The Influence of Beef Quality Characteristics on the Internalization and Thermal Susceptibility of Shiga Toxin-Producing *Escherichia coli* (STEC) in Blade-Tenderized Beef Steaks

By

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Mechanical tenderization is commonly used to increase value and palatability (flavor, tenderness, juiciness) and decrease variation of lower value or less tender cuts of beef (Luchansky et al., 2008). While this process increases value of beef cuts, it also poses a risk for internalization of bacteria and pathogens such as O157:H7 and non-O157 Shiga-toxin producing *Escherichia coli* (STEC; Luchansky et al., 2009). The risk of contamination with STEC has been a large concern for the meat industry since the early 1980s when the pathogen was brought to the attention of the public and the meat industry. Currently, *E. coli* O157:H7 is considered an adulterant in non-intact beef products. Although the USDA-FSIS has mandated intervention and screening procedures for O157:H7 in beef processing, concern for a group of non-O157 STEC serovars is rising due to rising illness associated with this group of serovars (Besser et al. 1999). These non-O157 serovars of STEC are commonly referred to as the 'Big 6' and include: O26, O45, O103, O121, O111, and O145 STEC. Prior research suggests these serovars are capable of producing illnesses that are equally as severe as their O157:H7 counterparts (Mathusa et al., 2010). Although large advances have been made in the control and reduction of O157:H7, a report published by Scallan et al. (2011) reported that illnesses associated with non-O157:H7 serogroups (including those considered as the 'Big 6') were four times more prevalent than previous estimates (Mead et al., 1999). Furthermore, the Centers for Disease Control and Prevention estimated that non-O157 STEC accounted for approximately 1,600 confirmed illnesses in 2010 and serovars within the 'Big 6' were responsible for over 70% of those cases. The importance of the

concern for these pathogens was highlighted in 2010 with the first documented outbreak of non-O157 STEC beef (O26) in the United States was reported (Mathusa et al. 2010). In response, the USDA Food Safety Inspection Service (FSIS) declared these six non-O157 STEC serovars as adulterants in ground beef (2011: 9 CFR) and in non-intact beef products (2012; 9 CFR sec. 417). Furthermore in 2012 an outbreak involving O157:H7 in blade-tenderized beef occurred in Canada; the outbreak, which caused illness in three provinces throughout Canada, was also the largest beef recall in Canadian history (Tarr, 2013). Further investigation suggested that the beef had been contaminated at slaughter due to unsanitary facilities and equipment; the bacteria were internalized by blade tenderization during further processing (Tarr, 2013, Goetz, 2012).

CHAPTER II

LITERATURE REVIEW

Escherichia coli O157:H7, the Big 6, and Blade Tenderized Beef

Mechanical tenderization of meat, namely beef, is a common practice in the meat industry. Mechanical tenderization constitutes the physical manipulation of muscle fibers, and is often used to add value and improve consistency and palatability of beef cuts. The National Cattlemen's Beef Association reports approximately 95% of beef processors utilize mechanical tenderization (Luchansky, Phebus, Thippareddi, and Call, 2008). While mechanical tenderization enhances palatability, previous studies provide evidence of increased risk of internal contamination from pathogen internalization during mechanical tenderization of previously intact beef products (Luchansky et al., 2009; Echeverry et al., 2010; Huang & Shen, 2011). Internalization of pathogens during tenderization occurs by the translocation of surface pathogens from the exterior to the interior portions of the meat. (Huang & Shen, 2011).

Pathogens of particular concern in fresh beef are *Escherichia coli* O157:H7 and a group of six non-O157:H7 serovars referred to as the 'Big 6' (O26, O45, O103, O111, O121, and O145). Shiga-toxin producing *E. coli* are of particular concern due to their potential to express genes which allow for attachment to intestinal epithelial cells (Abe et al. 1999), as well as genes which encode the production of a specific toxin (Shiga toxin) that can have lethal effects.

Shiga-toxin producing *Escherichia coli* (STEC) live in the rumen and intestines of beef cattle and may be transferred to the surface of beef cuts during slaughter and fabrication (CDC, 2012). Serogroups of Shiga-toxin producing *Escherichia coli* can be

classified as Enterohemorrhagic (EHEC) there are two acronyms commonly used in regards to STECs; STEC, (which is used in this study) is derived from the fact that these pathogens are similar to *Shigella* and are *Shigella-like Escherichia coli* (On et al. 2007). Also VTEC (Verotoxigenic *Escherichia coli*) is used and is derived from the pathogen affecting the vero cells in African monkeys kidneys (Smith et al. 1988).

Characteristics of Shiga-toxin Producing *Escherichia coli* (STEC)

Shiga-toxin producing *Escherichia coli* are facultative anaerobic gram-negative, rod shaped bacteria that are capable of producing a Shiga-toxin that may lead to serious illness or death. *Escherichia coli* was first discovered in 1885 by a pediatrician named Theodore Escherich; however, the pathogenic serotypes were not discovered until 1935, and were not considered a reportable disease until 1987 (Riley et al. 1983). *Escherichia coli* O157:H7 is probably the most well studied serotype of pathogenic *E. coli*–likely due to its relation to fresh beef and the declaration of O157:H7 as an adulterant in non-intact meat products in 1994. It is responsible for approximately 75% of STEC infections globally (Hussein, 2007). In addition to O157:H7, other non-O157 serogroups (O26, O45, O103, O111, O121, and O145) have recently been recognized as adulterants (Nguyen and Sperandio, 2012), which require control and monitoring in the United States. *Escherichia coli* naturally inhabit the intestines of farm animals, and are often spread by beef cattle to humans via food or a fecal to oral route (Nguyen and Sperandio, 2012; Hussein, 2007; Blanco et al., 2003). Currently, there is less research regarding the control and influence of non-O157 serogroups in red meats; however recent changes to U.S. federal laws and implementation of screening protocol, increased attention is being paid to this group of pathogenic STEC.

Pathogenicity of Shiga-toxin Producing *Escherichia coli* (STEC)

Shiga toxin producing *Escherichia coli* are inherently dangerous due to their low infective dose, which is thought to be less than 100 cells (Karmali, 2004). Nonetheless, the pathogenicity of STECs can be influenced by multiple factors. The pathogen must be able to survive and replicate in its environment (i.e. the gastrointestinal tract of beef cattle or humans). Once inside the GI tract of the host, pathogens attach to intestinal epithelial walls. Under these conditions, the pathogens is then able to not only grow in the tissues of the intestines, but also begin to produce toxins that lead to the progression of life threatening illnesses (i.e hemorrhagic colitis or hemolytic uremic syndrome; Grant et al., 2011).

Illnesses due to pathogenic *E. coli* result from the pathogen's ability to attach to the cell membranes in intestines of the host and form lesions causing bloody diarrhea. The pathogen has a type III protein secretion system that is vital to their pathogenicity. Type III secretion systems secrete effector proteins that are translocated to the host cells. This system allows for the transfer of valuable proteins related to pathogenicity. Specifically, the translocated intimin receptor (Tir) is translocated to the host cell early in the post-infection state. After Tir is inserted into the plasma membrane via the effector proteins translocation apparatus, it acts as a receptor for intimin, which is an outer membrane protein required for pathogen virulence (Donnenberg and Whittam 2001). After attachment using Tir and intimin, E. *coli* forms a cup-like pedestal that the bacteria sit on, this process is known as effacing. At this phase, the host (human) may become symptomatic. One of the most commonly reported illnesses related to STEC infections is hemorrhagic colitis. Approximately 15% of reported cases of hemorrhagic colitis will

develop into hemolytic uremic syndrome (HUS). Up to 12% of HUS cases result in death and approximately 25% of HUS survivors face lifelong complications. (Gill and Gill, 2010).

Prevalence of Escherichia coli O157:H7 and the 'Big 6' non-O157 Shiga-toxin Producing Escherichia coli in Beef Products

Shiga-toxin producing *E. coli* are estimated to cause 265,000 illnesses per year, 3600 hospitalizations, and 30 deaths (CDC, 2012). Recent estimates suggest that approximately 70% of illness from non-O157 STECs in humans were attributed to infections by the 'Big 6' *E.coli* serogroups (Tillman et al., 2012).

Meat industry interest in *Escherichia coli* O157:H7, commonly referred to as O157:H7, stemmed from an outbreak in the early 1980s (Tuttle et al. 1999) and one of the most well known outbreaks of O157:H7 is the Jack in the Box outbreak in the early 1990s (Koohmaraie, 2007) in which a large group of people (162 hopitalizations) became ill from the consumption of undercooked ground beef. Similar outbreaks, such as the 2003 outbreak of O157:H7, were attributed to blade tenderized (non-intact) beef steaks sold by a door-to-door vendor in Minnesota (MDH, 2003). This outbreak in particular affected consumers in three states prior to identification by the Minnesota Department of Health (MDH). Contaminated raw steaks were blade tenderized and injected with a marinade, which transferred the pathogen from the surface to the center portions of the steaks. The MDH confirmed the same subtype of *E.coli* O157 common among ill consumers were present in steaks that were recalled from the facility (Laine et al. 2005).

Serogroup O26 is one of the STEC serogroups capable of producing life-threatening illness, such as hemolytic uremic syndrome. Like O157:H7, O26 is

commonly found in the intestines and rumen of beef cattle and poses a food safety risk to the consumer. To date, there are few published investigations of O26 in blade tenderized beef cuts; however, in a 2005 investigation of non O157 cases of HUS, Brooks and others reported that between 1983 and 2002, 22% of the 940 human non-O157 STEC samples collected by health officials were confirmed as *E.coli* O26 (Brooks et al. 2005). When compared to O157:H7, O26 is known to cause serious illness; however it is less virulent and typically has a lower frequency of outbreaks than its O157 STEC counterpart (Patton & Patton, 1998). Regardless, *E. coli* O26 was recognized as the source of the cloverleaf sprouts outbreak in late 2011 and early 2012 (Foodborne Illness, 2014).

Escherichia Coli O45 is an additional 'Big 6' STEC of concern. Similarly to the other 'Big 6' STECs, O45 is not as life threatening as O157; however, it is still capable of producing the Shiga-toxins, which cause hemorrhagic colitis, and HUS. The first documented case of *E.coli* O45 in the United States was in a prison in New York State in 2005. Thirteen inmates reported bloody stool, investigations revealed *E.coli* O45 in the stool samples of the infected inmates (Foodborne Illness, 2014). Although not documented at the time, Brooks and others reported that between 1982 and 2002, 7% of the reported human non-O157 cases in the United States were later confirmed as O45 (Brooks et al., 2005).

Shiga-toxin producing *E. coli* O103 is also capable of invading the GI tract of its host and causing hemorrhagic colitis and HUS. Like other STECs, O103 is typically found in the GI tract of cattle and other farm animals and is spread to humans that consume contaminated and undercooked meat. In the early 2000s Brazil saw an increase in the prevalence of *E. coli* O103. Specifically, in 2002, three Brazilian children were

diagnosed O103 infections; prior to this outbreak the last reported illness from O103 in Brazil was in 1986 (Guth et al., 2005). Recent data regarding U.S. prevalence suggests that STEC O103 is found in less than 1% (3 of 308 samples) of retail ground beef in the U.S. (Wasilenko et al., 2014).

Escherichia coli O111 is another STEC serogroup in the Big 6, responsible for food borne infection in non-intact beef products; similarly to the other O groups of STEC, O111 will release a *Shiga-toxin* in the gastrointestinal tract of the infected host and can lead to HUS. In a study published in 2007 by H. Hussein that investigated the prevalence of O157 and other non-O157 STECS globally in the two decades, O111 was found in 1.7 to 58% of reported beef related food borne illnesses which is higher than O157 (.1-54%) (Hussein, 2007).

In a study conducted by Bosilivac and Mohammad in 2011, O121 was detected in retail ground beef in the U.S. Furthermore Bosilivac noted that O121 and the other non-O157 serogroups could be in approximately 24% of ground beef in the U.S. (Bosilivac et al., 2011). However, further investigation showed that only 0.24% of the presumptive positive samples of non-O157 carried virulence factors (attachment and type III protein secretion system), which are necessary for human illness.

Another of the 'Big 6', STEC O145, was confirmed in approximately 11% of STEC infections between 1982 and 2002 (Brooks et al., 2005). However, O145 is generally less virulent than O157. Nonetheless, it is capable of producing Shiga-toxins, bloody diarrhea, and can lead to HUS. In fact, in the summer of 2012, 18 people across nine states became ill from an *E.coli* O145 outbreak. Four hospitalizations and one death

were attributed to this outbreak, the outbreak was suspected from fresh beef but was not confirmed (USDA, 2014).

Mechanism and Factors Contributing to the Translocation of Pathogens During the Mechanical Tenderization of Beef

Mechanical tenderization of beef has been utilized for decades to improve palatability of beef. Blade tenderization disrupts muscle fibers and connective tissue in tough beef offering a more palatable experience to the consumer (Jeremiah et al. 1999, Davis et al., 1975). During mechanical needle tenderization, a blade or needle is pressed through the cut from surface to surface disrupting the muscle fibers, collagen and fat within the meat, needle tenderization is the most common form of mechanical tenderization in the United States and most of Europe (Gill et al. 2004). During the mechanical tenderization process, bacteria from the surface may be forced by the blade deep into the cut and result in contamination of previously sterile areas of the muscle (Chancey et al., 2013, Luchansky et al., 2008, Johnston et al., 1978). Pathogens translocated to the innermost portions of the beef may not be killed during cooking– especially if the cut does not reach required minimum internal temperature (Luchansky et al., 2012; Catford 2006).

Cooking Lethality of Internalized Shiga-toxin Producing *Escherichia coli* in Beef

The necessity of thermal-based destruction of *E. coli* upon cooking has resulted in numerous studies to validate the post-cooking lethality of O157:H7 (Luchansky et al., 2009; Echeverry et al., 2010; Huang & Shen, 2011). These studies investigate the cooking temperature needed to effectively kill O157 in beef at various levels of inoculation and length of time the meat was contaminated by the pathogen (Cassin et al.

1998, Ahmed et al. 1995, and Calicioglu et al. 1997). Prior to the USDA-FSIS categorizing non-O157 STECs as adulterants, there have been relatively few performed investigations regarding the cooking lethality of individual serogroups of non-O157 STECs.

Escherichia coli O157:H7 and Non O157 STEC Microbiology

Proficient isolation, identification, and confirmation of *E. coli* in foods is imperative for the fresh beef industry. Well-established and validated methods must be used to detect and confirm pathogenic *E. coli* in food samples. The USDA-FSIS method for confirming non-O157 STECs uses the BAX® System Real-time Polymerase chain reaction (PCR) to screen for the presence of the shiga toxin (*stx*) and intimin (*eae*) genes. BAX® positive samples are then subjected to culture isolation by immunomagnetic separation (IMS). Immunomagnetic separation utilizes beads coated with specific antibodies for specific serogroups. The isolated sample is then plated onto modified rainbow agar that is selective for STEC, (meaning it facilitates the grow of STEC only) and any subsequent colonies are subjected to agglutination to determine the presence of specific O antigens of the STEC. Agglutination positive samples are streaked onto tryptic soy agar (TSA) with 5% sheep blood (SBA). Isolated colonies from these plates are then confirmed using the BAX® Real-time PCR assays (Wasilenko et al. 2012).

MacConkey Agar is typically used to grow and isolate gram-negative enteric bacilli. Since O157:H7 and the 'Big 6' STEC are gram-negative enteric bacilli, MacConkey agar is a suitable medium for growth and isolation of these pathogens. MacConkey agar is a bile salt-neutral red lactose base modified with 0.5% sodium chloride for improved differential reactions of enteric pathogens (Neogen, 2005). If colonies form on MacConkey agar after incubation samples can be confirmed by using

polymerase chain reaction (PCR) software equipment such as the BAX® system. The BAX® system is capable of testing up to 96 samples in 4 hours or less by genetically confirming DNA of samples using real time PCR detection (BAX® system). Further confirmation of presumptive positive samples may include streaking on a STEC selective media such as CHROMagar (STEC), BAX® panel screens for individual serotype, or latex agglutination. CHROMagar (STEC) is a selective media with high detection sensitivity (98%) for *Shiga-toxin* producing *Escherichia coli* (Hirvonen et al. 2012). BAX® panel screens are conducted using a BAX® real time PCR assay STEC-suite that specifically screens for the Big 6 pathogenic STECs DNA (BAX® system). Latex agglutination uses latex beads coated with pathogen specific antigens; if the latex beads cluster together the sample is presumptive positive for the specific pathogen being investigated.

Beef Biochemical Traits and Their Effect on Pathogen Attachment

Beef cattle are prone to exhibit varying biochemical traits due to particular handling and stressors prior to the slaughter process. These stressors can alter the composition of the meat and cause the pH levels to rise within the muscle (Savell, 2013), which could create an optimum environment for the attachment and growth of pathogenic bacteria, such as STEC. When cattle are harvested the muscle attempts to maintain homeostasis and carry out regular functions by breaking down stored glycogen to adenosine triphosphate (ATP), which is an energy source for the muscle. Simultaneously, lactic acid accumulated during this process builds up in the muscle and drops the pH to a level near 5.6, which is normal for post slaughter meat. At this pH, beef exhibits expected visual quality properties (i.e. bright cherry red color upon oxygen exposure). In high pH

beef, the animal generally has been subjected to stress prior to slaughter and has utilized any glycogen stores within the muscle prior to harvest. Post slaughter the muscle begins to break down stored glycogen; however, the limited supply results in a minimal production of lactic acid and a post-mortem muscle pH near 6.0 (Forrest et al., 2001). Beef with a pH of 6.0 or higher is deemed "high pH" and typically takes on a light to dark purple/brown appearance (Savell, 2013). Additionally, high pH beef has a tacky surface and is more prone to bacterial spoilage and attachment (Sanz et al. 1996). It is hypothesized that the surface properties of high pH beef may make their products more prone to attachment of pathogenic bacteria attachment or internalization.

Conclusion

Shiga-toxin producing *Escherichia coli* can be present on the surface of beef products and may be translocated to the center of the meat during mechanical tenderization, and pose an immediate threat to the safety of blade tenderized beef products; these pathogens may attach to high pH beef more easily than to normal pH cutting beef. Although the 'Big 6' STECs appear to be less life threatening than *E.coli* O157:H7, these serogroups are capable of causing severe illness and long-term health repercussions and must thus remain a primary public health concern. Recent calls for further regulation of these pathogens, including suggestions of cooking recommendations to assure thermal destruction of all pathogenic STEC serogroups, requires that more information regarding the individual reactivity of each serogroup be developed.

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CHAPTER III

THE INFLUENCE OF BEEF QUALITY CHARACTERISTICS ON THE INTERNALIZATION AND THERMAL SUSCEPTIBILITY OF SHIGA-TOXIN PRODUCING *ESCHERICHIA* COLI (STEC) IN BLADE-TENDERIZED BEEF

Abstract

The risk of Shiga toxin-producing *Escherichia coli* (STEC) survival in blade-tenderized beef is a concern for beef processors. This study evaluated the internalization and post-cooking survival of individual STEC serogroups (O157:H7, O26, O45, O103, O111, O121, and O145) in blade-tenderized beef steaks with different quality traits. Strip loins representing four combinations of USDA Quality Grade (Choice or Select) and pH category (High pH or Normal pH) were inoculated (10^6 log CFU/cm^2 attachment) with individual STEC serogroups before storage (14 d), blade tenderization, and cooking (50, 60, 71, or 85°C). Serogroup populations on raw steak surfaces and internal cores were determined. Rapid-based methods were used to detect the internal presence of STEC in cooked steaks. Internalization and post-cooking survival varied among STECs. All serogroups, except O45 and O121, were detected in the internal cores of steaks cooked to 50°C, while O103, O111, and O145 STEC were detected in steaks cooked to 50, 60, and 71°C.

Key Words: beef, blade tenderized, cooking, internalization, non-intact, STEC

Introduction

Mechanical tenderization, which relies on the physical manipulation of muscle fibers, is a method commonly used to add value and improve consistency of beef cuts (Huang and Shen, 2011). In fact, the National Cattlemen's Beef Association reports approximately 95% of beef processors utilize mechanical tenderization (Luchansky, Phebus, Thippareddi, and Call, 2008).

Blade tenderization disrupts muscle fibers and connective tissue in tough beef offering a more palatable experience to the consumer (Jeremiah et al. 1999, Davis et al. 1975). During mechanical tenderization a blade or needle is pressed through the cut from surface to surface; during the tenderization process bacteria from the surface may be forced by the blade deep into the cut causing contamination of previously sterile areas of the muscle (Luchansky et al. 2008, Johnston et al. 1978). Though it is a valuable palatability-enhancing tool, research suggests a potential for an increased food safety risk due to pathogen internalization during mechanical tenderization of previously intact beef products (Luchansky et al., 2009; Echeverry, Brooks, Miller, Collins, Loneragan, and Brashears, 2010; Huang and Sheen, 2011). Furthermore (Gill et al., 2005) found *E.coli*, and other bacteria internalized in blade-tenderized beef in a retail setting; another study (Gill et al., 2009) found *E.coli* present in cooked beef (<70 C) previously injected with *E.coli* O157:H7 contaminated brine.

Pathogens translocated to the innermost portions of the beef may not be killed during cooking especially if the cut does not reach required minimum internal temperature (Luchansky et al. 2012, Catford 2006). In recent years, consumer groups and food safety advocates have filed petitions asking the United States Department of Agriculture Food

Safety and Inspection Service (USDA-FSIS) to "*promulgate regulations requiring…. these products to identify that they have been pinned, bladed, or otherwise mechanically manipulated*…" so that "*consumers know they must cook the product differently,*" (CFP, 2009; CFA, 2012). In response, the USDA-FSIS recently proposed a final rule, which will require on-package labeling and validated cooking methods for non-intact meat products (USDA FSIS, 2012).

Fresh meat contamination with STEC has been a preeminent concern of the U.S. meat industry for the past three decades. Until recently, the primary focus has been on *E. coli* O157:H7; however, concern for a group of six non-O157 STEC serogroups has risen significantly. These non-O157 STEC serogroups, specifically STEC O26, O45, O103, O121, O111, and O145 in the U.S., can produce illnesses that are equally as severe and impactful as their STEC O157:H7 counterparts. Furthermore, despite the advances in O157:H7 mitigation, management and understanding, a report published by Scallan, et al., (2011), evidenced that illnesses associated with non-O157 STEC serogroups were more than four times greater than previously published estimates (Mead et al., 1999). It should be noted that the discrepancies in illness could be due to improved detection methods, or the 2002 declaration of STEC illnesses as reportable diseases by the Centers for Disease Control. Regardless, although the studies utilized varying methods, both provide evidence that these STEC serogroups are a threat to public health.

Given the public health risk and current regulatory status of these STEC serogroups, a more comprehensive understanding of not only the pathogen's ability to migrate during mechanical tenderization, but also the susceptibility of each individual serogroup to temperatures that are considered lethal to STEC O157:H7 is necessary. Of

the available data, it is suggested that non-O157 STECs can exhibit patterns of survivability at internal temperatures representing a medium degree of doneness (71.1˚ C; Luchansky et al., 2012). However, investigations of specific parameters and characteristics that might influence internalization and thermal tolerance are sparse, yet needed. For example, prior research suggests meat composition (including but not limited to, fat, moisture, protein, and collagen) and biochemical traits (i.e. pH, water activity, etc.) can influence pathogen attachment, internalization, and thermal susceptibility (Ahmed, Conner, and Huffman, 1995; Doyle and Mazzotta, 2000; Carlson, et al. 2005). However, investigations regarding the influence of meat characteristics on STEC internalization and thermal susceptibility have yet to be performed. Furthermore, previous investigations have not determined the influence of carcass and meat quality variables, particularly USDA Quality Grade (QG) and pH, on the extent of pathogen internalization and thermal tolerance. Given the variable nature of the U.S. beef supply, we believe this data is imperative.

Thus, this project was performed with two primary objectives regarding the internalization and thermal susceptibility of STEC O157:H7 and non-O157 STEC in mechanically tenderized beef. First, we aimed to determine the influence of intramuscular fat (represented by QG) and pH (represented by High pH/Normal pH) on the attachment of pathogens to subprimal surfaces. Secondly, we evaluated the influence of QG and pH on the internalization and thermal susceptibility of individual STEC serogroups in steaks cooked to common end-point cooking temperatures.

Materials and Methods

Beef Subprimal Procurement and Processing

The objectives of this project include the evaluation of internalization and thermal tolerance in beef with targeted biochemical characteristics. As such, utilization of beef cuts from carcasses expressing distinct biochemical traits was imperative. The USDA Quality Grade system is a well-proven indicator of intramuscular fat (American Meat Science Association., 1995). Similarly, Page, Wulf, and Schwotzer (2001) indicated the discounts applied to High pH beef (i.e. carcasses with dark, firm, and dry lean) correlate strongly with intramuscular pH. Therefore, in order to meet the objective of selecting distinct biochemical parameters, USDA Quality Grade (QG) was used as an indicator of intramuscular fat and High pH status (High pH or Normal pH) as an indicator of intramuscular pH.

Trained university personnel were used to identify carcasses representing the following (n = 3 carcasses per combination per replication): USDA Choice and Normal pH (**CH-NpH**); modest to moderate marbling and High pH (**CH-HPH**); traces to slight marbling and Normal pH (**SEL-NpH**); traces to slight marbling and High pH (**SEL-HPH**). Marbling score and High pH status were evaluated on the loin surface of the 12th rib of each carcass. Carcasses were selected so that postmortem age would be no more than 7 d at the time of inoculation and processing. Paired strip loins (IMPS) #180 (USDA-AMS, 2014); n = 24 total per replication; replication = 3) were procured from each side of the carcasses meeting the four criteria combinations. The selected strip loins were immediately transported to the Gordon W. Davis Meat Laboratory at TTU (Lubbock, TX) and stored under refrigeration.

Upon arrival, paired strip loins from each carcass were fabricated using the Beef Alternative Merchandising method (Smith et. al., 2014) to longitudinally divide the

Longissimus dorsi into two equal halves. Each longitudinal half was halved again to produce eight equal sections per carcass. One raw, un-inoculated section was vacuum packaged for later biochemical analysis (described below). The remaining seven sections per carcass were assigned to inoculation (10^6 log CFU/mL) with one of seven STEC serovars (*E. coli* O157:H7, O145, O26, O111, O103, O45, or O121). Subprimal sections for each serogroup (n = 3 sections per pH and QG combination and serogroup; 84 total sections) were vacuum packaged and transported to the Texas Tech University BSL-II pathogen processing facility (Lubbock, TX) for inoculation within 24 h.

Raw and Cooked Meat Biochemical Properties.

Samples from each subprimal reserved for evaluation of un-inoculated samples were processed at the Texas Tech University Gordon W. Davis Meat Laboratory (Lubbock, TX). Subsequent evaluation of inoculated, raw and cooked samples was performed on the sample remaining after removal of the core for evaluation of pathogen internalization. All inoculated raw and cooked samples were processed in the BSL-II Food Microbiology Laboratory at Texas Tech University (Lubbock, TX).

Compositional Analysis (Raw, un-inoculated only). Compositional variables (% fat, moisture, protein, and collagen) were measured on raw samples using an AOAC-approved near infrared spectrophotometer (FOSS FoodScan, Hilleroed, Denmark; Anderson, 2007). Methods for sample preparation were the same as those described by Martin and colleagues (2013).

pH Analysis (Raw and cooked). The pH of raw and cooked steaks (for each cooking temperature) were evaluated using the procedures described by Luque and others

(2011) Samples were evaluated in duplicate and results averaged prior to statistical analysis.

Water Activity Analysis (Raw and cooked). Water activity was measured on raw and cooked steaks (for each cooking temperature) using a Hygrolab 3 bench-top water activity meter (Rotronic Inc., Huntington, NY).

Inoculum Preparation.

Cocktail mixtures of inoculation solutions for each STEC serogroup were prepared using strains from the TTU Food Microbiology Laboratory Stock Collection (Lubbock, TX). Three strains (Table 1) from each serogroup were utilized to form an inoculation cocktail for each serogroup. The frozen cultures were taken out of frozen storage (-80°C), and placed in an ice bath to thaw. Seven, one liter bottles of buffered peptone water (BPW, EMD Chemicals Inc. Gibbstown, NJ) were used and one inoculum loop of each culture were put into each bottle. Prior to formulation of each serogroup cocktail, frozen stock cultures of individual strains were added to 50 ml tryptic soy agar (TSA) and incubated 24 h at 37^0C before spread plating onto MacConkey agar (EMD Chemicals Inc., Gibbstown, NJ) plates and incubated for 18 to 24 h at 37˚C to ensure cultures were live and viable. After enumeration, the total concentration of the frozen cultures was calculated in CFU/ ml. From each of the individual strains (n = 3) within a serogroup, 50 ml BPW was inoculated and incubated for 18 to 24 h at 37˚C. From this, the amount needed to make an inoculum of 1.0×10^6 CFU/ ml in 5 L BPW was calculated using formula $C_1V_1=C_2V_2$. Shiga-toxin producing *E.coli* strains used, their sources, and actual inoculation concentrations (CFU/ml) are shown in Table 1.

Inoculation of Subprimal Sections.

One d after portioning subprimals into sections (within 7 d postmortem), individual subprimal sections were immersed in their respective STEC inoculation broth for approximately two min (one min per dorsal and ventral side). After the two min inoculation period, sections were removed from the inoculation broth and allowed to rest at 4°C for approximately 30 min to facilitate bacteria attachment. After sampling, the sections (n = 12 per serogroup; 84 total) were individually vacuum packaged (model MVS 45; Minipack-Torre, Dublin/Manchester) in high-barrier vacuum bags (Sealed Air, Cryovac, Bolingbrook, IL) and stored in the dark for 13 to14 d at 4°C.

Raw Subprimal Microbial Sampling In order to determine the attachment of STEC to subprimal surfaces, one 50-cm^2 swab sample was collected from the lean (ventral) side of an individual section from each treatment combination (QG and High pH classification; n = 4 sections per serogroup and replication) using a sponge moistened with 10 ml of BPW (World BioProducts, Mundelein, IL). The sponge was stomached for 2 min at 230 rpm using a commercial stomacher (Seward 400 Model:0400/001/AJ, Seward Medical Co., London, England) prior to formation of serial dilutions in 9 ml of BPW. The appropriate serial dilutions were spiral plated (Autoplate® 4000; Spiral Biotech Inc., Norwood, MA) onto MacConkey agar with a trypticase soy agar (TSA; Becton-Dickinson Co., Sparks, MD) overlay to allow for recovery of injured cells (Brashears, Amezquita, and Stratton, 2001). Plates were incubated for 18 to 24 h at 37°C. After incubation, STEC colonies were counted using a colony counter (QCount® 530; Advanced Instruments, Inc., Norwood, MA). Additionally, samples of the inoculation cocktail were obtained prior to inoculation for confirmation of pathogen populations;

each sample was serially diluted, spiral plated onto MacConkey agar, and incubated using the methods listed above.

Mechanical Tenderization and Steak Portioning.

After 13 or 14 d of dark storage, subprimal sections were removed from vacuum packaging, and subprimal pathogen populations were again enumerated using the methodology described previously (Section 2.4.1). After sampling, individual sections per STEC group (n = 28 sections per serogroup) were mechanically tenderized using a blade tenderization unit (Model H; Jaccard, Orchard Park, NY). Subprimal sections were passed through the blade tenderization unit only once, and the tenderization unit was thoroughly sanitized between each serogroup using Quadra-Quat™ a 7.5% active, four-way quaternary ammonium sanitizer/disinfectant (Birko corp., Henderson, Colorado). Following tenderization, each section was portioned into five 2.54-cm steaks, which were randomly assigned to either raw analysis, or one of four internal cooking temperatures (50, 60, 71, or 85°C). One 50-cm^2 swab sample was taken from the surface of one randomly chosen steak per subprimal section, and enumerated using the procedures described for subprimal sample enumeration. Steaks were immediately transported to the TTU Food Microbiology Laboratory (Lubbock, TX) for cooking and analysis of post-cooking STEC survival.

Steak Cooking

Prior to cooking, steaks for each serogroup (n = 336 steaks per replication; 7 serogroup × 4 QG/High pH categories × 4 temperatures × 3 subprimal sections per serogroup) were threaded with thermocouple wires (Type J; Cole Parmer, Vernon Hills, IL) attached to a remote temperature logging device (Model OMB-DAQ-56; Personal

Daq; OMEGA Engineering Inc., Stamford, CT) to allow for continuous internal temperature monitoring during the cooking process. After threading, steaks were placed on preheated (surface temperature of 195°C) clam-shell style grills (Model GRP99b; George Foreman, Russell Hobbs Inc. Miramar, Florida, United States) and cooked to their targeted internal temperature. Once the desired internal temperature was reached, the steak was removed from the heat source using sterile cooking tongs and allowed to rest at room temperature for three min. The start and stop time of cooking, as well as the temperature of the steak upon removal from the grill, peak temperature during rest, and temperature at the conclusion of rest period were recorded.

Microbial Sampling of Raw and Cooked Steaks.

Focus areas for microbial sampling included d 0 subprimal surface swabs, d 14 subprimal surface swabs, d 14 steak surface swabs, d 14 raw cores, and d 14 cooked cores. The extent of STEC internalization in raw and cooked steaks (after the three min rest period) was determined by aseptically obtaining a 2 cm × 5 cm × 2.54 cm core from each steak using an ethanol and flame sterilized knife for each slice going completely through the steak, the 2 cm × 5 cm × 2.54 cm core was removed from the center of the steak by following a pre-made template to ensure accuracy. After removal, the core surface was briefly flame sterilized to eliminate the possibility of inadvertent contamination to the external core surface. Following flame sterilization of the core surface, the core was comminuted in a sterile food processor (HC306; Black and Decker; Townson, MD) and the resulting homogenate was diluted in 90 ml of BPW prior to stomaching at 230 rpm for 2 min. Serial dilutions were made by placing 1 ml of diluted sample into 9 ml of BPW. The appropriate serial dilutions were spread plated onto

MacConkey agar with TSA overlay prior to incubation for 18 to 24 h at 37°C. Samples yielding plates with growth were visually counted following incubation.

Processing of non-enumerable plates. Samples that were not enumerable following incubation were subjected to rapid PCR-based detection of the *stx* and *eae* genes using the BAX® system (Dupont, Wilmington, DE). The original stomached samples were enriched with buffered peptone water (BPW) for 18 to 24 h at 37°C. After enrichment, 20 µl of the enriched sample was added to 200 µl of lysis reagent (a combination of lysis buffer and protease; Dupont) in a lysis tube. Each lysis tube was heated at 37°C for 20 min followed by 95°C for 10 min. After the heating cycles, lysis tubes were cooled at 5°C in a cooling block for 5 min. In a separate cooling block, PCR tubes were arranged and 30 µl of the lysate was transferred from the prepared sample. Samples were then subjected to PCR analysis on the BAX® system. Samples deemed to be negative by the BAX® system were discarded, while BAX®-positive samples were subject to further analysis.

Confirmation of BAX®-positive samples. Samples deemed as "BAX® positive" were subjected to confirmation by isolation of morphologically representative colonies on STEC Chromagar™ plates before further confirmation with agglutination (O157:H7) or BAX® panel screening (non-O157 STEC). The PCR based methodology of the BAX® system allows for the conservative detection of *E. coli* DNA fragments. In the case of cooked samples, this method may allow for the detection of destroyed or dead cells. Therefore, confirmation of BAX®-positive samples was performed by plating on a STEC selective media. Stomached samples were spread plated onto Chromagar™ (Chromagar™ STEC; DRG International Inc., Springfield, NJ) plates for isolation of

morphologically representative colonies (mauve colonies represent STEC, whereas other bacteria from the *Enterobacteriaceae* family are colorless or blue). After incubation at 37°C for 24 h, morphologically representative colonies on Chromagar™ were confirmed using latex agglutination (STEC O157:H7) or BAX® panel screens (for individual non-O157 STEC serogroups; Dupont).

Experimental Design and Statistical Analysis.

The experiment was designed as a 2 × 2 (marbling score x High pH category) factorial arrangement onto which a completely randomized block design was imposed. Inoculation serogroup was randomly assigned to strip loin sections from carcasses (blocks; n = 3/combination) representing each factor combination. Steaks from each strip loin section were randomly assigned to one of five analyses (raw or after cooking to 50, 60, 71, or 85°C). Independent variables included all carcass marbling score × High pH category combinations as well as internal cooking temperature and serogroup, when appropriate. Response variables of interest included the raw and cooked steak biochemical properties (composition, pH, and water activity) as well as the microbial populations of each of the following sample types: d 0 subprimal surface swabs, d 14 subprimal surface swabs, d 14 steak surface swabs, d 14 raw cores, and d 14 cooked cores. Cooking temperature data was summarized using the PROC MEANS procedure of SAS (v. 9.3, SAS Inst., Cary, NC). Microbial populations within a replication from each sample type were averaged and converted to either log CFU/g (cores) or CFU/cm^2 (surface samples) prior to statistical analysis. The influence of the independent variables of interest on microbial populations was assessed using a linear mixed model. Analysis of d 14 subprimal swabs incorporated d 0 subprimal surface populations as a covariate;

whereas analysis of d 14 steak cores included d 14 steak surface populations as a covariate in the model. Data were analyzed using a commercial statistical software program (SAS v. 9.3, Cary, NC) and model-adjusted estimates of concentration were computed for each level of the independent variables. Least squares means, generated using the LSMEANS option, were separated using the PDIFF statement and considered significant at an α of 0.05. Replication (carcass selection and inoculation) and carcass number served as blocks and were incorporated in all models as random effects.

Results and Discussion

Meat Quality Characteristics.

Carcass QG and High pH status influenced the composition, pH, and water activity values of beef strip loins prior to inoculation (Table 2 and 3). Strip loins originating from High pH carcasses had greater initial pH (Table 2; $P < 0.0001$) and water activity ($P = 0.0320$) than their Normal pH counterparts. Similarly, strip loins from USDA Choice carcasses had increased fat ($P < 0.0001$) and collagen ($P = 0.0016$), but less moisture ($P < 0.0001$) than USDA Select strip loins. Interestingly, the influence of High pH category on pH and water activity of inoculated strip loins was minimized after 14 d of vacuum storage (Table 4). No differences in pH were observed for any serogroup; however, the water activity values for STEC O26 inoculated USDA Select, High pH and Normal pH strip loins were greater ($P = 0.0201$) than values for USDA Choice High pH and Normal pH strip loins.

Carcasses classified as “high pH” are associated with a greater muscle pH (> 5.8) and high water holding capacity values (Page et al., 2001) These traits are generally associated with a more favorable environment for bacterial growth and decreased shelf-

life (Martin et al., 2013). As a result of this study, steaks from USDA Choice High pH and USDA Select High pH strip loins had a greater amount of pathogen populations on the surface (10^6 log CFU/cm^2 targeted attachment) as compared to their Normal pH counterparts. Gill, et al., (2004) and Johns, and colleagues (2011) reported that needle tenderization promotes translocation of surface pathogens to inner tissues, and some serogroups showed varied internalization.

Subprimal Surface Populations.

The populations of STEC serogroup colonies present on the subprimal surface after 30 min of attachment and 14 d of vacuum packaged dark storage are shown in Table 4. Aside from STEC serogroup O45, no differences ($P > 0.05$) in initial (d 0) attachment were observed due to combinations of carcass characteristics. However, a tendency ($P = 0.08$) for enhanced initial STEC attachment in USDA Choice Normal pH and USDA Select High pH strip loins was observed for serogroup O45. On the contrary, a tendency ($P = 0.07$) for enhanced increased subprimal surface colonies in USDA Choice High pH and USDA Select Normal pH strip loins was observed for serogroup O45 after 14 d of storage. When compared to previously performed studies (Chancey, Brooks, Martin, Echeverry, Jackson, Thompson and Brashears, 2013), these data do not comply with the expected reduction in bacterial populations due to vacuum storage; however, the extent of reduction in the current study may be influenced by the abbreviated (14 d) storage period. Furthermore, prior studies investigating pathogen internalization in mechanically tenderized products have included intervention technologies to reduce translocation (Echeverry, et al., 2009); thus, it is important to note that this study was conducted without the utilization of a subprimal intervention.

Translocation and Internalization of STEC.

The influence of carcass characteristic combinations on the populations of translocated (transfer from external subprimal surface to steak surface) and internalized (transfer from external subprimal surface to internal steak core) STEC are shown in Table 5. Generally, STEC populations were greater ($P < 0.05$) on the surface of steaks from USDA Choice High pH and USDA Select High pH strip loins when compared to their Normal pH counterparts. Carlson and colleagues (2005) also reported a correlation between pH and pathogen attachment.

The populations of internalized STEC (internal steak cores) were comparable to the populations on the steak surface; however, no differences were detected in steak core samples due to carcass characteristics ($P > 0.05$) for any serogroup. Among serogroups, populations of translocated and internalized STEC O157:H7 were among the fewest of all serogroups, whereas STEC O111 and STEC O45 were consistently among the greatest. Few studies exist that compare internalization rates of STEC O157:H7 and other STECs. These data suggest the validation data representing internalization of STEC O157:H7 may not accurately represent the non-O157 STEC serogroups tested.

Steak Cooking and Detection of STEC in Cooked Steak Samples.

Recent research (Luchansky et al., (2012) and Liao et al., (2012) have demonstrated that surviving non-O157 STEC cells can be found in the internal portions of mechanically tenderized beef steaks cooked to temperatures considered lethal to STEC O157:H7. Luchansky and colleagues (2012) found surviving cells post-cooking, it was reported that the survival of cells were presumably due to uneven heating, while Liao et al. (2012) also reported surviving cells post cooking with electric clam-shell style grills.

Regardless, investigations regarding the post-cooking survival of individual STEC strains are relatively limited.

The presence of STEC in the internal cores of beef strip loin steaks cooked to one of four internal temperature (50, 60, 71, and 85°C) following a three min rest period are shown in Table 6. Direct plating of cooked core samples yielded no enumerable plates, resulting in the utilization of PCR-based detection using the BAX® system. Samples deemed as "BAX® positive" were subjected to confirmation by isolation of morphologically representative colonies on STEC Chromagar™ plates before further confirmation with agglutination (STEC O157:H7) or BAX® panel screening (non-O157 STEC).

It is critical to note that a previous study in our laboratory using the BAX® system (Ortega et al. 2012) evaluated the detection of killed *E. coli* O157:H7 cells at concentrations from 1 to 9 log CFU/mL. The results indicated 100% of samples with 8 log CFU/ml or more of killed cells were deemed "positive", while and 9.5% to 14% of samples with less than 2.0 log CFU/ml of killed cells were classified as "positive". Therefore, in the current trial, the authors felt it was essential to not only utilized detection based methodology, but to also culturally confirm the presence of living STECs surviving after heat treatment.

Serogroups O45 and O121 were not detected in any samples, regardless of cooking temperatures. Further, a large portion of samples deemed as BAX®-positive were not confirmed to be viable STEC using Chromagar and agglutination/BAX® panel screens. Serogroups O26, O103, O111, O145, and O157:H7 STEC were confirmed in the core samples of strip loin steaks cooked to 50°C, regardless of carcass treatment

combination. Likewise, O103, O111, and O145 STEC were confirmed (BAX® positive, agglutinated and streaked on Chromagar) in the internal cores of steaks cooked to 71°C and O145 STEC was confirmed in a Select High pH steak cooked to 85°C. *Escherichia coli* O157:H7 was not detectable or confirmed in the cores of steaks cooked to 71 or 85°C. Similarly, Chancey et al. (2013) was unable to detect O157:H7 in the cores of steaks cooked to 71°C. However, Liao et al. (2012) reported enumerable colonies of STEC O26, O103, 0145, O45, and O121 in cooked beef samples.

A previous study by (Luchansky et al. 2011) suggest a variation in thermal resistance between O157:H7 and Non-O157 serogroups; data from this study are in agreement, however this study compared the non-O157 serogroups individually rather than as a group. Individual comparison data suggest that the individual serogroups behave differently when exposed to cooking.

The potential for variable thermal susceptibility among STEC serogroups in a broth model has been previously demonstrated in our lab. (Willems, Brooks, Parks, Jackson, and Brashears, 2012). Specifically, STEC O45 expressed resistance to destruction at temperatures ranging from 65.5 to 71°C and all non-O157 STECs (O145, O26, O111, O103, O45, and O121) had higher decimal reduction values at 60°C when compared to their STEC O157:H7 counterparts. These data, combined with the results of the current research, suggest there are likely inherent differences which make certain non-O157 STEC serogroups more or less tolerant of previously validated lethal processes, specifically cooking. The variation expressed by each serogroup suggest that Non-O157 and O157:H7 are unique and need to be viewed individually in blade tenderized beef rather than as a group.

Conclusions

Among observed STEC serogroups, populations of translocated and internalized STEC O157:H7 were among the lowest, whereas O111 and O45 were consistently among the greatest. These data suggest the validation data representing destruction of O157:H7 may not accurately represent all pathogenic STEC serogroups. Therefore, although STEC O157:H7 is often considered to be a suitable indicator for other pathogenic STECs, these data strengthen the suggestion that STEC serogroups act differently when exposed to similar stresses.

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Table 1. The inoculum level (log CFU/ml), strain identification, and origination[1] of Shiga-toxin producing *Escherichia coli* (STEC) serogroups represented in the investigation.

Serogroup	**Strains and strain origin used for Serogroup Inoculation Cocktail**						**CFU/ml**
O121	3.1065	Bovine	E1-158	Human	E1-159	Human	7.13 ± 0.79
O111	4.0005	Bovine	3.1009	Bovine	ATCC BAA-181	Human	7.32 ± 0.46
O145	95.0187	Bovine	9.0538	Grd Beef	E1-169	Human	6.99 ± 0.42
O45	2.0164	Bovine	92.0244	Bovine	E1-138	Human	7.17 ± 0.57
O26	7.1556	Grd Beef	0.1302	Bovine	ATCC BAA-1653	Stool	7.30 ± 0.69
O103	97.1241	Bovine	97.1377	Bovine	ATCC 23982	Stool	7.40 ± 0.65
O157:H7	A1-920	Bovine	A4-966	Bovine	A5-528	Bovine	7.14 ± 0.69

[1] Origination of each strain is listed in parentheses: *ATCC* is the American Type Culture Collection. *Bovine*-strains isolated from gastrointestinal tract of beef cattle. *Grd Beef*-Strains isolated from ground beef. *Human*- Strains isolated from human outbreaks and illnesses. *Stool*-Strains isolated from stool samples of individuals involved in STEC outbreaks.

[2] Cocktail strains were positive for eae, stx^1, or stx^2.

Table 2. The influence of High pH classification (High pH or Normal) and USDA Quality Grade on the pH, water activity, and composition (% moisture, fat, protein, and collagen) of beef strip loins prior to inoculation, storage, and blade tenderization.

Variable	*USDA Choice*		*USDA Select*		*Interaction*	*SEM*
	High pH	*Normal*	*High pH*	*Normal*	*P-Value*	
pH	5.88[b]	5.31[c]	6.24[a]	5.26[c]	<0.0001	0.13
Water Activity	0.976[a]	0.955[b]	0.962[ab]	0.954[b]	0.032	0.01
Moisture, %	68.47[b]	65.51[c]	73.03[a]	72.20[a]	<0.0001	0.81
Fat, %	9.44[a]	11.74[a]	3.39[b]	3.10[b]	<0.0001	1.04
Protein, %	22.29[b]	21.62[b]	22.17[b]	23.67[a]	0.0021	0.45
Collagen, %	2.14[b]	2.55[a]	1.76[c]	1.89[bc]	0.0016	0.19

[a-c] Least squares means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 3. The influence of High pH classification (High pH or Normal pH) and USDA Quality Grade on the pH and water activity of beef strip loins inoculated[1] with one of seven Shiga toxin-producing *Escherichia coli* (STEC) serogroups and stored for 14 d at 0 to 2°C.

Variable and STEC serogroup	*USDA Choice*		*USDA Select*		*Row P-Value*	*SEM*
	High pH	*Normal pH*	*High pH*	*Normal pH*		
pH						
O26	5.31	5.06	5.44	5.09	0.32	0.38
O45	5.21	4.94	5.47	4.98	0.13	0.39
O103	5.36	5.13	5.55	5.12	0.25	0.38
O111	5.12	4.95	5.40	4.91	0.20	0.41
O121	5.32	5.03	5.53	5.13	0.26	0.40
O145	5.16	4.94	5.36	4.95	0.37	0.44
O157:H7	4.91	4.72	5.13	4.67	0.26	0.43
Water Activity						
O26	0.957[b]	0.958[b]	0.975[a]	0.973[a]	0.02	0.005
O45	0.963	0.968	0.968	0.973	0.56	0.005
O103	0.982	0.973	0.979	0.976	0.56	0.01
O111	0.960	0.955	0.967	0.964	0.30	0.01
O121	1.007	1.000	1.023	1.015	0.13	0.02
O145	0.952	0.965	0.957	0.960	0.11	0.01
O157:H7	0.940	0.944	0.952	0.957	0.10	0.01

[1] Beef strip loins were inoculated (10^6 log CFU/cm^2 targeted attachment) with one of seven STEC serogroup cocktails.

[a-c] Least squares means within a row and variable (pH or water activity) lacking a common superscript letter differ ($P < 0.05$).

Table 4. The influence of High pH classification (High pH or Normal pH) and USDA Quality Grade on the concentration (log CFU/cm^2) of Shiga toxin-producing *Escherichia coli* (STEC) serogroups present on the external surface (50 cm^2) of inoculated[1] beef strip loins after 0 and 14 day of storage at 0 to 2°C.

Storage Day and STEC Serogroup	*USDA Choice*		*USDA Select*		*Row P-Value*	*SEM*
	High pH	*Normal pH*	*High pH*	*Normal pH*		
Day 0						
O26	5.41	5.23	5.42	5.30	0.55	0.38
O45	5.14	5.50	5.25	5.14	0.08	0.33
O103	5.25	5.27	5.63	5.49	0.18	0.32
O111	5.40	5.36	5.38	5.50	0.98	0.40
O121	5.31	5.49	5.41	5.37	0.76	0.39
O145	5.53	5.39	5.68	5.56	0.23	0.40
O157:H7	5.34	5.28	5.42	5.00	0.40	0.37
Day 14						
O26	4.90	4.82	4.84	4.95	0.77	0.60
O45	4.97	4.77	4.71	4.96	0.07	0.29
O103	5.07	5.00	4.97	5.03	0.89	0.38
O111	5.75	5.30	5.13	4.99	0.20	0.70
O121	5.09	4.88	5.00	5.24	0.37	0.34
O145	4.99	4.82	5.11	4.93	0.75	0.54
O157:H7	5.00	4.86	4.89	4.91	0.68	0.42

[1] Beef strip loins were inoculated (10^6 log CFU/cm^2 targeted attachment) with one of seven STEC serogroup cocktails.