Evaluating the efficacy of commonly used antimicrobials in the beef industry for controlling shiga toxin-producing *Escherichia coli* contamination on chilled beef subprimals and pre-rigor carcass sides

by

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Abstract

Shiga toxin-producing *E. coli* (STEC) are frequently associated with foodborne illness outbreaks, especially attributable to beef. Intervention methods, such as water washes and organic acid application, are widely implemented across the beef industry to combat this risk. This research evaluates the efficacy of intervention methods applied to chilled beef subprimal pieces and pre-rigor beef carcasses to reduce STEC contamination. Beef strip loins were inoculated (ca. 5 log CFU/cm^2) with a 7-serotype STEC cocktail and sprayed with increasing concentrations of peracetic acid (200-1800 ppm; ambient temperature), lactic acid (3-10%; 55°C), or a water control before being vacuum-packaged and stored for 24 h at 4°C. Meat surface excision samples and color readings (L*, a*, and b*) were obtained from each subprimal at three sampling points: post-inoculation, 5 min post chemical spray, and post-24 h vacuum packaged chilling. Peracetic acid spray and lactic acid spray reduced STEC populations by 0.5 - 1.3 log CFU/cm^2 and 0.2 – 0.7 log CFU/cm^2, respectively, across the incremental concentration increases. All concentrations of peracetic acid and lactic acid concentrations ≥3.5% reduced ($P \leq 0.05$) STEC populations compared to their respective control. Application of higher concentrations of lactic acid (7-10%) decreased ($P \leq 0.05$) L* and b* values compared to the control, indicating that quality attributes of the subprimals were negatively effected. Carcass intervention methods were evaluated using a three-stage commercial carcass washing cabinet (Chad Equipment). Four pre-rigor carcass sides were inoculated by electrostatically spraying with a 7-serogroup STEC cocktail (ca. 6.5 log CFU/100 cm^2). Three treatments were applied, in order, to each side: ambient water wash, hot water wash (82-92°C at the nozzle head), and antimicrobial mist. Meat surface excision samples were taken from the bottom, middle, and top

section of each carcass side at five sampling points: 30 min post-inoculation, post-ambient water wash, post-hot water wash, post-antimicrobial spray, and after 18 h spray chilling. The combination of the high-volume ambient water wash stage and subsequent hot water wash stage reduced STEC populations on sides by 3.5, 4.7, and 4.8 log CFU/100 cm^2 at the bottom, middle, and top of the carcass, respectively. Due to STEC populations declining to very low or undetectable levels after the hot water stage, minimal additional STEC reductions were observed after chemical spray application and chilling. Sequential antimicrobial treatments applied using a three-stage Chad carcass wash cabinet and a subsequent chill step reduced STEC populations on pre-rigor beef carcasses by 4.5 – 5.3 log CFU/100 cm^2.

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Chapter 1 – Introduction and Research Questions

Shiga toxin-producing *Escherichia coli* (STEC) are major pathogens that have garnered attention due to their association with foodborne disease outbreaks. These bacteria are estimated to cause 265,000 illnesses, 3,600 hospitalizations, and 30 deaths annually in the United States (CDC, 2012d). *Escherichia coli* O157:H7, the most prominent STEC strain, has long been a focus of food processors, researchers, and regulatory agencies; and is estimated to cause roughly 36% of STEC related illnesses each year (Ju et al., 2012; CDC, 2012d). Non-O157 STEC serotypes have gained notoriety and currently account for a much larger percentage of STEC related illnesses than *E. coli* O157:H7. Six non-O157 STEC strains (O26, O45, O103, O111, O121, and O145), also known as the "Big 6", account for at least 70% of the non-O157 STEC illnesses each year (FSIS, 2012a). Symptoms of STEC infection include nausea, mild diarrhea, severe bloody diarrhea, and diseases such as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), which can lead to kidney failure and death.

E. coli O157:H7 and the "Big 6" STEC strains have been implicated in numerous multistate foodborne outbreaks involving beef and veal products. STEC cells colonize within the gastrointestinal tract of cattle, presenting a risk of bacterial contamination during beef processing, primarily during the hide removal process. As a result, beef products are at a higher risk for STEC contamination compared to other food matrices. Approximately 55% of foodborne outbreaks caused by *E. coli* O157:H7 and 50% of foodborne outbreaks caused by non-O157 STEC are attributed to beef (Moxley and Acuff, 2014). In 1993, a landmark outbreak of *E. coli* O157:H7 in undercooked ground beef sold at a quick service restaurant chain in the Pacific Northwest led the United States Department of Agriculture's Food Safety and Inspection

Service (FSIS) to declare this pathogen an adulterant in raw ground beef products in September, 1994 (FSIS, 1999). In January 1999, the FSIS expanded this adulteration policy for *E. coli* O157:H7 to include raw non-intact beef products and intact cuts likely to be further processed into non-intact products before being distributed to the consumer. In response to multiple foodborne outbreaks involving the "Big 6" non-O157 STEC strains, the FSIS declared these additional serogroups to be adulterants in raw non-intact beef products in 2011 (FSIS, 2012b).

Beef processing plants have implemented intervention techniques to control STEC contamination at various steps throughout the slaughter and dressing processes. Carcass wash cabinets are widely used to apply ambient and hot water washes that have been proven effective for lowering *E. coli* O157:H7 contamination in beef products (Castillo et al., 1998a). Organic acids, such as lactic acid, peracetic acid (PAA), along with bromous acid and sulfuric acid blends, are popular intervention methods in the beef industry. Lactic acid has been the most widely used organic acid across the U.S. beef industry due to its low cost and effectiveness in reducing microbial loads. Application of peracetic acid to beef products has had varied efficacy for reducing microbial contamination depending on application method, concentration, and manufacturer (Gill and Badoni, 2005; King, 2005; Liao, 2015). Sulfuric acid, combined in an aqueous mixture with sodium sulfate, is believed to be an effective intervention against common pathogens, including STEC, on beef products (Zoetis, 2016); however, more scientific support is needed.

Parameters such as temperature, exposure time, application method, concentration, and type of meat have an impact on the efficacy of organic acids in reducing microbial loads. The FSIS also regulates lactic acid, peracetic acid, and sulfuric acid application to certain limits

(FSIS, 2017), which beef processing plants must adhere to in order to remain in compliance. The recent emergence of the "Big 6" STEC strains as foodborne pathogens has heightened the demand for effective and economical intervention strategies in the beef industry. Therefore, evaluating the efficacy of intervention methods to control STEC in beef products using various parameters becomes necessary.

The main objectives of the research reported in this thesis were to evaluate the efficacy of commercially utilized antimicrobial intervention methods for controlling STEC contamination in/on various raw beef products at the raw subprimal stage of processing and on beef carcasses during the slaughter process (final carcass wash). Increasing concentrations of peracetic acid (ZEE Company, Microtox Plus; Chattanooga, TN; ambient temperature) or lactic acid (88%, Birko Corporation; Henderson, CO; 55°C) were evaluated as antimicrobial spray interventions for reducing STEC populations on chilled beef subprimals immediately prior to vacuum packaging. Also, a high-volume ambient water wash, hot water wash (~85°C at the nozzle head), and antimicrobial mist were each evaluated sequentially as beef carcass sides passed through a three-stage commercial Chad spray cabinet to reduce STEC contamination immediately before entering the carcass cooler and after 24 hours of spray chilling of carcasses. Beef processors will benefit from this research as they seek validated antimicrobial interventions along the carcass to final raw product beef chain, including the application of substantially higher concentrations of peracetic acid and lactic acid than currently approved for chilled beef subprimals and the sequencing of standard ambient and hot water carcass washes followed by a final acid spray.

Research Questions

1. Are there any significant differences in mean reductions of STEC achieved when different concentrations of peracetic acid (from 0 to 1800 ppm) are applied as an antimicrobial spray on chilled beef subprimals immediately before vacuum packaging?
2. Are there any significant differences in mean reductions of STEC achieved when different concentrations of lactic acid are applied as an antimicrobial spray on chilled beef subprimals immediately before vacuum packaging?
3. Are organoleptic properties (i.e., surface color and lipid oxidation) of the chilled beef subprimals affected by application of higher concentrations of peracetic and lactic acids?
4. What mean reductions of STEC on pre-rigor carcass surfaces are achieved following a final ambient water wash, hot water wash, and antimicrobial mist step using a commercial three-stage Chad carcass wash cabinet?
5. Are there any significant differences in total mean reductions of STEC on pre-rigor beef carcass sides at the top, middle and bottom of the carcass sides after sequential ambient and hot water washes, followed by application of peracetic acid, lactic acid, or Centron™ sprays applied in a three-stage Chad carcass cabinet prior to chilling?
6. When ambient water wash, hot water wash, antimicrobial spray, and standard spray chill steps (using municipal water) are sequentially applied to pre-rigor beef carcass sides, what is the overall mean reduction of the inoculated STEC population beginning with very high contamination levels (ca. 6.5 log CFU/100 cm^2)?

Chapter 2 – Literature Review

2.1 *Escherichia coli*

Theodor Escherich first discovered *Escherichia coli* in 1885 during the process of isolating microorganisms from feces. Originally known as "*Bacterium coli",* it was found to be commonly isolated from intestinal samples. In 1919, it was proposed to name the genus "*Escherichia*" to honor Escherich's discovery (Janda and Abbott, 2006).

Escherichia coli (*E. coli*) is an original member of the Enterobacteriaceae family dating back to the 1930's (Janda and Abbott, 2006). *E. coli* is a Gram-negative rod that is facultatively anaerobic and motile, and is considered a coliform due to its ability to ferment lactose and the dark appearance of its colonies and display of a green sheen on eosin methylene blue agar (Batt, 2014). Optimal growth conditions for *E. coli* in relation to environmental parameters are shown in Table 2-1.

Table 2-1 Optimum growth conditions for *E. coli.*

Parameter	Growth Range	Optimum
pH	4-9	6-7
Temperature (°C)	8-46	37
Water Activity (a_w)	0.95-1.0	0.995

Formulated using data from Albrecht; ESR, 2001.

E. coli is a common component of the normal microflora in the intestinal tract of humans and warm-blooded animals (WHO, 2016). Commensal *E. coli* strains seldom cause

disease and are often beneficial to their host. They usually lack virulence factors found in pathogenic *E. coli* and cause infection only in immune-compromised patients or when normal gastrointestinal boundaries are breached (Janda and Abbott, 2006; Meng et. al., 2007). However, many strains of *E. coli* have acquired virulence attributes, which allow them to cause disease in humans (Bari and Inatsu, 2014; Meng et al., 2007).

Strains of *E. coli* contain three surface antigens, causing them to differ serologically, a distinction that requires serotyping of the bacteria. These antigens consist of O (somatic), H (flagella), and K (capsule). At this time, 180 O, 60 H, and 100 K antigens have been identified (White and McDermott, 2009). In order to identify *E. coli* strains associated with diarrheal disease, it is only necessary to identify the O and H antigens; the O antigen representing the serogroup and the H antigen representing serotype (Meng et. al. 2007).

Virulence, pathogenicity, clinical syndromes, and the O:H antigen serotypes categorize the six pathogenic groups of diarrheagenic *E. coli* (Janda and Abbott, 2006; Meng, 2007). The six categories are as follows:

1. Enterotoxigenic *E. coli* (ETEC)

ETEC strains cause infection through the fimbrial colonization pathway in the small intestine, leading to the release of enterotoxins. Two types of enterotoxins associated with ETEC exist; a heat-labile enterotoxin and a heat-stable enterotoxin. These may be produced together or singly depending on the strain and cause fluid accumulation leading to a diarrheal response in the host. ETEC are a major cause of diarrhea in infants and elderly people, especially in developing countries. They are also estimated to cause up to 75% of traveler's diarrhea cases worldwide (Janda and Abbott, 2006; Meng, 2007). While ETEC are associated

with acute diarrhea in travelers around the world, studies have shown that they are not associated with persistent diarrhea (Schultsz et al., 2000). The common serogroups falling within the ETEC pathogroup are O6, O8, O15, O20, O25, O27, O63, O78, O85, O115, O128ac, O148, O159, and O167.

2. Enteroinvasive *E. coli* (EIEC)

EIEC cause non-bloody diarrhea and dysentery due to their ability to invade and multiply in colonic epithelial cells. A large plasmid (ca. 140 MDa) is critical to EIEC invasiveness as it encodes outer membrane proteins. The bacteria localize in the colon, and breach and multiply in the epithelial cells (Meng et al., 2007). Symptoms of dysentery associated with EIEC include abdominal cramps, fever, watery diarrhea, and chills (Janda and Abbott, 2006). Serogroup O124 is the most common EIEC encountered along with the following strains often associated with illness: O28ac, O29, O112, O136, O143, O144, O152, O164, and O167.

3. Enteropathogenic *E. coli* (EPEC)

EPEC was the first group of diarrhea causing *E. coli* to be described. This pathogroup is affiliated with severe diarrhea in infants and outbreaks occurring in nurseries. EPEC invade epithelial cells by inducing attachment and effacing (A/E) lesions in cells. Common symptoms of EPEC infection are abdominal pain, diarrhea, malaise, and low-grade fever. Serogroups commonly associated with illness are O55, O86, O111, O119, O125ac, O126, O127, O128ab, and O124 (Meng et. al., 2007).

4. Enteroaggregative *E. coli* (EAEC)

EAEC commonly cause diarrheal disease around the world, especially in infants and children. They are unique from the other pathogroups, due to the production of an aggregative

adherence pattern on HEp-2 cells, which has a formation appearing like stacked bricks. EAEC serogroups associated with illness include O3, O15, O44, O77, O86, O92, O111, and O127 (Meng et. al. 2007).

5. Diffuse-adhering *E. coli* (DAEC)

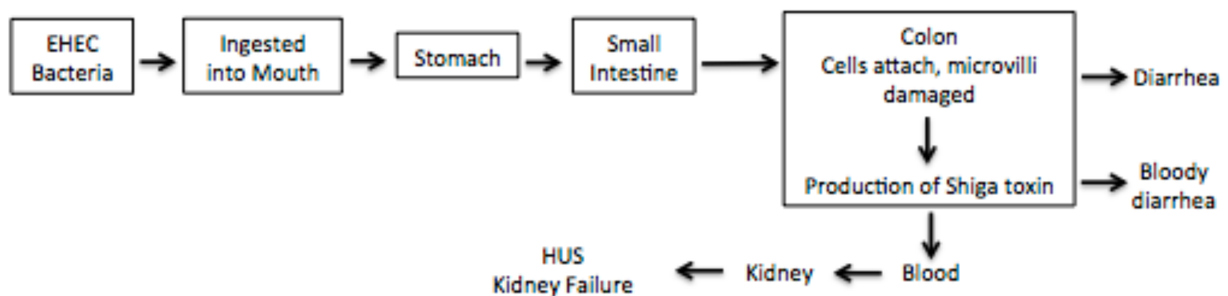
DAEC commonly cause diarrheal disease in young children older than 12 months of age. Children between ages 1 year to 5 years are at a higher risk for obtaining a DAEC-related illness than other groups and symptoms include acute mild non-bloody diarrhea. DAEC differ from other pathogroups due to their diffuse-adherent attachment pattern to HeLa cell lines or HEp-2. They are not known to produce elevated levels of Shiga toxin or enterotoxins and do not carry adherence factor plasmids. DAEC serogroups associated with illness include O1, O2, O21, and O75 (Meng et. al., 2007)

6. Enterohemorrhagic *E. coli* (EHEC)

EHEC were first recognized as pathogens after *E. coli* O157:H7 outbreaks occurred in the food supply. EHEC produce Shiga toxins (*stx*), also referred to as verotoxins (*vtx*), which are named due to their similarity to the toxin produced by *Shigella dysenteriae*. This toxin is associated with causing hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in humans, which is likely fatal to humans if untreated (Kuter, 2014). *E. coli* O157:H7 is the EHEC most often associated with human disease and is linked to numerous foodborne outbreaks. Non-O157 STEC are also associated with foodborne illness and outbreaks worldwide, with the following serogroups having the highest impact: O26, O45, O103, O111, O121, and O145. This group of pathogens, also known as the “Big 6”, has become a focal point of research and regulations over the past decade.

The Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*) genes are the primary virulence factors in STEC, and may be present together or independently, depending on strain. In infected hosts, *stx1* and *stx2* facilitate the release of Shiga toxins in the colon by binding to the globotriaosylceramide on target cells, cleaving rRNA, and inhibiting protein synthesis (Janda and Abbott, 2006). *Stx* then spreads throughout the bloodstream and travels to the kidneys, leading to inflammation and potentially causing HUS, which may result in kidney failure and death (Figure 2-1). *Stx2* is around 1000 times more toxic than *stx1* toward human renal endothelial cells; strains containing this gene are more likely to induce severe sickness than strains only carrying the *stx1* gene (Bertin et al., 2001).

Figure 2-1 Overview of EHEC disease causing mechanism



Formulated from Bari and Inatsu, 2014.

Intimin, an outer membrane protein encoded by the *eae* gene, is present in EHEC and helps facilitate attachment to epithelial layer cells (Fagan et al., 1999). The type III secretion system (TTSS) located in the locus of enterocyte effacement pathogenicity island (LEE PI) helps facilitate this process (Karmali, 2004). Attaching-and-effacing (A/E) lesions are formed when intimin interacts with the translocated intimin receptor “Tir”. These lesions, on the intestinal epithelial layer cells, are critical for the pathogenesis of STEC (Farfan and Torres, 2012).

2.2 *E. coli* O157:H7

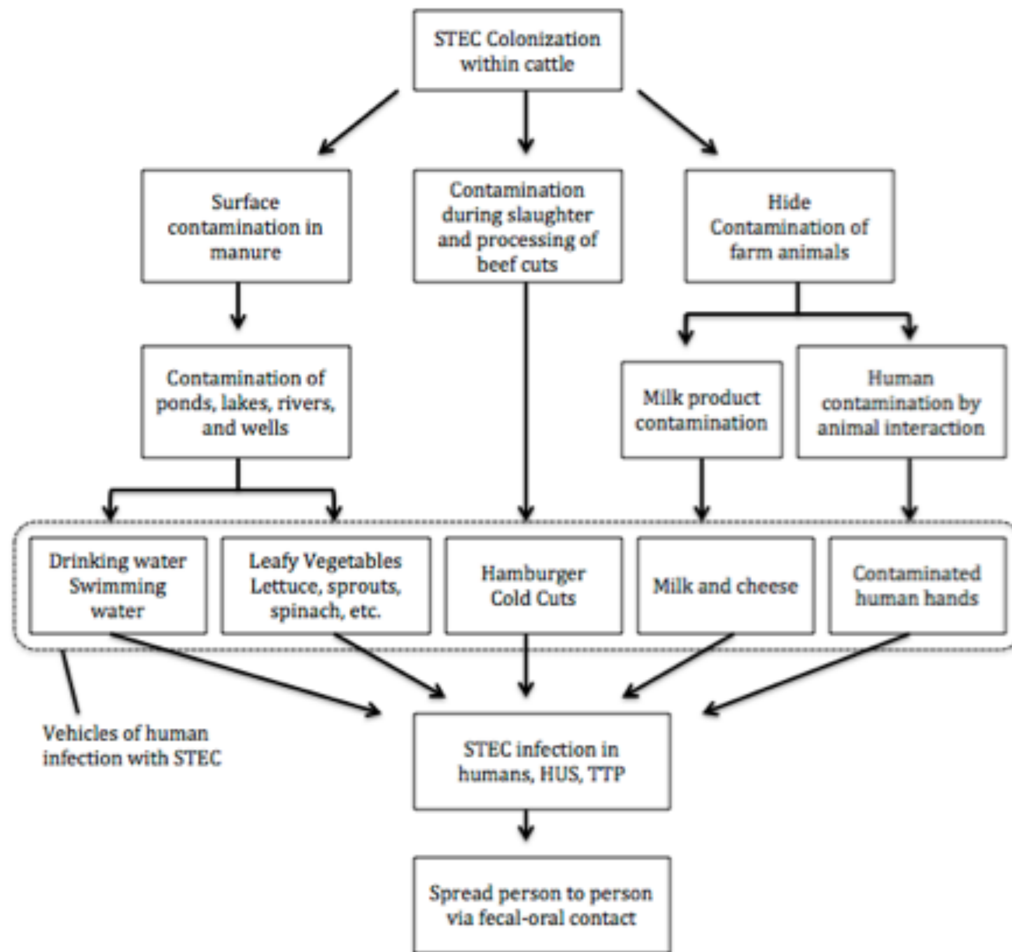
2.2.1 Introduction

Escherichia coli O157:H7 was first recognized as a human pathogen in the early 1980's due to its association with foodborne outbreaks in Michigan and Oregon (Lim et. al. 2010: Riley, 1983). It possesses all of the main virulence factors of STEC and is linked to cases of HUS dating back to 1983. Due to *E. coli* O157:H7's high pathogenicity and strong association with human outbreaks, it has long been a focus of research and government regulations. This pathogen expresses the O (somatic) antigen 157 and the H (flagella) antigen 7, giving it its name (Lim et. al. 2010).

2.2.2 Public Health Impact of *E. coli* O157:H7

E. coli O157:H7 has been involved in at least 19 major multistate foodborne outbreaks in the United States involving a variety of food products since 2006 (CDC, 2017). The Centers for Disease Control and Prevention (CDC) estimates that *E. coli* O157:H7 accounts for 96,534 infections each year in the United States (CDC, 2012d). From 1982 to 2011, 131 foodborne outbreaks of *E. coli* O157:H7 occurred, an estimated 75% of the total cases. These outbreaks caused 530 cases of HUS, resulting in 90 deaths. Common modes of *E. coli* O157:H7 transmission include waterborne, animals and their environment, and person-to-person (Figure 2-2). Non-foodborne modes of *E. coli* O157:H7 transmission accounted for the other 25% of cases from 1982-2011 and were responsible for 58 deaths (Bari and Inatsu, 2014)

Figure 2-2 Pathways of transmission of STEC



Formulated from Focosi, 2016

One of the United States' most important foodborne outbreaks in terms of media coverage and the overall impact on the approach to national meat regulation involved *E. coli* O157:H7. This outbreak took place in the early 1990's and involved contaminated hamburgers from the 'Jack in the Box' fast food restaurant chain. In January 1993, there was a collection of children in the Seattle, Washington area suffering from HUS. An increase in hospital emergency room visits for patients suffering from bloody diarrhea in the Seattle area was reported to the Washington Department of Health (WDOH). Soon afterwards, three children in the area died of

E. coli O157:H7 poisoning. The WDOH led an investigation and discovered that the regular and "jumbo" hamburger patties sold by 'Jack in the Box' were the outbreak source, due to undercooking of frozen patties, a result of overloading of restaurant grills. In total, the outbreak caused 600 patients to suffer bloody diarrhea, with 171 hospitalizations and 41 patients developing HUS. The outbreak spread to Idaho, Nevada and California, and led to a fourth child's death, which was reported in California (USDD, 1999; Flynn, 2009).

As a direct result of this outbreak, 'Jack in the Box' suffered a large financial burden that threatened the restaurant chain's future; projected losses reached between $20 million and $30 million. Eventually, the restaurant chain accepted full responsibility and took many steps to ensure the future safety of their food products (USDD, 1999). 'Jack in the Box' soon became a leader in food safety and this case helped pave the way for food safety programs, regulations, and practices that are still used today. One year after the 'Jack in the Box' outbreak, the USDA FSIS declared *E. coli* O157:H7 to be an adulterant in raw ground beef (FSIS, 1999; FSIS, 2013), which represented the beginning of stricter regulations, increased devotion of time and money for the research and prevention of STEC, and brought attention to ground beef as a potential bearer of STEC.

Advancement in research and improved regulatory controls has limited the amount of *E. coli* O157:H7 infections today, however, recent outbreaks have occurred. Beef and salad products are the most common foodborne sources of these recent outbreaks; cookie dough, frozen pizza, cheese, chicken, and hazelnuts have also been implicated (CDC, 2007a, 2009c, 2010a, 2011d, 2015). Together, these outbreaks have led to dozens of hospitalizations and cases of HUS within the United States, with some resulting in death.

Two recent multistate outbreaks within the beef industry were linked to *E. coli* O157:H7. A mid-western packing plant was identified as the source of contaminated ground beef in 2014 and two years later a New England based farm was forced to recall all beef, veal, and bison products due to *E. coli* O157:H7 contamination. Each outbreak led to 7 hospitalizations, with the 2016 outbreak causing a case of HUS (CDC, 2014; CDC, 2016).

A 2006 *E. coli* O157:H7 outbreak linked to fresh spinach became widespread across the United States. Hundreds of people became ill throughout 26 states, which led to 102 hospitalizations, 31 cases of HUS, and 3 deaths (CDC, 2006). Another salad related *E. coli* O157:H7 outbreak included ready-to-eat products, and impacted the global fast food chain 'Taco Bell', whose shredded lettuce caused an outbreak in 2006 with 8 resulting HUS cases (CDC, 2006). A 2015 outbreak of *E. coli* O157:H7 was spread in chicken salad that was sold in 'Costco' stores. Although a traceback investigation never identified a source of contamination, it was speculated that the celery used as an ingredient in the salad was a source (CDC, 2015). Oftentimes an official source of contamination for these outbreaks is never identified, meaning many ingredients must be recalled and corrective actions become harder to implement.

2.3 Non-O157 STEC

2.3.1 Introduction

The "Big 6" non-O157 STEC serotypes (O26, O45, O103, O111, O121, and O145) have been implicated in a growing number of foodborne illnesses and outbreaks over the past decade. Non-O157 STEC can be spread via the same pathways as *E. coli* O157:H7; foodborne, waterborne, animals and their environment, and person-to-person (Figure 2-2). The "Big 6" STEC do not always have all of the virulence factors exhibited by O157:H7, but often contain the

stx1 and/or *stx2* gene along with the *eae* gene (FSIS, 2012a). Due to the virulence factors that may be present, these strains can cause diarrhea and HUS in infected patients. Because of this, the FSIS declared the "Big 6" strains of STEC to be adulterants in non-intact raw beef products in June of 2012 (FSIS, 2012b).

Non-O157 STEC strains have likely caused foodborne illnesses long before recent outbreaks occurred, but went undetected due to various reasons. Less information was known about the genes of non-O157 STEC, therefore, detection methods were not as sensitive, more costly, and required more expertise than methods used to detect *E. coli* O157:H7 (Koohamariae et al., 2005). The CDC's Active Surveillance Network (FoodNet) reported that in the year 1999, 50% of clinical laboratories screened all stool samples for *E. coli* O157:H7, while only 3% had ever screened stool samples to detect non-O157 STEC (Griffin et al., 2001).

2.3.2 Public Heath Impact of Non-O157 STEC

The "Big 6" non-O157 STEC serotypes are estimated to account for over of 70% the 168,698 non-O157 STEC infections occurring each year in the United States (FSIS, 2012a). Since 2010, six major multistate outbreaks in the United States involving "Big 6" STEC strains, specifically O26, O121, and O145, have been documented and impacted a variety of food products (CDC, 2017). Although these outbreaks have led to dozens of hospitalizations and cases of HUS, only one death has been reported which was a result of an *E. coli* O145 outbreak throughout the southeastern United States in summer 2012, in which a foodborne source was never identified (CDC, 2012a). Another outbreak involving *E. coli* O145 in 2010 was linked to shredded romaine lettuce and led to 12 hospitalizations in the Great Lakes region of the US (CDC, 2010b).

Two *E. coli* O26 outbreaks have been associated with major fast food chains within the United States. A 2012 outbreak of *E. coli* O26 was attributed to raw clover sprouts consumed on sandwiches from 'Jimmy John's' Restaurants. This outbreak spread to 11 states across the United States, which led to 7 hospitalizations, although no patients developed HUS (CDC, 2012b). Shortly after the outbreak was discovered, the restaurant chain announced that they were permanently dropping sprouts as a menu item (Flynn, 2012). A 2015 outbreak of *E. coli* O26 was associated with 'Chipotle Mexican Grill', another national chain. Over 50 people became infected throughout the United States resulting in 22 hospitalizations, but no patients developed HUS from the outbreak. A single food ingredient was never identified as the cause of this outbreak, which was attributed to several ingredients being mixed or cooked together, complicating this process (CDC, 2016a). Reports of STEC infections related to flour emerged around the United States in spring 2016, with 46 cases reported and either *E. coli* O26 or O121 identified as the cause. Thirteen hospitalizations were associated with this outbreak, with one case of HUS, but no deaths were reported (CDC, 2016b). General Mills urgently responded by announcing a series of recalls, warning consumers about retail raw flour and certain cake mixes (General Mills, 2016).

E. coli O104 is not included in the USDA's "Big 6" adulterant strains, however, it was the cause of the largest and most deadly non-O157 STEC foodborne outbreak ever recorded. The source was raw sprouts from a farm in Germany that induced widespread illness. It was reported to cause 3,842 cases of illness, 855 cases of HUS and 35 deaths in Germany between May 8 and July 4, 2011 (Muniesa et al., 2012). The outbreak even spread to the United States, with 6 confirmed cases of infection; 5 of these patients having recently traveled to Germany.

One confirmed death from this outbreak in the United States highlighted the ability of foodborne outbreaks to spread internationally (CDC, 2011a).

Table 2-2. Overview of US multistate outbreaks of STEC over the past ten years (2006-2016)

Year	Strain(s)	Food Source	Cases	Hospitalizations
2016	O157:H7	Beef Products	11	7
2016	O121 & O26	Flour	63	17
2016	O157:H7	Alfalfa Sprouts	11	2
2015	O157:H7	Chicken Salad	19	5
2015	O26	Unidentified	55	21
2014	O121	Clover Sprouts	19	8
2014	O157:H7	Ground Beef	12	7
2013	O157:H7	RTE Salad	33	7
2013	O121	Frozen Food	35	9
2012	O157:H7	Spinach	33	13
2012	O145	Unidentified	18	9 (1 death)
2012	O26	Clover Sprouts	29	7
2011	O157:H7	Romaine Lettuce	58	33
2011	O157:H7	Bologna	14	3
2011	O157:H7	Hazelnuts	8	4
2010	O157:H7	Cheese	38	15
2010	O145	Romaine Lettuce	33	12
2010	O157:H7	Beef	21	9
2009	O157:H7	Beef	26	9 (2 deaths)
2009	O157:H7	Beef	23	12
2009	O157:H7	Cookie Dough	72	34
2008	O157:H7	Beef	49	27
2007	O157:H7	Frozen Pizza	21	8
2007	O157:H7	Beef Patties	40	21
2006	O157:H7	Shredded Lettuce	71	53
2006	O157:H7	Fresh Spinach	-	-

Derived from CDC, 2016a, 2016b, 2016c, 2015, 2014a, 2014b, 2013a, 2013b, 2012a, 2012b, 2012c, 2011b, 2011c, 2011d, 2010a, 2010b, 2010c, 2009a, 2009b, 2009c, 2008, 2007a, 2007b, 2006.

2.4 HACCP Validation in the Beef Industry

A year after the *E. coli* O157:H7 outbreak at Jack in the Box, the USDA FSIS declared *E. coli* O157:H7 to be an adulterant in raw non-intact beef products (FSIS, 1999; FSIS 2013). On July 25, 1996, a Final Rule was published by FSIS and titled Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems (FSIS, 1996). This rule required all meat and poultry plants to accept responsibility for identifying and controlling, reducing, or eliminating chemical, physical, and biological hazards (Keener, 2007). These requirements are described in the Code of Federal Regulations (CFR), Title 9, Part 417. All meat and poultry plants were given until the starting of the year 2000 to implement a HACCP program and also include a written sanitation program for the plant (FSIS, 1998, CFR, 1996). With the ultimate goal of pathogen reduction within food processing facilities, a new regulatory approach was implemented by the FSIS with four components: implement HACCP systems so products meet regulatory standards, train inspectors to ensure these standards are met, establish food safety performance standards for plants and ensure they are met, and strengthen enforcement to deal with plants who are unable to meet standards (FSIS, 1998). Essentially, the main focus of the government became the proper implementation of validation and verification procedures throughout industry.

The FSIS has released multiple documents to help clarify the expectations of validation within HACCP systems today. These documents emphasize the importance of validation of the entire HACCP system, including prerequisite programs, and breaks down validation into two parts: scientific support and initial in-plant validation. Supporting scientific documentation may come from one of five sources: published processing guidelines, peer-reviewed journal articles, inoculated pack studies, in-house data, or regulatory performance standards (FSIS, 2010; FSIS,

2015b). If this documentation uses a particular parameter, the same parameter must be used for the process. When validating a method in-plant, operational parameters such as time, temperature, pressure, or concentration are extremely important to define since laboratory conditions may often differ from those seen in a working environment. The next step of an in-plant validation is demonstration that the HACCP system is achieving the desired results, which are oftentimes presented by microbiological testing data. Samples need to be collected multiple times throughout the process, usually using surrogate organisms. For a successful validation to take place, the system must be theoretically sound, shown to reach the desired effect in-plant, and proven that the process can effectively operate on a daily basis (FSIS, 2010; FSIS 2015b).

2.5 Contamination of Beef with STEC

2.5.1 Introduction

Most Shiga toxin-producing *E. coli* are found in the intestines of cattle, making cattle a major reservoir for these pathogens. Cattle are not affected by STEC because they lack the vascular *stx* receptors (Ferens and Hovde, 2011). However, they do present a high risk of STEC contamination by way of pathogen shedding through feces or contamination during contact with hide. Due to these contamination pathways, STEC have the ability to spread to the surface of carcasses during cattle slaughter and eventually spread into the food supply. This results in STEC being directly related to numerous foodborne outbreaks related to beef products. Approximately 55% of foodborne outbreaks caused by *E. coli* O157:H7 and 50% of foodborne outbreaks caused by non-O157 STEC are attributed to beef (Moxley and Acuff, 2014).

Due to the association of STEC with beef, along with government regulations, intervention measures are implemented during processing to control pathogens. Intervention strategies begin with the application of good manufacturing practices (GMP's) in slaughter facilities. These include proper sanitation of all utensils and equipment throughout the process while using correct methods that avoid potential cross contamination (FSIS, 2015a). Other common beef industry intervention methods include steam vacuuming and hot carcass washing post-slaughter. The application of food-grade antimicrobials, primarily the use of organic acids, has also emerged as an effective intervention commonly used in the beef industry to control pathogens (Moxley and Acuff, 2014; Wheeler, 2014).

2.5.2 Commercial Beef Processing Flow

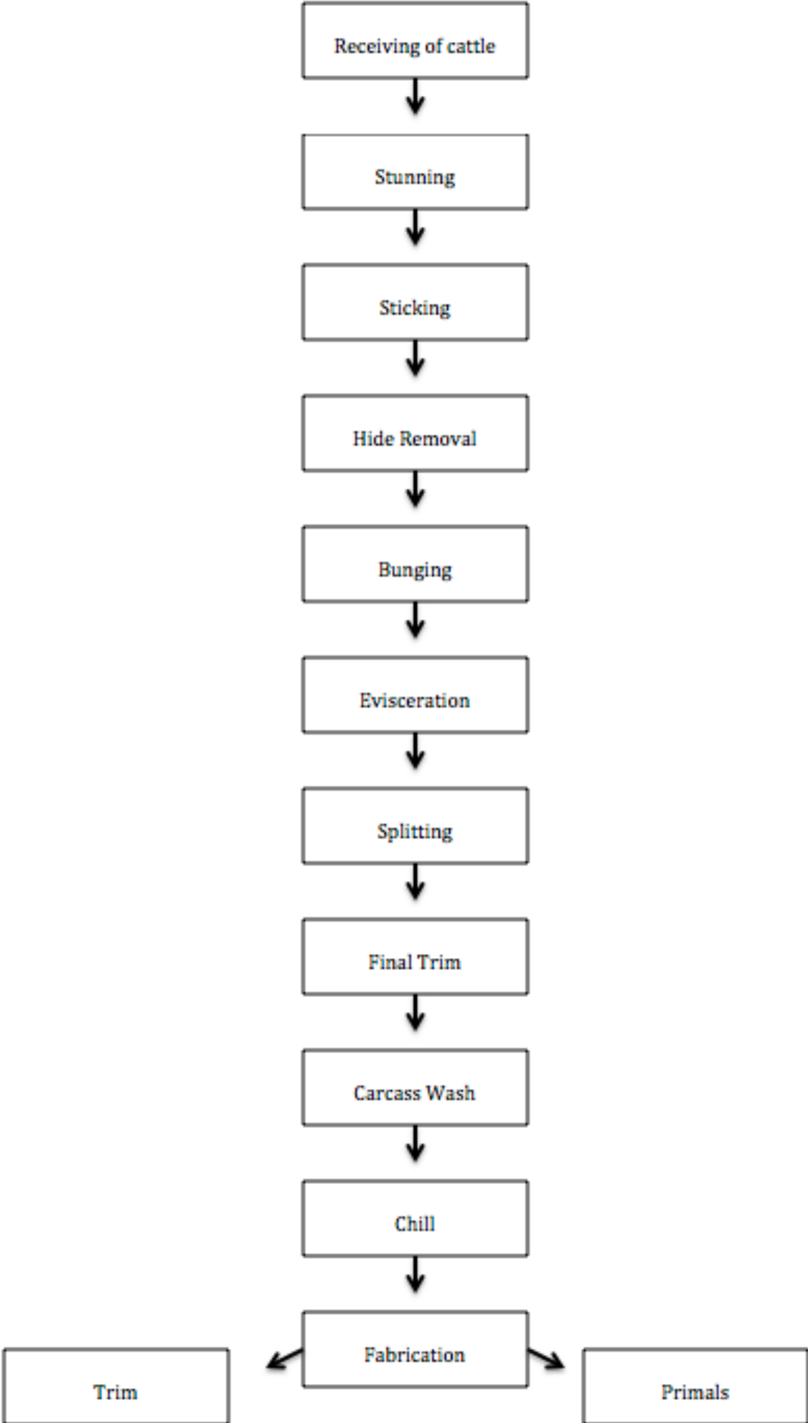
The FSIS provides descriptions of typical beef slaughter processing steps seen in large facilities. A flow diagram of this process is seen in Figure 2-3.

The following is a description of the steps commonly used during beef slaughter in commercial facilities along with a description of the best slaughter practices in order to meet regulatory guidelines for sanitation:

Receiving: Cattle are received from the trucks and placed into pens. They must be provided access to water at all times during this period. Prior to slaughter, the cattle are kept off of feed in order to fulfill requirements needed to facilitate dressing procedure (FSIS, 2015a).

All trailers and loading areas should be cleaned prior to each cattle loading period maintained in a safe condition. Facility personnel should observe the cattle unloading process to ensure proper handling of cattle. Any other equipment used in the process needs to be cleaned with pathogen-free water (Harris and Savell, 2003).

Figure 2-3 Flow chart of beef slaughter steps typically seen in large facilities.



Derived from FSIS, 2015a.

Stunning: Captive bolt is used throughout this process, as the industry norm. This mechanical technique is able to send the animal into an unconscious state with minimal discomfort. The stunning area should be maintained in good sanitary conditions.

Sticking: A blade is used to sever the carotid artery and jugular vein in the neck, which leads to death of the animal. The animal will be hung from its hind legs and head down to collect blood (FSIS, 2015a)

The knife used to cut the vein needs to be properly sanitized using hot water (180° F for 4-6 s) or a chemical sanitizer. A different knife should be used to make the initial cut into the hide and this opening should be kept as small as possible (Harris and Savell, 2003).

Hide Removal: In large processing facilities, this process is done using mechanical hide pullers. This equipment attaches to the hide near the hind section of the animal and pulls the hide off slowly toward the head. Due to the risk of cross contamination from hide, critical sanitation procedures are implemented.

The opening on the exterior of the hide must be done as cleanly as possible and visible contamination must be handled with air knives and vacuums. Multiple employees should be present to help skin the hide off the carcass. Knives need to be properly sanitized using hot water (180° F for 4-6 s) or a chemical sanitizer (Harris and Savell, 2003).

Bunging: This process involves detaching the muscles attached to the rectum and removing this part. This is a key step in preventing fecal contamination from spreading throughout the carcass. One incision should be made during the separation and the bung should be hung on a hook to prevent contamination to the carcass. The knife used should be properly sanitized with hot water (180° F for 4-6 s) or a chemical sanitizer. If any contamination

does occur, the carcass needs to be identified and corrective actions properly implemented to remove the contamination (Harris and Savell, 2003)

Evisceration: This involves the removal of all internal organs found in the carcass and is always done by hand. The contents of organs such as the stomach and intestines will contain potentially harmful bacteria. Good sanitation procedures need to be implemented on the viscera table and all viscera should remain intact throughout the process.

Splitting: Using a splitting saw the carcass is split into two halves. The saw should be rinsed with 180° F water throughout the process and saw housing dipped in between different carcasses. Any carcass identified as having contamination should be sent to the out rail for reconditioning.

Final Trim: This step provides another step to trim off any visible contamination to improve safety and quality of the carcass.

Carcass Wash: A carcass washing procedure is intended to remove any remaining blood, dust, hair, and provides a food safety and quality impact. A wash cabinet is often used and can use a variety of spray patterns, water pressures and temperatures, and durations of spraying. Typically, hot water (160 – 180° F) will be applied to the carcass for around 5 or more seconds. All drains need to be working properly and water pressure needs to remain low enough to not drive any contamination into the tissue and fat. During this step, a wash with an antimicrobial such as organic acids may also be performed. This intervention is used to reduce the likelihood of pathogens that remain on the carcass before the chilling process (Harris and Savell, 2003)

Chilling: A proper chill step will inhibit the growth of microorganisms on the carcass. Blast chilling or spray chilling are some of the many methods to chill the carcass down to an acceptable temperature range. Although the United States does not have a regulatory requirement for initial chilling, it is common practice for plants to implement the critical control point (CCP) of less than or equal to 4° C carcass surface temperature within 24 hours of slaughter (Savell, 2012).

The chilling process delays the conversion of muscle to meat, known as rigor mortis. Once the animal is dead, an oxygen supply is cut off and the reserve of adenosine triphosphate (ATP) becomes depleted. The bonds between actin and myosin can no longer be broken and the muscle becomes tough and inextensible. A build up of lactic acid occurs leading to a drop in the pH from around 7.0 to approximately 5.6. Lower chilling temperatures will slow the development of rigor mortis while higher temperatures cause rigor mortis to develop more rapidly. If the meat is chilled too rapidly before the onset of rigor mortis, a phenomenon known as "cold shortening" will result and have a negative impact on the meat toughness. Chilling is the last step of the carcass slaughter process before further processing of the meat into either primals or trim (Savell, 2012; Savell, 2016).

2.6 Intervention Strategies to Control STEC Contamination During Beef Processing

2.6.1 Introduction

In response to numerous foodborne outbreaks associated with beef products, many intervention strategies have been researched and implemented throughout the industry in food

safety plans. Intervention methods commonly used within the beef industry today to control STEC include ambient and hot water carcass washes and application of organic acids or other acid blends.

2.6.2 Hot Water Carcass Washing

Hot water carcass washing was the earliest form of intervention for reducing microbial contamination of beef carcasses. Early studies of this method showed that beef carcasses treated with steam and a subsequent 2 min hot water spray (80 – 96° C) would significantly reduce bacterial contamination compared to untreated carcasses (Patterson, 1969).

Castillo et al. (1998a) evaluated a carcass wash that included a water wash followed by a hot water spray (95°C). This method was used to decontaminate carcass surfaces inoculated with *E. coli* O157:H7. Application of the carcass wash followed by a hot water wash reduced the mean amount of *E. coli* O157:H7 by 3.7 log CFU/cm^2. A carcass wash only treatment led to a 2.1 log CFU/cm^2 reduction of *E. coli* O157:H7.

Kalchayanand et al. (2008) evaluated the efficacy of a hot water wash (74° C) for reducing *E. coli* O157:H7 contamination on bovine heads using a commercial spray cabinet. Application of this hot water wash led to a 3.0 log CFU/cm^2 reduction when application lasted 12 s and 3.5 log CFU/cm^2 reduction after a 26 s application. Other studies commonly evaluate a hot water carcass wash in combination with a lactic acid spray (2%) with mixed results. Bosilevac et al. (2006) showed that a hot water (74°C) carcass treatment or a 2% lactic acid treatment reduced *E. coli* O157:H7 contamination on pre-evisceration beef carcasses. However, treatments used in combination led to no additional reduction in pathogens compared to hot water washing alone. Castillo et al. (1998b) applied a hot water (95° C) wash with a 55° C lactic

acid (2%) spray to different areas of a pre-rigor carcass to measure the reduction of *E. coli* O157:H7. The combination of treatments led to a slightly higher reduction of pathogens present than each treatment alone; hot water carcass washing alone led to a 4.0 – 4.8 Log CFU/cm^2 reduction.

2.6.3 Peracetic Acid Components

Peracetic acid (PAA), also known as peroxyacetic acid, is an organic acid commonly used as an intervention strategy for controlling bacteria in both raw meat and fresh produce processing. Peracetic acid has been used for years as a disinfectant in healthcare facilities and as an antimicrobial for the following areas: food processing, water and wastewater industries, plumbing, and paper and pulp industries (Rutala and Weber, 2008; Kaya, 2010). Also, it has been commonly used as an effective antimicrobial intervention for poultry carcasses and parts in meat processing facilities. Peracetic acid use in the beef industry has gained popularity and there is interest to increase the allowable concentrations for application to beef carcasses and subprimals (personal communication with beef industry representatives). If higher concentrations are proven effective against pathogens and do not affect quality attributes of beef products, peracetic acid use will likely continue to grow as a low-cost alternative to the other interventions presently used in the industry.

There are many peracetic acid solutions available for use in beef processing, usually consisting of an aqueous mixture of the following components: peracetic acid, hydrogen peroxide, acetic acid, sulfuric acid, 1 – hydroxyethylidene-1, 1- diphosphonic acid (HEDP), and water (FSIS, 2017; FDA, 2014).

Peracetic Acid: Peracetic acid belongs to the peroxide compound family and is more potent than hydrogen peroxide as an antimicrobial agent. It is created by an equilibrium reaction between hydrogen peroxide and acetic acid (FDA, 2014; Kaya, 2010).

Figure 2-4 Equilibrium Reaction of Peracetic Acid

Derived from FDA, 2014

The mode of action of peracetic acid is similar to other oxidizing agents as it disrupts cell membranes and denatures proteins. The hydroxyl radical (OH-) comes into contact with and reacts with oxidizable compounds. This reaction damages the microorganism's macromolecules such as carbohydrates and amino acids. The transfer of electrons facilitates oxidation; therefore, a strong oxidizing agent will transfer electrons more rapidly to the microorganism and inactivate the microorganism quickly (CDC, 2008; Kaya, 2010).

Due to its capabilities as an oxidizer, peracetic acid has bactericidal and virucidal capabilities. According to the CDC, at less than 100 ppm, peracetic acid will inactivate gram-positive and gram-negative bacteria, yeast, and fungi, in less than 5 minutes. However, if there is organic matter present, a concentration of 200-500 ppm is needed to gain the same effect. The amount of peracetic acid needed to inactivate viruses ranges anywhere from 12-2250 ppm (CDC, 2008).

Hydrogen peroxide: This compound (H_2O_2) is a strong oxidizing agent that is unstable. It is non-flammable but can spontaneously combust when put in contact with organic material and will readily decompose into oxygen and water. Hydrogen peroxide is commonly found in households for medicinal purposes and has a growing number of industrial and environmental applications (NCBI, 2016a; Linley et al., 2012). Hydrogen peroxide has bactericidal capability, which has created a position for its use in the food industry. It has been used in low concentrations for commercial post-harvest washes, surface disinfectants, and even as an effective wash to decontaminate apples (Linley et al., 2012). Hydrogen peroxide is most applicable in areas where its decomposition into by-products that are non-toxic (oxygen and water) is important.

Acetic Acid: This is a simple carboxylic acid that has some antibacterial and antifungal properties and is found as the active ingredient in vinegar. It can inhibit metabolism of carbohydrates and therefore inactivate an organism (NCBI, 2016b). Acetic acid is commonly used in the food industry as an acidity regulator under food additive code E260.

Sulfuric acid: This acid has a strong affinity for water and is often used to dehydrate different compounds. It is used as a catalyst in the equilibrium reaction conversion of hydrogen peroxide and acetic acid to peracetic acid and water (FDA, 2014). Sulfuric Acid also has the potential to lower the pH of the solution, which could add antimicrobial value.

HEDP: This compound (1-hydroxyethylidene-1, 1-diphosphonic acid) belongs to the chemical class phosphonates, which increase the solubility of certain ions and inhibit the precipitation of mineral compounds. Common uses of HEDP include industrial water treatment, swimming pool applications, detergents and cleansers, and personal care products (USITC,

2008). HEDP is included in the mixture of peracetic acid components to act as a stabilizing agent (FDA, 2014).

2.6.4 Comparison of Peracetic Acid Manufacturers

A variety of peracetic acid solutions from different manufacturers exist and are listed in USDA Directive 7120.1 (USDA, 2016). Inspexx™ (Ecolab; St. Paul, MN), Spectrum (Peroxychem; Philadelphia, PA), and Birkoside MP-2 (Birko Corporation; Henderson, CO) are three widely used peracetic acid solutions in the poultry industry and have been studied within the beef industry. Microtox Plus (ZEE Company; Chattanooga, TN) has emerged as a widely used peracetic acid solution around the beef industry and has a much higher FSIS-allowed concentration limit (1800 ppm) for application to beef carcasses or subprimals than the competitors (220 - 400 ppm). The Microtox Plus formulation always includes sulfuric acid while the formulations of the other solutions do not. Manufacturers of Spectrum and Birkoside MP-2 may optionally include sulfuric acid into their solutions (FSIS, 2017), but have chosen only to do so during certain seasons (FDA, 2011). Sulfuric acid is included into these peracetic acid solutions to help catalyze the reaction of hydrogen peroxide and acetic acid, but also has a very low pH value which may impact the final solution. The majority of research investigating peracetic acid application to beef surfaces to control STEC has used either Inspexx™ or Birkoside MP-2. There are limited studies published on the STEC reduction capability of Microtox Plus, which is a main focus of this thesis.

2.6.5 Lactic Acid Components

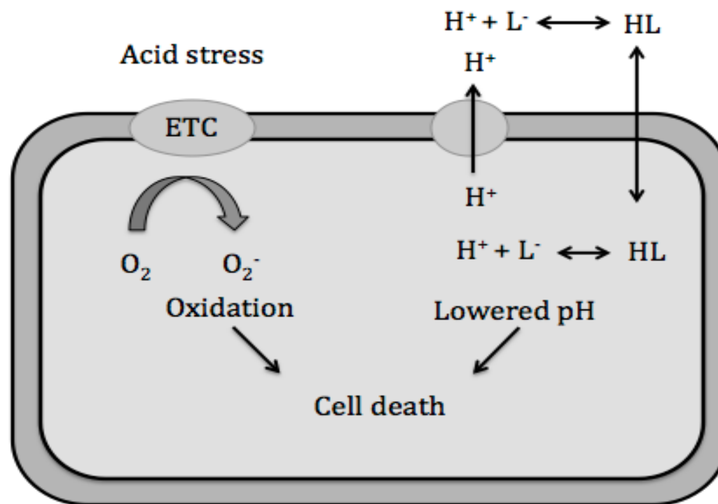
Lactic acid is an organic acid commonly used as an antimicrobial intervention on beef carcasses and subprimals. It is naturally produced by the human body, but can also be produced

synthetically. Common uses for lactic acid include moisturizing agent for cosmetic products, a chemical agent used in dying fabrics, and in the manufacturing of lacquers and inks. It is also widely used as a preservative in the food industry during the production of pickles, sauerkraut, yogurt, and cheese (Gotlib, 2016). In the beef industry it is the most commonly used organic acid intervention due to its low cost and effectiveness in controlling pathogens (Belk, 2001; Ransom et al., 2003).

L- lactic acid 88% (Birko Corporation, Henderson, CO) is the lactic acid concentrate widely used throughout the beef industry. It contains a mixture of 88% lactic acid and water, and is diluted with water to produce desired lactic acid concentrations following the formula $C_1V_1 = C_2V_2$. The mechanism of action for this antimicrobial is shown in Figure 2-5.

Lactic acid: This organic acid has the chemical formula $C_3H_6O_3$ and is able to inhibit microorganisms due to its ability to facilitate a low pH in and around a cell. Lactic acid is able to "shuttle" protons across cell membranes, which increases the acidity within the cell. Once this takes place, acid stress disrupts cell regulation and bacteria expend a great amount of energy trying to pump out acid and maintain their pH level. Also, the metabolism of the cell will be affected and acid stress will create damaging free radicals (Boomsma et al., 2015). An overview of this mechanism can be seen in Figure 2-5.

Figure 2-5 Overview of lactic acid mechanism of action to cause cell death



Derived from Boomsma, 2016

Lactic acid is manufactured either by microbial fermentation or chemically in an industrial setting. Batch fermentation of carbohydrates by bacteria is a widely used method to create lactic acid commercially. This process uses strains from the genus *Lactobacilli* that have been optimized and carbohydrates such as glucose, lactose, starch, or sucrose. This process is able to yield lactic acid at high rate (90-95 wt%) and takes 1-2 days to complete (Ghaffar et al., 2014).

2.6.6 Peracetic acid and Lactic Acid Use in the Beef Industry to Control STEC

The use of peracetic acid and lactic acid in the beef industry to control pathogens has had varied results and is dependent on factors such as product and/or solution temperature, application method, concentration, manufacturer of product, and type of meat. Due to the unstable characteristics of peracetic acid, it has been relatively difficult to pinpoint necessary concentrations needed to control pathogens on different food products. Research for both

peracetic acid and lactic acid in control of non-O157 STEC on beef products remains limited, especially on chilled beef surfaces.

Gill and Badoni (2005) evaluated the efficacy of peracetic acid and lactic acid on the microflora of chilled beef carcasses. Using the distal surfaces from brisket pieces of chilled beef quarters, 50 ml of peracetic acid was applied with a spray gun mist. Treatments were applied at 7±1°C and a concentration of 200 ppm for peracetic acid and 2% and 4% for lactic acid. The results showed that peracetic acid application was ineffective for aerobic, coliform, or *Escherichia coli* counts present on the samples. However, lactic acid application at both 2% and 4% achieved significant reductions of all three natural microbial populations.

King et al. (2005) evaluated peracetic acid and lactic acid as a post-chill intervention strategy to reduce inoculated rifampicin-resistant *E. coli* O157:H7 on beef carcasses. Peracetic acid was applied to carcasses pieces at three different concentrations (200, 600, and 1000 ppm) and two temperatures (45 and 55°C), while lactic acid was applied at 4% (55°C) for 15 s in a spray cabinet. Results showed that application temperature was an insignificant parameter and low levels of peracetic acid had no effect on the microbial counts of *E. coli* O157:H7 on this surface. Although peracetic acid concentrations up to 600 ppm had no effect, a concentration of 1000 ppm reduced rifampicin-resistant *E. coli* O157:H7 by up to 1.7 log CFU/cm^2 on chilled beef carcasses and application of 4% lactic acid achieved a 2.7 log CFU/cm^2 reduction. This experiment also evaluated the efficacy of peracetic acid when applied to the carcass surface prior to chilling. Peracetic acid (200 ppm) was applied to the outside round, plate, clod, and brisket sections of a beef carcass side prior to chilling using a hand-pump sprayer for 15 s, showing a 0.7 log CFU/cm^2 reduction of pathogens on this specific surface (ca. 6 log CFU/cm^2).

Ransom et al. (2003) compared 200 ppm peracetic acid and 2% lactic acid (55° C) to reduce *E. coli* O157:H7 on chilled beef cuts and trimmings, which were inoculated at a high (~6 log CFU/cm^2) and low (~4 log CFU/cm^2) level. The microbial reductions when plated on sorbitol MacConkey agar (SMAC) were 1.4 log CFU/cm^2 for both high and low inoculation levels after peracetic acid application compared to 3.3 (high inoculation) and 3.1 log CFU/cm^2 (low inoculation) after 2% lactic acid application.

Ellebracht et al. (2005) evaluated the efficacy of peracetic acid as a pre-grinding treatment on fresh beef trimmings to control inoculated rifampicin-resistant *E. coli* O157:H7. Three concentrations of peracetic acid (200, 500, and 1000 ppm) were applied to samples via submersion for 15 s and samples were evaluated post-treatment and post-grind. Samples treated with these three peracetic acid concentrations all exhibited reductions of at least 0.5 log CFU/cm^2 of *E. coli* O157:H7 with no significant difference between the treatments. Application of 2% lactic acid led to a 1.3 log CFU/cm^2 reduction of *E. coli* O157:H7. Post-grind data shows a slight increase in *E. coli* O157:H7, however, it was not significant.

Kalchayanand et al. (2012) evaluated commonly used intervention methods on fresh beef in order to control *E. coli* O157:H7 and the "Big 6" non-O157 STEC strains. Fresh beef flanks in the pre-rigor stage were inoculated with *E. coli* O26, O45, O103, O111, O121, O145, and O157:H7 before spray treatment with 200 ppm of peracetic acid or 4% lactic acid at 22-25°C for 15 s. Peracetic acid application led to reductions of 0.9 to 1.5 log CFU/cm^2 with no difference ($P > 0.05$) between *E. coli* O157:H7 and non-O157 STEC (except for O111). Lactic acid application led to significant STEC reductions of 1.6 to 2.7 log CFU/cm^2 with no differences ($P > 0.05$) between *E. coli* O157:H7 and non-O157 STEC strains.

Liao et al. (2015) evaluated numerous intervention methods for controlling *E. coli* O157:H7 and four non-O157:H7 STEC strains on chilled beef subprimals. Beef strip loins were inoculated with either *E. coli* O157:H7 or a cocktail containing *E. coli* O26, O103, O111, and O145 at a high (~6 log CFU/50 cm^2) and low (~2 log CFU/50 cm^2) level. Subprimals were subjected to a spray application of antimicrobials using a customized cabinet. Samples were treated with either 200 ppm peracetic acid (23°C) or 5% lactic acid (23°C), sampled, and then placed in vacuum packaged chilled storage for 14 days before a second sampling point. Results from the post-treatment sampling point showed that peracetic acid application reduced *E. coli* O157:H7 contamination by a significant level (0.4 log CFU/50 cm^2) when applied to subprimals inoculated at a high level. The 5% lactic acid application provided similar results with a 0.5 log CFU/50 cm^2 reduction of *E. coli* 0157:H7 on subprimals with high-level contamination. Low-level inoculated subprimals had an insignificant reduction of *E. coli* O157:H7 when treated with either peracetic acid or lactic acid. However, subprimals inoculated with the non-O157:H7 STEC cocktail provided differing results; peracetic acid and lactic acid application both led to significant reductions in pathogen contamination when inoculation was at the low-level (0.3 and 0.5 log CFU/50 cm^2, respectively), but not for high-level contamination. Analysis of the 14-day samples after vacuum packaged chilled storage demonstrated no significant reductions in pathogen contamination for any of the subprimals regardless of pathogen type or treatment.

Penney et al. (2007) evaluated the efficacy of peracetic acid as an intervention to reduce levels of *E. coli* O157:H7 on the exterior of pre-rigor beef and veal carcasses using a commercial spray apparatus. These carcasses were inoculated at either a high (~6.0 log CFU/cm^2) or low (~3.0 log CFU/cm^2) level with *E. coli* O157:H7. Peracetic acid treatment (180 ppm at 20°C)

resulted in >3 log CFU/cm^2 reduction and just under a 2 log CFU/cm^2 reduction of *E. coli* O157:H7 contamination for high-level and low-level inoculation, respectively.

Peracetic acid is a relatively new intervention method to the beef industry compared to lactic acid, therefore, more research findings have been published evaluating the efficacy of lactic acid against STEC, especially on chilled surfaces. Castillo et al. (2001a) applied 4% lactic acid at 55°C (at source) as an intervention method on non-inoculated chilled beef carcasses. Significant reductions of aerobic bacteria (3.0 - 3.3 log CFU/100 cm^2) were observed, while coliforms and *E. coli* counts were reduced to undetectable levels, albeit the populations of the later two were already near the detection limit of the counting method. A related study by Castillo et al. (2001b) applied 4.0% lactic acid at 55°C to chilled beef carcasses to control *E. coli* O157:H7; in this case, pathogens were reduced by 2.0 – 2.4 log CFU/cm^2 from an initial inoculation level of 8.0 log CFU/cm^2.

Pittman et al. (2012) evaluated the efficacy of lactic acid as an intervention for *E. coli* O157:H7 and non-O157 STEC on chilled beef subprimals. Samples were inoculated with either a cocktail containing strains of rifampicin-resistant *E. coli* O157:H7 or a cocktail containing rifampicin-resistant strains of *E. coli* O26, O45, O103, O111, O121, and O145 (to achieve an inoculation level of 6.0 log CFU/cm^2). When plated on Tryptic Soy Agar (TSA) supplemented with rifampicin, application of 5% lactic acid at 55°C using a commercial spray cabinet achieved a 1.6 log CFU/cm^2 reduction of *E. coli* O157:H7 and non-O157 STEC on the subprimals.

Wolf et al. (2012) compared the efficacy of lactic acid application through a dip or spray method to reduce *E. coli* O157:H7 and non-O157 STEC inoculated at 5.5 log CFU/cm^2 on chilled beef trim. When 4.4% lactic acid at ambient temperature was applied using immersion (5 s),

reductions of 0.91 to 1.41 log CFU/cm^2 were seen for *E. coli* O157:H7 and 0.48 to 0.83 log CFU/cm^2 for non-O157 STEC. When lactic acid was applied using a spray cabinet, there were no significant reductions of these pathogens.

Overall, research shows that certain concentrations of peracetic acid and lactic acid effectively reduce STEC on different beef surface types. Continued research evaluating the efficacy of these antimicrobials over a wide range of concentrations would be beneficial for the industry as regulatory requirements are often updated. As non-O157 STEC concerns grow, further research proving efficacy of peracetic acid and lactic acid against these pathogens is vital.

2.6.7 Centron™ Components and Use to Control Pathogens

Centron™, an antimicrobial product manufactured by the Zoetis Company (Parsippany-Troy Hills, NJ), is emerging as a possible intervention method for the beef industry. Centron™ is composed of an aqueous mixture of sulfuric acid and sodium sulfate to be used on meat surfaces as a spray, wash, or dip (FSIS, 2017). Although limited research has been completed using this specific antimicrobial, it is believed to be effective against common pathogens seen in meat such as STEC and *Salmonella spp.* (Zoetis, 2016). In 2012, the FDA designated Centron™ as a Substance Generally Recognized as Safe (GRAS) as an antimicrobial agent on meat surfaces at a pH range of 1.0 to 2.2, where it remains regulated today (FDA, 2012; FSIS, 2017). When applied at a low pH, Centron™ is believed to have an effect at reducing microbial levels and preventing the growth of microorganisms on the surface of meat. According to the manufacturer, this specially formulated mixture also does not cause any negative quality attributes to the product and has minimal impact on the organic load (Zoetis, 2016). These

characteristics could prove Centron™ to be an effective intervention against STEC in the beef industry, both alone and when used in combination with other antimicrobials to create a synergistic effect.

Various studies have evaluated Centron's efficacy as an antimicrobial intervention on beef products. Weinroth et al. (2015) compared the ability of Centron™ at a high (1.3) and low (1.05) pH to reduce natural microflora on whole beef carcasses. In both scenarios, APC counts were reduced by at least 1.0 log CFU/cm^2 with no significant differences between the different pH levels. Thus, Centron™ was proven to be an effective treatment to reduce natural microflora on a hot carcass.

Some studies evaluate this antimicrobial under its former marketing name, AFTEC 3000. Yang et al. (2014) compared the efficacy of 1.5% AFTEC 3000 and 4% lactic acid at heated and room temperatures to reduce rifampicin-resistant *Salmonella* strains on pri-rigor beef briskets. Each treatment was adjusted to either 21°C (ambient) or 52°C (heated) and sprayed on the beef briskets using a custom spray cabinet for 5 seconds. All treatments produced at least a 1.6 log CFU/cm^2 reduction of the *Salmonella* strains present (initial inoculation level of 6-7 log CFU/cm^2). The heated treatments for each antimicrobial produced slightly higher log CFU/cm^2 reductions, however, no significant ($P > 0.05$) differences were detected compared to the non-heated treatments. There were also no differences ($P > 0.05$) observed between AFTEC 3000 and lactic acid although the both effectively reduced ($P \leq 0.05$) the microbial loads compared to the control.

Geornaras et al. (2012) evaluated the ability of AFTEC 3000 (pH 1.2) to reduce *E. coli* O157:H7 and the "Big 6" STEC strains on chilled beef trimmings and compared it with other

antimicrobials such as acidified sodium chlorite, Bromitize Plus, peracetic acid, sodium metasilacate, and SYNTRx 3300. The beef trimmings were immersed in their respective treatments for 30 seconds and sampled after an hour. In this case, AFTEC 3000 only reduced STEC populations by 0.3 to 0.4 Log CFU/cm^2. Although these results were found to be statistically significant ($P \leq 0.05$) when compared with the control treatment, the reductions were much smaller than what was observed with other treatments such as peracetic acid, acidified sodium chlorite, and sodium metasilicate.

Chapter 3 - Efficacy of Peracetic Acid and Lactic Acid at Increasing Concentrations to Control Shiga Toxin-Producing *Escherichia coli* (STEC) Contamination on Chilled Beef Subprimals

3.1 Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are important foodborne bacterial pathogens that pose a serious public health risk. The Centers for Disease Control and Prevention (CDC) estimates that STEC cause 265,000 illnesses, 3,600 hospitalizations, and 30 deaths annually in the United States (CDC, 2012d). Symptoms of STEC infection include nausea, mild to severe bloody diarrhea, and severe diseases such as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) that lead to kidney failure (Kaper and O'brien, 1998). Cattle are known to carry STEC within their gastrointestinal tract (Moxley and Acuff, 2014), which presents a risk of bacterial contamination throughout the transformation process from live animal to fresh beef products (Ellebracht et al., 2005). Therefore, control of these pathogens in beef processing facilities is vital.

The United States Department of Agriculture Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7, the most prominent STEC strain, to be an adulterant in raw ground beef in 1994 (FSIS, 1999), which made it a main food safety related focus of the food industry, researchers, and regulatory agencies. *E. coli* O157:H7 remains an issue today due to its involvement in at least 19 major multistate foodborne outbreaks within the United States among a variety of food products since 2006 (CDC, 2017). Recently, non-O157 strains of STEC have garnered attention, with 2010 marking the first year that non-O157 STEC strains collectively caused more identified illnesses than O157:H7 (Shaw, 2012). Six STEC serogroups

(O26, O45, O103, O111, O121, and O145) account for at least 70% of reported and serogrouped non-O157 isolates from ill individuals where STEC are identified as the causative agent (Brooks et al., 2005*,* FSIS, 2012a). In 2012, the FSIS declared these serogroups, known as the "Big 6", to be adulterants in raw, non-intact beef products (FSIS, 2012b). Collectively, these STEC strains have a strong association with outbreaks in the beef industry. A reported 55.3% of O157 and 50% of non-O157 STEC outbreaks seen throughout all food commodities combined in the United States are attributed to beef (Moxley and Acuff, 2014).

Beef processing plants have implemented intervention techniques to control STEC contamination at different steps throughout the carcass dressing process (Gill and Badoni, 2004). Popular intervention methods used in the beef industry include application of organic acids such as lactic acid and peracetic acid. Lactic acid has been widely studied at different concentrations and application temperatures, showing an overall effectiveness in reducing *E. coli* O157:H7 populations on beef (Ransom et al., 2003). Peracetic acid has become widely utilized in the industry in terms of beef application, and has long been used as a sanitizer that effectively reduces *E. coli* O157:H7 on food contact surfaces (Farrel et al., 1998; Rassoni and Gaylarde, 2000). When applied to beef surfaces, peracetic acid has varying effectiveness in reducing STEC, depending on concentration, application method, and manufacturer (Gill and Badoni, 2005; King, 2005; Liao, 2015).

Although there is evidence that lactic acid and peracetic acid effectively lower STEC populations on fresh beef, there is question whether or not these organic acids exhibit the same effectiveness when applied to chilled beef surfaces. There is also limited research investigating the ability of these organic acids to reduce the "Big 6" non-O157 strains of STEC

on beef. Currently, FSIS regulates the limit for lactic acid applied to beef carcasses, subprimals, and trim to 5.0% (FSIS, 2017), while peracetic acid is regulated to a limit of 220 – 1800 parts per million (ppm) depending on the manufacturer of the product (FSIS, 2017). Interest continues in possibly updating the regulatory limits for organic acid application on beef (personal communication with beef processors), thus, evaluating the antimicrobial efficacy and beef product impacts of a wide range of concentrations becomes necessary.

The main objective of the research reported in this chapter was to evaluate the efficacy of increasing concentrations of Microtox Plus™, a stabilized peracetic acid and hydrogen peroxide blend (Zee Company, Chattanooga, TN) or lactic acid (88% concentrate, Birko Corporation, Henderson, CO) as antimicrobial sprays for reducing populations of STEC on chilled beef subprimals. This study also determined: 1) the impact of applying these antimicrobial sprays on subprimal color before and after vacuum packaged subprimal storage; and 2) the extent of lipid oxidation (TBARS) of vacuum packaged subprimals after 24-h storage.

3.2 Materials and Methods

3.2.1 Experimental Design

This study consisted of two experiments, one evaluating peracetic acid and one evaluating lactic acid, which were each repeated on three different days (replications). Vacuum packaged beef strip loins (~5.0 – 6.5 kg) were obtained through the Kansas State University Meat Laboratory. Each experimental replication for each antimicrobial spray utilized two strip loins that were cut into 10 pieces (0.9 - 1.3 kg) for the peracetic acid experiment and 12 pieces (0.8 – 1.2 kg) for the lactic acid experiment. These pieces were randomly assigned to their respective chemical treatments. In total, 30 and 36 subprimal pieces were used, respectively,

for the peracetic acid and lactic acid experiments. For each replication, a new STEC inoculum cocktail was propagated and fresh antimicrobial solutions were prepared from the original stock concentrate.

3.2.2 Bacterial Cultures

Rifampicin-resistant derivatives (100 µg/ml) of *E. coli* O157:H7 (ATCC 31150; human isolate) and non-O157 STEC strains O26 (H30, human isolate), O45 (CDC 96-3282, human isolate), O103 (CDC 90-3128, human isolate), O111 (JB1-95, clinical isolate), O121 (CDC 97-3068, human isolate), and O145 (83-75, human isolate) were obtained from Dr. John Luchansky (USDA Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA). Upon receipt, strains were propagated in 10 ml sterile tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) supplemented with 0.1 g/L rifampicin (TSB_{rif}) Tokyo Chemical Industry, Tokyo, Japan), with incubation at 37°C for 24 h. Each culture was streaked for isolation on tryptic soy agar (Difco) supplemented with 0.1 g/L rifampicin (TSA_{rif}) and incubated at 37° C for 24 h. Plates were sealed with Parafilm® and were stored at 4°C as working stock cultures. For long-term culture storage, 24-h broth cultures were placed onto protectant beads in glycerol (Pro-Lab Diagnostics Microbank Bacterial Preservation System, Fisher Scientific) and stored at -80°C.

3.2.3 Inoculum Preparation and Application

In preparation for trials, an isolated colony from each strain was transferred to 10 ml of TSB_{rif} and incubated at 37° C for 24 h. Inoculum was prepared for each of the three replications of each experiment by combining 10 ml each of rifampicin-resistant *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145 into a sterile container to create 70 ml of a 7-serogroup mixture. A 2-ml aliquot of the mixture was transferred to a handheld spray bottle containing 200 ml of

0.1% peptone water (Difco). For each replication, subprimal pieces were randomly assigned to a treatment and placed on a sanitized lunchroom style tray prior to inoculation. The handheld spray bottle containing 202 ml of inoculum was calibrated and approximately 3 ml was misted evenly over the top and bottom surfaces of each subprimal (total of 6 ml per piece). To control infectious aerosols during this misting process, each tray containing the subprimal piece was placed inside of a large biohazard bag within a biosafety cabinet and the technician utilized a plastic shoulder sleeve to enter the cabinet to operate the spray bottle. Each subprimal was placed into chilled storage (7°C) for 30 min to allow attachment of the STEC cells to the beef surface. For each replication, serial dilutions of the mixed culture inoculation solution were plated onto TSA_{rif} to verify STEC concentration applied to each meat surface.

3.2.4 Beef Subprimals Used for Studies

Beef strip loins used throughout the studies originated from cattle slaughtered (~3 weeks prior to research use) at National Beef Packing Company (Kansas City, Missouri) and were trimmed to leave 1/4" of fat on the outside surface of the subprimal. Previous carcass and subprimal antimicrobial applications cannot be specified, however, it is likely that typical industry interventions were applied at the processing facility before vacuum packaging and shipment. All subprimals were obtained and kept in vacuum packaged storage (4°C) no more than 24 h before experimental procedures. Subprimal pieces were used for research purposes within 2 h of removal from vacuum packaged storage.

3.2.5 Antimicrobial Solution Preparation

For the first experiment, solutions of peracetic acid (Microtox Plus™, Zee Company, Chattanooga, TN) were prepared according to manufacturer's recommendations. Peracetic

acid concentrations of 200, 400, 600, 800, 1000, 1200, 1400, 1600, and 1800 ppm were prepared along with a water control (0 ppm) by mixing the chemical with ambient tap water in a chemical fume hood. For the lactic acid procedure, solutions of *L*-lactic acid (88% lactic acid in water; Birko Corporation, Henderson, CO) were prepared at concentrations of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 9.0, and 10.0 % along with a water control (0%). The solutions were prepared by mixing the concentrated (88%) lactic acid in 55° C tap water and were held at 55° C after mixing until application. Peracetic acid concentrations were confirmed by titrating 5 ml of solution with 0.1 N sodium thiosulfate (Fisher Scientific, Waltham, MA) using a starch indicator (1% w/v; Fisher Scientific). Lactic acid concentrations were confirmed by titrating 5 ml of solution with 0.25 N NaOH (Fisher Scientific) using phenolphthalein (Fisher Scientific) as an indicator. Fresh antimicrobial solutions were prepared for each experimental replication from the original stock concentrate.

3.2.6 Application of Antimicrobials

All product treatments and packaging/storage were conducted within a biosafety level-2 pilot processing laboratory by trained laboratory personnel and utilizing University Research Compliance Office approved protocols, including appropriate personal protective equipment. Experimental replications consisted of each chilled subprimal receiving spray treatment with peracetic acid for the first experiment and lactic acid for the second experiment. After a 30-min inoculum attachment period at 7° C, each subprimal was placed on a wire rack and randomly assigned an antimicrobial spray treatment. Antimicrobial sprays were applied (6 seconds per side) using a 4.0 L hand-pump garden sprayer (Chapin International, Inc., Batavia, NY) that was calibrated before each treatment application. In total, 120 ml (30 ml per each of 4 sides) of

antimicrobial was applied to each subprimal piece, followed by a subsequent 5-min drip time at 7°C. Subprimal pieces were then placed into Cryovac bags (Cryovac, Inc., St. Joseph, MO) and vacuum packaged (240-260 mm Hg) using a MV45 vacuum packager (Minipack-torre, Dalmine, Italy) before placement in a dark cooler (4° C) for 24 h.

3.2.7 Microbial Sampling

Three sampling points were used during processing to determine rifampicin-resistant STEC populations on subprimals: post-inoculation after a 30-min attachment period, post-antimicrobial spray application after a 5-min drip time, and post-24 h of vacuum packaged chilling. Tissue excision samples were obtained from the top and bottom sides of each subprimal piece at each sampling point using a sterile corer (5.2 cm diameter), scalpel, and forceps. The surface area samples from the top and bottom were combined (42.45 cm^2 total) into a sterile filter stomacher bag containing 75 ml DE neutralizing broth (Difco) and stomached for 1 min in an AES Blue Line Smasher™ (Biomerieux, Marcy-l'Etoile, France). Each sample was then serially diluted in 0.1% peptone water and plated in duplicate on TSA_{rif} (selective plating), and tryptic soy agar containing no rifampicin supplement but overlaid with TSA_{rif} after 6 h of incubation to account for sublethally injured STEC cells. All plates were incubated at 37° C for 24 h and rifampicin-resistant *E. coli* colonies were counted. The direct plating detection limit for each study was -0.05 log CFU/cm^2.

3.2.8 Color Evaluation

Subprimal color was measured at three sampling points: post-inoculation after a 30-min attachment period, post-antimicrobial spray application after a 5-min drip time, and post-24 h chill (measured 10 min after exposure to air). A calibrated MSEZ 4500L spectrophotometer (D65

illuminant, 10° observer, and 0/40 geometry; Hunter Associated Laboratories Inc., Reston, VA) was used to obtain color data. Readings were taken based on the CIE Lab Color scale and L*, a*, and b* values were recorded at each sampling point from the left and right side of the subprimal piece (cut lean surfaces), with measurements taken from the middle of the loin muscle.

3.2.9 TBARS Analysis

A thiobarbituric acid (TBARS) test was performed according to the American Meat Science Association (AMSA) guidelines (AMSA, 2012) post 24-h chill to estimate lipid oxidation due to the organic acid spray treatments. Samples were obtained from the left and right side cut surface of the subprimal piece using a 5.2 cm diameter corer and scalpel, removing the surface area to a depth of ~3 mm. A 0.5 g surface tissue sample was combined with the TBA stock solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N HCl and mixed well. The tubes containing samples were placed in boiling water for 10 min before being centrifuged in a Fisher Scientific Marathon 21000R centrifuge (Fisher Scientific) at 5000 × *g* for 10 minutes at 10°C. The supernatant was placed into a cuvette and the absorbance was measured at 532 nm against a blank containing only the TBA stock solution. The final value was expressed as ppm malonaldehyde (AMSA, 2012). During the peracetic acid experiment, two replications were completed evaluating samples post-24 h chill. For the lactic acid experiment, three replications were completed evaluating samples post-24 h chill and post-72 h chill (day 3). Small pieces (~15 g) were sliced off the side of each subprimal piece (to a depth of 3 mm) and vacuum packaged separately to be used for the day 3 chilled storage samples.

3.2.10 Statistical Analysis

The peracetic acid and lactic acid experiments each consisted of three replications using 10 and 12 subprimal pieces per replication, respectively. All microbial counts were transformed into log CFU/cm^2 before statistical analysis was completed. Microbial counts, color values, and TBA values were analyzed in a linear mixed model using the SAS system (SAS Institute Inc., Cary, NC) PROC GlIMMIX function with the fixed effects of treatment concentration for the microbial and TBA analyses and treatment concentration, sampling time, and treatment concentration by sampling time for the color analysis. Treatment levels were compared at a significance of $P \leq 0.05$.

3.3 Results and Discussion

3.3.1 Effectiveness of Antimicrobial Sprays

Reductions in the rifampicin-resistant STEC levels on chilled beef subprimal pieces from post-inoculation to post-peracetic acid application are shown in Table 3-1. When plated on selective media, peracetic acid application resulted in a 0.5 – 1.3 log CFU/cm^2 reduction in STEC populations across the range of treatment concentrations compared to a 0.1 log CFU/cm^2 reduction observed using the water control spray. When plated on a non-selective media overlaid with a selective media, peracetic acid application resulted in a 0.5 – 1.1 log CFU/cm^2 reductions in STEC populations across treatment concentrations compared to a 0.4 log CFU/cm^2 reduction observed in the water control. When plated on the selective media, all concentrations of peracetic acid were different ($P \leq 0.05$) than the water control sample. There was no difference ($P > 0.05$) between concentrations of 400 – 1800 ppm peracetic acid except for 1600 ppm, which provided a slightly higher STEC reduction ($P \leq 0.05$) than all other

concentrations. When treated samples were plated on overlay media to detect sublethally injured STEC cells, no differences in population reductions ($P > 0.05$) were found across all concentrations of peracetic acid, including the water control. However, there was a trend of gradually increased reductions as peracetic acid concentrations increased up to 800 ppm. Concentrations of 1000 ppm and above produced fluctuating reductions when plated on overlay media, similar to what was observed when samples were plated on selective media, with a maximum STEC reduction of 1.1 log cycles observed for the 1600 ppm treatment.

Table 3-1. STEC reductions on chilled beef subprimal surfaces after spraying with increasing concentrations of ambient temperature peracetic acid[a]

Concentration PAA (ppm)	*Mean* ± SE *rifampicin-resistant* E. coli *reductions from post-inoculation to post-peracetic acid application (log CFU/cm²)*	
	Selective Media	Overlay Media[b]
0[c]	0.1 ± 0.23 a	0.4 ± 0.17
200	0.5 ± 0.13 b	0.5 ± 0.17
400	0.6 ± 0.08 bc	0.7 ± 0.07
600	0.7 ± 0.10 bc	0.8 ± 0.28
800	0.9 ± 0.05 c	0.9 ± 0.02
1000	0.9 ± 0.13 c	0.6 ± 0.36
1200	0.6 ± 0.02 bc	0.8 ± 0.15
1400	0.8 ± 0.03 bc	1.0 ± 0.25
1600	1.3 ± 0.11 d	1.1 ± 0.18
1800	1.0 ± 0.18 c	0.9 ± 0.12

[a] a to d, means within media type that do not share a common letter are statistically different ($P \leq 0.05$)

[b] Overall, no significant difference ($P > 0.05$) was found between peracetic acid concentrations when plated on an overlay media, therefore, letters expressing statistical distinctions are not included in this column.

[c] Water control sample, randomly assigned to a subprimal with the study and applied under same parameters as all peracetic acid concentrations.

Additional STEC reductions on subprimals were measured from post-peracetic acid application to post-24 h chill. Subprimals that had previously been treated with any concentration of peracetic acid did not show an increased STEC reduction ($P > 0.05$) compared to the STEC reduction observed from the water treated control during the chill period. This outcome held true when samples were plated on both selective and overlay media and indicates that any additional STEC reductions were due to the effect of vacuum packaging and chilled storage rather than residual concentrations of peracetic acid on the subprimal.

Reductions in rifampicin-resistant STEC levels from post-inoculation to post-lactic acid application are shown in Table 3-2. When plated on selective media, application of lactic acid resulted in a 0.2 – 0.7 log CFU/cm^2 reduction depending on the treatment concentration. Application of all lactic acid concentrations 3.5% and higher at 55°C resulted in a significant reduction ($P \leq 0.05$) of STEC compared to the water control, with no difference ($P > 0.05$) in STEC reductions across concentrations of 3.5 – 10%. Application of 3.0% lactic acid did not reduce STEC populations significantly ($P > 0.05$) compared to the control. Results from the overlay media analysis show that, although there is an increase in mean reduction of STEC as lactic acid concentrations increase, there was insignificant differences ($P > 0.05$) between all concentrations of lactic acid, including the water control, applied to the subprimal. STEC reductions measured on overlay media are all within ±0.2 log CFU/cm^2 to the corresponding values from the selective media and there is a trend of increasing pathogen reductions as lactic acid concentrations increase, despite the overall insignificant difference observed among concentrations evaluated. Data obtained from the post-24 h chill sampling point showed that additional STEC reductions (measured from the post-lactic acid spray sampling point) on

subprimals that were previously treated with any concentration of lactic acid were not different ($P > 0.05$) than the STEC reduction of the water treated control. Similar to the results of the peracetic acid study, this indicates that any additional STEC reductions were due to the effect of vacuum packaging and chilling rather than residual lactic acid concentrations present on the subprimals.

Table 3-2. STEC reductions on chilled beef subprimal surfaces after spraying with increasing concentrations of 55°C lactic acid[a]

	Mean rifampicin-resistant E. coli reductions from post-inoculation to post-lactic acid application (log CFU/cm²)	
Concentration LA (%)	Selective Media	Overlay Media[b]
0	0.0 ± 0.03 a	0.1 ± 0.03
3.0	0.2 ± 0.17 ab	0.4 ± 0.07
3.5	0.5 ± 0.10 bc	0.4 ± 0.04
4.0	0.4 ± 0.13 bc	0.4 ± 0.09
4.5	0.5 ± 0.02 bc	0.6 ± 0.07
5.0	0.4 ± 0.06 bc	0.4 ± 0.03
5.5	0.5 ± 0.05 bc	0.6 ± 0.06
6.0	0.5 ± 0.04 bc	0.7 ± 0.03
7.0	0.5 ± 0.11 bc	0.5 ± 0.16
8.0	0.6 ± 0.04 c	0.6 ± 0.06
9.0	0.6 ± 0.07 c	0.6 ± 0.12
10.0	0.7 ± 0.07 c	0.8 ± 0.04

[a] a to d, means within media type that do not share a common letter are statistically different ($P \leq 0.05$)

[b] Overall, no significant difference ($P > 0.05$) was found between lactic acid concentrations when plated on an overlay media, therefore, letters expressing statistical distinctions are not included in this column.

[c] Water control sample, randomly assigned to a subprimal with the study and applied under same parameters as all lactic acid concentrations.

Studies evaluating peracetic acid and lactic acid when applied to chilled beef subprimals and pre-rigor or chilled carcasses have shown varied efficacy at reducing microbial loads of

STEC (Ellebracht et al., 2005; Kalchayanand et al., 2012; King et al., 2005; Liao et al., 2015; Penney et al., 2007). Research evaluating peracetic acid application to chilled beef surfaces is limited, however, several studies have evaluated lactic acid in this scenario. Castillo et al. (2001a) applied 4% lactic acid (55°C at source) to chilled beef carcasses using a handheld compressed-air sprayer for 35 s and measured significant reductions in aerobic bacteria (3.0 – 3.3 log CFU/100 cm^2) and reduced coliforms and *E. coli* counts to undetectable levels (<1.4 log CFU/100 cm^2), although counts of each on untreated carcasses were already near the detection limit of the counting method. In an inoculated study, a lactic acid spray wash (2.0 or 4.0%; 55° or 65°C at source) was applied to chilled beef carcasses using a handheld compressed-air sprayer for 15 or 30 s, leading to a 2.0 – 2.4 log reduction of CFU/cm^2 of *E. coli* O157:H7 (Castillo et al., 2001b). Reductions observed in this carcass washing study were much higher in comparison to what was observed in the current study on chilled subprimals, which may be attributed to a longer spray time (15 or 30 s versus 5-7 s, respectively). Also, subprimals receive additional trimming of external fat, which could have impacted STEC attachment characteristics and antimicrobial spray contact. In studies involving lactic acid application to chilled beef subprimals and trim (Pittman et al., 2012; Wolf et al., 2012), varied efficacy against pathogens was observed. When 5.0% lactic acid (22 or 48°C) was applied to chilled beef subprimals using a spray cabinet, a 1.6 log CFU/cm^2 reduction was observed for both *E. coli* O157:H7 and non-O157 STEC (Pittman et al., 2012). After application of 4.4% lactic acid (ambient temperature) to chilled beef trim, significant reductions in *E. coli* O157:H7 (0.91 – 1.41 log CFU/g) and non-O157 STEC (0.48 – 0.82 log CFU/g) were reported using a dip method (5 s) (Wolf et al., 2012).

However, no significant reductions were reported from the same study when lactic acid was applied using a spray application method that would be more similar to the current study.

Two studies assessed the efficacy of peracetic acid and lactic acid on chilled beef surfaces. Gill and Badoni (2005) evaluated efficacy of these antimicrobials on native microflora of chilled beef carcass quarters. Application of 200 ppm peracetic acid (ambient temperature) resulted in no reduction of aerobic bacteria, coliform, or *E. coli* counts on the subprimal surfaces; however, application of 2% and 4% lactic acid (ambient temperature) led to reductions of all three microbial populations. King et al. (2005) evaluated peracetic acid and lactic acid as a post-chill intervention to control *E. coli* O157:H7 on beef carcasses and found that application of 200 and 600 ppm peracetic acid (43°C) had no effect on reducing microbial populations. However, when 1000 ppm peracetic acid (43°C) was applied to chilled beef carcasses, a 1.7 log CFU/cm^2 reduction of *E. coli* O157:H7 was observed. Application of 4% lactic acid (55°C) led to a 2.7 log CFU/cm^2 reduction of *E. coli* O157:H7, which was statistically greater than all other treatments except for 1000 ppm peracetic acid. The pathogen reductions observed at these concentrations were greater than what was observed in the current study, which may be attributed to a longer spray time and different application method to a carcass rather than subprimal. Ellebracht et al. (2005) compared application of 200 ppm peracetic acid (43°C) to 2% lactic acid (55°C) on chilled beef trim to control *E. coli* O157:H7. Although peracetic acid application led to a 0.7 log CFU/cm^2 reduction of *E. coli* O157:H7, the only significant reduction was observed with lactic acid application, which produced a 1.3 log CFU/cm^2 reduction of pathogens. Albeit these reductions are higher that what was measured using similar concentrations of these antimicrobials in the current study, this aforementioned

study used submersion rather than a spray application, had a longer contact time (15 s), and was evaluating a beef trim product rather than a loin cut.

Control of non-O157 STEC by peracetic acid or lactic acid has been evaluated, although research including chilled beef surfaces is limited. Kalchayanand et al. (2012) evaluated the ability of these antimicrobials to control *E. coli* O157:H7 and the "Big 6" STEC strains (O26, O45, O103, O111, O121, O145) on fresh beef flanks. Application of 200 ppm peracetic acid (22-25°C) was able to reduce pathogen contamination by 0.9 – 1.5 log CFU/cm^2 depending on strain. Application of 4% lactic acid (22-25°C) reduced pathogen contamination by 1.4 – 2.7 log CFU/cm^2 depending on strain. Although application of both of these antimicrobials resulted in significant reductions of STEC, application of hot water (85°C) produced the highest STEC reductions within the study (3.2 – 4.2 log CFU/cm^2). Liao et al. (2015) evaluated 200 ppm peracetic acid and 5% lactic acid application for controlling *E. coli* O157 and non-O157 STEC on beef strip loins. It was found that *E. coli* O157 was reduced when the sample was inoculated at a high level (~ 6 log CFU/cm^2) but not in samples with low-level inoculation (~ 2 log CFU/cm^2). Non-O157 STEC strains were not reduced in high-level inoculation samples, but were reduced in low inoculation level samples. Reductions of STEC observed in the study by Liae et al. were very similar to what was observed in the current study at the same concentrations, which may be attributed to them both using beef strip loins rather than larger carcass pieces.

3.3.2 Color Impact of Antimicrobial Treatments

The Hunter Miniscan used for each experiment measured L*, a*, and b* values using the CIELAB color scale. The L* scale ranges from 0 to 100, with low numbers representing darkness and high numbers representing lightness. The a* value measures red vs. green, with

positive values indicating red and negative values indicating green. The b* value measures yellow vs. blue, with positive values indicating yellow and negative values indicating blue (Hunter Laboratories, 2012). Subprimal L*, a*, and b* values were not different ($P > 0.05$) among all treatment concentrations of the peracetic acid experiment at each sampling point. However, there were significant differences ($P \leq 0.05$) between each sampling point throughout the experiment. All concentrations of peracetic acid and the water control became significantly ($P \leq 0.05$) lighter (L*) and less red (a*) at the post-treatment sampling point and again after a 24-h chill. Although there was a reduction ($P \leq 0.05$) in yellowness (b*) post-treatment, there were no changes ($P > 0.05$) after 24-h chill across all concentrations (Table 3-3).

Table 3-3. Color readings of chilled subprimals treated with peracetic acid[a]

	Mean ± SE color readings		
Sample[b]	L*	a*	b*
Post-inoculation[c]	44.80 ± 0.44 a	26.29 ± 0.23 a	20.74 ± 0.21 a
Post-PAA application[d]	43.58 ± 0.54 b	23.29 ± 0.29 b	18.96 ± 0.14 b
Post-24 h chill	41.89 ± 0.75 c	20.69 ± 0.54 c	18.83 ± 0.33 b

[a] L* measures light v. dark (100 = perfect white, 0= black); a* measures red (positive value) v. green (negative value); b* measures yellow (positive value) v. blue (negative value). Mean values for L*, a*, and b* (within columns) that do not share a common letter are statistically different ($P \leq 0.05$).

[b] Overall, there were no differences ($P > 0.05$) between all concentrations of peracetic acid within each sampling point, therefore, mean L*, a*, and b* were combined from each treatment.

[c] Readings were taken 30 min after inoculation.

Color analysis from the lactic acid experiment showed that post-inoculation, there were no differences ($P > 0.05$) between the L*, a*, and b* values across all concentrations. However, there were differences in the L* and b* values between treatment concentrations at the post-lactic acid application and post-24 h chill sampling points.

Table 3-4. Color readings of chilled subprimals treated with lactic acid at the post-treatment application sampling point[a]

LA concentration (%)	Mean ± SE color readings		
	L*	a*[b]	b*
0	43.73 ± 1.95 a	24.78 ± 0.22	20.00 ± 0.66 a
3	41.20 ± 2.50 ab	24.71 ± 1.13	19.96 ± 1.16 ab
3.5	39.11 ± 1.96 bc	25.00 ± 0.84	19.46 ± 1.11 ab
4	40.50 ± 2.97 bc	24.39 ± 0.89	19.12 ± 1.05 ab
4.5	39.96 ± 2.74 bc	25.04 ± 1.35	19.88 ± 0.67 ab
5	40.71 ± 2.45 abc	23.51 ± 0.34	18.86 ± 0.95 ab
5.5	39.45 ± 3.10 bc	23.76 ± 0.47	18.54 ± 1.06 abc
6	37.92 ± 1.12 c	24.75 ± 0.59	18.58 ± 0.33 abc
7	39.10 ± 1.08 bc	22.76 ± 1.27	18.06 ± 1.19 bcd
8	37.84 ± 2.05 c	22.20 ± 0.67	16.83 ± 0.41 cd
9	37.93 ± 0.26 c	22.24 ± 1.98	16.80 ± 1.63 cd
10	39.07 ± 1.22 bc	21.08 ± 1.13	16.38 ± 0.29 d

[a] L* measures light v. dark (100 = perfect white, 0= black); a* measures red (positive value) v. green (negative value); b* measures yellow (positive value) v. blue (negative value). Mean values for L*, a*, and b* (within columns) that do not share a common letter are statistically different ($P \leq 0.05$).

[b] Overall, no difference ($P > 0.05$) was found among a* values across all concentrations at this sampling point, therefore, letters expressing statistical distinctions are not included in this column.

Table 3-4 shows that post-lactic acid application, all concentrations of lactic acid 3.5% and above, except for 5.0%, significantly reduced ($P \leq 0.05$) the lightness (L*) of the subprimal compared to the water control sample. Subprimals treated with lactic acid concentrations of 7 – 10 % also showed a significant reduction ($P \leq 0.05$) in yellowness (b*) compared to the control sample. Subprimal evaluation after 24-h chill (Table 3-5) showed that lactic acid concentrations of 8.0 and 9.0 % led to a significant reduction ($P \leq 0.05$) in lightness (L*) compared to the control. For b* analysis at this sampling point, the control sample value had a low reading so it

was different ($P \leq 0.05$) than concentrations of 3.0, 4.5, and 5.0 % which reported higher b* values. All a* values among treatment concentrations were not significantly different than the control sample at each of the three sampling points. A decrease in lightness (L*) post-lactic acid application (5%) to beef trimmings has been observed in past research; however, readings were not taken until after a grinding step (Stivarius et al., 2002). Semler et al. (2013) reported a higher reduction of L* values on beef steaks treated with 4.17% lactic acid compared to other antimicrobials, but the values were not significantly different ($P > 0.01$) than the control.

Table 3-5. Color readings of chilled subprimals treated with lactic acid at the post-24 h vacuum packaged chill sampling point[a]

LA concentration (%)	Mean ± SE color readings		
	L*	a*[b]	b*
0	41.50 ± 1.44 ab	14.03 ± 0.86	14.73 ± 0.21 bc
3	41.33 ± 0.91 ab	16.79 ± 2.93	16.86 ± 1.37 a
3.5	41.91 ± 1.88 a	14.48 ± 0.49	16.03 ± 0.48 ab
4	41.28 ± 0.91 ab	13.67 ± 2.16	16.04 ± 1.13 ab
4.5	41.71 ± 1.51 ab	15.29 ± 0.23	16.68 ± 0.53 a
5	41.43 ± 1.33 ab	13.15 ± 2.18	16.68 ± 1.38 a
5.5	40.54 ± 1.71 ab	14.01 ± 2.06	16.38 ± 1.40 ab
6	39.75 ± 1.36 abc	15.11 ± 0.67	16.34 ± 1.07 ab
7	39.38 ± 1.47 abcd	13.95 ± 0.90	16.13 ± 1.15 ab
8	36.63 ± 0.59 d	13.42 ± 1.00	14.51 ± 0.17 bc
9	37.24 ± 1.16 cd	11.21 ± 0.96	13.25 ± 0.24 c
10	39.04 ± 0.54 bcd	13.95 ± 0.79	15.86 ± 1.78 ab

[a] L* measures light v. dark (100 = perfect white, 0= black); a* measures red (positive value) v. green (negative value); b* measures yellow (positive value) v. blue (negative value). Mean values for L*, a*, and b* (within columns) that do not share a common letter are statistically different ($P \leq 0.05$).

[b] Overall, no difference ($P > 0.05$) was found between a* values across all concentrations at this sampling point, therefore, letters expressing statistical distinctions are not included in this column

3.3.3 TBARS Analysis

TBARS values from beef subprimal pieces after treatment with each concentration of the peracetic acid spray were measured after 24-h vacuum packaged chilling. While values ranged from 0.21 to 0.34, there was no difference ($P > 0.05$) observed among all concentrations including the water control. For the lactic acid analysis, significant differences ($P \leq 0.05$) were observed among treatment concentrations at both the 24 and 72-h chill sampling points (Table 3-6).

Table 3-6. Thiobarbituric acid (TBARS) analysis of chilled subprimals treated with lactic acid[a]

	Mean ± SE TBARS value[b]	
LA Concentration (%)	24 – h Chill	72 – h Chill
0	0.23 ± 0.06 a	0.21 ± 0.04 a
3	0.30 ± 0.08 ab	0.24 ± 0.04 ab
3.5	0.24 ± 0.02 a	0.26 ± 0.02 abc
4	0.31 ± 0.01 ab	0.26 ± 0.01 abc
4.5	0.30 ± 0.05 ab	0.43 ± 0.05 e
5	0.51 ± 0.13 c	0.40 ± 0.05 de
5.5	0.30 ± 0.06 ab	0.39 ± 0.02 de
6	0.39 ± 0.01 abc	0.30 ± 0.03 bc
7	0.45 ± 0.11 bc	0.39 ± 0.07 de
8	0.40 ± 0.07 abc	0.32 ± 0.04 bcd
9	0.45 ± 0.06 bc	0.34 ± 0.02 cd
10	0.46 ± 0.06 bc	0.39 ± 0.02 de

[a] Mean values found within each sampling point (columns) that do not share a common letter are statistically different ($P \leq 0.05$).

[b] Final TBARS value is expressed in ppm malonaldehyde with higher values representing an increase of lipid oxidation on the product.

Post-24-h chill, subprimal pieces treated with 5.0, 7.0, 9.0, and 10.0 % lactic acid had a higher level ($P \leq 0.05$) of lipid oxidation compared to the water treated sample. Post-72 h

chilled samples treated with 4.5% lactic acid and above showed higher levels ($P \leq 0.05$) of lipid oxidation than the water sprayed control sample. Despite the fact that there were increased levels of lipid oxidation occurring at both sampling points during the lactic acid experiment, all values fall well below a TBARS value of 1.0, which is the threshold that represents detectable oxidized flavor and odor of beef products (AMSA, 2012). Research evaluating TBARS values of the subprimals over longer storage times would be beneficial.

In conclusion, the results from the current study show that all concentrations of peracetic acid and concentrations of ≥ 3.5% lactic acid (applied at 55°C) may be used as an effective control for STEC contamination on surfaces of chilled raw beef products. No differences ($P > 0.05$) existed in the STEC reductions of 400 – 1800 ppm peracetic acid (except 1600 ppm) or 3.5 – 10.0% lactic acid. However, lactic acid concentrations as low as 3.5% had an effect on subprimal color and higher concentrations (7 – 10%) may have had a negative effect on organoleptic properties of the raw beef product. Further research should be done to investigate the impact of each antimicrobial on the product quality and include sensory analysis panels. Further research evaluating these organic acids on different types of beef surfaces would also be beneficial. With the "Big 6" STEC strains classified as foodborne adulterants and antimicrobial regulatory limits continually changing, research investigating the efficacy of increased concentrations of antimicrobials to control these pathogens will continue to be valuable for industry use in food safety plans. This study suggests that beef processors could use a 400 ppm peracetic acid solution or a 3.5% lactic acid solution (55°C) as cost-effective intervention strategies to reduce STEC populations on chilled beef surfaces while not compromising the overall product quality.

Chapter 4 – Efficacy of an Ambient Water Wash, Hot Water Wash, and Application of Three Antimicrobial Sprays Using a Three-Stage Commercial Carcass Washing Cabinet for Reducing Shiga Toxin-Producing *Escherichia coli* Contamination on Beef Carcasses

4.1 Introduction

Beef cattle operations are major contributors to the food supply in the United States. In 2015, U.S. beef production was 23.7 billion pounds and total U.S. beef consumed was 24.8 billion pounds (NCBA, 2016). Shiga toxin-producing *Escherichia coli* (STEC) can be recovered from beef products and have resulted in numerous multistate outbreaks and recalls (CDC, 2017). From 2010 to 2015, over seven million pounds of beef was recalled due to STEC contamination (FSIS, 2016; FSIS, 2015c, 2015d, 2015e, 2015f, 2015g). STEC contamination in beef products has major economic implications for beef processors and the adulterated products have significant ramifications for public health.

The Centers for Disease Control and Prevention (CDC) estimates that STEC cause 265,000 illnesses, 3,600 hospitalizations, and 30 deaths annually in the U.S. (CDC, 2012). Humans infected with STEC show symptoms such as nausea, bloody diarrhea, or hemolytic uremic syndrome (HUS), which can lead to kidney failure and death. *E. coli* O157:H7 is the most prominent STEC strain due to the number of foodborne outbreaks and recalls linked to this serotype. In 1994, the United States Department of Agriculture Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7 an adulterant in ground beef and it has remained a focus of researchers and regulatory agencies over the past two decades. Six non-O157 STEC

serogroups (O26, O45, O103, O111, O121, and O145) have recently gained notoriety as foodborne pathogens and can cause similar illness to *E. coli* O157:H7. These six serogroups account for over 70% of non-O157 STEC infections (FSIS, 2012a) and were declared adulterants in non-intact raw beef by the FSIS in 2011 (2012b).

Beef is associated with 55% of O157:H7 and 50% of non-O157 STEC outbreaks across all food commodity groups in the United States (Moxley and Acuff, 2014). STEC bacteria are prevalent within the gastrointestinal tract of cattle and often contaminate the hide or are shed through feces. Beef processors face the risk of spreading STEC contamination during the slaughter and fabrication steps and combat this risk by implementing beef carcass intervention methods throughout the industry. Ambient water and hot water carcass washes are commonly used intervention methods that have been effective in reducing *E. coli* O157:H7 populations on beef carcasses (Castillo et al., 1998a, 2001a; Dorsa et al., 1996; Kalchayanand et al., 2012). Organic acids, such as lactic and peracetic acids, are also commonly used in the industry to reduce microbial contamination and have shown varied effectiveness in controlling STEC.

Commercial beef processing facilities often implement the intervention methods listed above as a multi-hurdle strategy to reduce pathogens at the carcass level. Limited research has been reported validating these intervention methods, particularly when applied in sequence, for reducing both *E. coli* O157:H7 and the non-O157 STEC serogroups on pre-rigor beef carcasses. The main objective of this study was to validate the antimicrobial effectiveness of intervention methods applied sequentially to pre-rigor beef carcass sides using a three-stage commercial spray cabinet (Chad Equipment) to reduce STEC contamination.

4.2 Materials and Methods

4.2.1 Experimental Design

Finished cattle (450-500 kg after dressing) were obtained from a local feed yard and transported by truck to the Kansas State University Biosecurity Research Institute holding unit. Using USDA-approved methods, each animal was slaughtered within 6 h of arrival and immediately used for research purposes. Common commercial slaughter protocol was utilized which included steam vacuuming of the dressed carcass along hide opening lines (pattern lines) and the midline after mechanical hide removal. Two cattle were slaughtered on three different days (replications) for a total of 6 animals or 12 carcass sides. For each replication, fresh STEC inoculum cocktails and antimicrobial solutions were prepared.

All experiments were conducted at the Kansas State University Biosecurity Research Institute, a biosafety level-3 biocontainment laboratory having full-scale slaughter and meat fabrication capabilities. All animal slaughter protocols were in compliance with USDA-FSIS standards and were approved by the university Animal Care and Use Committee. Inoculated studies were conducted under an Institutional Biosafety Committee approved protocol, ensuring laboratory personnel safety in a beef processing operation where infectious aerosols are likely to be encountered. All laboratory personnel undergo intensive biosafety training annually and rely on personal protective equipment and operational procedures (powered air purifying respirators, Tyvek suits, double gloving, and validated disinfection protocols) to ensure safety.

4.2.2 Bacterial Cultures and Inoculum Preparation

Rifampicin-resistant derivatives of *E. coli* O157:H7 (ATCC 31150; human isolate) and non-O157 STEC serogroups O26 (H30, human isolate), O45 (CDC 96-3282, human isolate), O103 (CDC 90-3128, human isolate), O111 (JB1-95, clinical isolate), O121 (CDC 97-3068, human isolate), and O145 (83-75, human isolate) were obtained from Dr. John Luchansky (USDA Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA) and used to inoculate carcass sides. Strains were propagated in 10 ml sterile tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) supplemented with 0.1 g/L rifampicin (TSB_{rif}); Tokyo Chemical Industry, Tokyo, Japan) with incubation at 37° C for 24 h. A loop of each of these solutions was transferred to tubes containing 10 ml TSB_{rif} and incubated at 37°C for 24 h. Subsequently, a loop of these seven solutions was transferred into centrifuge bottles containing 225 ml TSB_{rif} and incubated at 37°C for 24 h. Each of the 7 bottles were then centrifuged for 15 min at -4°C and 4960 x g and the supernatant decanted. Each bacterial pellet was reconstituted with 10 ml 0.1% peptone water (DIfco) and combined to make 70 ml of a 7-serogroup inoculum mixture. This STEC cocktail was diluted with 0.1% peptone water to reach a final volume of 10 L (at ~6.0 log CFU/ml) immediately prior to use as the inoculum solution.

4.2.3 Application of Inoculum

An electrostatic spray system (ESS) delivered the STEC cocktail to carcass sides during each replication. The ESS is a large, airtight stainless steel cabinet incorporating the overhead rail conveyance system. In each of the 4 corners of the cabinet (3.5 m tall X 1.8 m width X 1.8 m depth) an air-assisted, electrically powered spray nozzle (Electrostatic Spraying Systems, Watkinsville, GA) is installed. All eight ESS nozzles are plumbed to a solution reservoir located

outside of the sealed cabinet through a peristaltic pump. Prior to inoculum application, the ESS system operation was set to deliver a total of 200 ml of solution within 14 s to uniformly cover the entire exposed surface area of the carcass (established in preliminary trials), and operation was verified using a graduated cylinder to measure the flow rate, which was recorded at 90 – 175 ml/min at each nozzle. A digital multimeter (Amprobe; Everett, WA) was used to measure negative charge of the fluid at each nozzle and was used in combination with the flow rate to calculate a charge-to-mass ratio. A charge-to-mass ratio of -5.0 to -7.5 was measured at each nozzle throughout the study. Carcass sides were individually placed inside the sealed ESS cabinet and the STEC cocktail inoculum (200 ml) was applied for 14 s. After inoculum application, carcass sides remained inside of the sealed cabinet for 30-min (STEC attachment period) before removal from the cabinet. The target STEC inoculation level was ~7 log CFU/100 cm^2. Once carcasses were inoculated, a long rod was used to contact only the roller trolley hook to move carcasses along the rail to the Chad carcass wash cabinet without touching any of the inoculated carcass surfaces.

4.2.4 Antimicrobial Preparation

Solutions of 4.5% *L*-lactic acid (Birko Corporation, Henderson, CO), 200 ppm peracetic acid (Microtox Plus™; Zee Company, Chattanooga, TN), and 1.1 pH Centron™ (Zoetis, Madison, NJ) were all prepared according to manufacturers' recommendations. Lactic acid 88% is a concentrated mixture of lactic acid in water. Peracetic acid solution is a mixture of peracetic acid, hydrogen peroxide, acetic acid, sulfuric acid, and HEDP. Centron™ is an aqueous mixture of sulfuric acid and sodium sulfate. Peracetic acid and Centron™ were mixed with ambient tap water and lactic acid was mixed with heated tap water (54°C) to achieve the target

concentrations that fall within compliance of USDA-FSIS Directive 7120. Concentrations of lactic acid were confirmed by titrating 5 ml of the solution with 0.25 N NaOH (Fisher Scientific) using 1% phenolphthalein (Fisher Scientific) as an indicator. Peracetic acid concentrations were confirmed by titrating 5 ml of the solution with 0.1 N sodium thiosulfate (Fisher Scientific) using a starch indicator (1% w/v; Fisher Scientific). FSIS approval states that the pH of Centron™ for application should be 1.0-2.2, and this was confirmed using a calibrated pH meter (Oakton Instruments, Vernon Hills, IL) each time a solution was prepared. Fresh antimicrobial solutions were prepared from the original stock concentrates for each experimental replication.

4.2.5 Application of Treatments

Experimental replications consisted of four carcass sides sequentially receiving treatments using a three-stage commercial spray cabinet (Chad Equipment, Olathe, KS) following the 30-min inoculum attachment period. After each stage of the Chad cabinet, the long rod was used to pull the carcass side back out of the cabinet for sample collection (as defined in the next section). After each sample collection, the carcass side was returned to the next stage of the cabinet to resume sequential washing scenarios. In stage 1, an ambient high volume water wash (~23°C for 15 s) was applied to carcass sides via ninety-four 1/8" MEG 2510 nozzles (Chad Equipment) at 250 psi. This was followed in stage 2 by a hot water wash (82-92°C for 12 s) applied using forty-four H 3/8" U 5050 nozzles (Chad Equipment) at 15 psi. Thus, all carcass sides received the same ambient and hot water washes (stages 1 and 2) of the sequential treatment scenarios. As a final treatment (stage 3), each carcass side was randomly assigned one of four chemical spray treatments: control (no antimicrobial treatment), lactic acid, peracetic acid, or Centron. Antimicrobial treatments were applied as a mist (20 psi) for 12

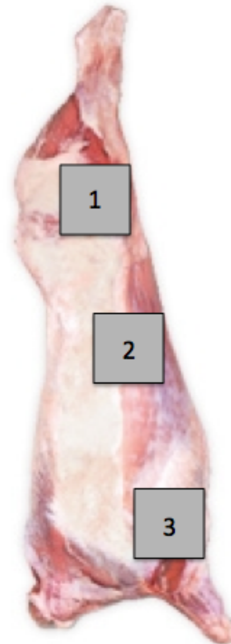
sec using ten H1/8VVSS110015 nozzles (Chad Equipment). Lactic acid was applied at 49-52°C (at nozzle) while peracetic acid and Centron™ were applied at ambient temperature (23°C at nozzle). Following this final antimicrobial spray treatment, carcass sides were moved to a carcass chill cooler for an 18 h chill cycle. The cooler remained at -2°C for 6 h and a 2°C water spray was applied to the carcasses for a 30 s duration at 29.5 min intervals; the subsequent 12 h was a dry chill at 2°C. Thirty-six 1/8" K-4 nozzles (9 per carcass) were used to apply the 2°C water spray at 40 psi.

4.2.6 Microbial Sampling

Five sampling points were used during processing to determine rifampicin-resistant STEC populations on carcass sides: post-inoculation, post-ambient water treatment (stage 1), post-hot water treatment (stage 2), post-antimicrobial chemical treatment (stage 3), and post-18 h chill cycle. Three anatomical locations, being the top (round), middle (flank), and bottom (neck/brisket) of the carcass side, were sampled at each processing point (Figure 4-1). Excised tissue samples were obtained by removing 42.45 cm^2 surface areas from each anatomical location at each sampling point using a sterile corer, scalpel, and forceps. Surface area samples were placed into a sterile filter-style Whirl-Pak bag (Nasco, Fort Atkinson, WI) containing 75 ml Dey-Engley neutralizing broth (Difco) supplemented with 0.1 g/L rifampicin and stomached for 1 min in an AES smasher (Biomerieux, Macry-l'Etoile, France). Each sample was serially diluted in 0.1% peptone water supplemented with 0.1 g/L rifampicin and subsequently plated in duplicate on ECC Petrifilm (3M, St. Paul, MN). Petrifilm plates were incubated at 37°C for 24 h and counted in compliance with manufacturer's instructions. Bags containing homogenized samples were stored at 4°C until results were obtained from direct plating. In cases where no

viable STEC was detected by direct plating, 25 ml of stored sample homogenate were transferred into 225 ml of TSB_{rif} and incubated at 37°C for 24 h, followed by streaking the enriched sample onto TSA_{rif} plates for qualitative detection of surviving populations below the direct plating detection limit (1.9 log CFU/100 cm^2).

Figure 4-1 Anatomical locations of sampling on carcass sides



4.2.7 Statistical Analyses

The experiment consisted of three replications (days), each using four carcass sides and three sampling locations. The microbial counts were transformed into log CFU/100 cm^2 format and were analyzed using the SAS system's (SAS Institute Inc., Cary, NC) PROC MIXED function with the fixed effects of sampling location, sampling time, and sampling location by sampling time. STEC counts for post-chemical spray treatment and post-spray chill samples were also analyzed with the fixed effects of treatment, location, and treatment by location. Sampling times and sampling locations, and treatment levels and sampling locations, were each compared at a significance of $P \leq 0.05$.

4.3 Results and Discussion

4.3.1 Introduction

Rifampicin-resistant STEC recoveries at each sampling point are shown in Table 4-1. Inoculation levels are different ($P \leq 0.05$) between sampling locations on the carcass. As a result, STEC recovery differences ($P \leq 0.05$) occur between sampling locations at each of the sampling points throughout the study. The sequential application of ambient water, hot water, chemical sprays, and 18-h chill cycle reduced STEC population means to at or below the direct plating detection limit (1.9 log CFU/100 cm^2) at each sampling location on the carcass.

Table 4-1 STEC recovery at each sampling point for each carcass treatment[a]

	Mean ± SE STEC recovery (log CFU/100 cm^2)		
Sampling Point	Bottom	Middle	Top
Inoculation	6.4 ± 0.15aw	7.0 ± 0.10bw	6.3 ± 0.13aw
Post-ambient water	5.5 ± 0.17ax	5.9 ± 0.19ax	4.8 ± 0.21bx
Post-hot water	2.9 ± 0.25ay	2.3 ± 0.31ay	1.5 ± 0.23by[d]
Post-antimicrobial treatment[b]	2.5 ± 0.25ay	1.5 ± 0.29bz[d]	1.6 ± 0.24by[d]
Post-spray chill	1.9 ± 0.21az[d]	1.7 ± 0.26abz[d]	1.1 ± 0.11bz[d]

[a]a and b, means within sampling point (within rows) that do not share a common letter are statistically different ($P \leq 0.05$); w to z, means within sampling location (within columns) that do not share a common letter are statistically different ($P \leq 0.05$).

[b]Antimicrobial treatment groups control, lactic acid, peracetic acid, and Centron showed no statistical differences ($P > 0.05$) between treatments, therefore, they were combined into the post-antimicrobial treatment group.

[c]Forty-seven samples reported below the detection limit (<1.9 log CFU/100 cm^2) and were reported at one-half the detection limit (1.0 log CFU/100 cm^2) for statistical analyses. Post enrichment process, four samples were shown to have no viable STEC cells present.

[d] Mean STEC recovery was at levels at or below the detection limit (<1.9 log CFU/100 cm^2)

Table 4-2 STEC reductions from previous step by ambient and hot water wash at different locations[a]

	Mean ± SE STEC reduction (log CFU/100 cm^2)	
Sampling location	Ambient water wash	Hot water wash[b]
Bottom	0.9 ± 0.11a	2.6 ± 0.23
Middle	1.1 ± 0.16a	3.6 ± 0.35
Top	1.5 ± 0.13b	3.3 ± 0.27[c]

[a] a to b, means within treatment (within column) that do not share a common letter are statistically different.

[b] No differences (*P* > 0.05) were observed between STEC reductions of the hot water wash overall, therefore, no letters were included in this column.

[c] The hot water wash reduced mean STEC recovery at the top of the carcass to below the detection limit (<1.9 log CFU/100 cm^2)

4.3.2 Antimicrobial Effectiveness of Ambient and Hot Water Washes

The cabinet's high-volume ambient water wash stage reduced the STEC population on inoculated carcass sides by 0.9, 1.1, and 1.5 log CFU/100 cm^2 at the bottom, middle, and top of the carcass, respectively. The STEC reductions observed from post-inoculation to post-ambient water application were higher ($P \leq 0.05$) at the top of the carcass compared to the other sampling locations (Table 4-2). In stage 1, the cabinet applies ambient water in four sequential zones, top to bottom, each for 15 s. Certain areas of the carcass may receive overlapping ambient water treatment from multiple zones, which increases the treatment time and could cause differences in pathogen reduction between sampling locations. The ambient water stage could have also relocated STEC contamination from the top of the carcass to the other locations through the downward washing effect. The cabinet's hot water stage (stage 2) reduced the STEC population on carcass sides by an additional 2.6, 3.6, and 3.3 log CFU/100 cm^2 at the

bottom, middle, and top of the carcass, respectively, with no overall difference ($P > 0.05$) observed between sampling locations (Table 4-2). In combination, sequential application of an ambient water wash and a hot water wash reduced STEC populations by 3.5, 4.7, and 4.8 log CFU/100 cm^2 at the bottom, middle, and top of the carcass, respectively.

Castillo et al. (1998a, 1998b) found that a warm water wash (35°C) could reduce *E. coli* O157:H7 counts by 1.7 – 2.9 log CFU/cm^2 on regions of a beef carcass (round, flank, clod, and brisket). Although *E. coli* O157:H7 reductions they observed at the round (top) region were slightly higher than other regions, which corresponds with the current study, no overall difference ($P > 0.05$) was observed between reductions at different anatomical locations. The higher STEC reductions observed from the warm water stage of the Castillo et al. studies compared to the ambient water stage of the current study may be due to differences in experimental parameters. Castillo et al. applied a 25°C hand spray wash (90 s at 10 psi) followed by a 35°C cabinet wash (9 s at 250 to 400 psi), whereas, the current study only applied a cabinet wash (15 s at 250 psi). Castillo et al. reported that a hot water treatment (95°C for 5 s) reduced the *E. coli* O157 population by an additional 0.8 – 2.2 log CFU/cm^2, and the sequential combination of a warm water wash and hot water wash reduced *E. coli* O157:H7 by 2.9 - 4.2 log CFU/cm^2, which is slightly less than results from the present study; however, the duration of their hot water application was only 5 s.

Dorsa et al. (1996) applied a warm and hot water wash to beef carcasses that each effectively reduced *E. coli* O157:H7 populations. The combination of a warm water wash (30°C) and hot water wash (72°C) reduced *E. coli* O157 populations by 3 log cycles, which is less than what was observed in the present study. This is likely due to the lower application temperature

of the hot water stage. Kalchayanand et al. (2012) applied a hot water wash (85°C for 15 s) to pre-rigor beef flanks contaminated with Shiga toxin-producing *E. coli* O26, O45, O103, O111, O121, O145, and O157:H7. The hot water wash reduced STEC populations by 3.2 – 4.2 log CFU/cm^2, which was more effective than any other antimicrobial treatment used in the study, including lactic and peracetic acid. Although this study restricted treatment applications to only the flank section (more exposed lean/fascia tissue) rather than entire beef carcasses (with substantial fat coverage), it demonstrated that hot water application was effective in controlling non-O157 STEC contamination.

4.3.3 Antimicrobial Effectiveness of Chemical Mist and Spray Chill Treatments

Antimicrobial mist treatments reduced ($P \leq 0.05$) STEC populations on the middle of the carcass but did not reduce ($P > 0.05$) populations at the bottom and top of the carcass. Post-spray chill treatments reduced ($P \leq 0.05$) STEC populations at the bottom and top of the carcass, while populations at the middle sampling point did not change ($P > 0.05$). No differences ($P > 0.05$) were observed between the three chemical sprays applied and the no treatment control (Table 4-3), therefore any differences observed after the antimicrobial mist and spray chill are only related to sampling location.

The three chemical sprays applied after the hot water wash resulted in additional STEC population reductions of 0.8, 0.4, and 0.2 log CFU/100 cm^2 for lactic acid, peracetic acid, and Centron™, respectively. Although STEC populations at the middle sampling point were significantly reduced ($P \leq 0.05$), no differences ($P > 0.05$) were observed between the three treatments and the non-treated control, at any of the three sampling locations. Insignificant STEC reductions by antimicrobial treatments compared to the control are likely due to STEC

contamination reaching very low or undetectable levels post-hot water wash; thus, residual STEC population levels were very low prior to chemical spray applications minimizing our ability to demonstrate further STEC reductions by the chemical sprays.

Table 4-3 STEC reductions by antimicrobial treatments[a]

	Mean ± SE STEC reductions (log CFU/100 cm^2)[b]
Control[c]	0.0 ± 0.00
Lactic acid	0.8 ± 0.26
Peracetic acid	0.4 ± 0.30
Centron	0.2 ± 0.50

[a] STEC reductions are from post-hot water treatment to post-antimicrobial treatment

[b] No differences (*P* > 0.05) were observed overall between treatment groups or sampling location of STEC reductions at this stage.

[c] The control carcass did not receive an antimicrobial spray treatment; therefore, mean STEC recoveries were reported to be the same as the previous sampling point, resulting in no STEC reduction.

Studies have evaluated the efficacy of lactic acid and peracetic acid on hot and chilled beef carcass surfaces. Castillo et al. (2001a) applied an initial carcass wash followed by a 2% lactic acid spray (55°C for 15 s), which together reduced the *E. coli* O157:H7 population by 5.2 log CFU/cm^2 compared to 2.4 - 3.3 log reductions from the carcass wash alone. This study further analyzed the efficacy of applying 4% lactic acid to chilled carcasses, showing additional *E. coli* O157:H7 reductions of 2.0 – 2.4 log CFU/cm^2 at this step. Higher inoculation levels (~8.0 log CFU/cm^2) than those of the current study, could have led to the greater observed reductions in *E. coli* O157:H7 populations. King et al. (2005) applied a heated 4% lactic acid

spray (55°C) and a 200 ppm peracetic acid spray (45 or 55°C) to chilled beef carcasses using a commercial wash cabinet. Lactic acid application reduced *E. coli* O157:H7 population by 2.7 log CFU/cm^2, however, peracetic acid application was ineffective on the chilled surface. The study further evaluated a 200 ppm peracetic acid spray on carcass sides prior to chilling, similar the to procedure followed in the current study, showing a 0.7 log CFU/cm^2 *E. coli* O157:H7 reduction on the pre-rigor surface.

Two studies have evaluated the efficacy of lactic and peracetic acid against non-O157 STEC contamination on beef surfaces. Kalchayanand et al. (2012) evaluated 4% lactic acid and 200 ppm peracetic acid in reducing *E. coli* O157:H7 and non-O157 serotypes (O26, O45, O103, O111, O121, and O145) on pre-rigor beef flanks. This study reported a 0.9 – 2.7 log CFU/cm^2 STEC reduction across all serogroups, which was substantially higher than what was observed in the current study. Liao et al. (2015) applied 5% lactic acid and 200 ppm peracetic acid to chilled beef strip loins inoculated with *E. coli* O157:H7 or a non-O157 STEC cocktail. Organic acid application reduced the STEC population by 0.2 – 0.5 log CFU/50 cm^2, similar to what was observed in the current study. Results also indicated that lactic and peracetic acids were effective against non-O157 STEC even at a low inoculation level (~2.0 log CFU/50cm^2).

The efficacy of Centron™ at different pH levels has been evaluated on beef surfaces. Weinroth et al. (2015) showed a >1.0-log reduction of aerobic bacteria on hot beef carcasses treated with a solution with a pH 1.05 or 1.3 using a spray cabinet. Geornaras et al. (2012) immersed beef trimmings contaminated with *E. coli* O157:H7 and non-O157 STEC strains in 1.2 pH Centron™. The results showed that Centron™ treatment only reduced STEC populations by 0.3 – 0.4 log CFU/cm^2 from an inoculation level of 3.0-4.0 log CFU/cm^2, which is similar to

results from the present study despite the different application methods. Weinroth et al. (2015) reported no differences in the microbial reductions by different pH levels of Centron™, a conclusion that corresponds with the results of Geornaras et al. (2012) and the current study. However, further research evaluating Centron™ at low pH levels (1.0 – 1.1) to reduce STEC would be beneficial.

Table 4-4 Samples reported under the detection limit at sampling points

	Number of samples reported below the detection limit (<1.9 log CFU/100 cm^2) out of 12 total at each point		
	Bottom	Middle	Top
Inoculation	-	-	-
Post-ambient water	-	-	-
Post-hot water	1	4	8
Post-antimicrobial[ab]	1	5	6
Post-spray chill[c]	4	7	11

[a] No control carcass samples were taken at this step, therefore, reported value is out of 9 possible samples.

[b] One post-antimicrobial sample (middle section), qualitatively showed no viable STEC cells post enrichment.

[c] Three post-spray chill samples qualitatively showed no viable STEC cells post enrichment, two from the top and one from the bottom.

Table 4-5 Comparison of samples reported under the detection limit between antimicrobial treatments

	Number of samples reported below the detection limit (<1.9 log CFU/100 cm^2) out of 9 total at each point			
	Control[a]	Lactic acid[b]	Peracetic acid[c]	Centron
Post-antimicrobial mist	-	5	4	3
Post-spray chill	5	6	7	4

[a] The control carcass did not receive an antimicrobial treatment; therefore, no sample was taken at this point

[b] Two lactic acid treated samples at the post-spray chill sampling point qualitatively showed no viable STEC cells post enrichment.

[c] Two peracetic acid treated samples qualitatively showed no viable STEC cells post enrichment; one post-antimicrobial mist and one post-spray chill.

The 18 h chill cycle provided STEC reductions ($P \leq 0.05$) at the bottom and top of the carcass, however, there was no difference between the STEC reductions on carcasses that had been previously treated with one of the three chemical sprays and the non-treated control. Therefore, it is unclear whether or not previous application of any of the chemical mist treatments helped in the STEC reduction potential during the spray chill treatment. This outcome may be due to STEC populations reaching very low or undetectable levels post-hot water wash or post-antimicrobial mist. In order to estimate the impact of these treatments, the number of samples reported below the detection limit (1.9 log CFU/100 cm^2) was analyzed at each sampling point and location (Table 4-4). The top section of the carcass had the most samples reported below the detection limit at each sampling point, followed by the middle section and bottom section, results that correlate with STEC recoveries listed at each sampling point in table 4-1. Although the top of the carcass had a higher number of samples reaching

undetectable levels, it is notable that this section was inoculated at the lowest level (6.3 log CFU/100 cm^2) and displayed high STEC reductions after treatment of ambient water (1.5 log CFU/100 cm^2) and hot water (3.3 log CFU/100 cm^2). Table 4-5 gives a comparison of samples reported below the detection limit after treatment with one of three chemical sprays or the control (no treatment). Lactic acid and peracetic acid treated carcass sides had more samples reported under the detection limit than Centron™. However, non-treated carcass sides reported similar numbers of samples at undetectable levels as the chemical sprays, showing little evidence that different treatments were more effective at reducing STEC contamination.

In summary, the results from the present study showed that sequential antimicrobial treatments applied using a commercial three-stage Chad carcass wash cabinet and subsequent chill step reduced STEC populations on pre-rigor beef carcasses by 4.5-5.3 log CFU/100 cm^2 at all anatomical locations on the carcass side. The cabinet's ambient and hot water stages reduced STEC significantly more than the antimicrobial mist and chill step. However, antimicrobial application and chilling may have provided additional antimicrobial benefit against low-level residual contamination, as an increased number of samples from these stages were reported below the detection limit. To understand the full capabilities of the chemical antimicrobial mist and chill steps, studies should inoculate carcass sides to a higher level (which would be difficult in the ESS cabinet used for the current study) or not apply an ambient and hot water stage prior to these steps. The data gathered in this study is useful to the beef industry as it provides evidence that ambient water washes and hot water washes in a commercial spray cabinet, which can be emulated by beef processors, effectively reduce *E. coli* O157:H7 and the "Big 6" STEC strains on pre-rigor beef carcasses. The overall STEC reductions

resulting from the sequential treatments in a Chad carcass wash cabinet and subsequent 18 h spray chill (4.5 – 5.3 log CFU/100 cm^2) shows that the process will likely reduce STEC risks substantially as products are further fabricated.

Chapter 5 – Conclusions

5.1 STEC Interventions on Chilled Beef Subprimals

Peracetic acid application at all concentrations (200-1800 ppm) effectively reduced ($P \leq 0.05$) STEC populations on chilled beef strip loins compared to the water spray control. There were no differences ($P > 0.05$) in the STEC reductions between concentrations of 400 – 1800 ppm peracetic acid, except for 1600 ppm, indicating that higher concentrations may provide little additional antimicrobial benefit on chilled beef subprimals. Lactic acid applications (55°C) at concentrations ≥ 3.5% for chilled subprimals reduced ($P \leq 0.05$) STEC populations to a greater extent than the water control. Similar to results from peracetic acid application, lactic acid concentrations of 3.5 – 10 % showed no difference ($P > 0.05$) in STEC reductions, implying that higher concentrations of lactic acid did not result in additional antimicrobial effect. High concentrations of lactic acid (7-10%) caused negative changes in color values and lipid oxidation, indicating that application of warm lactic acid sprays at these concentrations could negatively impact organoleptic properties of chilled beef subprimal products. Peracetic acid concentration for application to beef surfaces is currently approved by USDA FSIS up to ≤1800 ppm for the Microtox Plus™, as evaluated in the current study. Peracetic acid based antimicrobial products from other companies have different approved limits. The USDA FSIS has approved lactic acid applications at up to ≤5.0. Most beef processors currently apply peracetic acid and lactic acid washes at 400 ppm and 4.0-4.5%, respectively (personal communication with beef processors). These results will help beef processors determine the

appropriate concentrations of these chemicals to apply to chilled beef products in a commercial operation.

5.2 STEC Interventions on Pre-rigor Beef Carcasses

The ambient and hot water spray wash stages of the Chad carcass wash cabinet each significantly reduced STEC contamination to the bottom, middle, and top anatomical regions of pre-rigor (non-chilled) beef carcasses. There was a difference ($P \leq 0.05$) between STEC reductions at anatomical sampling locations during ambient water application, suggesting that the cabinet may provide an increased antimicrobial effect to the top of the carcass at this stage (or the gravity effect of water physically moves contamination downward on the carcasses to the neck/brisket region). Sequential application of ambient water and hot water (~88°C) reduced inoculated STEC populations by 3.5 – 4.8 log CFU/100 cm^2, indicating that these stages can be used as highly effective intervention methods on beef carcasses. Subsequent application of chemical acid spray antimicrobial treatments (lactic acid, peracetic acid, and Centron™) after the final carcass wash did not reduce ($P > 0.05$) STEC populations compared to the treatment control (no spray of any kind in stage-3 of the cabinet), likely due to the very low residual STEC population levels remaining after hot water washing (thus, unable to show an additional magnitude of reduction attributed to the chemical spray). The antimicrobial spray in stage-3 of the Chad cabinet following the final hot water wash, accompanied by a water spray chill carcass application during the first 6 hours of carcass cooling, helped reduce STEC contamination to undetectable levels. To better understand the antimicrobial effect of these chemical sprays on beef carcasses, further research must apply them to carcasses with higher STEC population levels, or to inoculated carcasses that have not been hot water washed. These results will help

beef processors understand the efficacy of these sequential pre-rigor carcass treatments commonly applied in commercial settings against *E. coli* O157:H7 and the "Big 6" non-O157 STEC serotypes, information that will be fundamental as food safety plans are created and updated.

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Appendix A – SAS Codes Used for Statistical Analyses

PAA Loin Micro Analysis – No Overlay

```
PROC IMPORT OUT= WORK.microno
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Micro NO OVERLAY - PAA loin - Matt Krug.xlsx"
            DBMS=xlsx REPLACE;
                 sheet="data";
RUN;

PROC IMPORT OUT= WORK.microwo
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Micro WITH OVERLAY - PAA loin - Matt Krug.xlsx"
            DBMS=xlsx REPLACE;
                 sheet="data";
RUN;

PROC IMPORT OUT= WORK.tbars
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\TBARS data - PAA loin - Matt Krug.xlsx"
            DBMS=xlsx REPLACE;
                 sheet="data";
RUN;

PROC IMPORT OUT= WORK.color
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Color data - PAA loin - Matt Krug.xlsx"
            DBMS=xlsx REPLACE;
                 sheet="data";
RUN;

data microno;
   set microno;
   lcfuwo=log;
   drop log;
run;

proc sort data=microno;
  by rep trt samp;
run;

data microwo;
   set microwo;
   lcfuw=log;
   drop log;
run;

proc sort data=microwo;
  by rep trt samp;
run;
```

```
proc transpose data=microno out=micronot;
  by rep trt;
  id samp;
run;
```

PAA Loin Micro Analysis – With Overlay

```
proc transpose data=microwo out=microwot;
  by rep trt;
  id samp;
run;

data micronot; set micronot;
  lreduc_trt= pre-post;
  lreduc_24hr= post-_24_hr;
run;

data microwot; set microwot;
  lreduc_trt= pre-post;
  lreduc_24hr= post-_24_hr;
run;

ods rtf file="C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\output_06-03-2016.rtf" style=journal;

title "Micro w/out Overlay -- Pre TRT Attachment";
proc glimmix data=micronot plots=all;
  class rep trt;
  model pre=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;

title "Micro w/out Overlay -- Post TRT Attachment";
proc glimmix data=micronot plots=all;
  class rep trt;
  model post=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;

title "Micro w/out Overlay -- Log Reductions Post TRT";
proc glimmix data=micronot plots=all;
   class rep trt;
   model lreduc_trt=trt;
   random rep;
   lsmeans trt/pdiff lines;
run;
```

```
title "Micro w/out Overlay -- Log Reductions Post 24 HR";
proc glimmix data=micronot plots=all;
   class rep trt;
   model lreduc_24hr=trt;
   random rep;
   lsmeans trt/pdiff lines;
run;

title "Micro with Overlay -- Pre TRT Attachment";
proc glimmix data=microwot plots=all;
  class rep trt;
  model pre=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;

title "Micro with Overlay -- Post TRT Attachment";
proc glimmix data=microwot plots=all;
  class rep trt;
  model post=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;

title "Micro with Overlay -- Log Reductions Post TRT";
proc glimmix data=microwot plots=all;
   class rep trt;
   model lreduc_trt=trt;
   random rep;
   lsmeans trt/pdiff lines;
run;

title "Micro with Overlay -- Log Reductions Post 24 HR";
proc glimmix data=microwot plots=all;
   class rep trt;
   model lreduc_24hr=trt;
   random rep;
   lsmeans trt/pdiff lines;
run;
```

PAA loin Color Analysis

```
title "Color Analysis -- L-star";
proc glimmix data=color order=data plots=all;
  class rep trt samp;
  model L=trt|samp;
  random rep;
  random _residual_/subject=rep*trt type=csh;
  lsmeans trt|samp;
  lsmeans samp/pdiff lines;
run;
```

```
title "Color Analysis -- a-star";
proc glimmix data=color order=data plots=all;
  class rep trt samp;
  model a=trt|samp;
  random rep;
  random _residual_/subject=rep*trt type=csh;
  lsmeans trt|samp;
  lsmeans samp/pdiff lines;
run;


title "Color Analysis -- b-star";
proc glimmix data=color order=data plots=all;
  class rep trt samp;
  model b=trt|samp;
  random rep;
  random _residual_/subject=rep*trt type=un;
  lsmeans trt|samp;
  lsmeans samp/pdiff lines;
run;
```

PAA Loin TBARS Analysis

```
Title "T-bar Analysis";
proc glimmix data=tbars plots=all;
  class rep trt;
  model tba=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;

ods rtf close;
```

Lactic Acid Loin Micro Analysis – No Overlay

```
PROC IMPORT OUT= WORK.microno
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Lactic_Acid_Study\Micro data (TSA w rif) - LA
loin.xlsx"
            DBMS=xlsx REPLACE;
                 sheet="Sheet1";
RUN;

PROC IMPORT OUT= WORK.microwo
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Lactic_Acid_Study\Micro data (OVERLAYS) - LA
loin.xlsx"
            DBMS=xlsx REPLACE;
                 sheet="Sheet1";
RUN;
```

```
PROC IMPORT OUT= WORK.tbars1
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Lactic_Acid_Study\TBARS Day 1 - LA loin.xlsx"
            DBMS=xlsx REPLACE;
                 sheet="Sheet1";
RUN;

PROC IMPORT OUT= WORK.tbars2
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Lactic_Acid_Study\TBARS Day 3 - LA loin.xlsx"
            DBMS=xlsx REPLACE;
                 sheet="Sheet1";
RUN;

PROC IMPORT OUT= WORK.color
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Lactic_Acid_Study\Color data - LA loin.xlsx"
            DBMS=xlsx REPLACE;
                 sheet="Sheet1";
RUN;

data microno;
   set microno;
   lcfuwo=log;
   drop log;
run;

proc sort data=microno;
  by rep trt samp;
run;

data microwo;
   set microwo;
   lcfuw=log;
   drop log;
run;

proc sort data=microwo;
  by rep trt samp;
run;

proc transpose data=microno out=micronot;
  by rep trt;
  id samp;
run;

proc transpose data=microwo out=microwot;
  by rep trt;
  id samp;
run;

data micronot; set micronot;
  lreduc_trt= pre-post;
  lreduc_24hr= post-_24_hr;
run;
```

```
data microwot; set microwot;
  lreduc_trt= pre-post;
  lreduc_24hr= post-_24_hr;
run;

ods rtf file="C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Lactic_Acid_Study\output_v1.rtf" style=journal;

title "Micro w/out Overlay -- Pre TRT Attachment";
proc glimmix data=micronot plots=all;
  class rep trt;
  model pre=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;

title "Micro w/out Overlay -- Post TRT Attachment";
proc glimmix data=micronot plots=all;
  class rep trt;
  model post=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;

title "Micro w/out Overlay -- Log Reductions Post TRT";
proc glimmix data=micronot plots=all;
   class rep trt;
   model lreduc_trt=trt;
   random rep;
   lsmeans trt/pdiff lines;
run;

title "Micro w/out Overlay -- Log Reductions Post 24 HR";
proc glimmix data=micronot plots=all;
   class rep trt;
   model lreduc_24hr=trt;
   random rep;
   lsmeans trt/pdiff lines;
run;
```

Lactic Acid Loin Micro Analysis – With Overlay

```
title "Micro with Overlay -- Pre TRT Attachment";
proc glimmix data=microwot plots=all;
  class rep trt;
  model pre=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;
```

```
title "Micro with Overlay -- Post TRT Attachment";
proc glimmix data=microwot plots=all;
  class rep trt;
  model post=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;

title "Micro with Overlay -- Log Reductions Post TRT";
proc glimmix data=microwot plots=all;
   class rep trt;
   model lreduc_trt=trt;
   random rep;
   lsmeans trt/pdiff lines;
run;

title "Micro with Overlay -- Log Reductions Post 24 HR";
proc glimmix data=microwot plots=all;
   class rep trt;
   model lreduc_24hr=trt;
   random rep;
   lsmeans trt/pdiff lines;
run;
```

Lactic Acid Loin Color Analysis

```
title "Color Analysis -- L-star";
proc glimmix data=color order=data plots=all;
  class rep trt samp;
  model L=trt|samp;
  random rep;
  random _residual_/subject=rep*trt type=un;
  lsmeans trt|samp/pdiff;
  lsmeans trt*samp/pdiff lines;
  lsmeans trt*samp/slice=samp lines;
run;


title "Color Analysis -- a-star";
proc glimmix data=color order=data plots=all;
  class rep trt samp;
  model a=trt|samp/ddfm=kr;
  random rep;
  random _residual_/subject=rep*trt type=un;
  lsmeans trt|samp;
  lsmeans samp/pdiff lines;
run;
```

```
title "Color Analysis -- b-star";
proc glimmix data=color order=data plots=all;
  class rep trt samp;
  model b=trt|samp;
  random rep;
  random _residual_/subject=rep*trt type=cs;
  lsmeans trt|samp/pdiff;
  lsmeans trt*samp/pdiff lines;
  lsmeans trt*samp/slice=samp lines;
run;
```

Lactic Acid Loin TBARS Analysis

```
Title "T-bar Analysis -- First Time Point";
proc glimmix data=tbars1 plots=all;
  class rep trt;
  model tba=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;


Title "T-bar Analysis  -- Second Time Point";
proc glimmix data=tbars2 plots=all;
  class rep trt;
  model tba=trt;
  random rep;
  lsmeans trt/pdiff lines;
run;

ods rtf close;
```

Carcass Study Inoculation

```
PROC IMPORT OUT= WORK.carc
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Antimicrobial_carcass_study\antimicrobial carcass
project.xlsx"
            DBMS=xlsx REPLACE;
                 Range="Sheet1$A1:F181";
                 GETNAMES=Yes;
RUN;

data pre; set carc;
   if samp="pre";
run;


proc sort data=pre out=pre;
  by rep trt loc;
run:
```

```
*ods rtf file="C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Antimicrobial_carcass_study\output1.rtf"
style=journal;

title 'Analysis of Innoculation by Location Where Treatment group is a
Blocking factor';
proc mixed data=pre plots=none;
   class trt rep loc;
   model log=loc/ddfm=kr;
   random rep trt(rep);
   lsmeans loc/pdiff;
run;

title 'Analysis of Post Innoculation by Trt & Location';

proc mixed data=pre plots=none covtest;
  class trt rep loc;
  model log=trt|loc/ddfm=kr;
  random rep rep*trt;
  lsmeans loc/pdiff;
run;
```

Carcass Study Post-Ambient Water

```
data postaw; set carc;
  if samp='post aw';
  rename log=logaw;
run;

title 'Analysis Post Ambient Wash by Trt & Location';
proc mixed data=postaw plots=none covtest;
  class trt rep loc;
  model logaw=trt|loc/ddfm=kr;
  random rep rep*trt;
  lsmeans loc/pdiff;
run;

title 'Analysis Post Ambient Wash by Location with Trt group is a blocking
factor';


proc mixed data=postaw plots=none covtest;
  class trt rep loc;
  model logaw=loc/ddfm=kr;
  random rep trt(rep);
  lsmeans loc/pdiff;
run;


proc sort data=postaw out=postaw;
  by rep trt loc;
run;
```

```
data awreduc; merge pre postaw;
  by rep trt loc;
  log_reduc=log-logaw;
run;

title 'Analysis of Log Reduction of Ambient Wash';
proc mixed data=awreduc covtest plots=none;
  class rep trt loc;
  model log_reduc=loc/ddfm=kr;
  random rep trt(rep);
  lsmeans loc/pdiff;
run;
```

Carcass Study Post-Hot Water

```
data posthw; set carc;
  if samp='post hw';
  rename log=loghw;
run;

proc sort data=posthw out=posthw;
  by rep trt loc;
run;

data hwreduc; merge postaw posthw;
  by rep trt loc;
  log_reduc=logaw-loghw;
run;

title 'Analysis of log cfu after Hot Wash by Trt & Location';
proc mixed data=posthw covtest plots=none;
  class rep trt loc;
  model loghw=trt|loc/ddfm=kr;
  random rep rep*trt;
  lsmeans trt|loc/pdiff;
run;

title 'Analysis of log cfu after Hot Wash by Location with Trt group as a
blocking factor';
proc mixed data=posthw covtest plots=none;
  class rep trt loc;
  model loghw=loc/ddfm=kr;
  random rep trt(rep);
  lsmeans loc/pdiff;
run;

title 'Analysis of Log Reduction from AW to HW by Trt & Location';
proc mixed data=hwreduc covtest plots=none;
  class rep trt loc;
  model log_reduc=trt|loc/ddfm=kr;
  random rep rep*trt;
  lsmeans trt|loc/pdiff;
run:
```

```
title 'Analysis of Log Reduction from AW to HW by Location with Trt as a
blocking factor';
proc mixed data=hwreduc covtest plots=none;
  class rep trt loc;
  model log_reduc=loc/ddfm=kr;
  random rep trt(rep);
  lsmeans loc/pdiff;
run;
```

Carcass Study Post-Antimicrobial

```
data postanti; set carc;
   if samp='post anti';
   rename log=loganti;
run;

proc sort data=postanti;
  by rep trt loc;
run;

title 'Analysis of log cfu after antimicrobial wash by TRT & Location';
proc mixed data=postanti covtest plots=none;
   class trt rep loc;
   model loganti=trt|loc/ddfm=kr;
   random rep rep*trt;
   lsmeans trt|loc/pdiff;
run;

data antireduc; merge posthw postanti;
  by rep trt loc;
  log_reduc=loghw-loganti;
run;

title 'Analysis of log reduction from Hot Wash to Post AntiMicrobial
Wash';
proc mixed data=antireduc covtest plots=none;
   class trt rep loc;
   model log_reduc=trt|loc/ddfm=kr;
   random rep rep*trt;
   lsmeans trt|loc/pdiff;
run;
```

Carcass Study Post-Spray Chill

```
data postsc; set carc;
  if samp='post sc';
  rename log=logsc;
run;

proc sort data=postsc;
  by rep trt loc;
run;
```

```
title 'Analysis of log cfu after spray chill by Trt & Location';
proc mixed data=postsc covtest plots=none;
   class trt rep loc;
   model logsc=trt|loc/ddfm=kr;
   random rep rep*trt;
   lsmeans trt|loc/pdiff;
run;

data screduc; merge postanti postsc;
   by rep trt loc;
   log_reduc=loganti-logsc;
run;

title 'Analysis of log reductions from Anti Microbial Wash to post Spray
Chill';
proc mixed data=screduc covtest plots=none;
   class trt rep loc;
   model log_reduc=trt|loc/ddfm=kr;
   random rep rep*trt;
   lsmeans trt|loc/pdiff;
run;
```

Carcass Study Overall

```
PROC IMPORT OUT= WORK.carc
            DATAFILE= "C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Antimicrobial_carcass_study\antimicrobial carcass
project.xlsx"
            DBMS=xlsx REPLACE;
                 Range="Sheet1$A1:F181";
                 GETNAMES=Yes;
RUN;


ods rtf file="C:\Users\Chris\Documents\KSU
Consulting\Matthew_Krug\Antimicrobial_carcass_study\output2.rtf"
style=journal;

proc mixed data=carc order=data plots=none;
   class samp rep sideID loc;
   model log=loc|samp;
   random rep sideID(rep) sideID*loc(rep) sideID*samp(rep);
   lsmeans loc*samp/pdiff;
   ods output diffs=diff;
run;
```

Appendix B – Raw Data Used for Statistical Analyses

PAA Loin Micro Analysis – No Overlay

Rep	Treatment	Micro Analysis Sampling point	Log CFU/cm2
1	0	pre	4.9
1	0	post	4.8
1	0	24 hr	4.5
1	200	pre	5.1
1	200	post	4.7
1	200	24 hr	4.0
1	400	pre	4.8
1	400	post	4.3
1	400	24 hr	4.4
1	600	pre	4.9
1	600	post	4.1
1	600	24 hr	4.1
1	800	pre	4.9
1	800	post	4.0
1	800	24 hr	3.9
1	1000	pre	4.9
1	1000	post	3.8
1	1000	24 hr	4.1
1	1200	pre	4.9
1	1200	post	4.3
1	1200	24 hr	4.0
1	1400	pre	5.1
1	1400	post	4.3
1	1400	24 hr	4.1
1	1600	pre	5.1
1	1600	post	3.5
1	1600	24 hr	4.0
1	1800	pre	5.0
1	1800	post	3.7
1	1800	24 hr	4.1
2	0	pre	4.9
2	0	post	4.4
2	0	24 hr	4.4
2	200	pre	5.1
2	200	post	4.3
2	200	24 hr	4.2
2	400	pre	4.8

2	400	post	4.0
2	400	24 hr	4.2
2	600	pre	5.0
2	600	post	4.1
2	600	24 hr	3.7
2	800	pre	4.8
2	800	post	4.0
2	800	24 hr	4.3
2	1000	pre	4.9
2	1000	post	4.2
2	1000	24 hr	3.9
2	1200	pre	4.7
2	1200	post	4.1
2	1200	24 hr	4.0
2	1400	pre	4.8
2	1400	post	3.9
2	1400	24 hr	3.7
2	1600	pre	4.9
2	1600	post	3.7
2	1600	24 hr	4.1
2	1800	pre	4.8
2	1800	post	4.1
2	1800	24 hr	3.8
3	0	pre	4.8
3	0	post	5.1
3	0	24 hr	4.3
3	200	pre	5.2
3	200	post	4.9
3	200	24 hr	4.1
3	400	pre	4.8
3	400	post	4.2
3	400	24 hr	4.2
3	600	pre	4.9
3	600	post	4.3
3	600	24 hr	3.9
3	800	pre	4.8
3	800	post	3.8
3	800	24 hr	3.8
3	1000	pre	5.2
3	1000	post	4.5
3	1000	24 hr	4.0
3	1200	pre	4.8
3	1200	post	4.1

3	1200	24 hr	3.7
3	1400	pre	5.1
3	1400	post	4.4
3	1400	24 hr	4.0
3	1600	pre	5.2
3	1600	post	4.0
3	1600	24 hr	3.7
3	1800	pre	5.0
3	1800	post	4.1
3	1800	24 hr	3.4

PAA Loin Micro Analysis – With Overlay

	Micro Analysis (overlay media)		
Rep	Treatment	Sampling Point	Log CFU/cm2
1	0	pre	5.1
1	0	post	5.0
1	0	24 hr	4.9
1	200	pre	5.1
1	200	post	4.8
1	200	24 hr	4.0
1	400	pre	5.0
1	400	post	4.4
1	400	24 hr	4.8
1	600	pre	5.1
1	600	post	4.3
1	600	24 hr	4.0
1	800	pre	4.9
1	800	post	4.1
1	800	24 hr	4.8
1	1000	pre	5.1
1	1000	post	4.0
1	1000	24 hr	4.0
1	1200	pre	5.1
1	1200	post	4.4
1	1200	24 hr	4.0
1	1400	pre	5.1
1	1400	post	4.5
1	1400	24 hr	4.1

1	1600	pre	5.2
1	1600	post	4.4
1	1600	24 hr	4.1
1	1800	pre	5.1
1	1800	post	4.0
1	1800	24 hr	4.1
2	0	pre	5.0
2	0	post	4.3
2	0	24 hr	4.9
2	200	pre	5.1
2	200	post	4.2
2	200	24 hr	4.1
2	400	pre	5.0
2	400	post	4.2
2	400	24 hr	4.1
2	600	pre	5.1
2	600	post	3.8
2	600	24 hr	3.7
2	800	pre	5.0
2	800	post	4.1
2	800	24 hr	4.1
2	1000	pre	5.0
2	1000	post	4.2
2	1000	24 hr	4.1
2	1200	pre	4.8
2	1200	post	4.1
2	1200	24 hr	4.0
2	1400	pre	4.9
2	1400	post	4.0
2	1400	24 hr	3.8
2	1600	pre	4.9
2	1600	post	3.5
2	1600	24 hr	3.8
2	1800	pre	5.0
2	1800	post	3.8
2	1800	24 hr	3.8
3	0	pre	5.3
3	0	post	4.9
3	0	24 hr	4.3
3	200	pre	5.2
3	200	post	4.9
3	200	24 hr	4.2
3	400	pre	5.1

3	400	post	4.4
3	400	24 hr	4.1
3	600	pre	5.7
3	600	post	6.0
3	600	24 hr	4.1
3	800	pre	4.8
3	800	post	3.9
3	800	24 hr	3.9
3	1000	pre	5.0
3	1000	post	5.1
3	1000	24 hr	4.2
3	1200	pre	5.0
3	1200	post	3.9
3	1200	24 hr	3.8
3	1400	pre	5.5
3	1400	post	4.0
3	1400	24 hr	4.1
3	1600	pre	5.3
3	1600	post	4.3
3	1600	24 hr	3.8
3	1800	pre	5.1
3	1800	post	4.1
3	1800	24 hr	3.7

PAA Loin Color Analysis

Rep	Treatment	Color Analysis Sampling Point	L*	a*	b*
1	0	pre	42.90	27.31	22.72
1	0	post	45.98	22.98	19.44
1	0	24 hr	48.24	26.56	22.69
1	200	pre	43.61	25.94	21.12
1	200	post	44.11	23.94	18.91
1	200	24 hr	47.30	22.41	20.18
1	400	pre	43.75	25.84	21.50
1	400	post	43.83	24.38	19.89
1	400	24 hr	47.50	23.19	19.62
1	600	pre	43.80	25.22	20.22
1	600	post	46.68	21.15	18.00
1	600	24 hr	47.55	19.54	18.20
1	800	pre	45.27	26.11	22.72
1	800	post	49.03	21.41	19.24
1	800	24 hr	53.28	23.18	22.78
1	1000	pre	45.50	26.31	22.54
1	1000	post	48.68	20.93	18.85
1	1000	24 hr	53.55	20.78	20.38
1	1200	pre	46.60	25.37	22.46
1	1200	post	48.13	22.68	20.46
1	1200	24 hr	50.61	17.89	18.67
1	1400	pre	47.57	24.93	22.76
1	1400	post	48.26	21.85	19.67
1	1400	24 hr	51.47	14.12	17.14
1	1600	pre	40.19	25.71	19.75
1	1600	post	43.01	22.64	18.15
1	1600	24 hr	46.17	19.66	18.14
1	1800	pre	44.83	24.95	21.96
1	1800	post	38.50	20.81	19.62
1	1800	24 hr	51.58	20.69	20.55
2	0	pre	40.27	28.47	21.28
2	0	post	42.49	26.47	20.46
2	0	24 hr	41.70	20.63	17.78
2	200	pre	38.22	26.10	19.40
2	200	post	40.94	24.35	18.50
2	200	24 hr	39.58	19.21	17.05
2	400	pre	41.93	23.64	19.78
2	400	post	45.38	20.60	17.85

2	400	24 hr	43.67	16.98	17.07
2	600	pre	40.17	27.73	20.77
2	600	post	42.43	25.64	19.55
2	600	24 hr	43.20	25.98	20.60
2	800	pre	41.49	23.71	19.16
2	800	post	41.61	22.36	17.99
2	800	24 hr	42.59	19.26	17.44
2	1000	pre	42.22	27.99	21.14
2	1000	post	40.51	25.14	18.55
2	1000	24 hr	41.92	22.62	17.78
2	1200	pre	39.44	25.27	19.09
2	1200	post	40.34	24.05	19.60
2	1200	24 hr	39.57	14.05	14.57
2	1400	pre	40.92	26.43	20.04
2	1400	post	42.48	24.11	18.14
2	1400	24 hr	42.07	23.45	19.77
2	1600	pre	40.08	27.26	20.75
2	1600	post	40.68	25.36	18.62
2	1600	24 hr	40.56	20.64	17.51
2	1800	pre	41.57	26.38	20.11
2	1800	post	43.81	23.39	18.60
2	1800	24 hr	42.57	23.57	19.39
3	0	pre	38.99	25.89	19.65
3	0	post	40.75	24.51	19.17
3	0	24 hr	40.67	21.86	18.35
3	200	pre	42.35	25.47	19.65
3	200	post	48.22	22.03	19.30
3	200	24 hr	44.92	20.60	18.91
3	400	pre	39.39	28.07	20.26
3	400	post	41.74	24.79	18.97
3	400	24 hr	40.83	23.28	18.82
3	600	pre	41.86	25.77	20.42
3	600	post	43.10	23.63	19.15
3	600	24 hr	42.21	22.39	22.44
3	800	pre	38.72	27.39	19.67
3	800	post	40.81	25.01	19.25
3	800	24 hr	41.24	20.01	17.75
3	1000	pre	39.18	27.22	19.95
3	1000	post	39.68	24.78	19.05
3	1000	24 hr	41.62	20.14	17.90
3	1200	pre	41.02	28.04	21.35
3	1200	post	43.52	21.73	19.10
3	1200	24 hr	43.52	21.73	19.10

3	1400	pre	41.25	26.09	20.14
3	1400	post	41.91	23.68	18.49
3	1400	24 hr	42.75	17.98	17.25
3	1600	pre	41.89	27.83	21.63
3	1600	post	47.06	22.43	18.92
3	1600	24 hr	46.74	16.54	17.99
3	1800	pre	41.64	26.33	20.39
3	1800	post	43.88	21.79	17.24
3	1800	24 hr	44.84	21.73	19.10

PAA Loin TBARS Analysis

	TBARS Analysis	
Rep	Treatment	TBA value
2	0	0.24
2	200	0.25
2	400	0.33
2	600	0.32
2	800	0.29
2	1000	0.22
2	1200	0.32
2	1400	0.26
2	1600	0.19
2	1800	0.19
3	0	0.18
3	200	0.28
3	400	0.17
3	600	0.36
3	800	0.25
3	1000	0.42
3	1200	0.22
3	1400	0.26
3	1600	0.46
3	1800	0.22

Lactic Acid Loin Micro Analysis – No Overlay

Rep	Treatment	Micro Analysis Sampling point	Log CFU/cm2
1	0	pre	4.7
1	0	post	4.8
1	0	24 hr	4.4
1	3	pre	4.8
1	3	post	4.8
1	3	24 hr	4.4
1	3.5	pre	4.9
1	3.5	post	4.4
1	3.5	24 hr	4.3
1	4	pre	4.7
1	4	post	4.7
1	4	24 hr	4.2
1	4.5	pre	4.7
1	4.5	post	4.4
1	4.5	24 hr	4.3
1	5	pre	5.0
1	5	post	4.5
1	5	24 hr	4.7
1	5.5	pre	5.1
1	5.5	post	4.5
1	5.5	24 hr	4.1
1	6	pre	4.9
1	6	post	4.5
1	6	24 hr	4.1
1	7	pre	4.9
1	7	post	4.5
1	7	24 hr	4.4
1	8	pre	5.0
1	8	post	4.3
1	8	24 hr	3.7
1	9	pre	4.9
1	9	post	4.3
1	9	24 hr	4.1
1	10	pre	4.7
1	10	post	4.4
1	10	24 hr	4.3
2	0	pre	4.6

2	0	post	4.6
2	0	24 hr	4.6
2	3	pre	4.6
2	3	post	4.5
2	3	24 hr	4.3
2	3.5	pre	4.7
2	3.5	post	4.3
2	3.5	24 hr	4.2
2	4	pre	4.7
2	4	post	4.4
2	4	24 hr	4.2
2	4.5	pre	4.7
2	4.5	post	4.1
2	4.5	24 hr	4.3
2	5	pre	4.8
2	5	post	4.3
2	5	24 hr	4.1
2	5.5	pre	4.7
2	5.5	post	4.2
2	5.5	24 hr	4.0
2	6	pre	4.7
2	6	post	4.2
2	6	24 hr	4.2
2	7	pre	4.7
2	7	post	4.0
2	7	24 hr	3.5
2	8	pre	4.7
2	8	post	4.2
2	8	24 hr	4.1
2	9	pre	4.7
2	9	post	4.2
2	9	24 hr	4.0
2	10	pre	4.7
2	10	post	3.9
2	10	24 hr	3.8
3	0	pre	4.8
3	0	post	4.7
3	0	24 hr	4.5
3	3	pre	4.9
3	3	post	4.3
3	3	24 hr	4.2
3	3.5	pre	4.9
3	3.5	post	4.2

3	3.5	24 hr	3.9
3	4	pre	4.9
3	4	post	4.1
3	4	24 hr	4.1
3	4.5	pre	4.8
3	4.5	post	4.3
3	4.5	24 hr	4.3
3	5	pre	4.5
3	5	post	4.2
3	5	24 hr	4.1
3	5.5	pre	4.8
3	5.5	post	4.4
3	5.5	24 hr	4.2
3	6	pre	4.8
3	6	post	4.1
3	6	24 hr	4.1
3	7	pre	4.6
3	7	post	4.2
3	7	24 hr	4.2
3	8	pre	4.7
3	8	post	4.1
3	8	24 hr	4.0
3	9	pre	4.9
3	9	post	4.1
3	9	24 hr	4.1
3	10	pre	4.8
3	10	post	3.9
3	10	24 hr	3.9

Lactic Acid Loin Micro Analysis – With Overlay

Micro Analysis (overlay media)

Rep	Treatment	Sampling Point	Log CFU/cm2
1	0	pre	4.9
1	0	post	5.0
1	0	24 hr	4.8
1	3	pre	5.0
1	3	post	5.0
1	3	24 hr	4.5
1	3.5	pre	5.0
1	3.5	post	4.5
1	3.5	24 hr	4.4
1	4	pre	4.9
1	4	post	4.9
1	4	24 hr	4.3
1	4.5	pre	5.1
1	4.5	post	4.6
1	4.5	24 hr	4.5
1	5	pre	5.1
1	5	post	4.8
1	5	24 hr	4.9
1	5.5	pre	5.2
1	5.5	post	4.5
1	5.5	24 hr	4.4
1	6	pre	5.1
1	6	post	4.4
1	6	24 hr	4.2
1	7	pre	5.0
1	7	post	5.0
1	7	24 hr	4.5
1	8	pre	5.2
1	8	post	4.4
1	8	24 hr	3.8
1	9	pre	4.9
1	9	post	4.4
1	9	24 hr	4.2
1	10	pre	4.9
1	10	post	4.4
1	10	24 hr	4.4
2	0	pre	4.7

2	0	post	4.5
2	0	24 hr	4.4
2	3	pre	4.8
2	3	post	4.3
2	3	24 hr	4.4
2	3.5	pre	4.7
2	3.5	post	4.4
2	3.5	24 hr	4.2
2	4	pre	4.9
2	4	post	4.4
2	4	24 hr	4.3
2	4.5	pre	5.1
2	4.5	post	4.4
2	4.5	24 hr	4.4
2	5	pre	4.9
2	5	post	4.4
2	5	24 hr	4.2
2	5.5	pre	4.6
2	5.5	post	4.2
2	5.5	24 hr	4.2
2	6	pre	4.9
2	6	post	4.3
2	6	24 hr	4.3
2	7	pre	5.0
2	7	post	3.9
2	7	24 hr	3.4
2	8	pre	4.9
2	8	post	4.4
2	8	24 hr	4.3
2	9	pre	4.7
2	9	post	4.3
2	9	24 hr	4.2
2	10	pre	4.9
2	10	post	4.1
2	10	24 hr	3.9
3	0	pre	5.1
3	0	post	4.9
3	0	24 hr	4.6
3	3	pre	5.1
3	3	post	4.5
3	3	24 hr	4.3
3	3.5	pre	5.0
3	3.5	post	4.5

3	3.5	24 hr	4.4
3	4	pre	5.0
3	4	post	4.3
3	4	24 hr	4.2
3	4.5	pre	4.9
3	4.5	post	4.4
3	4.5	24 hr	4.2
3	5	pre	4.8
3	5	post	4.3
3	5	24 hr	4.2
3	5.5	pre	5.0
3	5.5	post	4.4
3	5.5	24 hr	4.2
3	6	pre	5.0
3	6	post	4.2
3	6	24 hr	4.1
3	7	pre	4.8
3	7	post	4.3
3	7	24 hr	4.2
3	8	pre	4.8
3	8	post	4.2
3	8	24 hr	4.1
3	9	pre	5.0
3	9	post	4.2
3	9	24 hr	4.2
3	10	pre	4.9
3	10	post	4.0
3	10	24 hr	3.9

Lactic Acid Loin Color Analysis

Rep	Treatment	Sampling Point	Color Analysis L*	a*	b*
1	0	pre	37.42	27.14	20.25
1	0	post	40.07	24.73	18.77
1	0	24 hr	39.59	15.66	14.38
1	3	pre	38.85	27.34	19.99
1	3	post	36.91	25.55	19.25
1	3	24 hr	40.66	20.26	17.50
1	3.5	pre	35.94	28.32	19.74
1	3.5	post	36.60	24.79	18.46
1	3.5	24 hr	38.17	14.55	15.11
1	4	pre	36.66	25.87	19.03
1	4	post	34.62	24.02	17.09
1	4	24 hr	39.52	10.54	14.52
1	4.5	pre	36.10	29.78	21.84
1	4.5	post	34.42	26.99	19.61
1	4.5	24 hr	38.70	14.87	15.77
1	5	pre	37.04	27.93	20.06
1	5	post	36.13	23.65	17.27
1	5	24 hr	39.23	10.11	14.85
1	5.5	pre	36.70	26.20	19.22
1	5.5	post	34.18	23.94	16.74
1	5.5	24 hr	37.34	11.58	14.53
1	6	pre	36.98	27.41	19.21
1	6	post	35.83	25.65	17.98
1	6	24 hr	38.19	14.88	14.90
1	7	pre	39.06	25.38	18.82
1	7	post	37.19	21.28	16.36
1	7	24 hr	36.46	13.15	14.32
1	8	pre	38.27	29.63	21.58
1	8	post	35.08	22.61	16.48
1	8	24 hr	35.76	14.99	14.22
1	9	pre	37.10	26.29	18.98
1	9	post	37.43	18.76	14.07
1	9	24 hr	38.82	10.71	13.32
1	10	pre	39.73	26.51	20.89
1	10	post	37.34	22.58	16.28
1	10	24 hr	38.65	12.43	14.73
2	0	pre	41.98	29.02	23.15

2	0	post	44.37	25.18	20.24
2	0	24 hr	40.58	13.73	14.73
2	3	pre	42.48	25.25	20.66
2	3	post	41.13	22.47	18.42
2	3	24 hr	40.21	10.96	14.23
2	3.5	pre	40.07	23.75	18.58
2	3.5	post	37.75	23.65	18.26
2	3.5	24 hr	43.50	13.59	16.27
2	4	pre	43.36	25.37	21.65
2	4	post	44.10	23.07	19.69
2	4	24 hr	41.77	12.66	15.36
2	4.5	pre	45.97	25.27	21.65
2	4.5	post	43.13	22.45	18.90
2	4.5	24 hr	43.48	15.37	17.60
2	5	pre	44.76	26.58	22.39
2	5	post	41.48	22.87	18.81
2	5	24 hr	41.24	11.98	15.81
2	5.5	pre	42.20	27.22	22.04
2	5.5	post	39.25	22.87	18.46
2	5.5	24 hr	41.09	12.34	15.48
2	6	pre	39.43	27.65	21.69
2	6	post	38.27	24.97	19.12
2	6	24 hr	38.61	14.09	15.68
2	7	pre	43.39	25.26	21.67
2	7	post	39.21	21.71	17.46
2	7	24 hr	41.07	12.97	15.82
2	8	pre	40.46	27.67	21.58
2	8	post	36.59	23.11	16.37
2	8	24 hr	36.38	13.72	14.80
2	9	pre	41.09	29.25	22.55
2	9	post	38.04	25.60	19.70
2	9	24 hr	34.99	13.07	13.64
2	10	pre	44.60	25.94	21.25
2	10	post	38.46	21.81	16.97
2	10	24 hr	38.36	14.33	15.16
3	0	pre	44.61	27.86	22.64
3	0	post	46.75	24.43	21.02
3	0	24 hr	44.33	12.71	15.09
3	3	pre	46.20	28.37	24.51
3	3	post	45.57	26.11	22.23
3	3	24 hr	43.13	19.15	18.85
3	3.5	pre	43.89	28.02	22.51
3	3.5	post	42.99	26.55	21.68

3	3.5	24 hr	44.06	15.30	16.73
3	4	pre	42.90	28.02	23.86
3	4	post	42.79	26.09	20.59
3	4	24 hr	42.55	17.81	18.25
3	4.5	pre	42.19	26.81	21.26
3	4.5	post	42.01	25.70	21.17
3	4.5	24 hr	42.95	15.64	16.67
3	5	pre	48.58	25.35	23.38
3	5	post	44.51	24.01	20.55
3	5	24 hr	43.82	17.37	19.40
3	5.5	pre	45.09	27.43	23.60
3	5.5	post	44.93	24.46	20.43
3	5.5	24 hr	43.18	18.11	19.14
3	6	pre	44.87	26.10	22.17
3	6	post	39.66	23.63	18.65
3	6	24 hr	42.46	16.37	18.44
3	7	pre	45.49	26.28	23.39
3	7	post	40.92	25.28	20.35
3	7	24 hr	40.62	15.75	18.25
3	8	pre	47.56	25.17	23.05
3	8	post	41.85	20.89	17.65
3	8	24 hr	37.77	11.55	14.51
3	9	pre	43.32	26.37	21.23
3	9	post	38.33	22.36	16.63
3	9	24 hr	37.93	9.84	12.80
3	10	pre	47.06	25.23	23.02
3	10	post	41.43	18.86	15.98
3	10	24 hr	40.11	15.10	17.70

Lactic Acid Loin TBARS Analysis

	TBARS Analysis	
Rep	Treatment	TBA value
1	0	0.24
1	3	0.43
1	3.5	0.28
1	4	0.33
1	4.5	0.19
1	5	0.78
1	5.5	0.19
1	6	0.37
1	7	0.64
1	8	0.42
1	9	0.54
1	10	0.57
2	0	0.12
2	3	0.15
2	3.5	0.22
2	4	0.29
2	4.5	0.35
2	5	0.37
2	5.5	0.29
2	6	0.40
2	7	0.26
2	8	0.28
2	9	0.35
2	10	0.36
3	0	0.32
3	3	0.33
3	3.5	0.24
3	4	0.32
3	4.5	0.35
3	5	0.37
3	5.5	0.42
3	6	0.40
3	7	0.46
3	8	0.51
3	9	0.46
3	10	0.44

Carcass Study Raw Data

trt	samp	rep	sideID	loc	log
la	pre	1	1	top	6.82
la	pre	1	1	mid	7.53
la	pre	1	1	bot	6.7
la	post aw	1	1	top	5.08
la	post aw	1	1	mid	6.52
la	post aw	1	1	bot	5.21
la	post hw	1	1	top	0.97
la	post hw	1	1	mid	3.12
la	post hw	1	1	bot	2.25
la	post anti	1	1	top	0.97
la	post anti	1	1	mid	0.97
la	post anti	1	1	bot	0.97
la	post sc	1	1	top	0.97
la	post sc	1	1	mid	0.97
la	post sc	1	1	bot	0.97
cen	pre	1	2	top	6.47
cen	pre	1	2	mid	6.33
cen	pre	1	2	bot	5.87
cen	post aw	1	2	top	4.57
cen	post aw	1	2	mid	6.33
cen	post aw	1	2	bot	5.87
cen	post hw	1	2	top	0.97
cen	post hw	1	2	mid	3.55
cen	post hw	1	2	bot	4.05
cen	post anti	1	2	top	0.97
cen	post anti	1	2	mid	0.97
cen	post anti	1	2	bot	3.03
cen	post sc	1	2	top	0.97
cen	post sc	1	2	mid	0.97
cen	post sc	1	2	bot	2.25
ctrl	pre	1	3	top	6.19
ctrl	pre	1	3	mid	7.36
ctrl	pre	1	3	bot	5.36
ctrl	post aw	1	3	top	4.53
ctrl	post aw	1	3	mid	5.89
ctrl	post aw	1	3	bot	4.6
ctrl	post hw	1	3	top	2.25
ctrl	post hw	1	3	mid	0.97
ctrl	post hw	1	3	bot	0.97
ctrl	post anti	1	3	top	2.25

ctrl	post anti	1	3	mid	0.97
ctrl	post anti	1	3	bot	0.97
ctrl	post sc	1	3	top	0.97
ctrl	post sc	1	3	mid	2.43
ctrl	post sc	1	3	bot	0.97
paa	pre	1	4	top	6.33
paa	pre	1	4	mid	7.53
paa	pre	1	4	bot	6.7
paa	post aw	1	4	top	6
paa	post aw	1	4	mid	6.2
paa	post aw	1	4	bot	5.58
paa	post hw	1	4	top	2.43
paa	post hw	1	4	mid	2.25
paa	post hw	1	4	bot	3.65
paa	post anti	1	4	top	0.97
paa	post anti	1	4	mid	2.25
paa	post anti	1	4	bot	3.55
paa	post sc	1	4	top	0.97
paa	post sc	1	4	mid	0.97
paa	post sc	1	4	bot	2.25
paa	pre	2	5	top	5.69
paa	pre	2	5	mid	7.06
paa	pre	2	5	bot	5.82
paa	post aw	2	5	top	3.89
paa	post aw	2	5	mid	6.29
paa	post aw	2	5	bot	5.16
paa	post hw	2	5	top	0.97
paa	post hw	2	5	mid	3.09
paa	post hw	2	5	bot	2.9
paa	post anti	2	5	top	1.95
paa	post anti	2	5	mid	0.97
paa	post anti	2	5	bot	2.43
paa	post sc	2	5	top	0.97
paa	post sc	2	5	mid	3.31
paa	post sc	2	5	bot	0.97
ctrl	pre	2	6	top	6.06
ctrl	pre	2	6	mid	6.45
ctrl	pre	2	6	bot	6.62
ctrl	post aw	2	6	top	4.7
ctrl	post aw	2	6	mid	4.99
ctrl	post aw	2	6	bot	5.1
ctrl	post hw	2	6	top	2.9
ctrl	post hw	2	6	mid	0.97

ctrl	post hw	2	6	bot	3.2
ctrl	post anti	2	6	top	2.9
ctrl	post anti	2	6	mid	0.97
ctrl	post anti	2	6	bot	3.2
ctrl	post sc	2	6	top	0.97
ctrl	post sc	2	6	mid	0.97
ctrl	post sc	2	6	bot	2.25
la	pre	2	7	top	5.7
la	pre	2	7	mid	7.12
la	pre	2	7	bot	5.7
la	post aw	2	7	top	3.7
la	post aw	2	7	mid	6.22
la	post aw	2	7	bot	4.73
la	post hw	2	7	top	0.97
la	post hw	2	7	mid	3.09
la	post hw	2	7	bot	1.95
la	post anti	2	7	top	0.97
la	post anti	2	7	mid	2.55
la	post anti	2	7	bot	1.95
la	post sc	2	7	top	0.97
la	post sc	2	7	mid	0.97
la	post sc	2	7	bot	1.95
cen	pre	2	8	top	6.59
cen	pre	2	8	mid	6.94
cen	pre	2	8	bot	6.29
cen	post aw	2	8	top	4.96
cen	post aw	2	8	mid	6.12
cen	post aw	2	8	bot	5.22
cen	post hw	2	8	top	0.97
cen	post hw	2	8	mid	2.55
cen	post hw	2	8	bot	3.7
cen	post anti	2	8	top	3.03
cen	post anti	2	8	mid	0.97
cen	post anti	2	8	bot	3.49
cen	post sc	2	8	top	2.25
cen	post sc	2	8	mid	0.97
cen	post sc	2	8	bot	2.25
cen	pre	3	9	top	6.69
cen	pre	3	9	mid	6.96
cen	pre	3	9	bot	7.1
cen	post aw	3	9	top	5.06
cen	post aw	3	9	mid	5.35
cen	post aw	3	9	bot	6.27

cen	post hw	3	9	top	0.97
cen	post hw	3	9	mid	2.25
cen	post hw	3	9	bot	3.35
cen	post anti	3	9	top	2.55
cen	post anti	3	9	mid	3.25
cen	post anti	3	9	bot	2.73
cen	post sc	3	9	top	0.97
cen	post sc	3	9	mid	2.95
cen	post sc	3	9	bot	2.95
la	pre	3	10	top	6.41
la	pre	3	10	mid	6.69
la	pre	3	10	bot	6.65
la	post aw	3	10	top	4.97
la	post aw	3	10	mid	4.56
la	post aw	3	10	bot	6.52
la	post hw	3	10	top	2.65
la	post hw	3	10	mid	3.71
la	post hw	3	10	bot	2.55
la	post anti	3	10	top	0.97
la	post anti	3	10	mid	2.9
la	post anti	3	10	bot	2.25
la	post sc	3	10	top	0.97
la	post sc	3	10	mid	2.43
la	post sc	3	10	bot	2.25
paa	pre	3	11	top	5.64
paa	pre	3	11	mid	6.77
paa	pre	3	11	bot	6.77
paa	post aw	3	11	top	3.85
paa	post aw	3	11	mid	5.41
paa	post aw	3	11	bot	5.4
paa	post hw	3	11	top	0.97
paa	post hw	3	11	mid	0.97
paa	post hw	3	11	bot	3.35
paa	post anti	3	11	top	0.97
paa	post anti	3	11	mid	0.97
paa	post anti	3	11	bot	2.9
paa	post sc	3	11	top	0.97
paa	post sc	3	11	mid	0.97
paa	post sc	3	11	bot	0.97
ctrl	pre	3	12	top	6.86
ctrl	pre	3	12	mid	6.86
ctrl	pre	3	12	bot	6.77
ctrl	post aw	3	12	top	5.87

ctrl	post aw	3	12	mid	6.77
ctrl	post aw	3	12	bot	5.77
ctrl	post hw	3	12	top	0.97
ctrl	post hw	3	12	mid	0.97
ctrl	post hw	3	12	bot	2.25
ctrl	post anti	3	12	top	0.97
ctrl	post anti	3	12	mid	0.97
ctrl	post anti	3	12	bot	2.25
ctrl	post sc	3	12	top	0.97
ctrl	post sc	3	12	mid	2.25
ctrl	post sc	3	12	bot	2.55