DISSERTATION

PREHARVEST AND POSTHARVEST INTERVENTION STRATEGIES TO REDUCE PREVALENCE OF PATHOGENS IN BEEF AND BEEF PRODUCTS

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JUSTIN ROBERT RANSOM ENTITLED "PREHARVEST AND POSTHARVEST INTERVENTION STRATEGIES TO REDUCE PREVALENCE OF PATHOGENS IN BEEF AND BEEF PRODUCTS" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

PREHARVEST AND POSTHARVEST INTERVENTION STRATEGIES TO REDUCE PREVALENCE OF PATHOGENS IN BEEF AND BEEF PRODUCTS

Studies were conducted to determine the prevalence of *Escherichia coli* O157:H7 in fecal and hide samples from cattle in feedlots and on their carcasses at slaughter plants. Prevalence in pens (lots) ranged from 0 to 78% positive in feedlot pen-floor fecal samples, and from 0 to 5% on carcasses from those lots immediately after final intervention; however, too few positive samples were isolated from carcasses to develop a strong relationship between *E. coli* O157:H7-positive fecal samples and *E. coli* O157:H7-positive carcass samples. Nonetheless, data suggested that pens having 20% or more *E. coli* O157:H7-positive feedlot-floor fecal samples also had 22.5% hide, 46.3% colon, and 12.5%, 2.5% and 0.6% positive carcass samples at pre-evisceration, post-evisceration and after final intervention, respectively. Conversely, feedlot-floor samples that were less than 20% positive were associated with 5.7% hide, 7.1% colon, and 7.1%, 0% and 0% positive carcass samples at pre-evisceration, post-evisceration and post-final intervention, respectively.

A second study showed that preharvest pathogen mitigation strategies could aid in reducing prevalence of *E. coli* O157 in fecal and hide samples, as all treatments (i.e., *Lactobacillus acidophilus*-probiotic, neomycin sulfate-antibiotic and a prototype *E. coli* O157:H7 vaccine) resulted in fewer *E. coli* O157:H7 positive fecal and hide samples compared to controls. Although there was a significant lack of power, results from a preliminary antibiotic susceptibility study showed that there were no differences in

antimicrobial resistance patterns between *E. coli* O157 isolates recovered from cattle treated with nothing versus those treated with neomycin sulfate before harvest.

Finally, a series of studies comparing activated lactoferrin (2%) with nonactivated lactoferrin (2%), lactic acid (2%) and water were conducted to determine their effectiveness in reducing and/or inhibiting *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* populations on bologna and fresh beef products. Results from these studies showed that activated lactoferrin and lactic acid were effective in reducing initial populations and inhibiting growth of *E. coli* O157:H7 and *L. monocytogenes* during storage of bologna. Additionally, when activated lactoferrin and lactic acid were sequentially applied to beef adipose tissue, their effectiveness in reducing and inhibiting *E. coli* O157:H7, *L. monocytogenes,* and *S.* Typhimurium populations was enhanced.

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DEDICATION

This dissertation is dedicated to my God, Jesus Christ, my family and my closest friends.

Without each of them, this would not have been possible.

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CHAPTER I

OBJECTIVES OF DISSERTATION

The overall objective of the studies reported in this dissertation was to better understand relationships between prevalence of *Escherichia coli* O157:H7 in feedlot cattle and on their subsequent carcasses, to identify effective preharvest mitigation strategies to reduce prevalence of the pathogen on and in feedlot cattle as they are presented for slaughter, and to determine the effectiveness of activated lactoferrin in minimizing growth of pathogens on fresh and ready-to-eat beef products.

Specific Objectives:

1. To determine prevalence of *E. coli* O157 in feedlot cattle fecal samples and compare that prevalence with that on subsequent carcasses before and after application of final decontamination intervention technologies.
2. To evaluate preharvest *E. coli* O157 intervention strategies in the feedlot and to determine the antibiotic susceptibility of isolates recovered from antibiotic-treated cattle.
3. To determine the effectiveness of activated and non-activated lactoferrin, lactic acid and water in inhibiting and reducing *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium populations on fresh and ready-to-eat beef products.

CHAPTER II

LITERATURE REVIEW

INTRODUCTION

Meat products are a significant part of the diet for much of the world's population. Meat also provides important contributions to the economy of domestic and global agriculture. Due to increased incidence of virulent pathogens in the food supply, as well as enhanced detection methodologies and public awareness, foodborne illness has become a major topic of both public and scientific debate. The safety of meat can be compromised in various ways, including via contamination with chemical residues (pesticides, antibiotics), via incorporation of physical hazards (glass, metal), and, most importantly, with microbiological contamination. Estimates suggest that microbially contaminated food causes approximately 76 million illnesses, 325,000 hospitalizations and 5,000 deaths in the U.S. per year (Mead et al., 1999). Although researchers have made significant advances in food safety with decontamination intervention systems, food safety continues to be a challenging problem.

The safety of food is improved by minimizing contamination, killing microbial contaminants, denaturing toxins, and inhibiting growth of pathogenic organisms on food sources. In order for this to be accomplished, researchers, producers, packers, and consumers must work together to minimize microbiological contamination from "farm to fork" (Smith, 1999).

In the past 20 years, *Escherichia coli* O157:H7 has emerged as a pathogen of public health concern (Riemann and Cliver, 1998). Although most *E. coli* are normal

flora and reside in the colons of healthy people and other warm-blooded animals, certain serotypes are capable of causing significant human health problems. Diarrheagenic *E. coli* are gram-negative, facultatively anaerobic, nonspore-forming rods that are mostly motile (Doyle et al., 2001). There are four major categories of diarrheagenic *E. coli*: (1) Shiga toxin-producing *E. coli* (STEC), which includes Enterohemorrhagic (EHEC), (2) Enteropathogenic (EPEC), (3) Enterotoxigenic (ETEC), and (4) Enteroinvasive (EIEC). Diarrheagenic *E. coli* can grow in temperatures as low as 7°C and up to 44.5°C, but prefer 35 to 40°C (Doyle et al., 2000). In addition, they can grow at a pH from 4.4 to 9.0; however, they grow best at a pH of 6.0 to 7.0. Also, they require a a_w of at least 0.95 (Doyle et al., 2000).

Enterohemorrhagic *E. coli* strains have been transmitted via food and caused disease ranging from hemorrhagic colitis (bloody diarrhea) to Hemolytic Uremic Syndrome (HUS) (Buchanan and Doyle, 1997). Illness from EHEC exhibits disease symptoms similar to *Shigella* dysentery, and is most commonly found in developed countries (Riemann and Cliver 1998).

Escherichia coli O157:H7 was first recognized as a human pathogen following two hemorrhagic colitis outbreaks in 1982. The first outbreak took place in Oregon, where 26 human illnesses were reported and 19 were hospitalized; and the second, with 21 cases and 14 hospitalizations, three months later in Michigan (Food Quality, 1997). An outbreak occurred in the northwestern United States in 1992-1993 that ultimately was attributed to the consumption of undercooked ground beef, that resulted in illness in hundreds of people and the death of four children (Bell et al., 1994). Although both produce and meat serve as a vehicle for transmission of *E. coli* O157:H7, foods

specifically of bovine origin have been implicated in a majority of the outbreaks. It was estimated by Mead et al. (1999) that 62,000 cases of symptomatic *E. coil* O157:H7 infections occur annually, resulting in approximately 1,800 hospitalizations and 52 deaths. The Economic Research Service estimated that the annual total cost of *E. coli* O157:H7 due to medical costs, loss of productivity, and premature deaths totals $0.7 billion (ERS, 2002). In addition, Kay (2003) estimated that *E. coli* O157:H7 cost the beef industry approximately $2.7 billion over the last ten years.

Despite efforts, the origins of more than half of all foodborne illness outbreaks remain unknown. The World Health Organization predicted that there are 300 to 350 percent more outbreaks than those which are actually reported (Saucier, 1999). Some estimates suggest that hundreds of millions of people around the world suffer due to the incidence of foodborne illnesses. In addition, it is predicted that at least five to ten percent of the population is affected annually (Saucier, 1999).

Illnesses associated with strains of microorganisms such as *E. coli* O157:H7 have increased interest in pathogen intervention strategies and technologies to mitigate risks associated with such microbes throughout the entire food production system. Since the first large outbreaks of *E. coli* O157:H7 in the early 1990s, a tremendous amount of research funding has been allocated to identify effective risk-reduction strategies during harvesting and processing of beef cattle. These validated and implemented technologies include organic acid rinses, knife trimming, steam vacuuming and steam pasteurization (Sofos and Smith, 1998). Although these technologies have proven to be effective by both scientists and industry, prevalence of *E. coli* O157:H7 in cattle as they enter the harvesting facility remains problematic. Incidence of *E. coli* O157 is higher than

previously estimated, both on animals before slaughter and on the resulting carcasses following slaughter (Elder et al., 2000), and enhanced focus on preharvest pathogen intervention strategies has developed.

Studies before 2000 suggested a low prevalence of *E. coli* O157:H7 in the feces of cattle (Hancock et al., 1994; Faith et al., 1996). Specifically, prevalence of *E. coli* O157:H7 was reported to be 0.3% in dairy cattle and 0.7% in beef cattle, with a prevalence of 8.3% and 16% in dairy and beef herds, respectively. More recently, prevalence of the pathogen has appeared to increase, although the perceived increase may partially have been due to new techniques developed to isolate the pathogen from fecal, hide and carcass samples. For example, utilization of immunomagnetic bead capture and enhanced selective media likely played a role in a study by Elder et al. (2000) that reported: (a) of lots, 72% had at least one EHEC O157 positive fecal sample, 38% had at least one positive hide sample, and 87, 57 and 17% of lots had at least one EHEC O157 positive carcass sample at pre-evisceration, post-evisceration and post-processing, respectively; (b) prevalence of EHEC O157 in feces, hides, and on carcasses at pre-evisceration, post-evisceration and post-processing samples was 28%, 11%, 43.4%, 17.8% and 1.8%, respectively. Reduction in prevalence from pre-evisceration to post-processing suggested that plant sanitary procedures were effective; (c) EHEC O157 was recovered from 45.5% of carcasses; (d) fecal and hide prevalence were significantly correlated with carcass contamination ($P = 0.001$), indicating a role for control of EHEC O157 during live cattle production.

PREHARVEST PATHOGEN INTERVENTION STRATEGIES

Feedlot production systems have been the focus of most pre-harvest research as feeding is a critical step in controlling the pathogen in the beef production process. Multiple intervention strategies have been proposed and ultimately tested in the feedlot; however, to date, no definitive method for reducing prevalence of the pathogen in the feedlot to zero has been identified. Some proposed methods of controlling the pathogen in the feedlot include, use of probiotics, sodium chlorate, vaccines, antibiotics, and cattle washing at the harvesting facility.

Cattle Diet

Some of the earliest work in preharvest pathogen intervention strategies has shown that diet could play a potential role in the carriage and shedding of *E. coli* O157:H7 in the gastrointestinal tract of healthy cattle. Diez-Gonzalez et al. (1998), showed that cattle fed a feedlot-type ration had 3 log CFU/g higher generic *E. coli* populations than cattle fed only hay. When cattle were abruptly transferred from a 100% finishing diet to a 100% hay diet, *E. coli* populations in feces declined by 3 log CFU/g. Based on these results, the authors suggested that feedlot cattle be switched from high-grain finishing diets to hay before slaughter to reduce *E. coli* populations; however, negative effects on feedlot performance preclude the use of this intervention strategy by industry. Others (Russell et al., 2000) have shown similar results, in that brief periods of feeding hay can decrease the number of cattle shedding *E. coli*, and a similar trend was observed when cattle were fasted for a short period of time. Buchko et al. (2000) reported

contradictory results, indicating that forage (hay) feeding either had no effect or actually increased *E. coli* O157:H7 shedding. Other research (Dargatz et al., 1997) has shown an increased incidence of *E. coli* O157 in cattle consuming diets containing barley. Hancock et al. (1997) showed that calves on feed less than 20 days were more likely to have positive samples for *E. coli* O157 than market-ready cattle; however, it was speculated that stress incurred immediately after placement into the feedlot, rather than diet, was the likely cause of increased prevalence. Thus, it appears that no dietary changes or feeding management practices can generate consistent reductions of *E. coli* or *E. coli* O157:H7 populations.

Probiotics

The term probiotic has been loosely used by many researchers studying the effectiveness of bacterial compounds in inhibiting the attachment and growth of *E. coli* O157:H7 in intestines of cattle. In addition, much published research addressing probiotics in cattle does not describe the specific mode of action. Use of microflora to reduce pathogenic bacteria in the gut has been termed a "probiotic" strategy (Callaway et al., 2002). The overall goal of this strategy is to promote growth of groups of bacteria, such as lactobacilli, that are competitive with or antagonistic to pathogenic bacteria (Callaway et al., 2002). Microorganisms that are commonly referred to as probiotics include, lactobacilli, bifidobacteria, streptococci and yeast and molds.

The earliest research testing effects of probiotic bacteria as a means for reducing prevalence of *E. coli* O157:H7 in experimentally inoculated calves showed that, when probiotic bacteria were administered to calves before inoculation with the pathogen, the level of carriage and days of shedding of the pathogen were decreased over time

compared to controls (Zhao et al., 1998). Additional studies by the same research group showed that fecal shedding of *E. coli* O157:H7 was greatly reduced in all experimentally inoculated calves, but that there was no significant difference between probiotic-treated and control calves. However, probiotic-treated calves experimentally inoculated with *E. coli* O111:NM and *E. coli* O26:H11 resulted in significantly lower colonization and shedding of the pathogens than control calves which did not receive the probiotic treatment (Zhao et al., 2003). Tkalcic et al. (2003) showed that a three strain mixture of probiotic *E. coli* was effective in reducing shedding of *E. coli* O157 in experimentally inoculated weaned calves. In addition, Schamberger and Diez-Gonzalez (2002) demonstrated the effective isolation of colicinogenic *E. coli* strains inhibitory to *E. coli* O157:H7 in the laboratory; but, to date, these specific colicinogenic *E. coli* strains have not proven to be effective in reducing the carriage or shedding of the pathogen in cattle in applied field studies.

Other researchers were successful in identifying probiotic strains of *Lactobacillus acidophilus* to be fed to cattle to inhibit the growth and proliferation of *E. coli* O157:H7 (Brashears et al., 2003a). Subsequent studies demonstrated effectiveness of these probiotic compounds in the reduction of *E. coli* O157 shedding in fecal samples, in addition to a reduction in prevalence of the pathogen on the hide surface.

Most recently, Younts-Dahl et al. (2004) reported that cattle administered a high level of *L. acidophilus* strains (NP 51 and NP 45) were 57 and 79% less likely to have an *E. coli* O157-positive fecal and hide sample, respectively, than were controls, although prevalence of *E. coli* O157 was only 7.4 and 10.5% lower than controls in fecal and hide samples, respectively, on the day of harvest.

Sodium Chlorate

Some intestinal bacteria, such as *Salmonella* and *E. coli*, are able to anaerobically reduce nitrate to nitrite via the intracellular enzyme nitrate reductase (Callaway et al., 2002). Nitrate reductase also reduces chlorate to form the cytotoxic end-product chlorite. Thus, when *E. coli* is exposed to chlorate, cell lyses occurs, resulting in bacterial death. (Anderson et al., 2000). In a small study, cattle were experimentally infected with *E. coli* O157 and treated with sodium chlorate via drinking water. Sodium chlorate treatment for 24 h reduced *E. coli* O157:H7 populations by approximately 2 log CFU/g in the rumen and 3 log CFU/g in feces (Callaway et al., 2002). To date, use of sodium chlorate in food animals has not been approved by the Food and Drug Administration or the U.S. Department of Agriculture, and research on this feed ingredient has been deterred until sufficient toxicological studies have been conducted.

Vaccines

In order for cattle to be "shedders" of *E. coli* O157, rather than "transient carriers" of the pathogen, *E. coli* O157:H7 must colonize in the gastrointestinal tract. Vaccination may be an effective preharvest intervention strategy for reducing colonization and subsequent shedding of *E. coli* O157:H7. Mechanisms of action for vaccines either are to reduce susceptibility of cattle to colonization by *E. coli* O157:H7, or to decrease duration of colonization (Finlay et al., 2003). A number of vaccines have been and are being developed to prevent attachment (Finaly et al., 2003; Van Donkersgoed and Potter, 2003; Potter et al., 2004). Potter et al. (2004) recently reported that vaccination of cattle with proteins secreted by *E. coli* O157:H7 significantly reduced *E. coli* O157:H7 populations

shed in feces, the number of animals that shed, and the duration of shedding in cattle. In a larger trial consisting of 218 pens from nine commercial feedlots, cattle were either vaccinated or administered a placebo at arrival and at re-implant. Results from the study showed no differences in shedding associated with vaccine usage (Van Donkersgoed and Potter, 2003). Researchers from different laboratories continue to enhance current vaccines, as well as develop new vaccines; however, to date, there are no published data on the enhanced or new experimental vaccines (VanDonkersgoed, 2004).

Antibiotics

Antibiotics are known to be very effective in altering the microbial ecology of the intestinal tract; however, use of medically important antibiotics as growth promoters has become a highly controversial issue in recent years (Callaway et al., 2003). Due to concern over antibiotic resistance, it is likely that use of antibiotics as growth promoters in food animals will become even more highly regulated and, potentially, prohibited. It seems likely that new and/or modified uses of antibiotics, will only be allowed if they are proven to minimize risk of foodborne illness by reducing prevalence of pathogens before livestock enter a harvesting facility. Elder et al. (2002) demonstrated the effectiveness of feeding neomycin sulfate to reduce *E. coli* O157:H7 populations to non-detectable levels compared to controls, 72 h post treatment. Seven days post treatment, *E. coli* biotype I populations returned to normal levels, but *E. coli* O157:H7 populations remained undetectable in neomycin sulfate treated cattle. The bactericidal effectiveness of neomycin sulfate in Gram-negative bacteria is demonstrated by its ability to bind the 30S ribosomal subunit, resulting in stagnation of elongation, which causes protein synthesis to stop and subsequently cell death to occur. The use of neomycin sulfate to reduce *E. coli*

O157:H7 populations in feedlot cattle does not meet currently-approved label provisions as approved by the HHS-Food and Drug Administration.

Cattle Washing

Live animal contamination with pathogens, in addition to environmental plant contamination, is considered to be the most likely source of carcass and meat contamination (Chapman et al., 1993). A potential solution to controlling the amount of contamination on the surfaces of cattle entering a harvesting facility is to wash them with chemical solutions to either loosen debris from the hide or to destroy the microbial populations on the surface of the hide. Initial cattle washing research addressed the effectiveness of washing cattle with simple chemical solutions during lairage; however, this caused undue stress to the cattle and resulted in minimal reductions in pathogen populations (Byrne et al., 2000). In addition, results of Byrne et al. (2000) showed that cattle treated with chemical solutions did not exhibit contamination levels that were different from dirty (but dry) cattle, primarily due to excessive water remaining on the surface of hides.

More recently, Bosilevac et al. (2003) showed that washing cattle with 1% cetylpyridinium chloride (CPC) resulted in a lower prevalence of *E. coli* O157:H7 on cattle washed with CPC (56%) versus cattle washed with water (34%). In addition, samples from subsequent carcasses of cattle that were washed either with CPC or water, resulted in 3% and 23% *E. coli* O157:H7-positive samples, respectively.

Recently, a major U.S. packer installed a system that washes stunned and exsanguinated cattle in a three chamber washing system (Yovich, 2003). Cattle are

washed with a water/sodium hydroxide mixture, high pressure water, and then lactic acid; washed cattle are then vacuumed along the pattern-mark before the hide is first opened (Yovich, 2003).

An additional hide decontamination technology is chemical dehairing. Although it is a costly process, it effectively removes hair, mud, manure and other external contaminants that, in turn, minimizes carcass and plant contamination during the dehiding process (Sofos and Smith, 1998). Nou et al. (2003) recently evaluated a commercially-operating system and found reduced *E. coli* O157:H7 prevalence on carcasses of cattle chemically dehaired (1%) compared to those harvested conventionally (50%).

Production Best Practices

As previously described, targeted preharvest intervention strategies have been demonstrated to be effective in reducing prevalence of *E. coli* O157 in the gastrointestinal tract and on hides of cattle. Although a single management factor may not reduce (much less eliminate) *E. coli* O157 in the beef production system, a series of principle-based animal husbandry practices could potentially aid in the reduction of pathogen prevalence.

In a recent study conducted by Davis et al. (2003), 0.2% of feed samples and 0.4% of feed-mill samples were positive for *E. coli* O157:H7. Steps producers could take to prevent contamination of feed would be to identify pests and rodents that transmit pathogens to feed. Keen et al. (2002) proposed that flies captured in feedlots test positive for *E. coli* O157 and other Shiga-toxin-producing *E. coli*; however it is unclear if flies act as a vector for the pathogen in the feedlot. In addition, other research (Cizek et al., 2000) showed that both rats and pigeons that were experimentally infected with the pathogen

could harbor the pathogen for several days and excrete *E. coli* in the feces for 9.8 days. In addition, the researchers (Cizek et al., 2000) stored feces at temperatures ranging from 4 to 20°C and showed that *E. coli* O157:H7 was isolated from the feces for up to 34 weeks. Pigeons that were inoculated with the same dose of *E. coli* O157:H7 shed the pathogen for 20.2 days.

Sargeant et al. (2003) reported that 13.1% of all water tanks tested were culture positive for *E. coli* O157, and 60.3% of feedlots had at least one *E. coli* O157-positive water tank. In addition, the researchers showed that cattle were more likely to be positive if water tanks in the pens were positive for *E. coli* O157. Research by Rice and Johnson (2000) showed that *E. coli* O157:H7 could survive in experimentally inoculated water tanks at 5 and 15°C for up to 16 days. Besser et al. (2001) reported no effect on the prevalence of *E. coli* O157 in studies where water troughs were routinely cleaned. Chlorination of water for the disinfection of public water supplies has been widely used throughout the world for decades (Latshaw, 1994); however, the likelihood of chlorinating drinking water for livestock seems remote.

Logically, pen cleaning would aid in control of the pathogen in feedlots; however, Khaitsa et al. (2002) reported that cleaning pens by scraping the top sediment (manure) from pens did not influence prevalence of *E. coli* O157:H7. Identifying a pen cleaning/pathogen reduction technique could prove valuable in controlling the pathogen in the feedlot.

POST-HARVEST PATHOGEN INTERVENTION STRATEGIES

The interior muscle tissue of healthy animals is usually free from microorganisms (Sammarco et al., 1997). It has been well documented that microbial contamination of animal carcasses during the slaughter process is both undesirable and yet unavoidable (Dickinson and Anderson, 1992; Siragusa, 1995). Advances in engineering technology have allowed the meat industry to become modernized, streamlined, and more efficient (Saucier, 1999). Although technology enhances productivity, the delicate balance between efficiency and food safety cannot be compromised.

Carcass decontamination refers to the ability to reduce pathogen loads on carcass surfaces by applying organic (i.e., 2% lactic or acetic acid) sprays and washes, steam-vacuuming, hot-fat-trimming and quick-chilling, or a combination of treatments. It is not possible to reduce the level of microbial contamination on a carcass to zero using a single decontamination method (Siragusa, 1995; Delmore et al., 1998; Bacon et al., 2000).

As part of the FSIS Pathogen Reduction/HACCP regulation, FSIS recommended that all beef, pork, lamb and poultry slaughter establishments apply at least one antimicrobial treatment before carcass chilling. Proposed treatment recommendations included any antimicrobial compounds previously approved by FSIS, as well as hot water and chlorine compounds (FSIS, 1996).

Although this limited review of literature has focused on control *E. coli* O157:H7 during the production of market-ready cattle, other foodborne pathogens such as *Salmonella* Typhimurium and *Listeria monocytogenes* are important pathogens of concern in fresh and ready-to-eat food products.

The genus *Salmonella* also is a member of the *Enterobacteriaceae* family, which are classified on the basis of being gram-negative, non-sporeforming, motile bacilli. There are more than 2600 *Salmonella* serotypes in the genus, of which *S.* Typhimurium and S. Enteritidis are the most prevalent in the U.S. (Baumler et al., 1998). Today, *Salmonella* is the second leading cause of bacterial foodborne illness in the U.S. (Mead et al., 1999), and the third known cause of foodborne illness overall.

Salmonella Typhimurium may be present in the gastrointestinal tract of poultry and livestock that show no symptoms of sickness. In pork processing, Saide-Albonoz, et. al. (1995) noted that the stress of transportation and feed deprivation from farm to slaughterhouse increased the likelihood for pathogen shedding via feces, which ultimately passed *S. typhimurium* onto the slaughter floor and throughout the packing plant. Barham et al. (2002) demonstrated similar results in cattle when 6 and 18% of hide and fecal samples, respectively, were *Salmonella* spp.-positive before transport; however, upon arrival at the packing plant, prevalence increased to 89 and 46%, respectively. Ekperigin and Nagaraja, (1998) suggested that when beef, pork or poultry are infected with *S.* Typhimurium prior to slaughter, their carcasses test positive for the pathogen 0% to 90% of the time.

Although *S.* Typhimurium is resistant to several antimicrobial agents that other pathogens are not, there are ways the pathogen can be eliminated. *Salmonella* grows over a range of temperatures from 5°C to 47°C, with an optimum temperature of 37°C. Growth rates at below 10°C are very slow, but are significant if the shelf life of the product is prolonged. In addition, it grows over a range of pH from 4.5 to 9.0, with an optimum pH of 6.5 to 7.5 (Varnam, 1991).

The genus *Listeria* is composed of small motile, nonspore-forming, facultative anaerobic, gram-positive rods. These organisms are catalase positive and oxidase negative and ferment glucose (Cooper and Walker, 1998). There are seven species of *Listeria*; however, *L. monocytogenes* is the only one associated with human foodborne disease (Vela, 1997). *L. monocytogenes* is capable of growing at temperatures from < 3° to 45°C. *L. monocytogenes* is capable of existing as both a plant and animal pathogen. It enters the body by penetrating the epithelial barrier in the intestine and multiplies in the hepatic and splenic macrophages. It also enters through damaged mucosal surfaces, by inhalation or conjuctival contamination, and then invades the central nervous system via the neural sheath of peripheral nerve endings (Cooper and Walker, 1998).

Even with the low frequency of human exposure to *L. monocytogenes*, research suggests that listeriosis is a relatively rare disease. Studies conducted by the Center for Disease Control and Prevention (CDC) have established an incidence of listeriosis of 7.1 cases per one million people (Notermans, et. al, 1998). In 1999, the CDC reported that of all of the foodborne pathogens tracked by CDC, *L. monocytogenes* had the highest hospitalization rate (90%) and the second highest case fatality rate (20%) (Mead et al., 1999). People most susceptible to listeriosis are the immunocompromised, elderly, and infants (Notermans, et. al, 1998).

The first major outbreak of listeriosis occurred in Boston, MA, in 1979. Although the total number of cases was not definitely established, 5 people died after consuming raw vegetables that had been fertilized with fecal material from infected dairy cattle (Vela, 1997). In December 1998, *L. monocytogenes* was responsible for the largest meat recall ever. Thirty-five million pounds of ready-to-eat meat were recalled; ultimately, 101

people were sickened and 21 people died (CDC, 1999). Another *L. monocytogenes* outbreak in 2002 resulted in 50 illnesses and 7 deaths in 8 northeastern states, and was associated with the consumption of contaminated ready-to-eat poultry products (CDC, 2002). Public awareness of these outbreaks prompted government and industry to develop more effective control procedures to enhance product safety and improve consumer's confidence in the safety of ready-to-eat meat products (Sofos et al., 2003).

In 2003, USDA-FSIS published an interim final rule to control *L. monocytogenes* in ready-to-eat meat and poultry products (FSIS, 2003). According to this regulation, processing facilities that expose the product to the environment after lethality treatments are required to include in their HACCP plan one or more validated measures that prevent product adulteration with *L. monocytogenes*.

Organic Acid Solution Rinsing

A significant amount of research has been conducted to determine the effectiveness of organic acid solutions in controlling bacterial growth on carcasses. Short chain organic acids have been identified as the most logical agents to spray on carcasses. Lactic, acetic, citric, formic and propionic acids all have been used for this purpose (Siragusa, 1996; Castillo et al., 2001). Acetic and lactic acids are inexpensive, environmentally friendly, and occur naturally in nature. In general, organic acids reduce aerobic plate counts by 1 to 2 log CFU/cm^2, regardless of the acid type (Siragusa, 1995). More specifically, decontamination of beef surfaces with 2% lactic or acetic acid resulted in 2-3 log cfu/cm^2 reduction of *S.* Typhimurium DT104 and *E. coli* O157:H7 (Cutter et al., 2000). Acid concentration appears to be an important factor in the degree of pH decline and antimicrobial effect (Podolak et al., 1996; Tamblyn and Conner, 1997; Dorsa

et al., 1997; Cutter and Siragusa, 1994). When organic acids are applied at 55°C', their effectiveness is enhanced (Hardin et al., 1995). Concerns associated with use of organic acids are risk of the non-deliberate selection for acid-resistant spoilage and pathogenic organisms, which could cause both accelerated spoilage problems and enhanced public health dilemmas (Gill, 1998).

Novel Chemical Interventions

Although common organic acids continue to prove to be effective in reducing total bacterial and pathogen populations on beef carcass surfaces, concerns about the ability of *E. coli* O157:H7 to become acid tolerant have been expressed (Samelis et al., 2004); thus, application of novel intervention strategies to reduce bacterial populations on beef products could prove useful. An example of a novel intervention to recently obtain federal approval in poultry processing is cetylpyridinium chloride (CPC). CPC has proven to be effective in reducing pathogenic populations on beef adipose tissue by up to 6 log CFU/cm^2 (Cutter et al., 2000). In an additional study, CPC reduced *E. coli* O157:H7 populations by 4.8 and 2.1 log CFU/cm^2 on beef adipose tissue and lean tissue, respectively (Ransom et al., 2002).

Acidified (citric acid) sodium chlorite (ASC) is another antimicrobial that has received approval from the federal government for use in beef carcass decontamination systems. Castillo et al. (1999) showed that ASC combined with water effectively reduced *E. coli* O157:H7 populations by 4.5 log CFU/cm^2, whereas water alone reduced pathogen populations by only 2.3 log CFU/cm^2.

Finally, a relatively new antimicrobial compound recently receiving federal approval in 2003 is activated lactoferrin (GRAS Notice: GRN 000130). Activated

lactoferrin is an iron binding protein, and can reportedly be used to replace other products (Naidu and Bidlack, 1998). The patented process of producing activated lactoferrin includes the immobilization of milk lactoferrin, via its N-terminus region, on a food grade glycosaminoglycan, solubilized in a precalibrated citrate/bicarbonate buffering system containing sodium chloride and an excess of unbound lactoferrin. Activated lactoferrin acts as a microbial blocking agent that is able to prevent attachment and colonization of microorganisms to biological surfaces, and to inhibit microbiological growth and neutralize activity of neurotoxins (Naidu, 2004).

Sequential-Intervention Decontamination Systems

A combination of intervention strategies in food processing can result in synergistic or additive effects of interventions, causing inhibition or destruction of microorganisms. With this in mind, implementation of these strategies may prevent negative effects of processing on the product as well as decrease the likelihood of foodborne disease (Leistner, 1995).

There are four basic methods used to inhibit or destroy microorganisms (Leistner, 1978). Those methods are sterilization, freezing, chilling and adjustment of pH and a_w. The hurdle effect is the process of controlling growth, metabolic activity, resistance and survival of microorganisms in food. Each hurdle represents a method used for controlling microbial growth. In 1991, the idea of combining hurdles with HACCP systems offered a new dimension to food safety. Implementation of this concept has become known as hurdle technology (Leistner, 1995; Bacon et al., 2000).

Dorsa et al. (1998) found that meat treated with antimicrobial compounds in a hurdle technology system had residual efficacy and resulted in lower or no detectable

levels of pathogenic organisms 21 days later. Bacon et al. (2000) reported that use of multiple-sequential decontamination interventions (including steam vacuuming, pre-evisceration carcass washing, pre-evisceration organic acid solution rinsing, hot water carcass washing post-evisceration final carcass washing, and post-evisceration organic acid solution rinsing) resulted in *E. coli* counts that were reduced from a range of 2.6 to 5.3 $\log_{10}$ CFU/100 cm^2 to a range of 1.0 to 3.0 $\log_{10}$ CFU/100 cm^2. After carcass chilling, *E. coli* populations recovered from beef carcass surfaces were reduced further by 1.3 to 0.9 $\log_{10}$ CFU/100 cm^2.

Dickson and Anderson (1991) found that rinsing with distilled water before evisceration and using 2% acetic acid to sanitize after washing decreased the pH of the carcass surface and ultimately reduced the microbial population by 2 $\log_{10}$. Although not significant, slight reduction trends were observed when acetic acid was heated to 55°C prior to washing.

Combined treatments with a water wash, trimming, hot water washes and acid washes were shown to reduce pathogen populations on beef carcass tissue by 4.0 to 4.9 log CFU/cm^2, while individual treatments alone resulted in less reduction than the combined treatments (Castillo et al., 1998).

Castillo et al. (1999) studied the magnitude of microbial reduction when inoculated carcasses were treated with steam vacuuming or steam vacuuming plus sanitizing hot water or lactic acid sprays. Results showed that the combination of treatments were more effective in reducing pathogen loads (by 3 $\log_{10}$).

SUMMARY OF LITERATURE

Obviously, a multitude of opportunities exist at all segments of the beef production system to enhance the microbiological safety of beef products. If the beef industry has a genuine mission to provide consumers with safe, high quality beef, the industry must become committed to identifying the most effective way to mitigate risk at every level of operation (size and scope), from the cow/calf producer, through to the retail case. In addition, if the entire industry is to share the responsibility of providing the safest beef in the world, it cannot fall short of communicating the ultimate mission from the seedstock producer to the consumer.

CAPTER III

PREVALENCE OF *ESCHERICHIA COLI* O157 IN CATTLE AND BEEF FROM THE FEEDLOT TO THE COOLER STORAGE

ABSTRACT

Prevalence of *Escherichia coli* O157 on cattle entering the slaughter floor may range from 10 to >70%. This study was conducted to determine the effect of *E. coli* O157 prevalence in fecal samples collected from feedlot pen floors on subsequent *E. coli* O157 prevalence on carcasses at various points in the slaughter process. Fecal samples from the feedlot pen floor were collected within three days before slaughter. During cattle processing at the slaughter facility, additional samples were collected from the hide and colon, and from the carcasses before and after evisceration and after final decontamination. Of 15 lots (a group of cattle from a feedlot) sampled, 87% had at least one positive feedlot-floor fecal sample, 47% had a positive hide sample, 73% had a positive colon/fecal sample and 47% had a positive carcass sample pre-evisceration; however, only 7% of lots had a positive carcass sample post-evisceration or after final intervention. Of the total samples tested (N = 1,328), 24.7, 14.7, 27.6, 10.1, 1.4, and 0.3% of feedlot-floor, hide, colon, pre-evisceration, post-evisceration and final intervention samples, respectively, were positive for *E. coli* O157. Pens with greater than 20% positive feedlot-floor fecal samples had 22.5% hide, 46.3% colon, and 12.5%, 2.5% and 0.6% carcass samples positive at pre-evisceration, post-evisceration and after final intervention, respectively. However, feedlot-floor samples which contained less than 20% positive fecal samples showed a lower pathogen prevalence, with 5.7% hide, 7.1% colon, and 7.1%, 0% and 0% carcass positive samples at pre-evisceration, post-evisceration and post-final intervention, respectively. Data from this study can be used as part of risk assessment processes in order to identify mitigation strategies to minimize prevalence of *E. coli* O157 on fresh beef carcasses.

Introduction

Since 1982, more than 100 outbreaks of *Escherichia coli* O157:H7 have been documented (Mead et al., 1999). Of these outbreaks, 52% were attributed or linked to foods derived from cattle. *Escherichia coli* O157:H7 has been isolated from the oral cavity (Keen and Elder, 2002), from rumen contents (Van Donkersgoed et al., 1999), and from the hide and feces of cattle (Elder et al., 2000; Keen and Elder 2002). In 1994, the USDA National Animal Health Monitoring System (NAHMS) reported that *E. coli* O157 was isolated from 63% of 100 feedlots tested; however, incidence of the pathogen in individual fecal pats was 1.6% (Hancock et al., 1997). Later, NAHMS conducted an additional study, using more sensitive laboratory detection techniques, and found that *E. coli* O157 was present in 11.0% of fecal pats, with at least one positive culture from every feedlot (NAHMS, 2001). More recently, in a feedlot epidemiological study encompassing 10,662 fecal samples from 711 pens in 73 feedlots located in four states, prevalence of *E. coli* O157 overall and at the pen and feedlot levels was 10.2, 52.0 and 95.9%, respectively (Sargeant et al,. 2003).

Recently, questions have been raised about animal production practices and their resulting impact on the incidence of *E. coli* O157:H7 in beef. Many researchers believe that management practices at the feedlot play an important role in animal health, carcass merit and, potentially, food safety. Over the last five years, substantial research has been conducted to identify pathogen intervention systems and livestock management practices to reduce prevalence of *E. coli* O157 in and on market-ready feedlot cattle (Brashears et al., 2002a; Brashears et al., 2002b; Buchko et al., 2000; Moxley et al., 2003; Potter et al., 2004; Tkalcic et al., 2003). Such preharvest intervention systems include, but are not

limited to, cattle washing, probiotics, and *E. coli* O157 vaccines (Bosilevac et al., 2003; Younts-Dahl et al., 2004; Potter et al., 2004).

The objective of this study was to determine prevalence of *E. coli* O157 on feedlot pen floors and, subsequently, in samples obtained from the hides, colon, and carcasses after hide removal, splitting, and final intervention. Our intention was to generate sufficient data to be used in assessing the intervention measures and subsequently reduce the risk of contamination in the final meat products.

MATERIALS AND METHODS

Study Design. Samples were collected from 15 lots of cattle derived from 12 different feedlots in Eastern CO and Western NE. A lot was defined as a group of cattle from a common source (pen) harvested on the same day of production. Samples were collected at the feedlot and the cattle from the feedlot were followed to one of three commercial slaughtering facilities and subsequently sampled during processing.

Feedlot Sampling. Twenty-five samples were collected from the pen floor of each lot by obtaining approximately 25 g of feces from the inside of fresh fecal pats using a sterile wooden applicator. Fecal samples were placed in sterile Whirl Pak® (International Bioproducts, Bothell, WA) bags and transported to the Rocky Mountain Regional Animal Health Laboratory (RMRAHL), Colorado Department of Agriculture in Denver, CO, for culture.

In-Plant Sampling. Samples were collected from stunned animals and resulting carcasses at multiple locations throughout the harvesting process. Although all hide, colon and carcass samples were collected from animals for the same lot, no attempt was made to sample carcasses from the same animals from which hide and colon samples

were obtained. Hide samples were obtained by swabbing three 100 cm^2 areas (round, flank and brisket) using a sterile template and sterile sponge kit (International Bioproducts, Bothell, WA) as described by the Food Safety and Inspection Service of the U. S. Department of Agriculture (FSIS, 1996). Swabbing at each anatomical site consisted of 10 passes vertically (up and down being considered one pass) and 10 passes horizontally (side to side being considered one pass) with a pressure equivalent to that which would be used to remove dried blood. Sampling was performed aseptically using sterile latex gloves, which, in addition to the template, were changed between samples. Following hide removal and evisceration, colons were removed (approximately 750 cm proximal to the rectum) from the processing line; the contents (at least 25 g) were evacuated by massaging the feces through the anterior end of the colon and placed in a sterile Whirl Pak® bag. Carcasses were sampled (using the same previously described three-site sponge swabbing) at three different locations during the harvesting process: (1) pre-evisceration, (2) post-evisceration, and (3) post-final intervention. Opposite sides of approximately every fifth carcass were sampled at either pre-evisceration or post-evisceration and, subsequently, the remaining side of the carcass not previously sampled was then sampled following the final intervention. Samples were transported immediately to the RMRAHL (within 4 h) where isolation and confirmation of *E. coli* O157 occurred.

Laboratory Procedures. Techniques described by Elder et al. (2000) and Paton and Paton (1998) were followed for isolation and confirmation, respectively, of *E. coli* O157. For fecal samples, a 10% fecal suspension was prepared in an 18 oz Whirl Pak® bag by homogenizing 10 g of feces in 90 ml of GN broth containing vancomycin (8 mg/l,

Difco, Becton Dickinson & Co., Sparks, MD), cefixime (0.05 mg/l, Difco) , and cefsuludin (10 mg/l, Difco). Samples were incubated for 6 hr at 37°C. Carcass sample sponges were placed in 90 ml of Brilliant Green Bile (BGB, 40 g/l, Difco) broth and incubated for 6 h at 37°C. Sponge samples from hides were placed in 20 ml of BGB (60 g/l) and also incubated for 6 h at 37°C. Following the 6 h incubation, samples were briefly shaken and then allowed to settle for 1 min, following which, 1 ml of the enriched sample and 20 µl of anti-O157 immunomagnetic beads (Dynal Laboratories, Lake Success, NY) were combined in a 1.5 ml eppendorf tube and incubated at room temperature for 30 min on a rotating rack. Following attachment, the immunomagnetic bead suspension was washed 3 times in 1 ml of phosphate buffered saline (PBS, Difco)/0.05% Tween 20 (Sigma, St. Louis, MO) utilizing a magnetic separation rack (Dynal Laboratories). Following the final wash, beads were resuspended in 100µl of PBS/0.05% Tween 20. Fifty µl of the bead suspension was spread plated onto sorbitol MacConkey agar (Difco) plates containing cefixime (0.05mg/l) and potassium tellurite (2.5 mg/l) (ctSMAC). The remaining 50 µl was plated onto Chromagar O157 (Becton Dickinson & Co.) containing 0.0625 mg/ml potassium tellurite. After an 18 hr incubation at 37°C, up to three sorbitol-negative colonies exhibiting colony morphology typical of *E. coli* O157 were picked from either plate as suspect *E. coli* O157, and used to inoculate MacConkey broth (Difco) for an O157 ELISA. After an overnight incubation at 37°C, isolates were screened with an *E. coli* O157 indirect ELISA using monoclonal antibodies specific for O157 lipopolysaccharide (Elder et al., 2000). When a presumptive positive colony was identified, further tests were conducted with API 20E and rfb O157 PCR before confirmation of a sample was granted. Following confirmation, virulence factors

were characterized using *stx* 1, *stx* 2, *eaeA* and *hlyA* PCR described by Paton and Paton (1998).

Statistical analysis. The percentage of samples testing positive for *E. coli* O157 at each sampling location (feces, hide, colon, pre-evisceration, post-evisceration, post-final intervention) were used as the outcome/response variable. The Pearson's Correlation Coefficients (Statistical Analysis Software; Version 8.2e; Cary , NC), were used to determine relationships (with a 95% confidence level) between the percentage of *E. coli* O157-positive feedlot pen floor samples and the percentage of positive samples at each sampling location during the harvesting process. Data from the *E. coli* O157 analysis were reported as percentages of samples testing positive for the pathogen at each sampling location (feces, hide, colon, pre-evisceration, post-evisceration, post-final intervention). Differences in percentages of positive samples between sample locations were tested using a chi-square goodness of fit test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Overall, 14.4% (191 out of 1,328) of samples were positive for *E. coli* O157. Specifically, prevalence of *E. coli* O157 in samples from the feedlot pen floor, hides, colon and carcasses before evisceration, after evisceration and following final decontamination were 24.7, 14.7, 27.6, 10.1, 1.4 and 0.3%, respectively (Table 3.1). Prevalence of the pathogen in lots was 86.7, 46.7, 73.3, 46.7, 6.7 and 6.7% positive in the feedlot pen floor, on the hide during harvest, in the colon, and on the carcasses before evisceration, following evisceration and after final intervention, respectively (Table 3.1). Results from this study were similar to those described by Smith et al. (2001) in which 23% of rectal fecal samples were positive for *E. coli* O157:H7; however, our results

indicated a higher prevalence than that reported by Sargeant et al. (2003), in which *E. coli* O157 was isolated in only 10% of fecal samples from the pen floor.

For lots that had feedlot pen floor samples testing positive for *E. coli* O157 greater than twenty percent of the time, higher prevalence of *E. coli* O157 also was detected on the hides and in the colons of cattle at harvest. Although numerically higher (14.3 vs. 6.3% positive for *E. coli* O157), there was no statistical difference between the prevalence of positive *E. coli* O157 samples collected from carcasses before evisceration. Lack of positive *E. coli* O157 samples on carcasses after evisceration and after final intervention, 2 and 4%, respectively, resulted in no statistical differences between carcasses derived from cattle with a high or low prevalence in feedlot pen floor samples.

Overall, a correlation of $r = 0.67$ was observed between positive pen floor samples and positive hide samples collected during harvest. In addition, an analysis of the relationship between pen floor positive samples and colon samples collected during harvest showed a correlation of $r = 0.58$. Due to the fact that so few *E. coli* O157 positive carcass samples were isolated, no relationship existed allowing accurate prediction of the likelihood of finding a *E. coli* O157-positive carcass based on the prevalence of the pathogen in fecal pats in the feedlot. For lots with greater than 20% positive *E. coli* O157 pen floor samples, a correlation of $r = 0.79$ was observed between pen floor samples and hide samples; however, prevalence in colon samples and all carcass samples were not related to prevalence of *E. coli* O157 feedlot pen floor samples. Furthermore, in the feedlot pen samples with prevalence of 20% or lower, too few *E. coli* O157 positive samples were isolated to suggest a relationship among hide, colon and carcass samples.

In general, when prevalence of *E. coli* O157 in pen floor samples was greater than 20%, 25.7, 51.4, 14.3, 2.9 and 0.7% of hide, colon, pre-evisceration, post-evisceration and post-final intervention samples were positive, respectively (Table 3.2). Conversely, when prevalence of *E. coli* O157 was equal to or less than 20%, 7.5, 5.0, 7.5, 6.3, 0.0 and 0.0% of hide, colon, pre-evisceration, post evisceration and post-final intervention samples were positive, respectively (Table 3.2). Although prevalence of the pathogen in feedlot pen floor and hide samples was highly variable, overall prevalence of the pathogen was relatively low on all carcass samples, especially following application of decontamination intervention systems in each packing plant; these results supported use of decontamination intervention systems to help reduce the risk of *E. coli* O157 being transmitted to consumers via beef products. The hide is often implicated as a major source of *E. coli* O157 carcass contamination (Chapman et al., 1993); however in this study, no correlation existed between the percentage of *E. coli* O157:H7-positive hide samples and positive carcass samples.

It should be noted that the prevalence of *E. coli* O157:H7 in hide and fecal samples was similar to that reported by Elder et al. (2000); however, in the present study, prevalence of the pathogen on carcasses was much lower, resulting in the inability to establish definitive associations between prevalence of the pathogen in fecal and hide samples and prevalence on carcasses.

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Table 3.1. Summary statistics for *Escherichia coli* O157-positive samples collected from the feedlot pen floor, hide and colon of cattle, and from subsequent carcasses pre- and post-evisceration and post-final intervention.

				Carcass		
	Feedlot Fecal	Hide	Colon Fecal	Pre-evisceration	Post-evisceration	Post-Final Intervention
Total Samples Positive[1]	111/450	22/150	40/145	15/149	2/144	1/290
Lots Positive[2]	13/15	7/15	11/15	7/15	1/15	1/15
Percent of lots positive[3]	86.7	46.7	73.3	46.7	7.7	7.7
Mean positive/lot, %[4]	24.7	14.7	27.6	10.1	1.4	0.3
Range, %[5]	0-77.8	0-50	0-100	0-60	0-20	0-5

[1] Total *E. coli* O157-positive samples/total number of samples collected.
[2] Number of lots containing at least one *E. coli* O157-positive sample/number of lots.
[3] Percentages of lots with at least one *E. coli* O157-positive sample.
[4] Mean of *E. coli* O157-positive samples per lot.
[5] Range of percentage *E. coli* O157-positive samples within lots.

Table 3.2. Percentage of *Escherichia coli* O157-positive samples collected from the feedlot pen floor, hide and colon of cattle, and from subsequent carcasses pre- and post-evisceration and post-final intervention, when feedlot fecal samples were positive at different levels.

				Carcass		
	Feedlot Fecal	Hide	Colon Fecal	Pre-evisceration	Post-evisceration	Post-Final Intervention
Lots with > 20 % positive feedlot pen floor fecal samples						
Lots Sampled[1]	7/15	7/15	7/15	7/15	7/15	7/15
Total Samples[2]	81/210	81/70	34/68	10/70	2/70	1/63
Percent lots positive[3]	100	85.7	100	42.9	14.3	14.3
Mean positive/lot, %[4]	44.4[a]	25.7[a]	51.4[a]	14.3[a]	2.9[a]	0.7[a]
Range, %[5]	26.7-77.8	0-50	10-100	0-60	0-20	0-5
Lots with < 20 % positive feedlot pen floor fecal samples						
Lots Sampled[6]	8/15	8/15	8/15	8/15	8/15	8/15
Total Samples	18/240	4/80	6/77	5/79	0/75	0/153
Percent lots positive	75.0	12.5	50.0	50.0	0.0	0.0
Mean positive/lot, %	7.5[b]	5.0[b]	7.5[b]	6.3[a]	0.0[a]	0.0[a]
Range, %	0-20	0-40	0-20	0-20	0-0	0-0

[a,b] Mean positives/lot, at different levels of prevalence in the feedlot fecal samples (> or ≤ 20%), with the same superscripts in columns are not significantly different (P > 0.05).

[1] Number of lots sampled with greater than 20% of samples *E. coli* O157-positive/total number of lots.

[2] Number of *E. coli* O157-positive samples/samples collected.

[3] Percentages of lots with at least one *E. coli* O157-positive sample.

[4] Mean *E. coli* O157-positive samples per lot.

[5] Range of percentage *E. coli* O157-positive within lots.

[6] Number of lots sampled with less than 20% of samples *E. coli* O157-positive/total number of lots.

CHAPTER IV

CATTLE FEEDLOT MANAGEMENT PRACTICES TO REDUCE *ESCHERICHIA COLI* O157 CONTAMINATION

ABSTRACT

This study was conducted to determine the effectiveness of single and multiple preharvest intervention strategies on prevalence of *Escherichia coli* O157 on/in cattle before transport to harvest. Cattle from 24 pens [approximately 200 head of cattle (419 kg) per pen] were randomly allocated (3 pens/treatment) to one of eight treatments: Control (CT; No treatment), Bovamine (Bov; a *Lactobacillus acidophilus* NPC-747 dietary product), NEOMIX (Neo; feeding of neomycin sulfate), an *E. coli* O157:H7 bacterin vaccine (Vac) and all combinations of the single treatments. Treatment of cattle with Bov and Vac began 60 d preharvest, while Neo was administered for 3 d, followed by a 24 h withdrawal period, immediately before harvest. Fecal and hide samples were randomly collected from 25 head per pen; fecal samples by rectal palpation and hide samples by sponge-swabbing 500 cm^2 area over the dorsal-thorax region. All cattle were sampled within a 10 d time-period and samples were collected no more than 48 h before harvest. Results showed that CT cattle had the highest pathogen levels for *E. coli* O157 (45.8 and 40.3%, for fecal and hide samples, respectively), while treated cattle presented numerically lower prevalences of the pathogen than CT. Trends suggested that Neo was the most effective single intervention, reducing *E. coli* O157 prevalence levels to 0.0 and

8.5%, in feces and on hides, respectively. Bovamine and Vac, when used singly, were equally effective and pathogen prevalence was reduced to 13.3 and 14.7%, respectively, in fecal samples and to 22.7 and 20.0%, respectively, on hides. When Bov, Vac, and Neo were used in combination, pathogen prevalence in fecal and hide samples were 2.7 and 6.7%, respectively. In a preliminary antibiotic susceptibility study of *E. coli* O157 isolates recovered from feces of cattle treated with Neo showed no increased resistance to a panel of 21 antibiotics, when compared to isolates from untreated cattle; however due to a lack of power, differences were not likely to be identified. In general, combinations of interventions resulted in lower pathogen prevalence than when a single intervention was used or when cattle were not treated with a pre-harvest intervention technology. This preliminary study demonstrated that preharvest mitigation strategies used singly or in combination can be effective in reducing prevalence of *E. coli* O157 in market-ready feedlot cattle.

Introduction

Extensive research has identified post-harvest beef carcass decontamination strategies that have proven useful in minimizing prevalence of *Escherichia coli* O157:H7 on beef carcasses (Hardin et al., 1995; Reagan et al., 1996; Dorsa, 1997; Sofos and Smith 1998). Studies also have investigated intervention technologies that may control the pathogen in live cattle and the environment. Elder et al. (2000) reported that prevalence of *E. coli* O157 was much higher in fecal, hide, and carcass samples than previous studies had indicated (Chapman et al., 1993; Hancock et al., 2000), partially due to more sensitive *E. coli* O157 isolation methods.

Russell et al. (2000), Diez-Gonzalez et al. (1998) and Buchko et al. (2000) evaluated the effects of dietary shifts on *E. coli* O157:H7 populations immediately before slaughter; however, neither dietary changes nor feeding management practices generated statistically significant or consistent findings. Rice and Johnson (2000) indicated that *E. coli* O157:H7 can live in water troughs for up to two weeks; but, research to assess the influences of using chlorination to decontaminate livestock drinking water has been minimally effective and is not practical for application.

Other researchers (Byrne et al., 2000; Ransom et al., 2002) have reported that washing cattle with chlorinated water before slaughter may only be minimally effective in reducing pathogen loads on hide surfaces. Barham et al. (2002) reported that the incidence of *E. coli* O157:H7 on cattle after transport to the slaughter facility from the feedlot actually decreased, but that incidence of *Salmonella* spp. more than doubled after transport.

It may be possible to use feed additives (i.e., sodium chlorate) in cattle diets to effectively reduce prevalence of *E. coli* O157:H7 (Anderson et al., 2000; Callaway et al., 2002). Sodium chlorate, however, has not been approved for use in animal diets. Since 1971, neomycin sulfate has been licensed to be used to treat bacterial enteritis in cattle, horses, sheep, swine, goats, cats, turkeys, chickens, ducks, and mink (NADA 011-315); however, until recently, its effectiveness as a means of reducing *E. coli* O157:H7 in cattle feces was relatively unknown. Elder et al. (2002) showed a reduction in prevalence of *E. coli* O157:H7 in feces of cattle that received neomycin sulfate for 48 h and then were allowed a 24 h withdrawal; they shed significantly lower *E. coli* biotype I and *E. coli* O157:H7 populations in their feces. After 5 days of neomycin withdrawal, *E. coli* biotype I populations had returned to near pretreatment levels, but, *E. coli* O157:H7 populations remained almost undetectable.

Zhao et al. (1998) reported that probiotic bacteria could be effective in reducing prevalence of *E. coli* O157:H7 in cattle. In addition, Schamberger and Diez-Gonzalez (2002) demonstrated effective isolation of colicinogenic *E. coli* strains that were inhibitory to *E. coli* O157:H7. More recently, Brashears et al. (2003a) demonstrated methodology for developing competitive exclusion products (i.e., *Lactobacillus acidophilus*– based direct-fed microbials) to be fed to cattle to inhibit the growth and proliferation of *E. coli* O157:H7. In a feeding trial utilizing these competitive exclusion products, it was shown that shedding of *E. coli* O157:H7 in feces of finishing beef cattle was reduced (Brashears et al., 2003b). Most recently, Younts-Dahl et al. (2004) reported that cattle administered a high level of *Lactobacillus acidophilus* (a combination of

strains NP 51 and NP 45) were 57 and 79% less likely to have an *E. coli* O157-positive fecal and hide sample, respectively, than were controls.

Finally, vaccines also have been proposed as a new pathogen reduction strategy that would minimize colonization of *E. coli* O157:H7 in bovine intestines. Research in a typical cattle feedlot setting has shown that vaccination is effective in reducing prevalence of the pathogen from 21.3% in control cattle to 8.8% in vaccinated cattle (Potter et al., 2004).

The objective of the present study was to evaluate the effectiveness of various preharvest interventions designed to reduce carriage and shedding of *E. coli* O157 in market-ready commercial feedlot cattle.

MATERIALS AND METHODS

The Colorado State University Animal Care and Use Committee evaluated all proposed methodologies before initiation of this study and granted a food and fiber exemption.

Experimental Design. This study was conducted in a commercial feedlot in Eastern Colorado in early spring and utilized 24 pens of cattle [approximately 200 head of (419 kg) cattle per pen], that were randomly allocated to allow for eight treatment groups that were replicated three times. The eight treatments were as follows: (1) control (CT); (2) Bovamine Rumen Culture (Bov); (3) NEOMIX® AG 325 Medicated Premix (Neo); (4) a prototype Fort Dodge Animal Health Bovine *E. coli* O157:H7 Bacterin (Vac); (5) a treatment combination of Vac plus Bov; (6) a treatment combination of Vac plus Neo; (7) a treatment combination of Neo plus Bov; and (8) a treatment combination of Vac plus Bov plus Neo. For cattle receiving the CT treatment, no

interventions were administered. Bovamine Rumen Culture (treatment 2) is a *Lactobacillus acidophilus* probiotic produced by Nutrition Physiology Corporation (Amarillo, TX), which contains a minimum of 2 x 10^{10} log CFU/g of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii.* This particular mixture of Bovamine (blue label with red writing, no product code given) was portrayed by Nutrition Physiology Corporation to our research team as containing an elevated level of *Lactobacillus acidophilus* NPC 747, which was reported by Brashears et al. (2003b) to reduce shedding of *E. coli* O157:H7 in feces of cattle. Bovamine was fed to cattle for the duration of the study.

Neomycin sulfate (325 g/0.45 kg) (treatment 3) is the active ingredient of NEOMIX® AG 325 Medicated Premix, produced by Pharmacia & Upjohn Company (now Pfizer Animal Health; Exton, PA). The NEOMIX® AG 325 Medicated Premix was fed according to the label instructions for use in Type C medicated solid feed at slightly below the recommended dosage. In this study, cattle were fed 10 mg of NEOMIX per 0.45 kg of body weight, rather than 10 mg of neomycin sulfate per 0.45 kg of body weight. NEOMIX® AG 325 Medicated Premix was added to "hammermill" (ground) corn at a concentration of 19.2 kg of NEOMIX per 909.01 kg of medicated feed, or 1.6 kg of NEOMIX per 909.01 kg of complete feed. This neomycin sulfate concentration fell within the recommended range of 0.25 to 2.25 kg per 909.01 kg of neomycin sulfate in the complete feed diet. The NEOMIX® AG 325 Medicated Premix was fed for three days, four days before harvest, and was removed from the diet 24 h before harvest to meet withdrawal requirements.

An experimental *E. coli* O157:H7 vaccine produced by Fort Dodge Animal Health (Fort Dodge, IA) (treatment 4) was administered at the onset of the study and again 30 days later; USDA-FSIS granted slaughter permits for the cattle vaccinated with the experimental vaccine 30 days after the last vaccination was administered. The vaccine was described by Fort Dodge Animal Health as an experimental bacterin which is formulated utilizing a proprietary dual adjuvant system that stimulates a strong immune response while maintaining safety. Adjuvants were selected based on preliminary screening of several complex adjuvant systems. Experimental inactivated bacterin contained the immune dominant antigens of *E. coli* O157:H7, including intimin and lipopolysaccharides, and stimulated host immune system T cells and B cells to elicit humoral antibody and some cell mediated immunity (CMI) factors.

Sample Collection. Samples were collected within a time span of 10 days for all treatment levels. Samples were collected randomly from 25 cattle per pen 12 to 48 h before transport to a commercial slaughter facility. Fecal samples were obtained by palpating the rectum and collecting at least 10 g of feces from each animal using a clean, plastic palpation glove. Hide samples were collected from the same animal from which feces were obtained by swabbing approximately 500 cm^2 of the dorsal midline of the thorax (Keen and Elder, 2002) with a prehydrated sponge kit (International Bioproducts, Bothell, WA). All samples then were transported to the Pathogen Reduction Laboratory in the Department of Animal Sciences at Colorado State University.

Microbiological Analysis. Using the procedures of Barkocy-Gallagher et al. (2002), hide and fecal samples were suspended in 75 or 90 ml, respectively, of tryptic soy

broth (TSB; Difco, Becton Dickinson & Co., Sparks, MD) and then incubated for 2 h at 25°C, followed by 6 h at 42°C, and then left overnight at 4°C.

Escherichia coli O157 enrichment was followed by immunomagnetic bead separation, which consisted of a 30-min incubation (on a rocker at room temperature) of 1 ml aliquots of the enriched fecal and hide samples plus 100 µl protamine (50 µg/ml filter-sterilized solution; Sigma, St. Louis, MO), plus 20 µl of anti-O157 immunomagnetic beads (Dynal Laboratories, Lake Success, NY). The beads were washed three times with 1 ml of phosphate buffered saline (PBS)/0.05% Tween 20 (Sigma) on a magnetic separation rack, and then resuspended in 100 µl of PBS/0.05% Tween 20. Fifty microliters of the bead suspension was spread plated onto sorbitol MacConkey agar (Difco) plates supplemented with cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l, Dynal Laboratories, ctSMAC). The remaining 50 µl was plated on Rainbow-plus agar (Biolog, Inc., Hayward, CA) containing 0.8 µg/ml of potassium tellurite (Sigma-Aldrich, St. Louis, MO) and 20 µg/ml novobiocin (Sigma). The supplements were added to improve selectivity as suggested by the manufacturer.

Both ctSMAC and Rainbow-plus agar plates were incubated for 18 h at 35°C. Following incubation, up to five presumptive *E. coli* O157 colonies were picked from the ctSMAC and Rainbow-plus agar plates and screened with the Dry Spot O157 latex test (Oxoid, Ogdensburg, NY). Colonies generating a positive reaction using the latex test were subsequently streaked onto ctSMAC for purity (24 h; 35°C). From ctSMAC, morphologically typical sorbitol-negative colonies were tested for their indole reaction by inoculating them into 1% tryptone broth (Fischer Scientific, Fair Lawn, NJ, BAM) and incubating them at 35°C for 24 h, following which 0.3 ml of Kovac's reagent

(bioMérieux Vitek, Inc., Hazelwood, MO) was added to obtain an indole-positive or negative reaction .

Antibiotic Susceptibility. Isolates of *E. coli* O157 recovered from feces of cattle receiving no treatment (n=25, control) or from feces of cattle that were treated with Neo in any combination of treatments (n=21, treated), were tested to compare antimicrobial resistance characteristics.

No *E. coli* O157 isolates were recovered from cattle that were treated solely with NEOMIX (Table 3). Therefore, antimicrobial susceptibility of *E. coli* O157 isolates exposed to Neo was determined by using those isolates recovered from cattle treated with Neo in combination with Vac and Bov. Antimicrobial susceptibility was determined using the disk diffusion method in accordance with NCCLS (1999) standards.

Briefly, *E. coli* O157 isolates were transferred onto Trypticase soy agar containing 5% sheep blood (Difco) and then incubated for 24 h at 35°C. Following incubation, the BBL Prompt system was used to produce a standard inoculum of 1×10^8 CFU/ml (Becton Dickson Microbiology Systems, Sparks, MD). After standardization, the inoculum was spread onto two Mueller-Hinton agar (Difco) plates, at which time the antimicrobial disks were dispensed and tamed into place (BBL Sensi-Disc, Becton Dickinson). Antimicrobial susceptibility testing was conducting using the following 21 different antimicrobials (concentrations) and combinations: amikacin (30μg), ampicillin (10 μg), cefoxitin (30 μg), cephalothin (30 μg), ceftiofur (10 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), florfenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), netilmicin (30 μg), streptomycin (10 μg), sulfisoxazole (250 μg), tetracycline (30 μg), tilmicosin (15 μg),

tobramycin (10 μg), and combinations of amoxicillin/clavulanic acid (20 μg /10 μg) and trimethoprim/sulfamethoxazole (1.25 μg /23.75 μg). After incubation for 17 ± 1 h at 35°C, plates were removed and a computerized plate reader was used to measure the respective zones of inhibition for each of the antimicrobials tested.

Statistical Analysis. Data from the *E. coli* O157 analyses were reported as percentages of hide, fecal, or hide plus fecal (when either were positive) samples testing positive for the pathogen per pen. Since both the hide and fecal samples came from the same animal, the animal was considered positive if either the hide or the fecal sample was positive. Differences in percentages of positive samples among treatments were evaluated using a chi-square goodness of fit test. Data representing percentage prevalence of *E. coli* O157 isolates were analyzed using the Frequency Procedure of SAS (SAS Inc., Cary, NC, Version 8.2e, 2003). Differences between frequencies associated with the different pathogen intervention strategies were determined by calculating the chi-square statistic. Differences between means, standard deviations and the minimum and maximum zones of inhibition for each isolate were calculated with PROC GLM using the Hotellings T-test procedure of SAS (SAS Inc., Cary, NC, Version 8.2e, 2003).

RESULTS AND DISCUSSION

Escherichia coli O157 was isolated from 85 (14.5%), 95 (16.2%), and 166 (28.3%) of the 586 fecal, hide and hide plus fecal (when either the hide or fecal samples was positive, but not necessarily both) samples, respectively, in this study. Using pen as the experimental unit, the main effect of treatment did not prove to be statically relevant

($P > 0.05$; Table 4.1). Considering these findings, data are discussed in this report as reduction trends.

One of the difficulties in field studies that test for the prevalence of *E. coli* O157:H7 is an elusive dynamic of the pathogen. Smith (2003) reported that the proportion of cattle shedding *E. coli* O157:H7 within a single population during summer months can vary from 1 to 80%. With such large variation among pens in *E. coli* O157:H7 prevalence within any given time, one might conclude that variation observed among replicates for hide and fecal samples in this study was to be expected (Table 4.1). The problem of highly variable prevalence data in *E. coli* O157:H7 field studies might be resolved in future studies by enumerating pathogen levels in or on cattle, which would offer greater insight regarding effects of treatments on the reduction or proliferation of the pathogen.

When pen prevalence within treatments and between replicates (each individual pen) was averaged, mean *E. coli* O157 prevalences of 40.3, 45.8 and 56.9% were detected for hide, fecal, and hide plus fecal samples, respectively, for control groups (Table 4.2). Pre-harvest intervention treatments resulted in a lower prevalence of *E. coli* O157 by at least 17.6, 12.9 and 8.9%, respectively, for hide, fecal, and hide plus fecal samples, and by as much as 33.6, 45.8, and 48.9%, respectively. In addition, prevalence of *E. coli* O157 on hides appeared to be lowered compared to controls when cattle were treated with Neo, Vac +Neo, Neo + Bov, or Vac + Bov + Neo.

As a single intervention, or in combination with other treatments, Neo appeared to be the most effective for reducing pathogen prevalence in fecal samples when compared to controls. These data were similar to those reported by Elder et al. (2002), in which

neomycin treated calves resulted in undetectable levels of *E. coli* O157 in nearly all of the calves for up to 5 days.

When used singly, Bov and Vac were similar to each other in effectiveness, by exhibiting a 17.6 and 20.3% lower pathogen prevalence, respectively, on hide samples and reduced by 32.5 and 31.1%, respectively, in fecal samples compared to prevalence in cattle that were not treated. The combination of all three interventions (Vac + Bov + Neo) generated the lowest numerical pathogen prevalence on hides and in feces. Treatments with Bov in this study provided slightly greater differences from the controls in prevalence of *E. coli* O157 compared to those reductions reported by Brashears et al. (2003b) and Younts-Dahl et al. (2004). In addition, treatment with an *E. coli* O157:H7 vaccine provided slightly greater performance in reducing prevalence of *E. coli* O157 compared to controls than that reported by Potter et al. (2004).

In this study, no differences in antibiotic susceptibility/resistance patterns of *E. coli* O157 recovered from feces of control and Neo treated cattle (Table 4.3) were detected; however, it should be noted that due to the relatively low number of isolates and the high number of antibiotics tested, we lacked sufficient power to accurately identify statistical differences. In general, of the 21 antibiotics used in this study, 17 were effective in controlling growth of *E. coli* O157 recovered from the feces of feedlot cattle (Table 4.4). As expected, all but one *E. coli* O157 isolate from Neo treated cattle, and all isolates from control cattle were resistant to erythromycin. In addition, 60 and 76% of isolates from control and treated cattle, respectively, were resistant to tilmicosin. For cephalothin, intermediate resistance was shown in 12 and 9% of isolates from control and treated cattle, respectively. From the isolates and resulting susceptibility data collected in

this study, treatment with neomycin sulfate for three days before harvest did not appear to influence the antibiotic susceptibility/resistance patterns of *E. coli* O157 recovered from the feces of cattle that received neomycin sulfate immediately (4 days) before harvest.

Data from this preliminary study indicated that preharvest pathogen mitigation interventions appeared to reduce prevalence of *E. coli* O157 on hides and in the feces of treated cattle. Additional studies should be conducted to statistically validate their effectiveness in reducing prevalence of *E. coli* O157 on the hides and in the feces of cattle. More importantly, research should be conducted to determine if these preharvest interventions strategies impact the microbiological quality of carcasses post-processing. Further research should be conducted to identify supply-chain cattle management systems that could effectively minimize prevalence of *E. coli* O157 at all stages of beef cattle production. One potential disadvantage of applying preharvest intervention strategies in the feedlot is the added cost incurred by the producer.

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Table 4.1. Presumptive[1] prevalence (%) of *E. coli* O157 isolates by replicate and treatment from hide, fecal, or a combination of hide plus fecal samples collected from cattle exposed to one of eight preharvest intervention treatments.

Control or Treatment	Hide			Fecal			Hide plus Fecal		
	Rep[2] 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Control	66.7	8.7	44.0	87.5	4.3	44.0	95.8	13.0	60.0
Bovamine (Bov)	0.0	8.0	60.0	0.0	24.0	16.0	0.0	32.0	64.0
NEOMIX (Neo)	14.3	12.0	0.0	0.0	0.0	0.0	14.3	12.0	0.0
Vaccine (Vac)	16.0	16.0	28.0	16.0	0.0	28.0	28.0	16.0	52.0
Vac + Bov	4.2	44.0	4.0	91.7	8.0	0.0	91.7	52.0	4.0
Vac + Neo	12.0	4.0	4.0	80.0	0.0	0.0	84.0	4.0	4.0
Neo + Bov	23.8	0.0	0.0	0.0	4.0	0.0	23.8	4.0	0.0
Vac + Bov + Neo	12.0	0.0	8.0	0.0	4.0	4.0	12.0	4.0	12.0

[1] Isolates are considered presumptive positive when morphologically typical colonies are *E. coli* O157 latex positive and indole-positive.

[2] Pen served as the experimental unit (n = 3/treatment/sample type). This data represents the percentage of the 25 *E. coli* O157 presumptive-positive hide, fecal and hide plus fecal samples per pen.

Table 4.2. Percent prevalence (and, parenthetically, the difference from the control) of presumptive[1] positive *E. coli* O157 isolates from hide, fecal, or a combination of hide plus fecal samples collected from cattle exposed to one of eight preharvest intervention treatments.

Control or Treatment	% Presumptive Positive *E. coli* O157 isolates		
	Hide	Fecal	Hide or Fecal
Control	40.3	45.8	56.9
Bovamine (Bov)	22.7 (17.6)	13.3 (32.5)	32.0 (24.9)
NEOMIX (Neo)	8.5 (31.8)	0.0 (45.8)	8.5 (48.4)
Vaccine (Vac)	20.0 (20.3)	14.7 (31.1)	32.0 (24.9)
Vac + Bov	16.4 (23.9)	32.9 (12.9)	48.0 (8.9)
Vac + Neo	6.7 (33.6)	26.7 (19.1)	30.7 (26.2)
Neo + Bov	7.1 (33.2)	1.3 (44.5)	8.6 (48.3)
Vac + Bov + Neo	6.7 (33.6)	2.7 (43.1)	8.0 (48.9)

[1] Isolates are considered presumptive positive when morphologically typical colonies are *E. coli* O157 latex positive and indole-positive.

Table 4.3. Descriptive statistics for zones of inhibition (mm) relating to antibiotic susceptibility/resistance patterns for multiple classes of antibiotics against *E. coli* O157 isolates recovered from fecal samples collected from feedlot cattle receiving either no (control; n=25) or subsequent pre-harvest microbiological intervention strategies (Neo treated; n=21).

	Zones of Inhibition (mm)							
	Control[a]				Treated[b]			
Antimicrobial	Average	SD	Min	Max	Average	SD	Min	Max
Aminoglycosides:								
Amikacin	22.9	1.1	20	25	22.7	0.8	21	24
Gentamicin	22.6	0.7	21	23	22.1	0.7	21	23
Kanamycin	21.9	0.9	19	23	22.0	0.8	19	23
Neomycin	19.0	0.8	18	21	19.0	0.8	18	21
Netilmicin	26.0	1.1	22	27	26.0	1.0	23	27
Streptomycin	17.8	1.0	15	19	17.7	0.8	16	19
Tobramycin	22.2	0.9	19	23	22.3	0.8	20	23
β-Lactam/β-Lactamase inhibitors:								
Amoxicillin-clavulanic acid	21.2	1.1	19	23	21.4	1.1	18	23
Ampicillin	18.7	0.7	18	21	19.7	1.2	17	21
Cephalosporins:								
Cefoxitin	25.2	1.3	23	29	26.0	1.2	23	27
Ceftiofur	25.9	1.2	23	28	26.9	1.0	25	29
Ceftriaxone	31.2	1.3	28	34	31.7	1.4	28	35
Cephalothin	18.6	1.0	17	22	19.5	1.3	17	21
Chloramphenicol	21.6	1.4	19	25	21.0	1.4	19	25
Florfenicol (chloramphen. derive.)	22.2	1.7	19	25	20.4	1.7	18	26
Fluoroquinolones:								
Ciprofloxacin	33.6	3.5	24	39	34.0	2.1	28	37
Macrolides:								
Erythromycin	9.9	0.5	9	11	10.1	1.5	9	16
Tilmicosin	10.5	1.0	9	13	9.9	1.6	6	14
Sulfonamides:								
Sulfasoxazole	21.9	1.3	19	24	22.6	1.0	20	26
Tetracycline	22.3	1.1	19	25	23.3	1.4	20	26
Trimethoprim and Sulfamethoxazole	30.2	1.3	28	33	30.2	1.47	27	33

[a] *E. coli* O157 isolates recovered from cattle that did not receive a preharvest intervention treatment.

[b] *E. coli* O157 isolates recovered from cattle treated with either the experimental Fort Dodge Animal Health *E. coli* O157 vaccine and NEOMIX (neomycin sulfate) or from cattle treated with a combination of Bovamine (a product containing *Lactobacillus acidophilus*), Fort Dodge Animal Health *E. coli* O157 vaccine and NEOMIX (neomycin sulfate).

Table 4.4. Classification of antibiotic susceptibility (resistant, intermediate and susceptible) and number of *E. coli* O157 isolates, recovered from feedlot cattle receiving either no Neo (control; n=25) or that received Neo pre-harvest (Neo treated; n=21).

	Level of Resistance					
	Resistant		Intermediate		Susceptible	
Antimicrobial	Control	Treated[a]	Control	Treated[a]	Control	Treated[a]
Aminoglycosides						
Amikacin	0	0	0	0	25	21
Gentamicin	0	0	0	0	25	21
Kanamycin	0	0	0	0	25	21
Neomycin	0	0	0	0	25	21
Netilmicin	0	0	0	0	25	21
Streptomycin	0	0	0	0	25	21
Tobramycin	0	0	0	0	25	21
β-Lactam/β-Lactamase inhibitors						
Amoxicillin-clavulanic acid	0	0	0	0	25	21
Ampicillin	0	0	0	0	25	21
Cephalosporins						
Cefoxitin	0	0	0	0	25	21
Ceftiofur	0	0	0	0	25	21
Ceftriaxone	0	0	0	0	25	21
Cephalothin	0	0	3	2	22	19
Chloramphenicol	0	0	0	0	25	21
Florfenicol (chloramphen. derive.)	0	0	0	1	25	20
Fluoroquinolone						
Ciprofloxacin	0	0	0	0	25	21
Macrolides						
Erythromycin	25	20	0	0	0	1
Tilmicosin	15	16	10	4	0	1
Sulfonamide						
Sulfasoxazole	0	0	0	0	25	21
Tetracycline	0	0	0	0	25	21
Trimethoprim/sulfonamide						
Trimethoprim and Sulfamethoxazole	0	0	0	0	25	21

[a] *E. coli* O157 isolates recovered from cattle that did not receive a preharvest intervention treatment.

[b] *E. coli* O157 isolates recovered from cattle treated with either the experimental Fort Dodge Animal Health *E. coli* O157 vaccine and NEOMIX (neomycin sulfate) or from a combination of Bovamine (a product containing *Lactobacillus acidophilus*), Fort Dodge Animal Health *E. coli* O157 vaccine and NEOMIX (neomycin sulfate).

CHAPTER V

EFFECTIVENESS OF ACTIVATED AND NON-ACTIVATED LACTOFERRIN, LACTIC ACID AND WATER IN REDUCING *ESCHERICHIA COLI* O157:H7, *SALMONELLA* TYPHIMURIUM AND *LISTERIA MONOCYTOGENES* POPULATIONS ON BOLOGNA AND FRESH BEEF PRODUCTS

ABSTRACT

The objective of this study was to determine the antimicrobial effectiveness of activated lactoferrin (2%; ALF), lactoferrin (2%; LF), lactic acid (2%; LA), and water on *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* populations inoculated onto beef adipose tissue (BAT), lean tissue pieces (LTP), vacuum-packaged beef cuts (VPB), and bologna (RTE) stored at 10 to 12°C. Beef products were inoculated at a level of 3.5 to 4.0 log CFU/cm^2, and then dipped for two minutes in the appropriate antimicrobial solutions at 28°C, either singly or sequentially. For beef adipose tissue, ALF, LF, LA and water treatments were applied singly, and then sequential treatments of ALF followed by LA and LA followed by ALF also were tested for effectiveness in reducing pathogen populations initially, and during aerobic storage, at 12°C for up to 2 days. Lean tissue pieces, VPB, and RTE were inoculated either before (pre) or after (post) single treatment with ALF, LF, LA and water. Following treatment, LTP were stored under aerobic conditions at 12°C for up to 7 days, whereas VPB and bologna were vacuum packaged and stored at 12°C for up to 29 days (VPB) or at 10°C for up to 33 days (bologna). For all pathogens on BAT, LA was the most ($P < 0.05$) effective single intervention used; however, the sequential application of ALF followed by LA was more ($P < 0.05$) effective in inhibiting *E. coli* O157:H7 and *L. monocytogenes* populations than any other single or sequential intervention strategy used. In LTP, LA was most effective, and was the only treatment that inhibited pathogen levels at the end of the 7 day storage period. Similarly, in VPB, LA was more ($P < 0.05$) effective that all

other treatments in reducing initial populations and inhibiting growth of *S.* Typhimurium on VPB; however, for *E. coli* O157:H7, ALF and LA were more effective ($P < 0.05$) in inhibiting growth than all other treatments, both pre- and post-inoculation. In bologna inoculated with *L. monocytogenes*, ALF was more effective ($P < 0.05$) than all other treatments, inhibiting pathogen growth to 1.7 and 1.9 log CFU/cm^2 for pre- and post-inoculation, respectively, after 33 days of storage. Furthermore, ALF inhibited growth of *E. coli* O157:H7 populations on bologna to 0.3 and 1.0 log CFU/cm^2 on pre- and post inoculated product, respectively, while the bologna treated with LA allowed growth to levels of 2.7 to 3.2 log CFU/cm^2, respectively. For *S.* Typhimurium on bologna, LA was most effective in minimizing growth of the pathogen during storage. This study demonstrated the antimicrobial effectiveness of ALF and LA when applied individually and sequentially to bologna and fresh beef products.

Introduction

Many studies have described the antimicrobial effectiveness of lactic acid alone or in combination with other decontamination treatments (such as steam vacuuming, hot water washing and knife trimming) in reducing initial populations of pathogens on beef carcass surfaces and adipose tissue (Bacon et al., 2000; Castillo et al., 1998; Dorsa et al.,1998; Hardin et al., 1995). In the last five years, additional chemical compounds such as trisodium phosphate, acidified (citric acid) sodium chlorite, and cetylpyridinium chloride were shown useful in enhancing microbiological quality of fresh beef products (Cutter et al., 2000a; Castillo et al., 1999; Cutter et al., 2000b; Ransom et al., 2002). Before the USDA-Food Safety and Inspection Service Final Rule addressing control of *Listeria monocytogenes* in ready-to-eat meat and poultry products was published (FSIS, 2003), many researchers were identifying new ways to reduce and control growth of *L. monocytogenes* populations in ready-to eat products via post packaging pasteurization and inclusion of sodium lactate and sodium diacetate into the meat batter (Murianna et al., 2002; Seman et al., 2002; Glass et al., 2002)

Bovine apolactoferrin has been shown to have bacteriostatic activity against four strains of *Listeria monocytogenes* and an *Escherichia coli* strain at concentrations of 15 to 30 mg/mL in UHT milk (Payne et al., 1990). Lactoferrin from bovine colostrum is bacteriostatic by virtue of its ability to sequester iron ions, but also is bactericidal by means of mechanisms not depending on iron-binding alone (Arnold et al., 1980a,b) and can damage the outer membrane of Gram-negative bacteria (Yamaguchi et al., 1993). The bactericidal domain in lactoferrin helps explain earlier observations that lactoferrin

had rapid lethal activity unaffected by iron, as well as bacteriostatic activity counteracted by that element (Sofos et al., 1998). Growth and survival of many bacterial pathogens depends on the availability of iron ions; the iron-binding glycoproteins ovotransferrin and lactoferrin exert antimicrobial activity in biological fluids through their ability to withhold iron ions from microorganisms (Ekstrand, 1994). Naidu (2000) described a "microbial blocking agent" (MBA) in, among other things, milk, that was capable of preventing attachment of microorganisms to biological surfaces, preventing growth/multiplication of bacteria and neutralizing toxic proinflammatory agents such as endotoxins.

There is scientific evidence that lactoferrin has broad-spectrum antimicrobial properties (Rieter, 1983) and that gastric pepsin cleavage of lactoferrin generates peptides that are much more effective antimicrobials than lactoferrin itself (Tomita et al., 1992; Hoek et al., 1997). Bellamy et al. (1992a) isolated, identified and named (lactoferricin B) the active peptide from bovine lactoferrin; Bellamy et al. (1992b) examined the susceptibility of a variety of bacterial species to inhibition and inactivation by lactoferricin B (to elucidate its antibacterial spectrum) and identified some of the environmental factors that influence its activity. Bellamy et al. (1992b) reported that a physiologically diverse range of Gram-positive and Gram-negative bacteria was found to be susceptible to inhibition and inactivation by lactoferricin B. The list of susceptible organisms includes *E. coli, Salmonella* Enteritidis, *Klebsiella pneumoniae, Proteus vulgaris, Yersinia enterocolitica, Pseudomonas aeruginosa, Campylobacter jejuni, Staphylococcus aureus, Streptococcus mutans, Corynebacterium diphtheriae, L. monocytogenes*, and *Clostridium perfringens*. Concentrations of lactoferricin B required

to cause complete inhibition of growth varied within the range of 0.3 to 150 μg/ml, depending on the strain and the culture medium used; the peptide showed activity against *E. coli* O111 over the range of pH 5.5 to 7.5 and was most effective under slightly alkaline conditions (Bellamy et al., 1992b). Lactoferricin B was lethal, causing a rapid loss of colony-forming capability, in most of the bacterial species tested (Bellamy et al., 1992b). Wakabayashi et al. (1992) reported that lactoferricin B was lethal to four strains of *L. monocytogenes* at concentrations from 0.00003% to 0.0009%. All of these studies were based on laboratory testing and were not conducted with beef tissues, cuts or carcasses.

Efficacy of lactoferricin B was tested as an intervention technology for reducing counts of *E. coli* O157:H7 on beef carcass adipose tissue (BCT) and on fresh boneless beef short plates (BSP) by Ransom et al. (2003). That study compared abilities of water (at 25°C) plus seven chemicals (one of which was lactoferricin B) to reduce counts of *E. coli* O157:H7 on inoculated—at either high (5.8 to 6.4 log CFU/cm^2) or low (4.1 to 4.3 log CFU/cm^2) levels—BCT and BSP. In eight sets of statistical comparisons of water (at 25°C) and seven chemicals, lactoferricin B ranked 6^{th}-7^{th} in its effectiveness against *E. coli* O157:H7 on meat surfaces.

Because "activated lactoferrin" is different from "lactoferricin B," it is quite possible that the former (activated lactoferrin) might be efficacious against meatborne pathogens while the latter (lactoferricin B), at least in the Ransom et al. (2003) study, is not. The present study was designed to determine the effectiveness of activated lactoferrin against meatborne pathogens on ready-to-eat beef bologna, simulated beef

carcass surfaces (hot and chilled), and retail lean beef. In addition, water, non-activated lactoferrin, and 2% lactic acid were used as controls.

MATERIALS AND METHODS

Inoculum Preparation: Pathogen cocktails of either *Escherichia coli* O157:H7 [ATCC 43889, ATCC 43890, ATCC 43894, ATCC 43895 and EO 139 (isolated from venison jerky)] or *Salmonella* Typhimurium [ATCC 700408, ATCC 14028, UK1 (isolated from a horse wound) and two strains of *S.* Typhimurium DT104 var. Copenhagen (isolated from beef animal hides)] or *Listeria monocytogenes* [Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b) and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate) and PVM1, PVM2, PVM3 and PVM4 (pork variety meat isolates, serotype not known)] were used in the inoculation of beef products used in this study. All of the strains were available as frozen stock cultures (-70°C).

E. coli O157:H7 and *S.* Typhimurium strains were activated by transferring a loopful of the stock culture into 10 ml of Tryptic Soy broth (Difco, Becton Dickinson & Co., Sparks, MD; TSB) and incubating them at 35°C for 24 h. *Listeria monocytogenes* strains were activated by transferring a loopful of the stock culture into 10 ml of TSB with 0.6% yeast extract (Acumedia, Baltimore, MD; TSBYE) and incubating them at 30°C for 24 h. All pathogen strains were subcultured twice in TSB (*E. coli* O157:H7 and *S.* Typhimurium) or TSBYE (*L. monocytogenes*) before use in each experiment.

The TSB and TSBYE cultures were centrifuged (6000 rpm, 15 min, 4°C), washed with 10 ml of sterile phosphate-buffered saline (PBS), and then centrifuged again (6,000 rpm,

15 min, 4°C). The resulting pellet was resuspended in PBS and serially diluted to a concentration estimated to yield 3 to 4 log CFU/cm^2 on beef product surfaces.

Study I: Adipose Tissue Pieces. Beef adipose tissue (BAT) pieces were used to simulate beef carcass surfaces, and were collected from the brisket of carcasses as they exited a thermal decontamination spray cabinet at a large commercial packing plant. Samples were returned to the Pathogen Reduction Laboratory at CSU in a standard ice chest so that samples did not chill. Upon arrival at CSU, adipose tissue samples were immediately partitioned to an approximate area of 5 cm x 5 cm x 1 cm. Under a biological safety cabinet, one side of the BAT was inoculated with 0.1 ml of one of the three inocula, which was then spread over the entire surface with a sterile bent glass rod. Bacterial attachment was allowed to occur for 15 min at 5°C. Treatments involved single and combined applications of solutions of water, LA, LF and ALF to the inoculated adipose tissue pieces. For this study, treatments included: (1) no treatment (Control), (2) distilled water (Wal-Mart, Bentonville, AR), (3) 2% activated lactoferrin (ALF; N-terminus Research Laboratory, Pomona, CA;) (4) 2% non-activated lactoferrin (LF; N-terminus Research Laboratory, Pomona, CA;) or (5) 2% lactic acid (LA; 85% lactic acid, Sigma-Aldrich, St. Louis, MO). (6) sequential application of ALF followed by 2% LA, or (7) sequential application of 2% LA followed by ALF. The pH values for treatment solutions were 6.6, 7.4, 5.3 and 2.1 for water, activated lactoferrin, lactoferrin and 2% lactic acid, respectively. Treatments were applied by immersion of approximately 20 BAT pieces into 500 ml of the solution for 2 min, followed by draining. After treatment application, samples were placed on styrofoam trays and covered with oxygen-permeable

PVC film, then stored at 12°C for 2 days. Microbiological analyses were conducted on days 0, 1 and 2.

Each sample was added to 100 ml 0.1% buffered peptone water (BPW; Difco) and then shaken 30 times vertically. Samples were serially diluted and total bacterial populations were enumerated on Tryptic Soy agar plates (Difco; TSA; 25°C for 72 h) for BAT inoculated with *E. coli* O157:H7 and *S.* Typhimurium, whereas Tryptic Soy agar supplemented with 0.6% yeast extract (TSAYE; 25°C for 72 h) was used to quantify total bacterial populations from BAT inoculated with *L. monocytogenes*. *Escherichia coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* populations were enumerated on Sorbitol MacConkey (SMAC) agar (Difco) supplemented with cefixime and potassium tellurite (0.05 mg/l, Dynal Inc. Lake Success, NJ; ctSMAC), XLT-4 agar (Difco) plates, and Modified Oxford agar (MOX; Difco), respectively. Selective agars plates were incubated at 35°C for 24 h for *E. coli* O157:H7 and *S.* Typhimurium, and at 30°C for 48 h for *L. monocytogenes*. Following incubation, colonies were enumerated.

Study 2 – Lean Tissue Pieces. Beef inside rounds, with the cap removed (NAMP 169A), were purchased from a meat processor and transported to the Meat Science laboratory at CSU where they were frozen for slicing. Using a bandsaw (AEW-Thurne, Norwich , England), the inside rounds were sliced 1 cm thick, then portioned such that the final surface area was 40 cm^2 (2.5 cm x 5.0 cm x 1.0 cm). Following cutting, one side of the LTP was inoculated with 0.1 ml of one of the three inocula, which was then spread over the entire surface with a sterile bent glass rod. Following attachment, LTP were turned over with sterilized forceps and the second side was inoculated as described above.

Pre-inoculated LTP were subjected to one of the following treatments: (1) no treatment (Control), (2) distilled water, (3) 2% activated lactoferrin, (4) 2% non-activated lactoferrin, or (5) 2% lactic acid. Treatments were applied the same as described above, by immersion into treatment solution for 2 min, followed by draining. After treatment application, samples in this study were placed on styrofoam trays and covered with oxygen-permeable PVC film, then stored at 12°C for 7 days. Microbiological analyses were conducted on days 0, 1, 3, 5 and 7.

For post-inoculated samples, LTP (two replicates, three samples per sampling time per treatment) served as controls or were treated with one of the four treatments described previously, then inoculated in the same manner and with the same pathogen cocktails as described previously.

Study 3 – Vacuum Packaged Beef Cuts. Boneless beef short plates (NAMP 123) were purchased from a meat processor and transported to the Pathogen Reduction Laboratory in the Department of Animal Sciences at Colorado State University (CSU). The most anterior section (fat cap; approximately 15.24 cm) was removed so that the natural exterior lean surface tissue was exposed on the top and adipose tissue was exposed on the bottom of the subprimal. Pieces (one piece constituted one sample; a treatment consisted of two replicates of three samples per treatment) were cut (approximately 2.5 cm x 5.0 cm x 1.5 cm) to acquire an approximate 47.5 cm^2 piece of tissue. Methods used for inoculum preparation, boneless beef plate inoculation, and bacterial enumeration were the same as those previously described for Study 2. For this vacuum packaged beef cuts study, samples were immersed in one liter of treatment solution rather than 500 ml due to the size of the beef pieces. In addition, samples were vacuum packaged (2 pieces per

bag; bags were15 x 20 cm, 3 mil std barrier, Nylon/PE vacuum pouch, Koch) and stored at 12°C for up to 29 days, with microbiological analysis performed as previously described on days 0, 4, 8, 15, and 29.

Study 4 – Bologna. The bologna formulation consisted of (%wt/wt): beef trimmings (82.2; approximately 30% fat), ice (10.0), sodium chloride (2.0), dextrose (2.0), dry mustard (0.9), corn syrup solids (2.0), polyphosphate (0.4), sodium nitrite (0.0156), sodium erythorbate (0.05), paprika (0.25), onion powder (0.05), garlic powder (0.05), coriander (0.05) and white pepper (0.05). All spices and seasonings were purchased from A.C. Legg Co. (Birmingham, AL). Each replicate was prepared individually, 24 h in advance of the decontamination study. Ingredients were emulsified in a bowl chopper (RMF, Kansas City, MO) for 3 to 5 minutes until a final product temperature of 15.5°C was reached, then extruded into 65 mm diameter fibrous cellulose casings (Koch, Kansas City, MO). Product was cooked in dry air for 1 h, and then smoked for 38 min. After smoking, the product was cooked until the internal temperature reached 70°C, the bologna was showered with cool tap water for 5 min, and then cooled overnight at 4°C. Casings were removed and bologna was sliced into 5 mm slices using a Globe slicer (Mozley Manufacturing, Stamford, CT). Bologna slices were inoculated and treated as described above in Study 2. Following treatment, bologna slices were vacuum packaged and stored at 10°C for up to 33 days, with microbiological analysis performed as previously described on days 0, 8, 19, and 33.

Statistical Analysis. Microbiological counts were converted to $\log_{10}$ CFU/cm^2 before being analyzed. The study was designed as a two-way fixed effects factorial of treatment by day. Three samples per day were evaluated per treatment subclass in all

four studies, and all four studies were replicated. Due to different growth factors for each pathogen, and due to different product storage and packaging schemes, pathogens, media type (selective vs. non-selective) and beef products were analyzed independently. Analysis of variance (AOV) was conducted, and least-squares means and standard errors were computed for pathogen and total bacterial populations for each control and treatment group in each of the four studies using the General Linear Models (GLM) procedures of SAS (SAS Inc., Cary, NC, Version 8.2e, 2003). Least squares means were separated and differences were determined using an alpha-error level of 0.05 when AOV suggested a significant effect.

RESULTS AND DISCUSSION

On beef adipose tissue (BAT) inoculated with *E. coli* O157:H7 and *L. monocytogenes*, LA was the most effective ($P < 0.05$) single intervention used in reducing initial populations and inhibiting growth of pathogens; however, the sequential application of ALF and LA, resulted in a greater reduction on day 0 ($P > 0.05$), and greater inhibition 2 days after treatment application ($P < 0.05$) (Table 5.1). Two days after sequential treatment application of ALF and LA, *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* populations were inhibited to 0.2, 0.7, and 1.2 log CFU/cm^2, respectively, compared to 1.5, 1.8, and 2.0, log CFU/cm^2, respectively, for samples treated with only LA. In addition, total bacterial populations on BAT inoculated with *E. coli* O157:H7 and *L. monocytogenes* showed inhibition characteristics similar to those shown on the selective media (Table 5.2).

For LTP, *E. coli* O157:H7 populations were inhibited ($P < 0.05$) to 2.3 and 2.8 log CFU/cm^2 by LA, both pre- and post-inoculation; however, no treatments resulted in an

initial reduction of *E. coli* O157:H7 populations (Table 5.3). Lean tissue pieces treated with water, ALF, and LF (both pre- and post-inoculation) resulted in similarly consistent growth up to at least 5.7 log CFU/cm^2 after 7 days of storage. On non-selective media, no treatment effects were observed for any of the pathogens on lean tissue pieces (Table 5.6). No treatment effects were observed for *L. monocytogenes* and *S.* Typhimurium populations on lean tissue pieces (LTP) stored aerobically at 12°C; however LA treatment both pre- and post-inoculation resulted in greatest ($P > 0.05$) inhibition of growth for both pathogens (Table 5.3).

Activated lactoferrin and LA pre- and post-inoculation were more effective ($P < 0.05$) than all treatments in inhibiting growth of *E. coli* O157:H7 on vacuum packaged beef cuts (VPB) (Table 5.5). Lactic acid treatment on VPB resulted in an immediate 1.5 log CFU/cm^2 reduction in *E. coli* O157:H7 populations when compared to controls. Although not significantly different, *E. coli* O157:H7 populations were lower on VPB treated with ALF (by 1.6 and 1.4 log CFU/cm^2) than those treated with LA both pre-and post-inoculation after 29 days of storage at 12°C. Total bacterial populations showed no differences due to treatment (Table 5.6). This could suggest that either: (1) the inhibition of growth shown on the selective medium was a result of stress and not actual destruction of the pathogens due to treatment, or (2) due to the fact that the pathogenic bacteria were not viable, spoilage organisms took advantages of the favorable environmental conditions, and were allowed to grow quickly to high populations. For *S.* Typhimurium, LA application both pre- and post inoculation was the most ($P < 0.05$) effective treatment in initially reducing and inhibiting the growth of the pathogen on VPB product. Vacuum packed beef treated with water, LF, and ALF pre- and post-inoculation resulted in *S.*

Typhimurium populations of 6.0 log CFU/cm^2 or greater after 4 days of storage. No treatment effects were observed on non-selective media for *S.* Typhimurium or *L. monocytogenes* after 33 days of storage. Results from the VPB inoculated with *L. monocytogenes* showed no significant differences in growth inhibition due to treatment; however, LA and ALF resulted in the lowest numerical *L. monocytogenes* populations. *Listeria monocytogenes* populations grew to exceed 6 log CFU/cm^2 after 4 days of vacuum packaged storage at 12°C on VPB product treated with water or LF both pre- or post-inoculation.

On beef bologna, ALF was the most ($P < 0.05$) effective treatment, pre- and post-inoculation, in inhibiting the growth of *L. monocytogenes* and total bacterial populations (Tables 5.7 and 5.8, respectively) after 33 days of storage at 10°C; however, pathogen reductions were not evident until after 8 days of vacuum packaged storage at 10°C. In addition, although the main effect of treatment was not significant for *E. coli* O157:H7 on ready-to-eat beef product, data trends from both selective and nonselective agar plates suggested that ALF was more effective than other treatments in inhibiting growth pre- and post-inoculation after 19 and 33 days of storage. For bologna inoculated after (post) treatment, water appeared to be slightly more effective than LF and LA in inhibiting growth of *E. coli* O157:H7. Overall, *E. coli* O157:H7 populations remained relatively low in comparison to other pathogens after 33 days of storage, but this was anticipated due to low storage temperatures (10°C) applied during this phase of the study. For *S.* Typhimurium, no significant effect of treatment was observed on selective or non-selective growth media; however, application of lactic acid and lactoferrin resulted in the

greatest numerical reduction of *S.* Typhimurium immediately after application, and LA was more effective than ALF, LF and water, 8, 19, and 33 days after inoculation.

Possible explanations for the enhanced effectiveness of the sequential application of ALF and LA could be described by the Bardic (2003) report; those authors suggested that activated lactoferrin seeks out pathogenic bacteria that are already attached to the meat surface and binds with the pathogens to detach them. Once the activated lactoferrin has been applied, it is followed by a final rinse, allowing the detached bacteria to be safely washed away. Petrak (2002) reported that lactoferrin's job is to prevent the attachment of pathogens and to detach pathogens by eliminating the structure that binds pathogens to the meat process; as part of that process, lactoferrin prevents the growth of iron-dependent bacteria—specifically, by eliminating the hair-like structures called "fimbria" around bacteria that cause them to adhere to tissue surfaces. In its non-immobilized form, lactoferricin is a closed structure that is iron-saturated and less active; in its immobilized (activated) form, lactoferrin is an open structure that is iron-free and has maximum antimicrobial activity (Petrak, 2002).

Although the effectiveness of activated lactoferrin shows promise as an inhibitor of growth for *L. monocytogenes* in beef bologna, additional modeling studies should be conducted to determine if it offers a synergistic effect in reducing pathogen populations on bologna containing ingredients such as sodium lactate, or nisin. Also, further studies should be conducted to determine the effectiveness of ALF as an ingredient in bologna. In addition, industrial studies should be conducted to validate the effectiveness of the sequential application of ALF and LA in enhancing the microbiological quality of carcasses following treatment application.

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Table 5.1. Mean populations [log CFU/cm^2 (standard error), n=6] of *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes,* on selective media, recovered from the surface of beef adipose tissue inoculated before treatment with water, activated lactoferrin (ALF), lactoferrin (LF), 2% lactic acid (LA) or sequential combinations of LA and ALF, by immersion in treatment solution for two minutes, followed by aerobic storage at 12°C. Inoculated and untreated samples served as control.

Pathogen	Days of storage	Control	Water	ALF	LF	LA	ALF then LA	LA then ALF
E. coli O157:H7	0	3.5^{az} (.43)	3.0^{abz} (.21)	2.7^{bz} (.32)	3.3^{abz} (.14)	0.9^{cz} (.32)	0.5^{cz} (.22)	1.1^{cz} (.38)
	1	4.9^{azy} (.19)	3.8^{bzy} (.37)	3.7^{bz} (.25)	4.5^{az} (.09)	2.0^{cz} (.09)	0.6^{cz} (.21)	2.0^{cz} (.16)
	2	5.2^{ay} (.31)	5.0^{ay} (.24)	4.7^{az} (.11)	4.6^{az} (.19)	1.5^{bz} (.15)	0.2^{cz} (.14)	2.2^{bz} (.27)
Salmonella Typhimurium	0	3.7^{abz} (.29)	4.2^{az} (.18)	2.6^{bcz} (.22)	3.0^{abcz} (.41)	1.1^{dz} (.38)	1.0^{dz} (.44)	2.0^{cdz} (.52)
	1	5.1^{azy} (.31)	4.7^{az} (.36)	4.9^{ay} (.38)	4.2^{azy} (.17)	1.5^{bz} (.20)	0.0^{cz} (.00)	1.9^{bz} (.15)
	2	6.5^{ay} (.20)	5.8^{az} (.19)	5.9^{ay} (.20)	5.5^{ay} (.31)	1.8^{bcz} (.45)	0.7^{cz} (.26)	2.2^{bz} (.21)
Listeria monocytogenes	0	4.7^{az} (.18)	3.7^{bz} (.18)	3.5^{bz} (.19)	3.8^{bz} (.16)	2.1^{cz} (.23)	1.8^{cz} (.05)	2.3^{cz} (.19)
	1	5.8^{azy} (.05)	5.6^{aby} (.08)	5.4^{aby} (.08)	5.1^{by} (.09)	1.7^{dz} (.27)	1.4^{dz} (.20)	2.6^{cz} (.16)
	2	6.4^{aby} (.05)	6.8^{ay} (.16)	6.5^{aby} (.15)	6.0^{by} (.14)	2.0^{dz} (.27)	1.2^{ez} (.37)	3.1^{cz} (.16)

$^{a, b, c, d}$ Means within rows with a common superscript are not significantly different ($P > 0.05$).
$^{z, y}$ Means within columns with a common superscript are not significantly different ($P > 0.05$).

Table 5.2. Mean total aerobic plate counts [log CFU/cm^2 (standard error), n=6] recovered from the surface of beef adipose tissue inoculated before treatment with water, activated lactoferrin (ALF), lactoferrin (LF), 2% lactic acid (LA) or sequential combinations of LA and ALF, by immersion in treatment solution for two minutes, followed by aerobic storage at 12°C. Inoculated and untreated samples served as control.

Pathogen	Days of storage	Control	Water	ALF	LF	LA	ALF then LA	LA then ALF
E. coli O157:H7	0	4.3^{az} (.23)	4.4^{az} (.10)	3.7^{bcz} (.30)	3.5^{cdz} (.20)	3.2^{cdz} (.20)	3.0^{dz} (.15)	3.0^{dz} (.28)
	1	5.1^{az} (.27)	4.9^{az} (.22)	5.2^{az} (.17)	5.1^{az} (.11)	3.5^{bz} (.58)	2.2^{cz} (.18)	2.3^{cz} (.43)
	2	5.6^{az} (.35)	6.0^{az} (.16)	5.8^{az} (.16)	4.9^{bz} (.23)	2.8^{cz} (.09)	1.7^{dz} (.19)	3.3^{cz} (.21)
Salmonella Typhimurium	0	4.3^{az} (.26)	3.8^{az} (.30)	4.2^{bcz} (.03)	4.3^{abcz} (.05)	2.5^{dz} (.26)	2.0^{dz} (.34)	2.7^{cdz} (.10)
	1	5.5^{az} (.10)	5.4^{az} (.33)	5.1^{azy} (.14)	5.4^{az} (.32)	1.9^{bz} (.20)	1.6^{cz} (.12)	2.9^{bz} (.22)
	2	6.8^{az} (.13)	6.5^{az} (.13)	6.4^{ay} (.15)	5.9^{az} (.36)	3.3^{bcz} (.27)	3.2^{cz} (.18)	2.9^{bz} (.43)
Listeria monocytogenes	0	4.6^{az} (.10)	3.6^{bz} (.23)	3.7^{bz} (.31)	3.8^{bz} (.10)	2.7^{cz} (.27)	2.8^{cz} (.23)	2.9^{cz} (.21)
	1	5.9^{az} (.06)	5.6^{aby} (.11)	5.4^{aby} (.22)	5.1^{bz} (.09)	2.0^{dz} (.17)	2.1^{dz} (.14)	2.9^{cz} (.31)
	2	6.3^{az} (.09)	6.9^{ay} (.19)	6.7^{ay} (.18)	5.9^{bz} (.19)	1.9^{dz} (.25)	2.4^{dz} (.29)	3.7^{cz} (.19)

$^{a, b, c, d}$ Means within rows with a common superscript are not significantly different (P > 0.05).

$^{z, y}$ Means within columns with a common superscript are not significantly different (P > 0.05).

Table 5.3. Mean populations [log CFU/cm^2 (standard error), n=6] of *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes,* on selective media, recovered from the surface of lean tissue pieces inoculated before (Pre) or after (Post) treatment with water, activated lactoferrin (ALF), lactoferrin (LF) or 2% lactic acid (LA) by immersion in treatment solution for two minutes, followed by simulated retail storage in air-permeable packages and at 12°C. Inoculated and untreated samples served as control.

Pathogen	Days of storage	Control	Pre Water	Pre ALF	Pre LF	Pre LA	Post Water	Post ALF	Post LF	Post LA
E. coli O157:H7	0	3.4^{az}(.06)	3.1^{az} (.13)	2.9^{az} (.17)	3.0^{az} (.14)	2.7^{az} (.10)	3.5^{az} (.06)	3.4^{az} (.05)	3.3^{az} (.02)	3.3^{az} (.23)
	1	3.2^{az} (.07)	3.1^{az} (.12)	3.0^{az} (.11)	3.0^{az} (.07)	2.9^{az} (.15)	3.5^{az} (.07)	3.6^{az} (.14)	3.4^{az} (.06)	3.1^{az} (.09)
	3	3.9^{az}(.05)	3.8^{az} (.21)	3.8^{az} (.09)	4.2^{az} (.13)	2.2^{az} (.10)	4.1^{az} (.14)	4.0^{az} (.09)	4.5^{az} (.13)	2.7^{az} (.12)
	5	4.5^{abz} (.20)	5.0^{abzy} (.34)	4.7^{abzy} (.15)	4.9^{abzy} (.28)	2.2^{bz} (.36)	5.2^{abzy} (.15)	5.0^{abzy} (.19)	5.3^{azy}(.03)	2.4^{abz} (.32)
	7	5.5^{abz} (.41)	5.7^{aby} (.49)	6.1^{ay} (.53)	6.0^{ay} (.26)	2.3^{cz} (.38)	6.3^{ay} (.28)	6.0^{ay} (.51)	6.8^{ay} (.28)	2.8^{bcz} (.03)
Salmonella Typhimurium	0	3.4^{az} (.05)	2.9^{az} (.11)	3.0^{az} (.04)	3.1^{az} (.13)	2.6^{az} (.08)	3.3^{az} (.11)	3.3^{az} (.06)	3.2^{az} (.11)	3.1^{az} (.17)
	1	3.2^{az} (.14)	2.9^{az} (.04)	3.1^{az} (.07)	3.0^{az} (.09)	2.8^{az} (.07)	3.3^{az} (.08)	3.3^{az} (.05)	3.4^{az} (.06)	2.8^{az} (.17)
	3	4.1^{az} (.17)	3.5^{az} (.07)	3.9^{az} (.05)	3.6^{az} (.17)	2.2^{az} (.11)	3.9^{az}(.11)	4.1^{az} (.07)	4.2^{az} (.09)	2.6^{az} (.13)
	5	4.5^{azy} (.15)	4.5^{azy} (.38)	4.4^{azy} (.16)	4.2^{az} (.07)	2.3^{az} (.25)	5.0^{azy} (.20)	5.1^{ay} (.06)	5.2^{azy} (.32)	3.4^{az} (.39)
	7	5.0^{ay} (.70)	5.0^{ay} (.76)	5.1^{ay} (.37)	4.6^{az} (.09)	2.3^{az} (.25)	5.8^{ay} (.47)	5.3^{ay} (.58)	5.5^{ay} (.80)	2.3^{az} (.17)
Listeria monocytogenes	0	3.5^{az} (.09)	3.2^{az} (.14)	2.8^{az} (.10)	3.1^{az} (.09)	2.9^{az} (.09)	3.5^{az} (.12)	3.6^{az} (.12)	3.5^{az} (.10)	3.2^{az} (.16)
	1	3.3^{az} (.04)	3.3^{az} (.04)	2.8^{az} (.08)	3.2^{az} (.06)	2.4^{az}(.01)	3.7^{az} (.09)	3.7^{az} (.07)	3.2^{az} (.03)	2.5^{az} (.05)
	3	4.0^{az} (.06)	3.9^{az} (.14)	3.9^{az} (.13)	3.8^{az} (.10)	2.3^{az} (.10)	3.9^{az} (.12)	4.2^{az} (.18)	4.5^{az} (.09)	2.6^{az} (.15)
	5	4.9^{az} (.24)	4.5^{az} (.27)	4.7^{azy} (.33)	4.6^{az} (.19)	2.2^{az} (.32)	5.0^{az} (.29)	4.7^{azy} (.30)	4.6^{az} (.51)	2.8^{az} (.09)
	7	4.9^{az} (.50)	5.3^{az} (.38)	5.4^{ay} (.49)	5.0^{az} (.08)	2.3^{az} (.61)	5.2^{az} (.16)	5.6^{ay} (.72)	6.0^{ay} (.63)	2.3^{az} (.07)

$^{a, b, c}$ Means within rows with a common superscript are not significantly different (P > 0.05).

$^{z, y, x}$ Means within columns with a common superscript are not significantly different (P > 0.05).

Table 5.4 Mean total aerobic plate counts [log CFU/cm^2 (standard error), n=6] recovered from the surface of lean tissue pieces inoculated before (Pre) or after (Post) treatment with water, activated lactoferrin (ALF), lactoferrin (LF) or 2% lactic acid (LA) by immersion in treatment solution for two minutes, followed by simulated retail storage in air-permeable packages and at 12°C. Inoculated and untreated samples served as control.

Pathogen	Days of storage	Control	Pre Water	Pre ALF	Pre LF	Pre LA	Post Water	Post ALF	Post LF	Post LA
E. coli O157:H7	0	3.5^{az} (.12)	3.3^{az} (.09)	3.1^{az} (.14)	3.1^{az} (.17)	3.2^{az} (.19)	3.9^{az} (.04)	3.8^{az} (.04)	3.6^{az} (.12)	3.8^{az} (.06)
	1	3.5^{az} (.10)	3.5^{az} (.09)	3.2^{az} (.23)	3.6^{az} (.19)	3.1^{az} (.10)	3.8^{az} (.10)	3.8^{az} (.14)	3.8^{az} (.08)	3.4^{az} (.07)
	3	5.5^{ay} (.13)	5.4^{ay} (.06)	5.5^{ay} (.13)	5.8^{ay} (.06)	3.5^{az} (.10)	5.5^{ay} (.07)	5.6^{ay} (.08)	5.5^{ay} (.06)	3.6^{az} (.22)
	5	6.4^{axy} (.09)	6.5^{axy} (.14)	6.5^{axy} (.23)	6.5^{axy} (.19)	4.8^{ay} (.07)	6.7^{axy} (.08)	6.2^{axy} (.31)	6.7^{ay} (.19)	5.0^{ay} (.10)
	7	7.9^{ax} (.41)	7.8^{ax} (.43)	7.6^{ax} (.39)	7.9^{ax} (.52)	6.0^{ay} (.14)	7.3^{ay} (.12)	7.8^{ax} (.46)	8.2^{av} (.27)	5.9^{ay} (.08)
Salmonella Typhimurium	0	3.6^{az} (.13)	3.1^{az} (.08)	3.1^{az} (.09)	3.4^{az} (.10)	3.5^{az} (.09)	3.9^{az} (.11)	3.7^{az} (.03)	3.8^{az} (.07)	3.6^{az} (.09)
	1	3.5^{az} (.05)	3.2^{az} (.15)	3.3^{az} (.16)	3.5^{az} (.08)	3.3^{az} (.07)	3.7^{az} (.06)	3.9^{az} (.13)	3.9^{az} (.11)	3.2^{az} (.12)
	3	5.6^{ay} (.14)	5.3^{ay} (.14)	5.6^{ay} (.12)	5.2^{ay} (.18)	3.5^{az} (.33)	5.6^{ay} (.15)	5.6^{ay} (.08)	5.5^{ay} (.10)	3.6^{az} (.15)
	5	6.9^{ax} (.14)	7.0^{ax} (.25)	6.9^{axy} (.10)	6.5^{ay} (.38)	5.0^{ay} (.06)	7.0^{ax} (.05)	6.8^{axy}(.12)	6.2^{ay} (.44)	4.8^{azy} (.23)
	7	7.8^{ax} (.48)	8.0^{ax} (.48)	8.1^{ax} (.47)	8.3^{ax} (.50)	6.5^{ax} (.10)	8.3^{av} (.36)	8.0^{ax} (.52)	8.0^{ax} (.63)	6.4^{ay} (.23)
Listeria monocytogenes	0	3.5^{az} (.07)	3.2^{az} (.08)	3.1^{az} (.07)	3.0^{az} (.10)	3.0^{az} (.04)	3.6^{az} (.07)	3.7^{az} (.10)	3.4^{az} (.15)	3.2^{az} (.11)
	1	3.7^{az} (.09)	3.4^{az} (.13)	3.2^{az} (.13)	3.3^{az} (.11)	2.5^{az} (.10)	3.8^{az} (.04)	3.7^{az} (.24)	3.5^{az} (.14)	3.1^{az} (.33)
	3	5.6^{ay} (.16)	5.7^{ay} (.11)	5.6^{ay} (.08)	5.4^{ay} (.06)	3.5^{az} (.25)	5.6^{ay} (.15)	5.6^{ay} (.07)	5.6^{ay} (.07)	3.8^{az} (.16)
	5	6.9^{axy} (.08)	6.9^{axy} (.23)	6.4^{ay} (.21)	7.4^{ax} (.20)	4.8^{azy} (.24)	7.2^{ax} (.07)	6.5^{axy} (.27)	7.4^{ax} (.25)	5.0^{ay} (.15)
	7	7.9^{ax} (.46)	8.0^{ax} (.37)	7.2^{ay} (.63)	8.3^{ax} (.49)	6.1^{ay} (.11)	8.3^{ax} (.28)	7.8^{ax} (.53)	8.5^{ax} (.41)	6.1^{ay} (.15)

[a] Means within rows with a common superscript are not significantly different (P > 0.05).
[z, y, x] Means within columns with a common superscript are not significantly different (P > 0.05).

Table 5.5. Mean populations [log CFU/cm^2 (standard error), n=6] of *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes,* on selective media, recovered from the surface of vacuum packaged beef cuts inoculated before (Pre) or after (Post) treatment with water, activated lactoferrin (ALF), lactoferrin (LF) or 2% lactic acid (LA) by immersion in treatment solution for two minutes, followed by vacuum packaging and storage at 12°C. Inoculated and untreated samples served as control.

Pathogen	Days of storage	Control	Pre Water	Pre ALF	Pre LF	Pre LA	Post Water	Post ALF	Post LF	Post LA
E. coli O157:H7	0	3.0^{az} (.18)	3.0^{az} (.25)	2.7^{ay} (.11)	2.9^{az} (.28)	1.6^{az} (.25)	3.4^{az} (.10)	3.1^{ay} (.18)	3.2^{az} (.11)	3.7^{az} (.45)
	4	6.2^{ay} (.06)	6.2^{ay} (.17)	2.4^{by} (.04)	6.3^{ay} (.16)	2.2^{bz} (.06)	6.4^{ay} (.07)	1.5^{bz} (.14)	6.2^{ay} (.09)	2.8^{abz} (.04)
	8	6.6^{ay} (.14)	6.5^{ay} (.28)	2.8^{by} (.14)	6.9^{ay} (.30)	1.9^{bz} (.23)	6.6^{ay} (.25)	2.8^{by} (.29)	6.7^{ay} (.12)	2.8^{bz} (.22)
	15	6.6^{ay} (.25)	6.6^{ay} (.27)	2.1^{bzy} (.11)	6.8^{ay} (.33)	1.7^{bz} (.14)	7.2^{ay} (.16)	2.3^{bzy} (.36)	7.1^{ay} (.22)	2.8^{bz} (.19)
	29	5.7^{abcy} (.11)	6.1^{aby} (.10)	1.1^{dz} (.07)	6.4^{ay} (.43)	2.7^{bcdz} (.30)	6.3^{ay} (.20)	1.0^{dz} (.06)	6.4^{ay} (.18)	2.4^{cdz} (.52)
Salmonella Typhimurium	0	3.4^{az} (.08)	3.2^{az} (.12)	2.7^{az} (.03)	2.8^{az} (.11)	1.0^{az} (.07)	3.7^{az} (.11)	3.2^{az} (.08)	3.3^{az} (.04)	1.6^{az} (.46)
	4	6.3^{ay} (.10)	6.2^{ay} (.17)	6.7^{ay} (.26)	6.4^{ay} (.28)	1.5^{bz}(.30)	6.1^{ay} (.06)	6.0^{ay} (.04)	6.5^{ay} (.23)	1.9^{bz} (.12)
	8	6.5^{ay} (.11)	7.2^{ay} (.15)	7.0^{ay} (.15)	7.2^{ay} (.29)	2.0^{bz} (.23)	7.1^{ay} (.07)	7.0^{ay} (.13)	6.9^{ay} (.22)	1.9^{bz} (.25)
	15	6.5^{ay} (.11)	7.0^{ay} (.06)	6.8^{ay} (.13)	6.4^{ay} (.47)	2.4^{bz} (.13)	6.9^{ay} (.19)	7.1^{ay} (.27)	6.4^{ay} (.20)	4.1^{aby} (.18)
	29	5.5^{ay} (.31)	6.4^{ay} (.16)	6.1^{ay} (.33)	6.3^{ay} (.62)	2.3^{bz} (.32)	6.3^{ay} (.21)	6.4^{ay} (.14)	6.0^{ay}(.44)	3.1^{aby} (.22)
Listeria monocytogenes	0	3.5^{az} (.05)	3.4^{az} (.05)	3.1^{az} (.05)	3.2^{az} (.10)	2.4^{az} (.07)	3.5^{az} (.04)	3.5^{az} (.06)	3.5^{az} (.05)	3.0^{azy} (.07)
	4	5.8^{ay} (.16)	6.6^{ay} (.10)	2.6^{bz} (.20)	6.1^{ay} (.24)	1.6^{bz} (.22)	6.9^{ay} (.18)	2.8^{bz} (.18)	6.4^{ay} (.06)	2.2^{bz} (.26)
	8	7.2^{ax} (.39)	6.8^{ay} (.06)	2.4^{bz} (.33)	7.0^{ay} (.24)	1.9^{bz} (.18)	7.0^{ay} (.09)	2.7^{bz} (.08)	6.9^{ay} (.28)	2.0^{bz} (.14)
	15	7.2^{ax} (.24)	6.7^{ay} (.12)	2.6^{bz} (.15)	7.1^{ay} (.35)	1.5^{bz} (.20)	7.3^{ay} (.09)	2.8^{bz} (.18)	7.2^{ay} (.34)	3.6^{by} (.71)
	29	7.2^{ax} (.27)	6.8^{ay} (.46)	2.6^{bz} (.19)	7.2^{ay} (.30)	1.5^{bz} (.24)	7.2^{ay} (.21)	3.9^{bz} (.58)	7.1^{ay} (.21)	1.6^{bz} (.45)

$^{a, b, c, d}$ Means within rows with a common superscript are not significantly different (P > 0.05).
$^{z, y, x, v}$ Means within columns with a common superscript are not significantly different (P > 0.05).

Table 5.6. Mean total aerobic plate counts [log CFU/cm^2 (standard error), n=6] recovered from the surface of vacuum packaged beef cuts inoculated before (Pre) or after (Post) treatment with water, activated lactoferrin (ALF), lactoferrin (LF) or 2% lactic acid (LA) by immersion in treatment solution for two minutes, followed by vacuum packaging and storage at 12°C. Inoculated and untreated samples served as controls.

Pathogen	Days of storage	Control	Pre Water	Pre ALF	Pre LF	Pre LA	Post Water	Post ALF	Post LF	Post LA
E. coli O157:H7	0	3.7^{az} (.04)	3.6^{az} (.05)	3.4^{az} (.05)	3.4^{az} (.06)	2.9^{az} (.09)	3.8^{az} (.05)	3.6^{az} (.03)	3.5^{az} (.08)	3.5^{az} (.05)
	4	6.5^{ay} (.10)	6.7^{ay} (.17)	5.7^{ay} (.29)	6.8^{ay} (.09)	4.4^{azy} (.63)	6.4^{ay} (.17)	5.8^{ay} (.20)	6.5^{ay} (.09)	3.3^{az} (.12)
	8	7.4^{ay} (.11)	7.1^{ay} (.08)	7.1^{ay} (.40)	7.4^{ay} (.07)	4.8^{azy} (.62)	7.2^{ay} (.15)	6.9^{ay} (.26)	7.4^{ay} (.09)	4.4^{azy} (.37)
	15	7.3^{ay} (.17)	7.4^{ay} (.09)	7.4^{ay} (.08)	7.5^{ay} (.10)	4.9^{azy} (.73)	7.7^{ay} (.09)	7.6^{ay} (.10)	7.4^{ay} (.20)	5.0^{ay} (.53)
	29	7.2^{ay} (.10)	7.2^{ay} (.12)	7.3^{ay} (.09)	7.3^{ay} (.07)	6.1^{ay} (.28)	7.4^{ay} (.22)	7.5^{ay} (.05)	7.5^{ay} (.16)	6.4^{ay} (.28)
Salmonella Typhimurium	0	3.8^{az} (.04)	3.6^{az} (.05)	3.4^{az} (.08)	3.5^{az} (.04)	2.2^{az} (.22)	3.5^{az} (.12)	3.7^{az} (.03)	3.7^{az} (.05)	3.5^{az} (.04)
	4	6.5^{ay} (.22)	6.4^{ay} (.15)	6.7^{ay} (.21)	6.7^{ay} (.14)	2.7^{bz} (.16)	6.4^{ay} (.16)	6.1^{ay} (.20)	6.7^{ay} (.23)	3.3^{bz} (.19)
	8	7.3^{ay} (.10)	7.6^{ay} (.12)	7.6^{ay} (.05)	7.8^{ay} (.07)	5.3^{aby} (.12)	7.5^{ay} (.10)	7.4^{ay} (.09)	7.5^{ay} (.07)	4.2^{bz} (.44)
	15	7.4^{ay} (.11)	7.6^{ay} (.06)	7.3^{ay} (.09)	7.2^{ay} (.09)	5.4^{ay} (.33)	7.7^{ay} (.10)	7.5^{ay} (.04)	7.5^{ay} (.10)	6.4^{ay} (.13)
	29	6.9^{ay} (.15)	7.6^{ay} (.17)	7.1^{ay} (.05)	7.3^{ay} (.23)	6.8^{ay} (.03)	7.4^{ay} (.13)	7.5^{ay} (.15)	7.4^{ay} (.30)	5.8^{ay} (.28)
Listeria monocytogenes	0	3.6^{az} (.04)	3.5^{az} (.03)	3.3^{az} (.07)	3.2^{az} (.03)	2.9^{az} (.03)	3.7^{az} (.03)	3.4^{az} (.06)	3.4^{az} (.05)	2.7^{az} (.25)
	4	6.8^{ay} (.15)	7.0^{ay} (.12)	5.5^{ay} (.47)	6.5^{ay} (.32)	2.9^{az} (.43)	7.0^{ay} (.22)	5.2^{azy} (.14)	6.7^{ay} (.17)	2.9^{az} (.50)
	8	7.6^{ay} (.12)	7.7^{ay} (.31)	6.3^{ay} (.42)	7.5^{ay} (.10)	3.9^{az} (.68)	7.3^{ay} (.09)	6.5^{ay} (.29)	7.2^{ay} (.09)	4.1^{ay} (.72)
	15	7.6^{ay} (.11)	7.3^{ay} (.06)	6.6^{ay} (.38)	7.5^{ay} (.06)	4.5^{azy} (.22)	7.6^{ay} (.10)	7.1^{ay} (.32)	7.8^{ay} (.13)	6.3^{ax} (.34)
	29	7.4^{ay} (.17)	7.4^{ay} (.19)	7.2^{ay} (.19)	7.6^{ay} (.07)	5.6^{ay} (.38)	7.5^{ay} (.14)	7.1^{ay} (.21)	7.5^{ay} (.11)	6.2^{ax} (.42)

[a] Means within rows with a common superscript are not significantly different (P > 0.05).

[z, y, x] Means within columns with a common superscript are not significantly different (P > 0.05).

Table 5.7. Mean populations [log CFU/cm^2 (standard error), n=6] of *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes,* on selective media, recovered from the surface of beef bologna slices inoculated before (Pre) or after (Post) treatment with water, activated lactoferrin (ALF), lactoferrin (LF) or 2% lactic acid (LA) by immersion in treatment solution for two minutes, followed by vacuum packaging and storage at 10°C. Inoculated and untreated samples served as control.

Pathogen	Days of storage	Control	Pre Water	Pre ALF	Pre LF	Pre LA	Post Water	Post ALF	Post LF	Post LA
E. coli O157:H7	0	3.9^{az} (.07)	3.2^{az} (.04)	3.0^{ay} (.07)	2.0^{az} (.13)	2.9^{az} (.52)	3.9^{ay} (.06)	4.0^{ay} (.03)	4.0^{az} (.05)	4.1^{az} (.05)
	8	2.9^{az} (.16)	2.1^{az} (.22)	0.1^{az} (.28)	2.3^{az} (.06)	2.8^{az} (.24)	2.1^{az} (.16)	2.5^{az} (.17)	2.6^{az} (.24)	3.5^{az} (.10)
	19	2.0^{az} (.22)	2.2^{az} (.17)	0.4^{az} (.27)	2.4^{az} (.48)	2.1^{az} (.25)	2.5z^{a} (.18)	1.9^{az} (.13)	2.8^{az} (.22)	3.4^{az} (.12)
	33	1.7^{az} (.30)	2.7^{az} (.11)	0.3^{az} (.24)	2.9^{az} (.34)	2.7^{az} (.48)	2.3^{az} (.37)	1.0^{az}(.10)	3.2^{az}(.33)	2.9^{az} (.18)
Salmonella Typhimurium	0	4.0^{ay} (.02)	3.3^{az} (.02)	3.1^{az} (.04)	2.0^{az} (.08)	2.0^{az} (.51)	4.0^{az} (.02)	4.0^{az} (.06)	3.9^{az} (.05)	3.9^{az} (.04)
	8	2.9^{azy} (.25)	4.6^{azy} (.37)	2.7^{az} (.37)	2.7^{az} (.06)	1.9^{az} (.16)	3.8^{az} (.40)	3.8^{az} (.42)	4.6^{az} (.28)	4.0^{az} (.30)
	19	3.0^{azy} (.07)	4.5^{azy} (.07)	3.8^{az} (.45)	4.0^{azy} (.40)	3.4^{ay} (.51)	4.2^{az} (.57)	3.9^{az} (.49)	4.0^{az} (.50)	4.2^{az} (.70)
	33	2.6^{az} (.35)	5.7^{ay} (.16)	3.7^{az} (.53)	5.6^{ay} (.52)	3.0^{azy} (.26)	6.3^{ay} (.31)	5.0^{az} (.58)	6.6^{ay} (.14)	4.5^{az} (.27)
Listeria monocytogenes	0	3.3 ^{az} (.02)	2.6^{az} (.04)	2.5^{az} (.03)	2.6^{az} (.05)	2.6^{az} (.07)	3.3^{az} (.02)	3.3^{az} (.04)	3.3^{az} (.02)	3.2^{az} (.02)
	8	6.5^{aby} (.30)	7.2^{ay} (.20)	2.2^{cdz} (.03)	7.0^{aby} (.10)	1.9^{dz} (.32)	8.1^{ax} (.06)	2.6^{bcdz} (.03)	7.7^{ax} (.20)	4.2^{abzy} (.40)
	19	8.5^{ax} (.07)	8.4^{ay} (.13)	2.0^{bz} (.21)	8.3^{ay} (.33)	4.7^{aby} (.04)	6.6^{aby} (.15)	2.2^{bz} (.09)	5.7^{aby}(.90)	5.5^{aby} (.70)
	33	8.1^{ax} (.11)	8.0^{ay} (.07)	1.7^{bz}(.08)	8.3^{ay} (.26)	6.7^{ax} (.06)	8.1^{ax} (.08)	1.9^{bz} (.05)	8.2^{ax} (.04)	6.9^{ax} (.26)

$^{a, b, c, d}$ Means within rows with a common superscript are not significantly different (P > 0.05).

$^{z, y, x}$ Means within columns with a common superscript are not significantly different (P > 0.05).

Table 5.8. Mean total aerobic plate counts [log CFU/cm^2 (standard error), n=6] recovered from the surface of beef bologna slices inoculated before (Pre) or after (Post) treatment with water, activated lactoferrin (ALF), lactoferrin (LF) or 2% lactic acid (LA) by immersion in treatment solution for two minutes, followed by vacuum packaging and storage at 10°C. Inoculated and untreated samples served as control.

Pathogen	Days of storage	Control	Pre Water	Pre ALF	Pre LF	Pre LA	Post Water	Post ALF	Post LF	Post LA
E. coli O157:H7	0	4.2^{az} (.02)	3.4^{az} (.08)	3.4^{ay} (.09)	3.4^{az} (.04)	3.2^{az} (.08)	4.2^{azy} (.02)	4.2^{az} (.02)	4.0^{az} (.06)	4.1^{az} (.08)
	8	3.5^{az} (.09)	2.8^{az} (.10)	2.6^{azy} (.30)	3.0^{az} (.09)	3.1^{az} (.06)	3.3^{az} (.10)	2.8^{az}(.20)	3.8^{az} (.12)	3.9^{az} (.07)
	19	3.5^{az} (.10)	3.9^{az} (.28)	1.8^{az} (.17)	3.2^{az} (.46)	3.1^{az} (.20)	3.8^{azy} (.38)	2.3^{az} (.11)	4.2^{az} (.45)	3.7^{az} (.02)
	33	4.7^{az} (.25)	3.8^{az} (.67)	1.7^{az} (.08)	5.0^{ay}(.80)	4.5^{az} (.33)	4.9^{ay} (.35)	2.7^{az} (.47)	4.9^{az} (.75)	4.5^{az} (.33)
Salmonella Typhimurium	0	4.2^{az} (.02)	3.5^{az} (.05)	3.4^{az} (.08)	3.1^{az} (.12)	2.9^{az} (.07)	4.2^{az} (.03)	4.2^{az} (.05)	4.1^{az} (.02)	4.2^{az} (.04)
	8	4.3^{az} (.21)	5.0^{azy} (.13)	3.6^{az} (.43)	3.9^{az}(.21)	2.6^{az} (.10)	5.0^{azy} (.29)	4.5^{az} (.42)	5.3^{azy} (.18)	4.8^{az} (.34)
	19	4.2^{az} (.44)	5.5^{ay} (.67)	4.3^{azy} (.64)	4.8^{azy}(.56)	4.0^{az} (.49)	5.8^{ay} (.48)	4.7^{az} (.20)	5.9^{ay} (.54)	3.9^{az} (.23)
	33	4.8^{az} (.28)	6.2^{ay} (.22)	5.1^{ay} (.42)	5.7^{ay} (.34)	4.0^{az} (.53)	6.7^{ay} (.25)	5.7^{az} (.60)	6.9^{ay}(.09)	5.3^{az} (.07)
Listeria monocytogenes	0	3.4^{az} (.02)	2.7^{az} (.02)	2.7^{az} (.04)	2.5^{az} (.08)	2.7^{az} (.03)	3.3^{az} (.04)	3.3^{az} (.05)	3.4^{az} (.07)	3.3^{aa} (.06)
	8	6.5^{aby} (.25)	7.1^{aby} (.18)	2.5^{dz} (.06)	7.1^{aby} (.30)	2.0^{dz} (.07)	8.1^{ay} (.03)	3.0^{cdz} (.06)	7.8^{aby}(.22)	4.2^{bcda} (.42)
	19	8.4^{ax} (.09)	7.4^{ay} (.96)	2.5^{bz} (.14)	8.3^{ay} (.05)	4.9^{aby} (.40)	8.5^{ay} (.09)	2.7^{bz} (.10)	8.2^{ay} (.05)	6.0^{aby} (.47)
	33	8.3^{ax} (.04)	8.3^{ay} (.13)	2.5^{bz} (.17)	8.4^{ay} (.05)	6.7^{ax} (.25)	8.1^{ay} (.10)	2.8^{bz} (.23)	8.1^{ay} (.04)	6.8^{ay} (.33)

$^{a, b, c, d}$ Means within rows with a common superscript are not significantly different (P > 0.05).

$^{z, y, x,}$ Means within columns (among the same pathogen) with a common superscript are not significantly different (P > 0.05).

CHAPTER VI

DISSERTATION SUMMARY

Results of research included in this dissertation regarding prevalence of *E. coli* O157:H7 in fecal and hide samples from cattle in feedlots and on their carcasses at slaughter plants were used to obtain a better understanding of the relationship that exists between presence of *E. coli* O157:H7 in the feedlot and subsequently on resulting carcasses. Results showed that prevalence of the pathogen in pens (lots) ranged from 0 to 78% positive in feedlot pen floor fecal samples, and from 0 to 5% on carcasses from those lots, immediately after final intervention; however, too few positive samples were isolated from carcasses to develop a strong relationship between *E. coli* O157:H7-positive fecal samples and positive carcass samples. Future research should be conducted to determine the populations of pathogens and not just the prevalence of *E. coli* O157:H7 on cattle and subsequent carcasses as they enter the slaughter and processing environment. Such studies could offer more insight into the relationship that might exist between *E. coli* O157:H7-positive samples on live cattle and on their subsequent carcasses. In a second study, data showed that preharvest pathogen mitigation strategies (i.e., *Lactobacillus acidophilus*-probiotic, neomycin sulfate-antibiotic and a prototype *E. coli* O157:H7 vaccine and combination of treatments) could aid in minimizing prevalence of *E. coli* O157 in fecal and

hide samples on/in cattle, as samples collected from treated cattle resulted in fewer *E. coli* O157:H7-positive fecal and hide samples when compared to cattle that did not receive treatments. Additionally, *E. coli* O157 isolates recovered from feces of cattle which received neomycin sulfate did not result in an enhanced antimicrobial resistance. Future studies could be targeted to identify the effectiveness of preharvest treatments during earlier stages of production and the resulting impact of the prevalence and populations of *E. coli* O157 in the environment. In addition, preharvest intervention strategies studies should be conducted to determine the impact of preharvest interventions on other pathogens, such as *S.* Typhimurium, in the feedlot environment.

In addition to preharvest research, a series of food safety studies comparing the effectiveness of activated lactoferrin (2%) and nonactivated lactoferrin (2%), lactic acid (2%) and water was on inhibiting and/or reducing *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* populations on fresh and ready-to-eat beef products showed that activated lactoferrin and lactic acid were effective in inhibiting and reducing growth of *E. coli* O157:H7 and *L. monocytogenes* in beef bologna. Additionally, when activated lactoferrin and lactic acid were sequentially applied to beef adipose tissue, their effectiveness in inhibiting and reducing *E. coli* O157:H7, *L. monocytogenes,* and *S.* Typhimurium populations was enhanced. Additional studies should be conducted to validate the effectiveness of the sequential application of activated lactoferrin and lactic acid in the beef processing environment. Additional large-scale studies should be conducted to determine the effectiveness of activated lactoferrin as a post-processing treatment to control *Listeria monocytogenes* in ready-to-eat beef products. This

information would aid government and industry personnel in producing end products of enhanced microbiological quality.

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