acetic acid and citric acid for 60 s and determined that the 5.0% acetic acid solution was the most effective in reducing inoculated *E. coli* populations. This study also reported that the 3.0% and 5.0% acetic acid solutions reduced *S.* Typhimurium populations by 0.9 and 0.7 log CFU/g, respectively (*70*).

Acetic acid was also evaluated for its efficacy against pathogens in ground beef products to determine its value within a multiple hurdle system. Harris et al. (*57*) evaluated use of 2.0% and 4.0% acetic and lactic acids, and 1,200 ppm acidified sodium chlorite against inoculated *E. coli* O157:H7 and *S.* Typhimurium populations on beef trim destined for ground beef. Trim samples were spray treated on one side, then mixed together during grinding after treatment. Acetic acid successfully reduced bacterial populations by approximately 2.0 log CFU/g immediately after treatment. Additionally, researchers reported that both tested concentrations of acetic acid were the most effective at minimizing pathogen growth 24 h after grinding and throughout cold storage (*57*). This suggested that acetic acid is an effective antimicrobial against *E. coli* O157:H7 and *Salmonella* at multiple stages of beef processing.

Peroxyacetic Acid

Peroxyacetic acid (PAA), also known as peracetic acid, is commonly used within the beef industry at various stages throughout beef processing (*149*). It is approved for use up to 400 ppm, but is most commonly used at 15 to 400 ppm as a beef carcass wash and in spray chill systems (*74*, *142*, *149*). Peroxyacetic acid is not an organic acid, but is considered an organic peroxide (a combination of acetic acid and hydrogen peroxide), and therefore has a different mode of action than that of lactic and acetic acids. The mode of action in PAA has not been extensively studied, but Kitis et al. (*76*) describes PAA as being similar to other peroxides and oxidizing agents and determined that PAA likely has similar oxidizing effects. The decontamination effect of PAA occurs by releasing oxygen which disrupts the chemiosmotic function of the lipoprotein cytoplasmic membrane, ultimately rupturing cell walls and destroying the functionality of the cell (*76*).

Varying application parameters of PAA have been extensively studied for use within the beef industry (*47*, *74*, *91*, *108*). Kalchayanand et al. (*68*) spray treated prerigor beef flanks inoculated with non-O157 STECs and O157:H7 STECs with a 4.0% solution of lactic acid and a 200 ppm PAA solution for 30 s. The researchers reported a reduction of 0.9 to 1.5 log CFU/cm^2 for STEC strains after treatment with PAA (*68*). Ransom et al. (*108*) observed a 1.0 to 1.4 log CFU/g decrease of inoculated *E. coli* O157:H7 populations on beef carcass adipose tissue and trimmings when samples were immersed in PAA (200 ppm; 55°C) for 30 s. Though, in another study (*74*), it was determined that PAA (200 ppm) minimally reduced inoculated *E. coli* O157:H7 and *S.* Typhimurium populations on beef carcasses, by 0.7 log CFU/cm^2 when used on cold tissue.

Other Chemical Treatments

Other chemicals have been evaluated for use in beef processing systems in an attempt to discover cost effective alternatives to current chemical interventions. These chemicals include hyprobromous acid, citric acid, a lactic acid and citric acid blend, chlorine, a sulfuric acid and sodium sulfate blend, and trisodium phosphate; all were reported as potentially appropriate additions to a multiple hurdle system (*47*, *48*, *70*, *77*, *91*, *119*, *121*).

1.7 Chemical Decontamination of Poultry Carcasses

In recent years, the poultry industry has put more emphasis on decontamination strategies to control presence of pathogens in poultry products (*93*). There are many physical and chemical interventions that have been developed and multiple hurdle systems have been implemented in poultry processing facilities to aid in controlling these pathogens (*9*, *93*). Chlorine, or chlorine dioxide, have been considered the golden standard for decontamination of poultry carcasses, but have recently become a concern due to improper application. Therefore studies were conducted

to evaluate alternatives (*96*). Other antimicrobials that are commonly utilized in the poultry industry are cetylpyridinium chloride (CPC), PAA, and lactic acid (*9*, *96*).

Chlorine

Chlorine was previously the most widely used antimicrobial intervention in the poultry industry (*9*, *32*, *99*). However, research has shown that failure to optimize the appropriate application parameters of chlorine (pH, concentration, or composition of incoming water) can drastically affect the antimicrobial efficacy of the solution and can result in harmful odors on the product (*79*, *93*, *96*). Chorine gas utilized in water is generally recognized as safe and is approved by USDA-FSIS (*142*) for use in water as a spray or immersion treatment up to 50 ppm. Chlorine can be used at various steps in the processing system, but is most commonly used in an immersion chilling system (*93*). The mode of action for chlorine is not fully understood, but research has suggested that chlorine may disrupt the bacterial membrane leading to cell death, while others have suggested that it inhibits protein synthesis (*12*). There also is a possibility that chlorine may use a combination of factors, such as "oxidation of enzymes and amino acids, ring chlorination of amino acids, loss of intracellular contents, decreased uptake of nutrients, inhibition of protein synthesis, decreased oxygen uptake, decreased ATP production, breaks in DNA, and depressed DNA synthesis" (*115*).

Nagel et al. (*93*) immersed whole chilled broiler carcasses inoculated with *S.* Typhimurium (approximately 5.1 log CFU/ml) into a post-chill immersion tank (4°C, 20 s) to evaluate the antimicrobial efficacy of chlorine (40 ppm), PAA (1,000 ppm) and lysozyme (1,000 ppm or 5,000 ppm). Chlorine resulted in < 1.0-log reduction of *S.* Typhimurium, while PAA was the most effective treatment with a 2.02 to 2.14 log reduction of *S.* Typhimurium (*93*). In another study (*151*), broiler skin samples were removed from the breast, inoculated with either *S.*

Typhimurium, *Campylobacter jejuni*, or *E. coli,* and were then subjected to scalding, followed by immersion in chilled water (0 h and 8 h) with various levels of chlorine added (0, 10, 30, or 50 ppm). Even with the varying parameters in place with this study, the researchers reported < 1.0-log reduction of *S.* Typhimurium and *Campylobacter jejuni* regardless of application (*151*). Additionally, Purnell et al. (*106*) reported no differences between the control and chlorine dioxide (approximately 9 ppm) when spray treated on broiler carcasses for 15 or 30 s. These reports validated the industry's desire to utilize an antimicrobial treatment that will be more effective against pathogens on poultry carcasses.

Cetylpyridinium Chloride

Cetylpyridinium chloride (CPC) has been reported as the most utilized postchill antimicrobial agent for drench cabinets in poultry processing (*32*). The USDA-FSIS Directive 7120.1 (*142*) indicates that CPC is GRAS and is approved for use on raw poultry carcasses, giblets, or parts (skin-on or skinless) as a spray treatment (not to exceed 0.8% by weight) or as an immersion treatment (not to exceed 0.8% by weight with a 10 s dwell time); both treatment applications must be followed by immersion in a chiller or by a rinse with potable water. Cetylpyridinium chloride is considered a cationic surfactant and its mode of action involves an interaction between the cetylpyridinium ions and the acidic groups of bacteria, which form ionized compounds that inhibit the functional mechanisms of the bacteria (*73*).

Chen et al. (*32*) immersed broiler carcasses inoculated with *S.* Typhimurium or *Campylobacter jejuni* in a chill tank (4°C potable water) containing either 0.35% CPC, or 0.6% CPC for approximately 23 s, followed by grinding of the samples. The researchers reported that both concentrations of CPC reduced the pathogen load by approximately 0.8 log CFU/g for each pathogen (*32*). Kim and Slavik (*73*) conducted a study to evaluate the effects of CPC against

Salmonella on poultry skin. A spray treatment of CPC (0.1%) at 15°C and 50°C, and an immersion treatment of CPC for 1 min, 1 min with a 2 min dwell time, and 3 min were used. Results showed that regardless of application parameter, CPC treatment resulted in a 1.0-log reduction of *Salmonella* (*73*). Further, in another study (*153*), researchers spray treated whole broiler carcasses with a 0.5% solution of CPC for 17 s and reported that the treatment reduced inoculated *Salmonella* populations from 5.63 log CFU/carcass to 3.62 log CFU/carcass.

Peroxyacetic Acid

PAA is a mixture of acetic acid combined with hydrogen peroxide to minimize the negative color and flavor changes that can often result from antimicrobial application of organic acids (*9*). The combination of acidic and oxidizing properties of PAA have been found to be effective against bacteria, bacterial spores, fungi, and yeast (*9*). PAA is considered GRAS by the USDA-FSIS and is approved for use as a spray or immersion treatment on poultry carcasses, parts, and organs at a maximum of 2,000 ppm (*142*).

Nagel et al. (*93*) evaluated the antimicrobial efficacy of PAA (400 ppm and 1,000 ppm) as a post-chill dip, rather than an immersion chill system. They reported that PAA was the most effective antimicrobial evaluated in their study, reducing inoculated populations of *Salmonella* by 2.0 to 2.1 log CFU/ml from the control (5.1 log CFU/ml) (*93*). Purnell et al. (*106*) spray treated broiler carcasses with 400 ppm PAA and observed a 0.97 to 1.15-log reduction of natural *Campylobacter* populations. Additionally, Scott et al. (*122*) immersed (20 s) whole, skin-on chicken wings in PAA (700 ppm), and reported a 1.5 log CFU/ml reduction from initial populations of 5.5 log CFU/ml of inoculated *Salmonella* populations immediately post treatment and a 1.7 log CFU/ml reduction (from 5.5 log CFU/ml) of the pathogen 24 h after treatment.

Other Chemical Treatments

Poultry processing is highly automated. Therefore there are many places in the process where cross-contamination may occur and the poultry industry has put an extensive amount of effort into finding alternative antimicrobial interventions for their systems (*9*). Additionally, since USDA-FSIS issued their new prevention-based regulations, the industry has determined it is necessary to reevaluate their intervention methods (*32*). Other antimicrobials that have been evaluated for use in the poultry industry are various organic acids, a sulfuric acid and sodium sulfate blend, trisodium phosphate, acidified sodium chlorite, lysozyme, among others (*9*, *32*, *47*, *93*, *106*, *122*).

1.8 Surfactants as Antimicrobial Interventions

The uneven surfaces of meat and poultry create a challenge for the chemical and physical decontamination of these products. The high pH level, nutrient composition, and water activity of raw meat and poultry inherently supports the survival and growth of bacterial populations (*85*). For example, the high lipid content of poultry skin creates a favorable environment for pathogens to attach and survive, thus making it difficult to remove or kill these contaminants (*154*). Surfactants have the ability to decrease the surface tension of the antimicrobial and enhance the attractiveness of chemicals to surfaces of meat and poultry, as well as assisting in physically removing pathogens(*154*). There are a number of industries that utilize surfactants, such as the petroleum, pharmaceutical, food, biotechnology, cosmetic, and paint industries (*128*). Use of surfactants in the meat industry have not been extensively studied, but various anionic, cationic, and nonionic surfactants have been evaluated for use as antimicrobials. In food, these surfactants include cetylpyridinium chloride and tween 80, among others (*44*, *83*, *152*). Alkyl polyglycosides are nonionic surfactants that are synthesized by combining a polyfunctional sugar component (often a glucose molecule) with a nucleophile, most commonly a fatty alcohol, but

also with a carbohydrate or protein (*148*). These are commonly utilized within the food industry due to their biodegradability and antimicrobial properties.

Mohan & Pohlman (*90*) used various organic acids, including peroxyacetic acid (PAA), with and without the addition of a 0.5% solution of a non-ionic surfactant (ethoxylated glycerol; EG) to evaluate the decontamination efficacy of these acids, in combination with EG, on frozen beef trimmings inoculated with *E. coli* O157:H7. The investigators rinsed the inoculated trimmings for 15 s in 100 ml of solution; the PAA solution was applied at 0.2 g/L, and EG was added at 5g/L. They reported that the PAA treatment decreased the *E. coli* O157:H7 populations by 0.33 log CFU/g, but the reduction of the *E. coli* populations after treatment with PAA and EG was 0.89 log CFU/g (*90*). Additionally, Zaki et al. (*154*) evaluated the effects of adding Sodium Dodecyl Sulfate (SDS), a transdermal surfactant, to three different organic acids: lactic acid, levulinic acid, and acetic acid. Sterilized chicken breast skin pieces were inoculated with *Salmonella* by immersing the pieces in the inoculum mixture and immersed in one of 20 antimicrobial solutions, with and without surfactant addition, for 1 min or 3 min with gentle agitation. The investigators (*154*) found that by adding SDS to the antimicrobial, there were significant ($P < 0.05$) reductions in *Salmonella* survival after all organic acid treatments.

CHAPTER 2

Effect of Surfactant Addition on the Antimicrobial Effects of Chemical Interventions on Inoculated Bacterial Populations Applied to Beef Tissue and Chicken Parts

Summary

Three studies were conducted to determine if addition of a nonionic alkyl polyglycoside surfactant to the formulation of various chemical solutions would enhance their antimicrobial efficacy against bacterial populations on beef tissue and chicken parts. In part A, a 0.4% mixture of one alkyl polyglycoside (AP) was added to each solution and a 0.5% solution of a different alkyl polyglycoside (DB) was added to the solution in part B. In Part A – study 1, 80% lean beef trimmings were inoculated (~ 6 log CFU/g) with nonpathogenic *Escherichia coli* (5-strain mixture) and were left untreated or were treated in a spray cabinet with one of six antimicrobial treatments including, a sulfuric acid and sodium sulfate blend (SSS; pH 1.1), peroxyacetic acid (PAA; 400 ppm), and PAA (400 ppm) acidified with pH 1.1 SSS (aPAA), without or with the addition of AP (0.4%) to the chemical solution. In Part A – study 2, whole, skin-on chicken wings inoculated (~ 6 log CFU/ml of rinsate solution) with *Salmonella* (5-strain mixture) resistant to novobiocin and naladixic acid were left untreated or were immersed for 15 s in either SSS (pH 1.2), SSS with AP (0.4%), PAA (500 ppm), PAA with AP, aPAA (500 ppm PAA acidified with pH 1.2 SSS), or aPAA with AP. In Part B, warm, prerigor beef carcass surface tissue was inoculated (~ 6 log CFU/cm^2) with nonpathogenic *E. coli* (5-strain mixture) and was left untreated or was spray treated with one of eight treatments: water (ambient temperature), DB (0.5%), PAA (400 ppm), PAA with DB, SSS (pH 1.2), SSS with DB, aPAA (400 ppm PAA acidified with pH 1.2 SSS), or aPAA with DB. In all studies, treated samples were analyzed for

surviving inoculated bacterial populations 5 min post treatment. In Part B, samples were also evaluated 24 h post treatment. In both Part A studies, all antimicrobials were effective at reducing ($P < 0.05$) inoculated bacterial populations from that of the untreated control; however, the main effect of surfactant addition to the chemical solution did not affect ($P \geq 0.05$) efficacy of the antimicrobials in either of the two studies. PAA-containing treatments were the most effective in both studies of part A, reducing initial *E. coli* populations by 0.5 to 0.6 log CFU/g on beef trimmings (from 6.5 log CFU/g), and initial *Salmonella* populations by 1.7 to 1.8 log CFU/ml of rinsate solution for the chicken wings (from 6.0 log CFU/ml of rinsate solution). In Part B, all spray treatments reduced ($P < 0.05$) *E. coli* populations on prerigor beef carcass surface tissue, though PAA-containing treatments were the most effective, reducing ($P < 0.05$) initial bacterial populations by 1.4 to 1.7 log CFU/cm^2 from the intial inoculation level of 6.1 log CFU/cm^2. The interaction between treatment and surfactant addition in Part B was significant ($P < 0.05$), such that surviving *E. coli* populations after treatment with PAA and aPAA plus DB added was 0.3 log-units greater than the same treatments without surfactant added. Therefore, it can be concluded that the addition of a nonionic alkyl polyglycoside may not increase the efficacy of PAA, SSS, or aPAA at the tested parameters.

Introduction

Presence of foodborne pathogens remains an ongoing concern for the meat and poultry industries (*6*, *22*, *132*). Foodborne illness from bacterial contaminants such as *Escherichia coli* O157:H7, *Salmonella*, and *Campylobacter* are responsible for more than 3.6 million illnesses, nearly 36,000 hospitalizations, and 861 deaths each year (*118*). Microbial contamination is inevitable during the harvest and processing procedures. Often, carcasses become contaminated during hide or feather removal, the evisceration process, or unsanitary handling (*22*, *69*).

Effective intervention systems are required to minimize the potential for an outbreak of foodborne illness (*31*, *63*, *91*, *132*).

Since the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) introduced the concept of the hazard analysis and critical control point (HACCP) system in July 1996, many chemical and physical intervention systems have been tested and validated as effective (*6*, *109*). These interventions may include wash cabinets, steam vacuuming, and the application of chemical interventions, such as organic acids, chlorine dioxide, peroxyacetic acid, and cetylpyridinium chloride (*48*, *91*, *121*). Additionally, some studies have evaluated addition of various surfactants, antioxidants, and other compounds to chemical interventions to increase their efficacy (*31*, *91*, *112*). Regardless of the number of decontaminating treatments utilized by the meat industry (*6*), foodborne illness due to the consumption of contaminated meat and poultry products continue to occur (*31*, *86*, *122*).

Peroxyacetic acid (or peracetic acid; PAA) has been evaluated extensively across all sectors and is commonly utilized within the beef and poultry industries (*9*, *46*, *88*, *107*, *118*, *119*, *148*). A commercially available blend of sulfuric acid and sodium sulfate (SSS) has been evaluated as a chemical intervention system on beef and poultry products (*47*, *121*, *122*, *152*).

The uneven surfaces of meat and poultry can create a challenge for physical and chemical decontamination. Fresh meat and poultry products inherently support survival of bacterial populations because of their pH level, nutrient composition, and water activity (*84*). Zaki et al. (*154*) explained that the high lipid content of chicken skin creates a favorable environment for microorganisms to attach, making it challenging for interventions to remove these contaminants. Surfactants have the ability to enhance the permeability of the antimicrobial interventions applied to surfaces of meat and poultry, therefore, it is expected that antimicrobial compounds

should be more likely to penetrate and adequately coat these surfaces when a surfactant is included in the formulation (*154*).

Continued investigation of post-harvest intervention systems is still needed across all sectors of the food industry. Therefore, the overall objective of these three studies (Part A – studies 1 and 2 and Part B) was to determine if addition of two different alkyl polyglycoside surfactants (AP and DB; nonionic surfactant) to the formulation of SSS and PAA would enhance their antimicrobial efficacy against inoculated *E. coli* and *Salmonella* on beef tissue and chicken parts, respectively.

Materials and Methods

Part A – AP Surfactant

Bacterial strains and preparation of inocula. Two inoculum mixtures were used for part A: (i) a 5-strain mixture of non-pathogenic *E. coli* biotype I (ATCC-BAA 1427, ATCC-BAA 1428, ATCC-BAA 1429, ATCC-BAA 1430, ATCC-BAA 1431), considered surrogates for pathogenic *E. coli* O157:H7, non-O157 Shiga toxin-producing *E. coli* (STEC), and *Salmonella* (*20*), was used to inoculate the beef trimmings in study 1, and (ii) a 5-strain mixture of *Salmonella* serotypes of poultry origin (*Salmonella* Montevideo, *Salmonella* Typhimurium, *Salmonella* Heidelberg, *Salmonella* Enteritidis, and *Salmonella* Newport; obtained from Dr. Thomas Edrington, USDA, Agricultural Research Center, College Station, TX), was used to inoculate the chicken wings (study 2).

In the first study, the *E. coli* strains were individually cultured and subcultured (35ºC, 22 h; stationary phase) in 10 ml of tryptic soy broth (TSB; Difco, Becton Dickson and Co. [BD], Sparks, MD), following which broth cultures of all five strains were combined and cells harvested via centrifugation (5,590×*g*, 15 min, 4ºC; J2-MC centrifuge, Beckman Coulter, Inc.,

Pasadena, CA). The resulting cell pellet was washed with 10 ml of phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich, St. Louis, MO), re-centrifuged as described above, and then resuspended in 50 ml of PBS. The concentration of the cell suspension was approximately 8-9 log CFU/ml. A 3-ml aliquot of the inoculum cell suspension in 47 ml of PBS was used to inoculate each 1.4 kg (3 lb.) batch of beef trimmings needed for each treatment.

For the second study, *Salmonella* strains were selected for resistance to novobiocin and naladixic acid to allow for selection and differentiation of the inoculum from any potentially naturally-occurring *Salmonella* populations associated with the poultry parts. Strains were cultured and subcultured (35°C, 22 h; stationary phase) in 10 ml TSB supplemented with novobiocin (25 µg/ml) and naladixic acid (20 µg/ml). Harvesting and washing of cells followed the same procedure outlined for study 1. A 50-ml volume of PBS was added to the washed cell pellet and a concentration of 8-9 log CFU/ml of the inoculum suspension was obtained.

Sample procurement and inoculation. Beef trimmings (80% lean) were obtained from a commercial beef harvest facility in northern Colorado and immediately transported to the Center for Meat Safety & Quality at Colorado State University (CSU; Fort Collins, CO). Product was stored at 0-4ºC and used within 24 h (Trial 1) or 72 h (Trial 2). On each experiment day, trimmings were divided into seven 1.4 kg (3 lb.) batches and were inoculated using synthetic paint brushes (one per treatment) that had been sterilized the previous day by immersing them into 95% ethanol and then air drying them overnight under a biosafety cabinet. Each 1.4 kg batch of trimmings was laid on a tray and brushed with approximately half of the 50 ml of inoculum suspension. After 1 min, trimmings were turned over and inoculated in the same manner on the opposite side with the remaining suspension. The target inoculation level was 6 log CFU/g.

Inoculated trimmings were allowed a 15-min cell attachment time before antimicrobial treatment or sampling of the untreated trim for determination of initial bacterial populations.

Skin-on, whole chicken wings (all joints attached) were purchased from a commercial poultry processing facility and shipped fresh and refrigerated (not frozen) to the Center for Meat Safety & Quality at CSU. Upon arrival, they were immediately refrigerated in the same manner as the beef trim. On each of the two experiment days, samples were separated into seven batches of eight whole wings (five wings for microbial analysis and three for pH), then the batches were randomly assigned to one of six treatment groups or the untreated control group. Eight wings per batch were placed onto trays lined with alcohol-sanitized foil (70% alcohol) and were inoculated under a biosafety cabinet. Samples were inoculated by depositing a 100 µl aliquot of the *Salmonella* inoculum on one side of the wing and spread using a disposable spreader. After a 10-min attachment time, wings were turned over and the opposite side was inoculated using the same method. Untreated samples were utilized to obtain initial *Salmonella* counts. The target inoculation level was approximately 6 log CFU/ml of rinsate solution.

Antimicrobial treatment of samples. Inoculated beef trimming samples in study 1 were randomly assigned to one of seven treatment groups: untreated control, SSS pH 1.1 (Zoetis, Parsippany, NJ), SSS pH 1.1 with AP addition (0.4%, Kroff Food Services, Inc., Pittsburgh, PA), PAA (400 ppm, Kroff Food Services), PAA (400 ppm) with the addition of AP (0.4%), PAA (400 ppm) acidified with pH 1.1 SSS (aPAA), and aPAA with AP added (0.4%). Antimicrobial treatments were applied using a custom-built spray cabinet (CHAD Co., Olathe, KS) specifically designed for trim and subprimal cuts. The cabinet had 18 floodjet spray nozzles (0.1 gallons per minute; Grainger Industrial Supplies, Fort Collins, CO); 10 nozzles above the product belt and eight nozzles below. Solutions were applied at a pressure of 20 psi and a contact time of

approximately 10 s. Following treatment, the trim samples were placed onto sanitized racks and allowed to drip for approximately 3 min before being transferred to the Food Safety & Microbiology laboratory for sampling (2 min for transfer). On each experiment day, five samples were collected for microbial analysis and three samples for pH analysis.

In study 2, batches of eight inoculated chicken wings were randomly assigned to one of seven treatment groups: untreated, SSS pH 1.2 (Zoetis, Parsippany, NJ), SSS pH 1.2 with AP addition (0.4%, Kroff Food Services, INC., Pittsburgh, PA), PAA (500 ppm, Kroff Food Services), PAA (500 ppm) with the addition of AP (0.4%), aPAA (500 ppm PAA, SSS pH 1.2), and aPAA (500 ppm PAA, SSS pH 1.2) with AP added (0.4%). Treatments were applied by placing individual wings into a 24-oz sterile Whirl-Pak bag (Nasco, Atkinson, WI) with 350 ml of the test solution. A different Whirl-Pak bag with fresh solution was used for each wing. Samples were aseptically removed from the Whirl-Pak bag after a 15-s treatment time, then allowed to drip for 5 min on a sterile rack before sampling. On each experiment day, five samples were analyzed for microbial survival and three samples were used for pH analysis.

Microbial analysis. In study 1, 25-g composite samples of surface tissue from the whole pieces of untreated or treated trim were excised using sterile disposable scalpels and placed into a filtered Whirl-Pak bag (24-oz) with 50 ml of Dey/Engley (D/E) neutralizing broth (Difco, BD). Samples were mechanically pummeled for 2 min using a stomacher (Stomacher 400 Circulator, Seward Laboratory Systems, Inc., Bohemia, NY). Samples were serially diluted (10-fold) in 0.1% buffered peptone water (BPW; Difco, BD) and appropriate dilutions were plated, in duplicate, onto Petrifilm *Enterobacteriaceae* Count plates (EB; 3M, St. Paul, MN). Colonies were counted after incubation at 35ºC for 24 ± 2 h. Uninoculated trim samples were also

analyzed for counts of any naturally present *Enterobacteriaceae populations*. The detection limit of the microbiological analysis was 0.5 log CFU/g.

Whole wings in study 2 were placed into a Whirl-Pak bag (55-oz) containing 150 ml of neutralizing buffered peptone water (nBPW; Acumedia-Neogen), and were vertically shaken by hand with a strong downward force for 60 s to recover cells. Sample rinsates were serially diluted (10-fold) in 0.1% BPW and appropriate dilutions were plated, in duplicate, onto tryptic soy agar (TSA; Acumedia-Neogen) supplemented with novobiocin (25 µg/ml) and naladixic acid (20 µg/ml; TSANN) for enumeration of surviving inoculated *Salmonella* populations. Colonies were counted after incubation at 35ºC for 24 ± 2 h. Uninoculated chicken wing samples were analyzed for naturally present novobiocin- and naladixic acid-resistant *Salmonella* populations. The detection limit of the microbiological analysis for chicken wing samples was 0.0 log CFU/ml of rinsate solution.

pH analysis. In study 1, 10 g composite samples were excised from whole trim pieces, and pH was determined by diluting samples 1:10 with deionized water (1 part sample and 9 parts water). Samples were mechanically pummeled for 2 min using a Masticator (IUL instruments, Barcelona, Spain). The pH of the untreated and treated samples was measured with a calibrated pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO). In study 2, wing samples were placed into a Whirl-Pak bag, weighed, and then diluted 1:4 with DI water. The same shaking method was used as described above, and the pH of the rinsate was measured with the same pH meter used in study 1.

Statistical analysis. Both studies were designed as randomized complete blocks (a 2x2 factorial) with experiment day serving as a block effect. Each study (i.e., study 1 and study 2) was replicated on two different days, with n = 10 per treatment. Bacterial populations were

expressed as least-squares means for log CFU per g of trim or ml of wing rinsate solution (studies 1 and 2, respectively) under the assumption of a lognormal distribution for plate counts. Data from both studies 1 and 2 were analyzed using the mixed procedure in SAS (SAS 9.4, Cary, NC) with independent variables including antimicrobial treatment, surfactant addition, and their respective interaction. Differences in least squares means were separated using a significance level of $\alpha = 0.05$.

Part B – DB Surfactant

Bacterial strains and preparation of inoculum. The same five nonpathogenic *E. coli* biotype I strains used in Part A – study 1 were utilized for Part B of this study. The preparation procedures are the same as previously described for Part A – study 1.

Sample procurement and inoculation. On two separate days, 60 sections of prerigor (warm) beef carcass surface tissue were collected from the plate region of carcasses after electrical stimulation during harvest at a commercial beef processing facility in northern Colorado. Beef tissue samples were immediately placed into insulated containers and transported to the Center for Meat Safety & Quality at CSU (Fort Collins, CO).

Each beef tissue section was divided into two or three 10 x 10 cm pieces and these were then assigned randomly to either an untreated control or one of eight spray treatment groups. For each treatment, ten-10 x 10 cm pieces were placed on a sanitized tray and were inoculated on the external adipose side. Samples were inoculated by depositing a 0.2 ml aliquot of the *E. coli* inoculum mixture on the tissue surface and then spreading it over the entire surface using a disposable spreader. The target inoculation level was approximately 6 log CFU/cm^2. Inoculated samples were allowed a 15 min cell attachment period at room temperature (approximately 4-

7°C) before application of the water or antimicrobial spray treatments, or before sampling of the untreated control tissue samples for determination of initial counts.

Antimicrobial treatment of samples. The inoculated prerigor beef tissue samples in Part B were randomly assigned to one of nine treatment groups: untreated control, water (ambient temperature), DB (0.5%; BASF Corporation, Ludwigshafen, Germany), PAA (400 ppm, Kroff Food Services), PAA (400 ppm) with the addition of DB (0.5%), SSS pH 1.2 (Zoetis), SSS pH 1.2 with DB addition (0.5%), PAA (400 ppm) acidified with SSS pH 1.2 (aPAA), and aPAA with DB added (0.5%). Part B utilized the same custom-built spray cabinet as in Part A – study 1. Solutions were applied at a pressure of 15 psi and a contact time of approximately 10 s. Following treatment, the beef tissue samples were placed onto sanitized racks and allowed to drip for approximately 3 min before being transferred to the Food Safety & Microbiology laboratory for microbial analysis within 2 min or a 24 h chilled storage period.

Microbial analysis. There were a total of 10 samples per treatment on each experiment day (trial). Five out of the 10 samples were analyzed within 10 to 15 min post-treatment (designated as 0 h samples), while the remaining five samples were placed into individual Whirl-Pak bags and were analyzed after a 24 h storage period at 4°C. For microbial analysis, each 10 x 10 cm piece was placed into a filtered Whirl-Pak bag (55-oz) containing 175 ml of D/E neutralizing broth (Difco, BD) and mechanically pummeled for 2 min (Masticator). Samples were then serially diluted (10-fold dilution) in 0.1% BPW and appropriate dilutions were plated, in duplicate, on violet red bile glucose agar (Difco, BD). Colonies were counted after incubation of plates at 35°C for 24 ± 2 h. Uninoculated prerigor beef samples were also analyzed for counts of naturally present *Enterbacteriaceae* populations. The detection limit for the prerigor beef carcass surface tissue samples was 0.24 log CFU/cm^2.

Statistical analysis. The study was designed as a randomized complete block (2 x 2 factorial) with trial (experiment) day serving as the block effect. It was replicated on two separate days, with n = 10 per treatment and sampling time. Bacterial populations are expressed as least squares means for log CFU/cm^2 of prerigor beef carcass surface tissue under the assumption of a lognormal distribution for plate counts. Data were analyzed using the Mixed procedure in SAS (SAS 9.4, Cary, NC) with independent variables including antimicrobial treatment, surfactant addition, and sampling time, as well as their respective interactions. Least squares means were separated using a significance level of $\alpha = 0.05$

Results and Discussion

Part A – AP Surfactant

Study 1: 80% Lean beef trimmings. The natually present *Enterobacteriaceae* populations recovered from the uninoculated samples were 1.0 log CFU/g, which is below the inoculation level utilized for this study (6.5 log CFU/g). An initial analysis of surviving *Enterobacteriaceae* counts showed that the main effects of antimicrobial treatment were significant ($P < 0.001$), but that the effect of surfactant had no impact on surving plate counts ($P = 0.3868$). Additionally, the interaction between antimicrobial treatment and surfactant addition was not significant ($P = 0.2335$). Therefore, least squares means are presented only for the main effect of antimicrobial treatment, with each antimicrobial plus surfactant analyzed as a separate treatment (Table 2.1). When compared to the untreated control, all treatments effectively reduced ($P < 0.001$) inoculated *E. coli* populations (6.5 log CFU/g) by 0.3 to 0.6 log CFU/g. PAA-containing treatments (PAA and aPAA, with and without the addition of AP) lowered ($P < 0.05$) initial counts by 0.5 to 0.6 log CFU/g. No differences in efficacy were obtained between the

PAA and aPAA treatments (Table 2.1). Application of SSS and SSS with AP only reduced inoculated *E. coli* counts from 6.5 log CFU/cm^2 to 6.2 and 6.1 log CFU/cm^2, respectively.

Previous research has evaluated the efficacy of SSS and PAA as an intervention on beef surface tissue, but to the best of our knowledge, there are no publications discussing the efficacy of aPAA. Geornaras et al. (*47*) inoculated beef trimmings by distributing a 0.1 ml aliquot of *E. coli* O157:H7 on each side of the tissue. Samples were then immersed in SSS (pH 1.2) for 30 s before microbiological analysis. Results showed a 0.3 log CFU/cm^2 reduction (3.0 log CFU/cm^2) of inoculated *E. coli* O157:H7 populations on selective media (*47*). The study by Geornaras et al. (*47*) and the present study evaluated differing application parameters, but still observed similar reductions for inoculated *E. coli* populations. However, Yang et al. (*152*) inoculated 10 × 10 cm prerigor beef tissue pieces with *Salmonella* (6-strain mixture). Samples were treated in a custom-built spray cabinet (15 lb/in^2, 33 mL/s flow rate), hanging on a hook, for 5 s, with either heated (52°C) or unheated (21°C) SSS pH 1.1. Immediately following treatment, Yang et al. (*152*) observed a 2.0 and a 2.3 log CFU/cm^2 reduction (6.2 to 6.3 log CFU/cm^2) of the inoculated *Salmonella* populations.

King et al. (*74*) utilized a high pressure automated spray system (0.85 MPa, 15 s) to assess the efficacy of a 200 ppm solution of PAA when applied to chilled beef carcass surface tissue inoculated with rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium. They reported a 1.8 log CFU/cm^2 reduction when compared to the inital inoculation level for both pathogens evaluated in their study, and further observed that a 48 h chill after antimicrobial application did not affect the *E. coli* O157:H7 populations, while the *S.* Typhimurium populations further decreased by another 1.2 log CFU/cm^2 after chilling of the carcasses (*74*).

Mohan and Pohlman (*91*) used various organic acids, including PAA, with and without the addition of a 0.5% solution of a nonionic surfactant (ethoxylated glycerol; EG), to evaluate the decontamination efficacy of these acids, in combination with EG, on frozen beef trimmings inoculated with *E. coli* O157:H7. The investigators (*91*) rinsed the inoculated trimmings for 15 s in 100 ml of solution; each organic acid was applied at 30g/L, with the exception of PAA (applied at 0.2 g/L), and EG was added to each at 5 g/L. They reported that the PAA treatment decreased *E. coli* O157:H7 populations by 0.33 log CFU/g, but that the reduction of the *E. coli* populations after treatment with PAA and EG was 0.89 log CFU/g (*91*). This suggested that addition of a surfactant to the antimicrobial treatments may have increased the efficacy of the treatment and had a greater decontamination effect.

Study 2: Chicken wings. The natually present *Salmonella* populations recovered from the uninoculated poultry wing samples was 2.5 log CFU/ml of rinsate solution, which is below the inoculation level utilized for this study (6.0 log CFU/ml of rinsate solution). Initial analysis of the data showed similar results to those reported for study 1. The main effect of antimicrobial treatment was significant ($P < 0.001$), surfactant addition did not effect ($P = 0.0589$) efficacy of the antimicrobials, and the interaction between antimicrobial treatment and surfactant was not significant ($P = 0.3097$). Table 2.2 presents the least squares means by antimicrobial treatment. All antimicrobial treatments effectively reduced ($P < 0.05$) inoculated *Salmonella* populations by 1.3 to 1.8 log CFU/ml of rinsate solution compared the untreated control (6.0 log CFU/ml). The PAA- and aPAA-containing treatments lowered the *Salmonella* counts from 6.0 log CFU/ml of rinsate solution to 4.3 and 4.2 log CFU/ml, respectively (Table 2.2). No differences ($P \geq 0.05$) in efficacy were found between the PAA and aPAA treatments (Table 2.2). Scott et al. (*122*) observed a 0.8 to 0.9 log CFU/ml and 1.1 to 1.2 log CFU/ml reduction of inoculated *Salmonella*

populations when whole, skin-on chicken wings were immersed in SSS (pH 1.8) for either 10 s or 20 s, respectively. The differences between the study conducted by Scott et al (*122*) and the present study show that application time and pH of SSS can have significant effects on the efficacy of the treatment. To the best of our knowledge, the application of SSS to poultry products has not been extensively studied, but studies have shown that SSS can be an effective antimicrobial intervention on beef (*47*, *119*, *152*).

Nagel et al. (*93*) immersed whole chicken carcasses into a post-chill immersion tank (20 s) containing two different concentrations of PAA (400 ppm or 1000 ppm). Skin-on chicken breasts were inoculated with *S.* Typhimurium and *Campylobacter jejuni*. The researchers reported a 2.02 and 2.14 log CFU/ml reduction of *Salmonella* (5.1 log CFU/ml) after immersing the carcasses in the 400 ppm and 1000 ppm solutions of PAA, respectively (*93*). In the present study, immersion of chicken wings in 400 ppm PAA resulted in a 1.3 to 1.8 log CFU/ml reduction of inoculated *Salmonella* populations (6.0 log CFU/ml).

Zaki et al. (*154*) evaluated effects of adding sodium dodecyl sulfate (SDS), a transdermal surfactant, to three different organic acids: lactic acid, levulinic acid, and acetic acid. Sterilized chicken breast skin pieces were inoculated (8.72 log CFU/cm^2) with *Salmonella enterica* Kentucky by immersing the pieces in the inoculum mixture for 20 min. The samples were then immersed in one of 20 antimicrobial solutions, with and without surfactant addition, for 1 min or 3 min with gentle agitation. The investigators (*154*) found that by adding SDS to the antimicrobial, there were significant ($P < 0.05$) reductions in *Salmonella* survival.

pH: Beef trimmings and chicken wings. The pH of the trim samples (Table 2.1) treated with SSS-containing treatments (pH 5.06 to 5.25) were lower ($P < 0.05$) than those of the untreated controls (pH 5.74), suggesting that SSS reduced the surface pH of beef trim. Trim

treated with PAA or PAA with surfactant had pH values that were not different ($P \geq 0.05$) from the control (Table 2.1). Georgnaras et al. (*47*) reported similar pH results when they immersed beef trim in PAA and SSS; PAA did not change ($P \geq 0.05$) the pH of the beef trim, but SSS did lower ($P < 0.05$) the pH of the trim samples by 0.79 units after immersion (control was 5.47 to 6.04 units).

The initial pH of the chicken wings was 6.96 pH units and all immersion treatments lowered ($P < 0.05$) the pH values of treated chicken wings (Table 2.2). The SSS and aPAA, both with and without AP, had the most significant impact on pH, decreasing the pH of treated samples by 2.78 to 2.85 and 2.56 to 2.65 pH units, respectively. In a similar study (*122*), investigators reported that treatment with SSS (pH 1.8) lowered ($P < 0.05$) the pH of the chicken wings from 6.30 units to 4.24 and 4.31 pH units. Additionally, treating the wings with PAA lowered ($P < 0.05$) the pH of the samples by 0.63 units (*122*). The present study showed similar effects on pH.

Part B – DB Surfactant

Enterobacteriaceae populations were not recovered (0.24 log CFU/cm^2 detection limit) from the prerigor beef tissue samples before inoculation, but after 24 h at 4°C *Enterobacteriaceae* populations were recovered from three of the five samples (1.6 log CFU/cm^2). The remaining samples were below detection limit (0.24 log CFU/cm^2. An initial statistical analysis of the *Enterobacteriaceae* counts recovered from untreated and treated prerigor beef surface tissue samples showed that the three way interaction between antimicrobial treatment, surfactant addition, and sampling time was not significant ($P = 0.0971$). Also not statistically significant ($P \geq 0.05$) were the antimicrobial treatment × sampling time ($P = 0.1848$) and surfactant addition × sampling time ($P = 0.3493$) interactions. The only significant ($P <$

0.05) interaction was that of antimicrobial treatment type × surfactant addition ($P = 0.0026$), as well as the main effects of antimicrobial treatment ($P < 0.0001$), surfactant addition ($P = 0.0003$), and sampling time ($P = 0.0009$). Therefore, least squares means are presented for the antimicrobial treatment × surfactant interaction pooled across sampling time (Table 2.3) and for the main effect of sampling time (Table 2.4).

When compared to untreated controls, all spray treatments reduced ($P < 0.05$) inoculated *E. coli* populations (Table 2.3). The PAA-containing treatments were the most effective and reduced ($P < 0.05$) initial populations (6.1 log CFU/cm^2) by 1.4 to 1.7 log CFU/cm^2. No differences ($P \geq 0.05$) were observed between the PAA and aPAA treatments. Inoculated *E. coli* counts of the two SSS-containing treatments were 0.4 log CFU/cm^2 lower ($P < 0.05$) than that of the controls (Table 2.3). The reductions for PAA-containing treatments in the present study are similar to that reported by Kalchayanand et al. (*68*), where prerigor beef flank muscles inoculated with *E. coli* O157:H7 and non-O157 STECs (4 log CFU/cm^2) were spray treated (20 psi) with PAA (200 ppm) using a model spray cabinet. They (*68*) reported a reduction of 1.0 to 1.5 log CFU/cm^2 for the *E. coli* strains used in their study after treatment with PAA (200 ppm).

Yang et al. (*152*) spray treated (15 psi) prerigor beef carcass surface tissue suspended on a hook for 5 s to evaluate the efficacy of SSS (pH 1.1). The chemical compound was tested heated (52°C) and unheated (21°C) for effectiveness against inoculated *Salmonella* populations. The researchers (*152*) reported that unheated SSS and heated SSS reduced inoculated *Salmonella* populations by 2.0 and 2.3 log CFU/cm^2, respectively. As previously explained, there was no known published work describing the antimicrobial efficacy of aPAA.

Statistical differences ($P < 0.05$) in bacterial counts were noted between the PAA and PAA + surfactant, and aPAA and aPAA + surfactant treatments. Specifically,

Enterobacteriaceae counts of surfactant-containing PAA and aPAA treatments were 0.3 log units higher ($P < 0.05$) than corresponding treatments that did not include the surfactant (table 2.3). It should be noted, however, that although statistical differences were detected, a 0.3-log unit difference is not considered a biologically meaningful difference. Similarly, the 0.1-log unit statistical difference ($P < 0.05$) obtained between the pooled counts of samples analyzed immediately post-treatment (0 h) and those analyzed after a 24 h storage period at 4°C (Table 2.4), were not considered to be a biologically meaningful difference. To the best of our knowledge, there is minimal research evaluating antimicrobial effects of surfactant addition to meat surfaces. As previously described, Mohan and Pohlman (*90*) concluded that the addition of a nonionic surfactant (tween 80) to various chemical solutions could increase their antimicrobial efficacy against *E. coli* O157:H7 populations on chilled beef trimming. Again, results suggested that the addition of a nonionic surfactant to the antimicrobial solution may influence the efficacy of the chemical, which was different result from that reported here.

In conclusion, results of the three studies conducted indicated that spray treatment of beef trimmings or prerigor beef carcass surface tissue with 400 ppm PAA, SSS pH 1.1 or pH 1.2 (respectively), or aPAA (400 ppm, pH 1.1 or 1.2) can effectively reduce pathogen contamination. Additionally, immersing chicken wings in 500 ppm PAA, SSS pH 1.2, or aPAA (500 ppm, pH 1.2) is effective against inoculated *Salmonella* populations. However, all three studies suggest that the addition of a nonionic alkyl polyglycoside did not increase the efficacy of the tested antimicrobials against *E. coli* or *Salmonella* at the application parameters used.

Table 2.1. Adjusted least squares mean *Enterobacteriaceae* counts (log CFU/g ± standard deviation) and pH values for beef trimmings inoculated with a 5-strain mixture of *Escherichia coli* biotype I that were left untreated (control) or were spray-treated with a sulfuric acid-sodium sulfate blend (SSS), peroxyacetic acid (PAA), or PAA acidified with SSS (aPAA), with and without the addition of an alkyl polyglycoside (AP) surfactant.

Treatment	Adjusted Least Squares Mean	
	Microbial Counts	pH
Control	6.5 ± 0.2^{a}	5.74^{a}
SSS (pH 1.1)	6.2 ± 0.1^{b}	5.25^{b}
SSS + AP (0.4%)	6.1 ± 0.1^{bc}	5.06^{b}
PAA (400 ppm)	5.9 ± 0.2^{c}	5.68^{a}
PAA + AP	5.9 ± 0.2^{c}	5.73^{a}
aPAA (pH 1.1, 400 ppm)	5.9 ± 0.1^{c}	5.16^{b}
aPAA + AP	6.0 ± 0.1^{bc}	5.20^{b}

[a-c] Least squares means in the same column without a common superscript letter differ ($P < 0.05$).

Table 2.2. Adjusted least squares mean S*almonella* counts (log CFU/ml of rinsate solution ± standard deviation) and pH for poultry wings inoculated with a 5-strain mixture of *Salmonella* that were left untreated (control) or were treated by immersing individual wings for 15 s in a sulfuric acid-sodium sulfate blend (SSS), peroxyacetic acid (PAA), or PAA acidified with SSS (aPAA), with and without the addition of an alkyl polyglycoside (AP) surfactant.

Treatment	Adjusted Least Squares Mean	
	Microbial Counts	pH
Control	6.0 ± 0.1[a]	6.96[a]
SSS (pH 1.2)	4.6 ± 0.1[b]	4.18[c]
SSS + AP (0.4%)	4.7 ± 0.2[b]	4.11[c]
PAA (500 ppm)	4.3 ± 0.2[c]	6.49[b]
PAA + AP	4.3 ± 0.2[c]	6.50[b]
aPAA (pH 1.2, 500 ppm)	4.2 ± 0.4[c]	4.31[c]
aPAA + AP	4.2 ± 0.3[c]	4.40[c]

[a-c] Least squares means in the same column without a common superscript letter differ ($P < 0.05$).

Table 2.3: Adjusted least squares mean *Enterobacteriaceae* counts (log CFU/cm^2 ± standard deviation) for prerigor beef carcass surface tissue inoculated with a 5-strain mixture of *Escherichia coli* biotype I that was left untreated (control) or was spray-treated with water, peroxyacetic acid (PAA), a sulfuric acid and sodium sulfate blend (SSS), or PAA acidified with SSS (aPAA), alone or in combination with the surfactant, Disponil DB (DB). Least squares means are presented as treatment, with and without the surfactant, pooled across sampling time (0 h and 24 h).

Treatment	**Adjusted Least Squares Mean *Enterobacteriaceae* Counts (log CFU/cm^2 ± SD)**
Control (untreated)	6.1 ± 0.1[a]
Water	6.0 ± 0.1[b]
Disponil DB (DB; 0.5%)	6.0 ± 0.1[b]
PAA (400 ppm)	4.4 ± 0.2[e]
PAA (400 ppm) + DB (0.5%)	4.7 ± 0.2[d]
SSS (pH 1.2)	5.7 ± 0.1[c]
SSS (pH 1.2) + DB (0.5%)	5.7 ± 0.1[c]
aPAA (400 ppm; pH 1.2)	4.4 ± 0.2[e]
aPAA (400 ppm; pH 1.2) + DB (0.5%)	4.7 ± 0.3[d]

[a-e] Least squares means without a common superscript letter differ ($P < 0.05$)

SD: standard deviation

Table 2.4. Adjusted least squares mean *Enterobacteriaceae* counts (log CFU/cm^2 ± standard deviation) for the main effect of sampling time pooled across treatment for prerigor beef carcass surface tissue inoculated with a 5-strain mixture of *Escherichia coli* biotype I that was left untreated (control) or spray-treated with water, peroxyacetic acid (PAA), a sulfuric acid and sodium sulfate blend (SSS), or PAA acidified with SSS (aPAA), alone or in combination with the surfactant, Disponil DB. Least squares means are presented as sampling time pooled across antimicrobial treatment, with and without surfactant addition.

Sampling Time (h)	**Adjusted Least Squares Mean *Enterobacteriaceae* Counts (log CFU/cm^2 ± SD)**
0	5.4 ± 0.1[a]
24	5.3 ± 0.1[b]

[a-b] Least squares means without a common superscript letter differ ($P < 0.05$)

SD: standard deviation.

CHAPTER 3

Antimicrobial Efficacy of Peroxyacetic Acid Acidified With Different Chemicals Against *Escherichia coli* Biotype I When Applied to Prerigor Beef Carcass Surface Tissue

Summary

Two studies were conducted to evaluate antimicrobial effects of blends of peroxyacetic acid (PAA) acidified with various acids against inoculated populations of nonpathogenic *Escherichia coli* biotype I surrogates for pathogenic *E. coli* and *Salmonella,* on warm, prerigor beef carcass surface brisket tissue. In study 1, 10 × 10 cm pieces (n = 10) of warm, prerigor beef carcass surface brisket tissue were inoculated (6 to 7 log CFU/cm^2) with a five-strain mixture of the nonpathogenic *E. coli* biotype I surrogates. Samples were either left untreated (control) or were immersed for 10 s in PAA (400 ppm) acidified with lactic acid (3.5%), PAA (400 ppm) acidified with acetic acid (2%), PAA (400 ppm) acidified with citric acid (1%), PAA (400 ppm) acidified with a sulfuric acid and sodium sulfate blend (pH 1.2 and pH 1.8; SSS), or PAA (300 ppm) acidified with SSS (pH 1.2). All samples were analyzed 5 min post-treatment for surviving *Enterobacteriaceae* populations. In study 2, 10 × 10 cm pieces (n = 10) of prerigor beef tissue inoculated (6-7 log CFU/cm^2) with the same five-strain mixture of nonpathogenic *E. coli* surrogates were either left untreated or were spray-treated (10 s), in a spray cabinet, with water, PAA (350 ppm), PAA (400 ppm), PAA (400 ppm) acidified with acetic acid (2%), PAA (400 ppm) acidified with SSS (pH 1.2), or PAA (350 ppm) acidified with SSS (pH 1.2). As in study 1, untreated and treated beef tissue samples were analyzed 5 min post-treatment for inoculated *E. coli* counts. All immersion treatments evaluated in study I effectively ($P < 0.05$) reduced inoculated *E. coli* populations on the prerigor beef carcass surface tissue by at least 2.3 log

CFU/cm^2. The 400 ppm PAA treatments acidified with lactic acid, SSS pH 1.2, or acetic acid were the most ($P < 0.05$) effective treatments, lowering inoculated bacterial counts from 6.2 log CFU/cm^2 to 3.4, 3.4, and 3.7 log CFU/cm^2, respectively. In study 2, all of the tested antimicrobial spray treatments effectively ($P < 0.05$) lowered initial inoculated *E. coli* counts (6.4 log CFU/cm^2) by 1.7 to 1.9 log CFU/cm^2. No ($P \geq 0.05$) differences in efficacy were observed between the five antimicrobial treatments.

Introduction

In the United States, an estimated 48 million people experience foodborne illness each year (*97*). *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* (STEC) are responsible for approximately 100,000 illnesses each year and nearly 90 deaths; The top 6 STECs are now labeled adulterants in non-intact beef products by USDA-FSIS, along with *E. coli* O157:H7 (*149*). Additionally, *Salmonella* is estimated to be the leading cause of foodborne illness from a bacterial agent, being responsible for about 35 percent of hospitalizations and 28 percent of all deaths related to foodborne illness (*26*, *97*). Therefore, *E. coli* and *Salmonella* are considered pathogens of concern in fresh beef products (*121*).

Cattle are known reservoirs for STEC and *Salmonella*, and it has been reported that fecal contamination of the hide is likely the primary cause of STEC contamination of the beef carcass (*8*, *152*). Due to this, the industry has expended considerable effort since the early 1990's to control these pathogens in beef products (*18*, *47*, *104*, *119*, *121*, *149*, *152*). These efforts have resulted in a multiple hurdle technology that is commonly utilized throughout the industry to control pathogen contamination (*104*). These sequential intervention systems include both physical and chemical decontamination methods, including steam pasteurization, hot water washes, and antimicrobial solution treatments (*149*). The efficacy of various chemical

interventions, such as lactic acid, citric acid, and peroxyacetic acid (PAA), for reducing STECs and *Salmonella* have been extensively studied (*6*, *13*, *48*, *77*, *90*, *108*). Still, the beef industry continues to seek out new chemical interventions for use in a multiple hurdle system to reduce pathogen contamination of beef carcasses.

Chemical interventions should easily be implemented into existing systems and be inexpensive, while meeting regulatory standards (*121*). Use of PAA has been shown to effectively reduce *STEC* and *Salmonella* contamination in a beef carcass wash (*47*, *74*, *76*, *90*), and utilizes an oxidative mechanism for killing bacteria (*76*). Adding additional chemicals that utilize different mode of action may increase the antimicrobial efficacy of the PAA solution. To the best of our knowledge, no studies have evaluated efficacy of an acidified PAA product against pathogens on beef tissue. Therefore, the objective of these two studies was to evaluate the antimicrobial effects of PAA acidified with different acids against inoculated nonpathogenic *E. coli* biotype I surrogates on warm, prerigor beef carcass surface brisket tissue.

Materials and Methods

Bacterial strains and preparation of inoculum. In both studies, a five-strain mixture of nonpathogenic *E. coli* biotype I (ATCC-BAA 1427, ATCC-BAA 1428, ATCC-BAA 1429, ATCC-BAA 1430, and ATCC-BAA 1431), considered surrogates for *E. coli* O157:H7, non-O157 STEC, and pathogenic *Salmonella* (*20*), were used to inoculate of the warm, prerigor beef carcass surface tissue pieces. Strains were individually cultured and subcultured (35°C, 22 h) in 10 ml of tryptic soy broth (Difco, Becton Dickson and Co. [BD], Sparks, MD). Following subculturing, all five strains were combined and harvested via centrifugation (6,000×*g*, 15 min, 4˚C; Sorvall Legend X1R, Thermo Scientific, Waltham, MA). Resulting cell pellets were then washed in 10 ml of phosphate buffered saline (pH 7.4, PBS; Sigma-Aldrich, St. Louis, MO), re-

centrifuged as previously described, and resuspended in 50 ml of PBS. The concentration of the inoculum mixture was approximately 8 to 9 log CFU/ml. In both studies, a 0.2 ml aliquot of inoculum was used to inoculate each of the 10 x 10 cm pieces of prerigor beef tissue.

Sample procurement and inoculation of prerigor beef carcass surface tissue. For each of the two studies, 40 sections of warm, prerigor beef carcass surface tissue were collected from the brisket area of carcasses on two separate days each. These sections were collected on the harvest floor, after electrical stimulation but before the final acid washing system, from a commercial beef processing facility located in northern Colorado. Tissue samples were then placed in insulated containers and immediately transported to the Center for Meat Safety & Quality at Colorado State University (CSU; Fort Collins, CO).

Each section of beef surface tissue was divided into one or two 10 x 10 cm portions and each piece was randomly assigned to either an untreated control group or one of six acid treatment groups for study 1. In study 2 the 10 × 10 cm pieces were randomly assigned to either an untreated control, a water treatment, or one of five acid treatment groups. For each treatment, five 10 × 10 cm portions were placed onto sanitized, foil lined trays and were inoculated under a biosafety cabinet. Samples were spot inoculated with 0.2 ml of the *E. coli* inoculum on the external adipose side of the tissue and spread over the surface using a disposable spreader. The target inoculation level was approximately 6 log CFU/cm^2. Inoculated samples were allowed 15 min at room temperature (4-7°C in study 1 or 20 to 25°C in study 2) for cell attachment, before application of antimicrobial treatments or sampling of the untreated control for determination of initial counts.

Application of antimicrobial treatments. In the first study, beef tissue pieces were randomly assigned to one of seven treatment groups: untreated control, PAA (400 ppm; Kroff

Food Services, Inc., Pittsburgh, PA), PAA (400 ppm) acidified with acetic acid (2%; Fisher Scientific, Fair Lawn, NJ), PAA (400 ppm) acidified with citric acid (1%; Fisher Scientific), PAA (400 ppm) acidified with SSS pH 1.2 (Zoetis, Parsippany, NJ), PAA (400 ppm) acidified with SSS pH 1.8, and PAA (300 ppm) acidified with SSS pH 1.2. The antimicrobial treatments were applied by placing individual 10 × 10 cm pieces of beef tissue into sterile Whirl-Pak bags (55-oz; Nasco, Modesto, CA) containing 350 ml of the test solution. A different Whirl-Pak bag with fresh, unused solution was used for treatment of each piece. Pieces of tissue were aseptically removed from the bag after a 10 s treatment time with gentle agitation, and allowed to drip for 5 min on a sterile wire rack before sampling for surviving inoculated populations. On each of the two trial days of this study, five samples per treatment were analyzed.

Antimicrobial treatments in the second study were determined from the results of study 1. Therefore, the 10 × 10 cm beef tissue pieces were randomly assigned to one of seven treatment groups: untreated control, tap water, PAA (350 ppm), PAA (400 ppm), PAA (400 ppm) acidified with acetic acid (2%), PAA (400 ppm) acidified with SSS pH 1.2, and PAA (350 ppm) acidified with SSS pH 1.2. Water and antimicrobial treatments were applied using a custom-built spray cabinet (Chad Co., Olathe, KS) that had 18 floodjet spray nozzles (0.1 gallons per minute [gpm]; Grainger Industrial Supplies); 10 nozzles above the product belt and eight nozzles below. Solutions were applied at a pressure of 15 psi with a product contact time of 10 s. After treatment, sample pieces were placed onto sanitized racks, with the adipose tissue side down, and allowed to drip for 5 min before being transferred (within 2 to 3 min) to the Food Safety & Microbiology laboratory for microbial analysis. The experiment was repeated on two separate days, with five samples analyzed per treatment on each day.

Microbiological analysis. In both studies, each untreated or treated 10 × 10 cm sample was placed into a filtered Whirl-Pak bag (55-oz; Nasco, Modesto, CA) containing 175 ml of Dey/Engley neutralizing broth (Difco, BD). Samples were mechanically pummeled for 2 min (Masticator, IUL Instruments, Barcelona, Spain) and then serially diluted (10-fold dilution) in 0.1% buffered peptone water (Difco, BD). Appropriate dilutions were plated, in duplicate, onto Petrifilm *Enterobacteriaceae* Count plates (3M, St. Paul, MN). Colonies were counted after incubation of plates at 35˚C for 24 ± 2 h. Uninoculated prerigor beef tissue samples were also analyzed for naturally occuring *Enterobacteriaceae* populations; the detection limit of the microbial analysis was 0.2 log CFU/cm^2.

Statistical analysis. Both studies were designed as a randomized complete block design with trial (experiment) day serving as the block effect. Both were replicated on two separate days, with n = 10 per treatment. *Enterobacteriaceae* counts were transformed to base-10 logarithms and expressed as least squares means for log CFU per cm^2 of prerigor beef surface tissue under the assumption of a lognormal distribution for plate counts. Data were analyzed using the lsmeans package in R (Rstudio, 2015, Boston, MA) with antimicrobial treatment serving as the independent variable. Least-squares means were separated using a significance level of $\alpha = 0.05$.

Results and Discussion

Study 1: Immersion treatment of beef tissue samples. *Enterobacteriaceae* counts recovered from untreated and treated prerigor beef surface tissue samples are shown in Table 3.1. Least squares means are presented for the main effect of antimicrobial treatment. When compared to the untreated control, all treatments were effective ($P < 0.05$) in reducing inoculated *E. coli* populations (Table 3.1). Specifically, all treatments lowered ($P < 0.05$) initial counts by at

least 2.3 log CFU/cm^2. The 400 ppm PAA treatments acidified with lactic acid, pH 1.2 SSS, or acetic acid were the most effective ($P < 0.05$) treatments, reducing the initial bacterial counts from 6.2 log CFU/cm^2 to 3.4, 3.4, and 3.7 log CFU/cm^2, respectively. The levels of *Enterobacteriaceae* populations recovered from the surface of the uninoculated samples was approximately 1.7 log CFU/cm^2.

Minimal research has evaluated the antimicrobial efficacy of PAA in combination with another chemical, though Kassem et al. (*70*) evaluated efficacy of a combination of lactic acid with acetic acid and lactic acid with citric acid compared to that of single chemical treatments. They did not observe a significant difference between the combination treatments and those of the single chemical treatments of lactic acid, acetic acid, and citric acid. Kassem et al. (*70*) immersed beef tissue samples for 60 s in either a 3.0% or 5.0% solution of lactic acid, acetic acid, or citric acid against inoculated STECs or *Salmonella* Typhimurium. They (*70*) reported that all treatments reduced *E. coli* populations by more than 1.1 log CFU/cm^2 and the 5% acetic acid treatment was the most effective against inoculated *E. coli* populations, reducing initial counts from 6.2 log CFU/g to 4.7 log CFU/g. Additionally, the 5.0% lactic acid treatment was the most effective against inoculated *Salmonella* Typhimurium populations with a 1.5 log CFU/g reduction from the intial 6.3 log CFU/g populations, though all treatments effectively reduced inoculated populations.

In another study, researchers (*119*) evaluated the efficacy of lactic acid (2.5% and 5%), SSS (1%), and PAA (220 ppm) as immersion treatments against inoculated STECs and *Salmonella* on beef cheek meat. Cheek meat samples were inoculated with either *E. coli* O157:H7 (3.9 log CFU/cm^2), non-O157 Shiga toxin-producing *E. coli* (4.0 log CFU/cm^2), or *Salmonella* (4.1 log CFU/cm^2) and were immersed for either 1 min, 2.5 min, or 5 min. Schmidt

et al. (*119*) reported that 5% lactic acid was the most effective treatment against all pathogens, with reductions of 1.7 to 2.1 log CFU/cm^2. Additionally, they (*119*) observed reductions of 1.0 to 1.3 log CFU/cm^2 and 1.1 to 1.5 log CFU/cm^2 for the PAA and SSS immersion treatments, respectively. Geornaras et al. (*46*) utilized a similar treatment method to that of the present study to evaluate the efficacy of individual treatments of PAA (200 ppm) and SSS (pH 1.2) against inoculated rifampin-resistant *E. coli* O157:H7 and non-O157 STECs on beef trimmings. They (*46*) reported that treatment with 200 ppm PAA reduced inoculated bacterial populations (3.4 to 3.9 log CFU/cm^2) by 0.6 to 1.0 log CFU/cm^2 and treatment with SSS (pH 1.2) reduced pathogen counts by 0.3 to 0.4 log CFU/cm^2.

Study 2: Spray treatment of beef tissue samples. *Enterobacteriaceae* counts recovered from untreated and treated prerigor beef surface tissue samples are shown in Table 3.2. Least-squares means are presented for the main effect of antimicrobial treatment. The water treatment was not effective ($P \geq 0.05$) in reducing inoculated bacterial populations. However, all of the tested acid treatments effectively ($P < 0.05$) lowered the inoculated *E. coli* populations, when compared to untreated control and water treatments. Specifically, counts of all acid-treated samples were 1.7 to 1.9 log CFU/cm^2 lower ($P < 0.05$) than those of the untreated control tissue samples (6.4 log CFU/cm^2). No differences ($P \geq 0.05$) in efficacy were obtained between the five acid treatments, nor between the acidified and non-acidified PAA treatments; therefore, it cannot be concluded that one acid treatment was more effective than another. *Enterobacteriaceae* populations were not recovered (0.2 log CFU/cm^2 detection limit) on EB petrifilms for any of the uninoculated beef samples analyzed; therefore the *Enterobacteriaceae* counts shown in table 3.2 are those of the inoculated populations. Podolak et al. (*103*) evaluated the antimicrobial effects of combining organic acids against inoculated *E. coli* O157:H7 populations on lean beef tissue

when used as a dip treatment for 5 min; specifically, the researchers combined fumaric acid with lactic acid and acetic acid. The study (*103*) found that the combinations of acids had similar efficacies against *E. coli* populations to those in which only using a single chemical treatment of fumaric acid, acetic acid, or lactic acid were utilized. Additionally, they (*103*) reported that a 1.0% acetic acid treatment reduced bacterial populations from 5.12 log CFU/cm^2 to 4.36 log CFU/cm^2, which was statistically similar to the combination of 1.0% acetic acid and 1.0% fumaric acid.

King et al. (*74*) applied a 200 ppm PAA solution as a pre-chill carcass wash intervention (15 s) to prerigor lean tissue obtained from the outside round, plate, clod, and brisket regions of beef carcasses inoculated with STEC and *Salmonella* contaminated fecal material. The authors (*74*) reported that the mean reduction of the bacterial populations was 0.7 log CFU/cm^2, from the 4.1 log CFU/cm^2 that remained after the application of a water wash. Additionally, Yang et al. (*152*) evaluated the efficacy of SSS pH 1.1 at two different temperatures (21°C and 52°C) against inoculated *Salmonella* populations on prerigor beef carcass surface tissue. They (*152*) reported reductions of 2.2 to 2.3 log CFU/cm^2 after spray treating hanging samples for 5 s at 15 psi, but found no significant differences in the solution temperature.

Overall, all of the PAA combinations and single treatments reduced inoculated nonpathogenic *E. coli* biotype I populations on prerigor beef carcass surface tissue at the operation parameters specified in both studies. However, acidifying PAA with either acetic acid or SSS at the described solution concentrations were not more effective than using PAA alone at 400 or 350 ppm, therefore it may not be more beneficial to acidify PAA with another chemical. Additionally, applying the antimicrobials as an immersion treatment for 10 s can be a more effective treatment than spray treating the prerigor beef tissue at the tested parameters.

Table 3.1: Adjusted least squares mean *Enterobacteriaceae* counts (log CFU/cm^2 ± standard deviation) for prerigor beef surface tissue inoculated with *Escherichia coli* biotype I, before (control) and after treatment with peroxyacetic acid (PAA) acidified with various acidulants.

Treatment	Bacterial Counts ± SD
Control (untreated)	$6.2^{a} \pm 0.0$
PAA (400 ppm) acidified with 3.5% lactic acid	$3.4^{c} \pm 0.1$
PAA (400 ppm) acidified with 2% acetic acid	$3.7^{bc} \pm 0.1$
PAA (400 ppm) acidified with 1% citric acid	$3.9^{b} \pm 0.1$
PAA (400 ppm) acidified with SSS (pH 1.2)	$3.4^{c} \pm 0.1$
PAA (400 ppm) acidified with SSS (pH 1.8)	$3.9^{b} \pm 0.1$
PAA (300 ppm) acidified with SSS (pH 1.2)	$3.9^{b} \pm 0.1$

[a-c] Least squares means without a common superscript letter differ ($P < 0.05$).

Table 3.2: Adjusted least squares mean *Enterobacteriaceae* counts (log CFU/cm^2 ± standard deviation) for prerigor beef surface tissue inoculated with *Escherichia coli* biotype I, before (control) and after treatment with water, peroxyacetic acid (PAA), or PAA acidified with acetic acid or a sulfuric acid and sodium sulfate blend (SSS) .

Treatment	Bacterial Counts ± SD
Control (untreated)	6.4 ± 0.3[a]
Water	6.0 ± 0.1[a]
PAA (350 ppm)	4.7 ± 0.2[b]
PAA (400 ppm)	4.5 ± 0.2[b]
PAA (400 ppm) acidified with 2% acetic acid	4.7 ± 0.2[b]
PAA (400 ppm) acidified with SSS (pH 1.2)	4.5 ± 0.3[b]
PAA (350 ppm) acidified with SSS (pH 1.2)	4.6 ± 0.3[b]

[a-b] Least squares means without a common superscript letter differ ($P < 0.05$).

CHAPTER 4

Review of Literature – Part 2

3. 1 Pork Quality Factors

Traditionally, the food animal industry has associated meat quality with terms such as freshness, grade, color, eating satisfaction, or processing attributes (*21*). Consumer satisfaction in relation to price ultimately is the most important factor in pork production, and with a growing international market for pork the pressure of producing high quality products to compete with other export markets is only increasing (*146*). Pork quality is typically determined by water-holding capacity, color, fat content, oxidative stability, and uniformity (*113*). These quality attributes are influenced by pre- and post-harvest factors such as genetics, nutrition, and handling and shipment, as well as stunning procedures, dehairing methods, chilling, and postmortem handling (*21*). Carcass defects can be caused by each of these factors and may affect the value of the carcass, resulting in lost revenue for the industry (*21*).

One major quality defect that has been studied extensively in the United States is the incidence of pale, soft, and exudative (PSE) meat (*71*, *146*). PSE is characterized by a pale color, soft texture, and being watery in appearance, which is not desirable by consumers (*81*). Incidence of PSE in pork is associated with muscle size, pre-harvest stress from handling, protein denaturation, and accelerated postmortem metabolism (*124*), and, it can be heavily influenced by genetics, ante mortem stress, and nutrition. Short-term pre-harvest stress is a major contributor to the development of PSE carcasses; this stress causes increased glycolysis, producing more lactic acid early post mortem, resulting in a low pH and high temperature situation, and increasing the rate of protein denaturation (*146*). This process causes increased light scattering and a low water

holding capacity, giving these pork products the light color and watery appearance previously described (*71*, *81*, *146*).

Pre-harvest factors

In recent years, the pork industry has made a push for lean, fast growing pigs that have a high feed conversion efficiency. Unfortunately, selection for these phenotypic traits initially increased the presence of the halothane gene and porcine stress syndrome (PSS) in the US swine herd (*117*). Heterozygotes for the halothane gene bring forth these desired traits, but unfortunately are also four times more likely to result in PSE carcasses (*30*). The PSS and halothane genes are inherited, recessive traits that cause a mutation of the ryanodine receptor responsible for malignant hyperthermia (*81*). Therefore, pre-harvest stress can have detrimental effects on the quality of the carcass, and in severe cases, may result in death of the animal (*81*).

Nutrition can have a dramatic effect on the carcass composition of pork. In recent years, the industry has started feeding pigs beta-adrenergic agonists, growth promoting compounds that are effective in increasing the lean muscle while decreasing fat deposition and improving feed efficiency (*21*, *23*). This class of growth promotants include clenbuterol, cimaterol, ractopamine, salbutamol, and L-644,969, though ractopamine is the most commonly used beta-adrenergic agonist compound fed to finishing hogs in the United States (*21*). It has been reported that feeding beta agonists to finishing pigs before slaughter either has no effect or slightly improves the color, firmness, and marbling score of pork carcasses (*126*). Additionally, some beta agonists may reduce tenderness, due to their effect on muscle fiber type and growth, but ractopamine specifically does not have a negative effect on pork tenderness (*21*).

Pre-harvest handling and transportation has a significant impact on pork quality due to the short term stress levels that can influence the development of quality defects. This handling

includes comingling of unfamiliar pigs, the number of pigs loaded on a truck, distance for transport, and lairage time at the processing facility (*43*, *100*). Studies have found that stress level and glycolytic potential are closely related and can determine the variation in water holding capacity and meat color (*54*). The effects of handling and transport have been extensively studied in regards to pork quality due to the high incidence of PSE pork in recent years (*38*, *43*, *54*, *123*, *146*). Hambrecht et al. (*54*) and Gajana et al. (*43*) have reported that minimal stress, shorter transport, and longer lairage are considered optimal handling protocols to minimized stress and potential for quality defects.

Post-harvest factors

Immobilization and stunning of pigs can have adverse effects on pork quality if not performed correctly and effectively. Immobilization techniques currently utilized to stun livestock are captive bolt, electrical stunning, and carbon dioxide (CO_2); these are highly effective in humanely rendering the animal unconscious and ensuring optimal muscle quality when done correctly (*21*). Captive bolt stunning is rarely used to immobilize pigs and is most commonly utilized to stun cattle (*72*). Electrical stunning induces an epileptic seizure that will render the animal unconscious, though this is dependent on the amount of current passing through the brain and may cause more kicking by the animal making it more difficult to shackle and effectively exsanguinate (*29*). Due to the nature of electrical stunning, there is a greater chance for blood splash (pinpoint hemorrhages that develop when small capillaries burst) and PSE to occur in these carcasses (*19*). However, CO_2 stunning is an alternative immobilization method that has been evaluated that can decrease the incidence of PSE and blood splash in pork carcasses because the pigs remain motionless for up to 60 s, making them easier to stick and bleed more efficiently (*29*, *144*). Additionally, scalding of carcasses can lead to quality defects,

such as PSE, due to the high temperature (60°C) used to remove the hair from the carcass (*143*). This high temperature may lead to protein denaturation early post mortem when the pH of the carcass is beginning to decrease. It has been suggested that decreasing the amount of time the carcass is in the scald tank may minimize the protein denaturation that can occur in this step and allow the carcass to enter the cooler earlier postmortem (*44*).

Since pork carcasses reach full rigor earlier than other species, rapid chilling (or blast chilling) is widely used within the pork industry to rapidly decrease the internal temperature of the carcass and increase the final pH (*21*, *125*). By decreasing the temperature more quickly, postmortem glycolysis is slowed, resulting in less shrink and a lower chance of PSE (*116*). The recommended time from stunning to chilling is less than 45 min in order to maximize the effects of blast chilling (*38*). Studies have shown that there is no effect on tenderness between blast chilled carcasses and conventionally chilled carcasses, but blast chilling can have a positive impact on objective color scores and muscle firmness (*53*, *125*).

3.2 Small Processing Facilities

The United States Department of Agriculture defines large meat processing facilities as having more than 500 employees, small facilities as those having more than 10 employees but less than 500, and very small meat processing facilities as having less than 10 employees or less than $2.5 million in annual sales (*25*). Often, small and very small processors are considered "local" processing facilities and can either process livestock out of established infrastructures or can also include mobile slaughter units (*2*, *52*). The smaller facilities often offer custom processing options and harvest animals for individual customers that do not sell products through retail (*36*).

In the United States, any meat or poultry sold via retail to customers that are not the owner of the harvested animal, requires that the animal be slaughtered and processed in a state- or federally-inspected facility (*2*). Additionally, many of these very small meat processors are classified as custom exempt within their state inspection agency. The Federal Meat Inspection Act (FIMA) exempts from inspection any animal that is harvested solely for the use of the owner or non-paying guests (*66*), thus allowing these facilities to be considered custom exempt. If the owner chooses to sell these animals, they must be sold live and still meet certain labeling requirements (*52*, *66*). Custom exempt facilities are not required to have a state or federal inspector present to inspect each carcass, but are still reviewed at least annually to ensure the processor is in compliance with record keeping and sanitation requirements (*66*, *136*).

Many custom exempt processors utilize a mobile slaughter unit which allows processors to harvest the animal onsite at the farmer's home. These units can also be state or federally-inspected if the processors choose to offer services to their customers that fall under the inspection regulations (*66*, *140*). The United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) (*140*) defines mobile slaughter units as "a self-contained slaughter facility that can travel from site to site." These units allow processors to travel to farms and remote locations, offering harvest services to small producers that may otherwise be unaffordable or even unavailable in those areas (*66*). Additionally, these units can minimize the stress on the animal because they require less handling and no transportation before harvest (*140*). Mobile units (regardless of inspection status) are still required to abide by state and federal regulations for animal handling and welfare, as well as sanitation protocols (*36*). All processing facilities, regardless of size, are required to implement a hazard analysis critical control points

(HACCP) protocol for their harvest and processing procedures in order to minimize the risk of foodborne illness and appropriately follow state and federal regulations (*131*).

These custom harvest facilities will often not reject livestock due to the nature of harvesting for individual customers rather than for retail sales. Therefore, the quality of the pigs that are harvested in small and very small facilities is variable because the pre-harvest factors differ by farm and are uncontrolled by the processor (*21*). The differences in time from stunning to the cooler will also vary by processing facility; many of the small processors do not have a rapid chill cooler and therefore cannot create a rapid decline of temperature or pH in the carcass (*38*). These variations in harvesting pigs could have a drastic effect on quality of the pork produced and, therefore, it is important to understand the harvest process of these facilities and how the quality of the product may be affected.

3.3 Pork in Colorado

Colorado is the 15th largest hog producing state in the country, with marketable hog production numbers of about 3 million head in 2015, including custom harvest animals (*138*). This only gives Colorado approximately 0.8% of the national pork market share (*138*). The greatest percentage of the marketable pigs produced in Colorado are raised in the northeastern corner of the state on large-scale production farms owned by Seaboard Foods and Smithfield Foods, Inc. Approximately 60% of the national swine herd currently resides in the Midwestern region of the United States due to its proximity to corn and soybean (*1*), which is likely the reason that there are only small numbers of hogs produced in Colorado. Additionally, legislative measures have also contributed to the decrease of large-scale hog farming in the state. The Hogs Rule was passed in 1998 in Colorado, requiring large hog operations to reduce the odor from waste lagoons and ensure that the water supplies are not polluted by their facilities (*75*).

Regulations such as this have made it more challenging for large, corporate operations to reside in the state, allowing small, family-owned operations to overtake the swine production scene in Colorado, producing predominately feeder hogs for home use.

There are currently no large pork processing facilities in Colorado. Therefore small and very small processors are harvesting most pigs that are not transported outside of Colorado. There are a few small processors in the state that utilize federal and state inspection agencies in order to sell their products via retail, but the majority of the very small processors are considered custom exempt (*66*). The facilities that are state and federally inspected have permanent infrastructure and follows the building and sanitation protocols that are required by USDA-FSIS, as well as having a USDA inspector present at all times (*2*). As previously described, custom exempt processors are still expected to follow the animal handling, sanitation, and HACCP compliance guidelines, regardless of utilizing permanent infrastructure or a mobile slaughter unit (*36*). Again, custom exempt processers are required to only harvest for the owner of the animal and may not use any non-inspected meat product for retail.

3.4 Rationale for a Survey of the Small Processors in Colorado

Minimal research has been conducted to characterize small and very small processing facilities, especially in the state of Colorado. The most recent list of meat processors provided by the Colorado Department of Agriculture (*35*) was compiled in May of 2015; of these, many processors have either sold their business, changed contact information, or are no longer a company. Since the majority of the pigs that are being raised in Colorado are individual animals that are custom harvested by small processors, it becomes important for the state Department of Agriculture and the various associations, such as the Colorado Pork Producers Council, to be aware of where these processors are located. Many small processors are located in rural

communities and serve those livestock producers that are unable to transport their market animals to inspected facilities (*36*, *120*). Additionally, providing educational materials to small processors can only enhance their business and allow them to share valuable information with their customers.

Over the past 20 years, the beef industry has established a valuable quality and value assessment system; every five years, the National Beef Quality Audit (NBQA) is conducted to audit the quality of slaughter steers and heifers, and less frequently, market cows and bulls, for quality shortfalls and to identify targets for desired quality levels (*58*, *64*). This audit has three phases: phase one includes face-to-face interviews with those who make purchasing decisions in various sectors of the beef industry, phase two includes in-plant audits of the slaughter floor and grading coolers, and phase three is a strategy workshop to develop a strategy to improve the beef industry (*58*). The NBQA has been extremely effective in helping to identify opportunities for improving the beef value, and therefore would likely be effective in other sectors of the meat industry.

The Michigan State University Extension group (*120*) conducted a survey similar to phase one of the NBQA to determine which facilities harvest livestock and if these companies utilize an inspection agency. The survey was mailed to approximately 400 processors in the state and the extension services received 111 of these surveys back, resulting in a 28% response rate. This survey found that approximately 41% of the respondents actually harvested livestock; the remaining portion of respondents only processed meat for their customers. Additionally, the results showed that the majority of respondents were small and very small processors that were either a custom exempt slaughter facility or retail exempt retail establishment (*120*). The survey

data in Michigan have been valuable to their meat industry relative to providing in regards to educational opportunities and could possibly do the same in Colorado.

CHAPTER 5

A Survey of the Colorado Pork Processors

Summary

A survey was conducted with the small and very small processors in Colorado to determine how many harvested pigs, the desired traits for live pigs, and to determine processors views of the local pork industry; to our knowledge, this survey is the first of its type in the state. An online survey was designed to establish definitions for various quality factors, determine relative importance for these factors, and assess the image, strengths, weaknesses, and potential threats to the Colorado pork producers from a small processors' perspective. Those making purchasing decisions, or were knowledgeable in the daily activities of each company, were asked to complete the survey. Initial contact was made via telephone in February 2018, and surveys were desseminated and completed during a three month period of time (February to April 2018). Using a dynamic routing software system (Qualtrics), a survey was designed, that routed the processors based on their initial response of whether they did or did not harvest pigs. If they did not harvest pigs, further questions were asked to determine the primary reasons for processors to not harvest them. If the processor taking the survey did harvested pigs, they were routed to questions pertaining to their business. Definitions for predetermined quality factors of 1) how and where the pigs are raised, 2) weight and size, 3) conformation, 4) food safety, and 5) quality were recorded and analyzed to assist in determining the perceived meaning for each quality factor and interpret importance of these factors to the processors. Financial considerations were the most common reason for small processors to not harvest pigs; the input costs of updating their facilities, purchasing additional equipment to harvest or further process, and the costs of

updating their HACCP plans or abiding by regulatory standards outweighed the minimal profit margins they would receive from harvesting pigs and processing pork products. Custom exempt meat processors were the most common type of facility and most harvested less than five head per week. How and where the pigs are raised was the factor that was most likely (42.7%) to be selected as a must have, followed by "quality" of the live animal (35.5%). Respondents believed that a strength of the industry is the number of small and local producers, though, they were concerned about the lack of swine numbers and the low quality of the livestock that are harvested. Responses by the processors surveyed suggested that there is a positive image of the pork industry in Colorado, likely due to the number of small and local producers which would allow for the processors to have a relationship with their customers. Overall, the results of the study were able to characterize the size and scope of the small pork processors in Colorado and provide initial information to further improve the pork industry.

Introduction

Pork production in Colorado is considered an extremely small commodity, with less than one percent of the national pork market share (*138*). Colorado is the 15th largest hog producing state in the country, but the majority of the marketable pigs are produced at large, corporate owned farms in the northeastern corner of the state (*138*). The pigs produced by these companies are typically shipped to large finishing units in the Midwest and are ultimately harvested by large processing facilities in those locations. Therefore, the pork that is produced in Colorado is often harvested and processed at small, local processors. Many of these small processors are located in rural areas of the state and harvest for individual farmers with few hogs in order to service the customers who are unable to transport their animals to state and federally inspected facilities (*66*).

Studies have shown that interviews and surveys of those who are knowledgeable about a company's day-to-day operations can provide insights on production size and scope of that facility (*58*, *64*, *120*). Understanding demographics and live pig traits that are desired by small processors in Colorado would be beneficial to the industry from an educational and profitability standpoint.

Minimal research has been conducted to characterize and locate the small processors in Colorado, resulting in an outdated list of processors available from the Colorado Department of Agriculture (*35*). Therefore, the objectives of this survey were to identify how many small processors harvest pigs or process pork products, to determine the importance of various quality factors that processors would prefer from customers and establish definitions for these factors, and to assess the images, strengths, weaknesses, and potential threats to the Colorado pork industry from the small processors perspective.

Materials and Methods

An online survey was administered to representatives of small and very small processing facilities in Colorado from February to April of 2018 using a dynamic routing software system (Qualtrics 2016, Provo, UT). Two contact lists were accessed from the Colorado Department of Agriculture and from the Colorado Association of Meat Processors. The survey targeted individuals who make purchasing decisions, are knowledgeable about the daily operation of the company, and/or a technical personnel employed by the company (e.g., owner and/or plant manager). An attempt was made to contact all small meat processors in the state of Colorado, regardless of whether or not pork was part of their business.

Computer-assisted survey software. A computer-assisted, dynamic-routing survey was developed using the Qualtrics software platform (Qualtrics 2016; Provo, UT). This program

administered the survey questions in a manner that prevented "leading" of the interviewees to biased answers, while still routing the respondent through the appropriate sequence of questions. The routing of these questions was dependent upon the interviewee's answers and provided multiple choice questions, as well as open-ended questions, to quantify the respondent's views. This survey tool was able to solicit information to determine approximately how many processors harvest pigs in Colorado, to determine the most desirable market swine traits for the local processors, and to assess their views on the image of the pork industry, as well as a SWAT analysis. This approach has been successful in completing the two most recently conducted National Beef Quality Audits in 2011 and 2016, as well as an international pork study (*58*, *64*, *92*).

Survey structure. The survey began with demographic questions designed to characterize the respondent's company, specifically focused on if they did or did not have pork as part of their business model. Companies not associated with harvesting pigs were asked a series of questions pertaining to why they did not, including the challenges and limitations that would restrict their ability or desire to harvest pigs. Additionally, those processors that did not harvest pigs but did process pork products were routed to respond to questions regarding why they did not harvest pigs, as well as questions regarding their pork business. These processors were also asked to respond to open-ended questions regarding their company's views of the image, strengths, weaknesses, potential threats, and changes in the pork industry in Colorado. companies responded that they do in fact harvest pigs, they were routed to questions regarding the size and scope of their business. The size and scope of each company was determined through questions about the preferred size and weight of the pigs to be harvested, as well as how far the pigs travel and if the company ever rejects pigs on arrival. Questions regarding the

frequency of rejecting pigs and various carcass defects were determined through a series of questions about live animal handling and travel.

Must-have questions immediately followed, which may not truly be "must-haves," but describe the highly desired traits that processors prefer in the pigs they harvest. If a respondent chose a category as a must-have, they then were asked: "if this trait could not be guaranteed, would you still purchase the pig at a discount?" If the respondent selected "no" as the answer, then this was determined to be a highly desirable trait. If the respondent agreed that they would purchase the animal at a discount, a follow up question was asked to determine at what percentage discount they would be willing to pay for that specific trait, and the trait was determined to be less desirable. This was followed by questions to evoke an individual definition for each quality factor. Interviewees were allowed to give an open-ended response (150 characters maximum) to the questions "What does [quality factor] mean to your company in regard to the pork products that you purchase and/or sell?" These open-ended responses were then categorized into groups that were similar in response for analysis.

Industry image, strengths, weaknesses, potential threats, and changes to the Colorado pork industry were the last questions asked on the survey. These were also open-ended responses, limited to 150 characters. These responses were then grouped into five or six similar categories of responses for analysis. Response categories for each question were determined based on the open-ended responses from those who participated in the survey.

Data collection. A team of two Colorado State University personnel made an attempt to contact 101 small meat processors in Colorado; those that only processed wild game species were excluded before contact. Initial contact was made via a telephone call, and each processor was individually asked to participate in the survey. The primary means of distribution of the

survey, after agreeance to participate, was through an email link which was sent immediately after the telephone conversation. The respondents were then given a week to respond to the survey before a secondary distribution of the survey was made via U.S. postal service. An online link was uploaded to a USB drive and mailed to each processor that agreed to participate. Additionally, in a final attempt to contact processors to participate, an in-person visit was made to the Colorado Association of Meat Processors annual meeting in April 2018 to solicit responses from those who had not yet completed the survey. Initial contact was made in February 2018, and surveys were completed February through April 2018. There were a total of 41 respondents, all with varying engagement in the Colorado pork industry.

Statistical analysis. A binary logit model using the Glimmix Procedure in SAS (SAS 9.4, Cary, NC) was used to estimate the statistical probabilities that a respondent would select one of the five preselected quality factors as a "must have." If the quality factor was selected as a "must have," then the respondents were asked if they would be willing to still purchase the animal at a discount, if the factor could not be guaranteed. Probabilities were calculated and means were separated using $\alpha = 0.05$.

Survey Results and Discussion

Of the 101 small and very small processors that were initially contacted via telephone, 62 verbally agreed to participate in the survey. After both distribution methods were completed, the survey achieved a 66% response rate (n = 41) from the small processors who received the survey. Locations of the surveyed processors evenly represented the entire state of Colorado: approximately 27% (11/41) represented the front range and I-25 corridor, while 19.5% (8/41) responded from the northeast, 22% (9/41) from the northwest, 22% (9/41) from the southwest,

and 9.5% (4/41) from the southeast. Of these, 51.2% confirmed that their company does harvest pigs.

Pork Was Part of The Company's Business

Did harvest pigs. As previously mentioned, 51.2% (21/41)of the respondents indicated that they did harvest pigs. These respondents were then prompted to answer questions regarding the nature of their business. Only two of these processors harvested more than 30 pigs, on average, per week, while 10 of the 21 of these harvested less than five head per week. Additionally, only two companies responded that they were federally inspected for the harvest and processing of pork products; this company harvested over 30 pigs per week. Of the remaining responses, 57.8% (11/19) were custom exempt and only harvested pork for individual customers. The remaining 42.1% (8/19) were state inspected and were approved for retail sales of pork products.

Demographics of Pork Processors. A concern with only having a few small processors in Colorado is the distance that the live animals may have to travel before slaughter and the defects that travel may have on carcass quality. Therefore, processors were asked how far, on average, pigs travel to reach their facility. Only one processor responded that they have pigs that traveled more than 100 miles for harvest, while 30% (6/20) of the remaining respondents processed pigs that traveled up to 100 miles; 50% (10/20) have pigs travel up to 50 miles, while the remaining 20% (4/20) of pigs traveled less than 25 miles to reach their facility.

Only 47.6% (10/21) of respondents said that they rejected pigs when they reached their facility, for various reasons, including that the animals were lame, are exhibiting signs of illness or disease, or are too large to harvest. The frequency of those that rejected live pigs before harvest was evenly distributed amongt the various distances traveled. Though, the respondents

that reported observing carcass defects due to travel were those that harvested pigs that traveled more than 25 miles (up to 50, up to 100, and more than 100 miles). There were no reported carcass defects due to travel in pigs that travel less than 25 miles. Carcass defects were also seen due to handling prior to harvest; approximately 57% (12/21) of processors reported that they see these defects. Of these, one processor reported that handling defects occurred in 6 – 10% of the hogs they harvest, while the remaining processors saw these defects less than 5% of the time. Only 33.3% (7/21) of processors reported carcass defects due to their harvest process. Furthermore, 19% of the pork processors (n = 4) reported that they have condemned at least one carcass after harvest in the past year.

Processing of pork products. Those processors who responded that they did have retail sales or custom processing were 75.6% (31/41) of all respondents. Only two (6.5%) companies reported that they soley processed pork products for retail sales in their local facilities. Most (64.5%; 20/31) of the respondents who process pork products processed both fresh and cured products, though 16.1% (5/31) of the processors only processed fresh (un-cured) pork products. Additionally, 48.4% (15/31) sold their products both fresh and frozen, while the remaining 51.6% (16/31) of respondents only sold their products frozen. Only 9.7% (3/31) of the respondents did not purchase boxed product, but the remaining 90.3% (28/31) companies purchased boxed product to supplement the processing needs from food service providers, wholesale clubs, and distributors. Finally, only 29% (9/31) of those who processed pork products did not utilize any marketing claims to market their products. The claims that processors reported using were "natural," "antibiotic free," and "hormone free." All processors reported using word of mouth and social media or the internet to market their products.

Relative importance of quality factors. The relative importance of the five predetermined quality factors were determined by finding the statistical probabilities that a respondent would select one as a must have for those who have pork as part of their business (those that harvested pigs and those that processed pork products). These probabilites can be found in Table 5.1. "How and where the pigs are raised" was the most important (42.7% likely to be selected) quality factor to the small processors and deemed to be highly important. When asked to define this term (Table 5.2), respondents most commonly described how and where the pigs are raised as "feeding and handling" (35.7%; 15/42) and "a relationship with the producer" (16.7%; 7/42). Though, 26.2% (11/42) of the respondents said that they are not interested in how and where the pigs are raised. "Quality," defined as "consumer satisfaction" (42.5%; 17/39; Table 5.2), was the second most important (37.5% likely to be selected) to the respondents. "Quality" was deemed as highly important to the processors, who are still willing to purchase pigs at a 27.5% discount if it could not be guaranteed.

The third most important factor to the small processors was the "weight and size" of the animal (32.4% likely to be selected). When asked to define "weight and size," 37.5% (12/32) of the respondents described it as "live weight of the animal" (Table 5.2). The remaining two factors, "conformation" and "food safety," were equally likely to be selected as must haves (27.3% chance of selection). Nearly 50% (16/33) of the respondents replied that they were not interested in conformation in pigs (Table 5.2). When asked to define what the term "conformation" means to their company, 27.3% (9/33) responded that they would define the term as uniformity of cuts. "Food safety" was most commonly defined as "sanitation" (30%; 11/40) and "post-harvest handling" (15%; 6/40; Table 5.2).

Images, strengths, weaknesses, potential threats. At the end of the survey, open-ended questions were asked regarding what the processors believed the image of the pork industry in Colorado was, and what they believed the strengths, weaknesses, and potential threats were to the industry (Table 5.3). Over 34.4% (11/32) of the respondents replied that they believed that the image of the Colorado pork industry was improving, while an additional 31% (10/32) believed that the image is good. Approximately 12% (4/32) of the processors who participated believe that Colorado has a reputable image, and only 3% (1/32) of respondents said that they see the industry as "needing improvement" or being "nonexistent." This is a positive sign for the pork industry in Colorado.

When the interviewees were asked what they believed the strengths of the Colorado pork industry were (top three responses found in Table 5.3) it became very clear that they were proud of the small processors that reside in the state (28.6%; 9/32) and that they were able to market "local" pork products (22.2%; 7/32). The predominant response to this question was that there were no large pork producers or processors in the state and that they were able to market local products. In contrast, when asked what they believed the weaknesses of the industry were (Table 5.3), there was a concern for the short supply of pigs in Colorado (38.5%; 12/32). Responses suggested that the majority of the pigs they harvested came from farms that only raised one or two hogs at a time, therefore there were very few numbers of pigs in the state. One processor even asked "is pork raised in Colorado?" Additionally, the other predominant weakness that these small processors expressed as a concern was the poor quality of the pigs they are harvested (26.9%; 9/32) which they attributed to poor producer education; descriptions included the terms "junk hogs" and "trash hogs." It was requested that educational material on how to appropriately produce higher quality pigs be decimated to small producers.

Finally, when the question "what does your company believe the potential threats are to the Colorado pork industry?" was asked, the responses varied greatly among the respondents. The top three responses are presented in Table 5.3 However, the responses suggested that the most significant threat to the industry was related to the supply and price of pigs (25.9%; 8/32). It was determined that there is a decreasing supply of quality pigs and that the price is increasing, ultimately decreasing the processors profit margins. The second most common response was that animal rights activites and "uneducated consumers" (22.2%; 7/32) are a potential threat to the industry. Additionally, there were several processors who expressed a concern regarding the regulatory measures they must meet (18.5%; 6/32).

Past and future changes. The processors who responded that they did harvest pigs were given the opportunity to share the changes they have seen in the past five years and what they would like to see change in the next five years. Nearly 30% (6/21) of the respondents replied that they did not know what had changed or did not keep track. Aside from this, the top responses for the changes that processors saw included a decreased supply of pigs in Colorado (22.2%; 7/32) and increased quality of pork (18.5%; 6/32). Improved pork quality was a positive for the industry, but decreasing profit margins and less pig numbers could create a challenge for the next five years. Though, the top response (29.6%; 9/32) was that the processors did not know what they would like to see change, there was concern with the growing number of show pigs that were raised in Colorado. There were processors that were concerned that show pigs have ruined the industry because they "are not palatable," while others believed these pigs improved the overall quality of Colorado pork but the kids who raised these animals were "beat up" financially. Additionally, when asked what they would like to see change in the next five years, nearly every respondent had a different answer. The responses varied from wanting to see more

local and organic swine producers in the state that are transparent with their customers so they could build a relationship, while others would like to see more medium to large pig producers. Some processors believed that there needs to be more encouragement for 4-H and FFA participants to become more involved in the meat industry, while other processors believe that show pigs are ruining the industry. Furthermore, other respondents wanted more educational opportunities for producers, others wanted to find more versatility for pork products. Clearly, the survey results show that the small processors have widespread views of the industry that may make it challenging to meet their individual needs in the coming years.

Did Not Harvest Pigs

Of the 41 respondents, 48.8% (20/41) verified that they did not harvest live pigs. The survey then routed these individuals to respond to questions to determine the reasons that they did not. The top four responses for why processors did not harvest pigs are presented in Table 5.4. The responses that interviewees were able to select included that they did not have the facilities to harvest pigs, the additional processing for smoked and cured pork products (i.e. processing of bacon, hams, etc.) was either a burden or required more equipment that they did not have, they did not want to update their HACCP plans or go through the inspection responsibilities, there was not enough of a supply of pigs in their region to make harvesting pigs profitable, or "other." Regardless of the response, all respondents were then prompted to input an open-ended answer for each reason they selected. Individuals were allowed to select more than one response in this section of the survey.

According to the responses from the 20 interviewees that do not harvest pigs, "facilities" (38.5%; 8/20) was the top reason that they did not. Further responses determined that "facilities" means that these processors would have to update their facilities to accommodate harvesting pigs

or they would have to invest in more equipment. These processors expressed a concern that the input costs would not be worth the minimal profit they would receive. The second (22%; 4/20) and third (22%; 4/20) reasons were "additional processing is required" and "regulatory restrictions." Of the interviewees that selected "additional processing is required," most did not have a smokehouse and therefore found it difficult to do the additional curing and smoking that most pork products require. Additionally, the additional time, space, and packaging requirements that are necessary for further processing were determined to be undesirable for these companies. Those that selected "regulatory restrictions" determined that the necessity for an additional HACCP plan restricted them from harvesting pigs. Furthermore, 15.4% (3/20) of processors that did not harvest pigs responded with "other." These reasons varied from not having enough personnel to needing more space to either process or hold pork carcasses. Only one processor responded that there is not enough of a supply of pigs in the area to make harvesting them profitable.

Conclusions and Recommendations

The results of this survey concluded that over half of the small and very small processors in Colorado did harvest pigs and they were evenly distributed throughout the state. Custom exempt processors was the most common type of facility and the majority of the processors harvested fewer than five pigs per week. Since there were only a few small processors that did harvest pigs, producers would have to transport their pigs long distances to reach these facilties, which may have resulted in carcass defects due to stress and handling. Additionally, the importance of various quality factors were determined; "how and where the pigs are raised" was the most important factor, which was further validated when the processors described "local" and "small producers" as the top strengths of the industry. Though, the respondents did see several

weaknesses with the quality of the pigs that were raised in Colorado and would like to see more producer education. Even with a small swine industry in the state, there still seemed to be a positive outlook by these processors of the industry as a whole in Colorado. Though, based on the responses from the interviewees, there are multiple educational opportunities that could enhance the pork industry in the state.

Of those processors that did not harvest pigs, many did not do so because they did not want to update their HACCP plans or have to abide by additional regulatory standards. These processors would not only have needed a HACCP plan for slaughtering pigs, but also for processing the carcasses and for further processing (curing, smoking, etc.) of pork products. This becomes challenging for these small processors and was not worth the extra time and financial considerations when there were minimal profit margins associated with harvesting only a few pigs a week. More small processors may be willing to harvest pigs if there were more in-depth educational opportunities for small processors to learn how to create a HACCP plan in a more efficient manner, or if there were a low-cost (or even free) outside source that could collaborate with these processors to help create a HACCP plan for slaughtering pigs and processing pork products. Additionally, many of these processors expressed a concern for needing further inspection or having to abide by the state and federal regulatory standards.

In the additional comments section, some processors expressed the desire for more educational materials to share with their customers and the producers that raise these pigs. These include information regarding the health of pigs and pre-harvest factors that may affect pork quality (genetics, feeding, handling, transportation, etc.). One processor requested a pamphlet or handout regarding the occurrence of *Trichinella spiralis* in pork because his company has many customers who refused to raise pigs or purchase pork due to the potential of contamination in the

product. Others requested similar flyers on the overall health and pre-harvest handling of pigs to share with their customers in order to improve the quality of the product. These could be beneficial for both producers and for processors in order to continue to improve the image of the pork industry in Colorado.

Overall, this study was able to appropriately summarize the small and very small pork processing facilities in Colorado. The responses from those who completed the survey are beneficial to understanding the size and scope of the small pork industry in the state and will provide the initial background information necessary to further improve the quality of the pigs raised by small producers.

Table 5.1: Statistical probabilities (± standard error) that each predetermined quality factor will be selected as a "must have" quality trait for processors to purchase a pig for harvest.

Quality Factor Category	Probability for Selection (SE of Mean)
How and Where Pigs were Raised	0.427[a] (0.12)
Quality	0.375[a] (0.12)
Conformation	0.273[a] (0.11)
Weight and Size	0.324[a] (0.11)
Food Safety	0.273[a] (0.11)

[a] There are no statistical differences between the quality factors ($P \geq 0.05$).

Table 5.2. Categorized responses from the surveyed processing facilities for explaining what the pre-identified quality categories mean to their company as it relates to the harvest and processing of pork products in Colorado.

How and Where the Pigs Were Raised		Quality		Weight and Size		Conformation		Food Safety	
Most Freq.[1]	Definition	Most Freq.	Definition	Most Freq.	Definition	Most Freq.	Definition	Most Freq.	Definition
35.7%	Feeding and handling	42.5%	Consumer satisfaction	37.5%	Live weight	48.5%	Not interested	30.0%	Sanitation
26.2%	Not interested	15%	Not interested	31.3%	Not interested	27.3%	Uniformity	15.0%	Handling
16.7%	Relationship with farm	12.5%	Lean/fat Meat color[2]	18.6%	Quality	9.1%	Yield	15.0%	Not interested

[1] Most Freq.: Top three most frequent responses to define the five predetermined quality factor categories.

[2] "Lean/fat" and "Meat color" are two separate categories with the same response rate.

Table 5.3. Categorized responses from the surveyed processing facilities for their company's views of the image, strengths, weaknesses, potential threats to, and the changes they have seen of the Colorado pork industry.

Image		Strengths		Weaknesses		Potential Threats		Changes	
Most Freq.[1]	Definition	Most Freq.	Definition	Most Freq.	Definition	Most Freq.	Definition	Most Freq.	Definition
34.4%	Improving Image	29.6%	Small Producers	38.5%	Supply	25.9%	Supply/Price	29.6%	Unknown
31.3%	Good Image	25.9%	Unknown	26.9%	Education	22.2%	Consumer Education & Activists	22.2%	Decreased Supply
12.5%	Reputable & Unknown[2]	22.2%	Local	23.1%	Unknown	18.5%	Regulations	18.5%	Increased Quality

[1] Most Freq.: Top three most frequent responses to the open-ended response questions of each companies views of the Colorado pork industry's image, strengths, weaknesses, potential threats, and the changes the processors have seen in the past five years.

[2] "Reputable & Unknown" are two separate categories with the same response rate.

Table 5.4: Categorized responses from the surveyed processing facilities that do not harvest pigs.

Category[1]	Frequency of Response
Facilities	38.5%
Further Processing	19.2%
Regulatory Reasons	19.2%
Other	15.4%

[1] Top four most frequent responses for the reasons that companies do not harvest pigs in Colorado.

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