

**ASSESSING BEEF HIDE INTERVENTIONS AS A
MEANS TO REDUCE CARCASS CONTAMINATION**

A Thesis

by

BRIDGET ELAINE BAIRD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2005

Major Subject: Animal Science

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ABSTRACT

Assessing Beef Hide Interventions as a Means to Reduce Carcass Contamination.

(December 2005)

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Chair of Advisory Committee: Dr. Jeffrey W. Savell

Food safety is a critical issue for beef harvest operations. There are multiple interventions available for treating carcasses; however, this project was designed to evaluate an intervention capable of reducing bacterial counts on the hide prior to opening in order to minimize carcass contamination. In Trial I, fresh beef hides (n = 12) were cut into sections and assigned to serve as either clipped (hair trimmed) or non-clipped sections. Sections were inoculated with a bovine fecal slurry and sampled following a water wash. Treatments (distilled water, isopropyl alcohol, 3% hydrogen peroxide, 2% L-lactic acid, 1% cetylpyridinium chloride (CPC), and 10% Povidone-iodine) then were applied to each section and sampled for aerobic plate counts (APCs), coliform, and *Escherichia coli* counts. Within clipped samples, 1% CPC and 3% hydrogen peroxide caused the greatest reductions in aerobic plate counts, and 1% CPC, 2% L-lactic acid, and 3% hydrogen peroxide showed among the greatest reductions in coliform counts.

In Trial II, beef carcasses with hides on were sampled initially and clipped, and then antimicrobials (2% L-lactic acid, 3% hydrogen peroxide, and 1% CPC) were applied before sampling again for APC, coliform, and *E. coli* counts. This procedure was replicated in Trial II utilizing a non-pathogenic *E. coli* Type I indicator strain transformed to produce a green fluorescing protein (GFP). In Trial II, though few

differences existed between antimicrobial treatments, all three (1% CPC, 2% L-lactic acid, and 3% hydrogen peroxide) resulted in approximately a 2- \log_{10} CFU/100- cm^2 GFP reduction when applied to clipped hide surfaces in the brisket region of the carcass. In Trial III, 1% CPC produced the greatest reduction on the hide surface for APCs.

In Trial IV clipped beef hide sections were sampled initially and then antimicrobials (2% L-lactic acid, 3% hydrogen peroxide, and 1% CPC) were applied before sampling again to determine reduction. Trial IV also involved the use of the *E. coli* GFP indicator strain. In Trial IV, non-clipped samples had a mean reduction of 2.8 \log_{10} CFU/100 cm^2 , and clipped samples had a mean reduction of 2.2 \log_{10} CFU/100 cm^2 . Within the antimicrobials tested, 1% CPC and 3% hydrogen peroxide produced the greatest reductions.

DEDICATION

I dedicate this work to my family. I have the most supportive parents in this world, and I love and thank them for all they have done for me. Thank you for believing in me and for being there every single time I needed to you. Thank you for the excellent example you set for me. I admire more than anything how hard you both work every day. Thanks to my little brother for just being you. Thanks also to my extended family for all of your support and encouragement.

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TABLE OF CONTENTS

	Page
ABSTRACT	iii
DEDICATION	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS.....	viii
LIST OF FIGURES.....	ix
LIST OF TABLES	x
 CHAPTER	
I INTRODUCTION AND REVIEW OF LITERATURE.....	1
II MATERIALS AND METHODS.....	11
2.1. Trial I.....	11
2.2. Trial II.....	13
2.3. Trial III.....	14
2.4. Trial IV.....	15
2.5. Statistical analysis.....	17
III RESULTS AND DISCUSSION	18
3.1. Trial I.....	18
3.2. Trial II.....	21
3.3. Trial III.....	22
3.4. Trial IV.....	23
IV SUMMARY AND CONCLUSIONS.....	25
REFERENCES.....	26
VITA.....	31

LIST OF FIGURES

FIGURE		Page
1.	Least squares means for the treatment effect of hair removal (clipped vs. non-clipped) on the effectiveness of antimicrobial agents on <i>E. coli</i> reduction. Standard error of the least squares means (SEM) = 0.17.	20
2.	Least squares means for the treatment effect of hair removal (clipped vs. non-clipped) on the effectiveness of antimicrobial agents on GFP reduction. Standard error of the least squares means (SEM) = 0.24.	24

LIST OF TABLES

TABLE		Page
1.	Least squares means for the interaction of clipping × antimicrobial agent on reductions in aerobic plate count (APC).....	18
2.	Least squares means for the interaction of clipping × antimicrobial agent on reduction of coliform bacteria.....	19
3.	Least squares means for the treatment effect of antimicrobial agent on <i>E. coli</i> reduction.....	20
4.	Least-squares means for green fluorescing protein (GFP) indicator bacteria reductions on inoculated (10^6 CFU/g fresh bovine feces) brisket areas of hides clipped and treated with antimicrobial agents	21
5.	Least squares means for APCs, coliform, and <i>E. coli</i> counts and log reductions on brisket area of before and after clipping and treatment with antimicrobial agents.....	22
6.	Least squares means for the treatment effect of antimicrobial agent on GFP indicator bacteria reduction on hide sections inoculated with 10^6 CFU/g fresh bovine feces	23

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

According to the Centers for Disease Control and Prevention (CDC), approximately 76 million cases of foodborne illness occur each year in the United States, and approximately 14 million of these can be attributed to known pathogens (Mead et al., 1999). Foodborne diseases are also to blame for approximately 325,000 hospitalizations and 5,000 deaths in the United States each year (Mead et al., 1999). Nontyphoidal *Salmonella* causes approximately 1,400,000 human cases each year, with 95% of these cases linked to foodborne transmission (Mead et al., 1999). More than 100 outbreaks of *Escherichia coli* O157:H7 have occurred since 1982, and over half (52%) of those outbreaks have been linked to beef (Barham, Barham, Johnson, Allen, Blanton, & Miller, 2002). According to data from the 2001 to 2002 USDA school lunch ground beef-purchasing program, within a total of 1,491 samples collected, 1.01% and 3.96% were reported positive for *E. coli* O157:H7 and *Salmonella*, respectively (USDA, 2002; Huffman, 2002). Microbial contamination occurs inevitably in the conversion of live animals to meat products (Ellebracht et al., 2005). As a result, USDA-FSIS (1996) has recognized in its guidance materials that a decontamination step should be considered part of the slaughter and dressing process (Huffman, 2002).

Cattle are a known reservoir for *E. coli* O157:H7, and it has been estimated that

This thesis follows the style of *Meat Science*.

15.7% of all cattle carry this organism in their rumen and colon (Chapman, Siddons, Cerdan-Malo, & Harkin, 1997). *E. coli* is a natural, harmless inhabitant of cattle intestines, but some strains of this organism, particularly serotype O157:H7, are pathogenic and can cause serious illness in humans (Smith et al., 2001). Sofos et al. (1999) reported that the incidence of *E. coli* O157:H7 could be ten times greater on the hide than in the feces of cattle at slaughter. Prevalence rates for *E. coli* O157:H7 in feces of cattle have been reported to range from 1.0% to 28% (Reid, Small, Avery, & Buncic, 2002; Chapman et al., 1997; Cizek, Alexa, Literák, Hamřík, Novák, & Smola, 1999; Elder, Keen, Siragusa, Barkocy-Gallagher, Koohmaraie, & Laegreid, 2000) with up to 60.6% on the hide surface itself (Barkocy-Gallagher et al., 2003). Elder et al. (2000) reported a 10.7% incidence of *E. coli* O157:H7 contamination on cattle hides in the United States. Prevalence levels of *Salmonella* on the external surfaces, or hides, of cattle have been determined to range from 15.4% (Bacon, Sofos, Belk, Hyatt, & Smith, 2002) to 71.0% (Barkocy-Gallagher et al., 2003) pre-slaughter. As suggested by Barkocy-Gallagher et al. (2003), hides are the primary source of beef carcass contamination with *E. coli* O157:H7 and *Salmonella*.

Typical prevalence rates for *Salmonella* spp. have been reported at approximately 5.5%, and typical prevalence rates for *Campylobacter* spp. range from 5.0% to 53.0% (Reid et al., 2002; Hancock, Besser, Rice, Herriott, & Tarr, 1997). Prevalence rates, however, can be affected by multiple factors, including seasonal variation (Reid et al., 2002b; Hancock et al., 1997; Barkocy-Gallagher et al., 2003), with the highest incidence of *E. coli* O157:H7 seen in spring and late summer (Chapman et al., 1997) and in the fall

(Barkocy-Gallagher et al., 2003), and the highest incidence of *Salmonella* seen in the summer and fall (Barkocy-Gallagher et al., 2003).

Hides are considered an important source of pathogenic organisms during slaughter because of fecal contamination that occurs during holding (Castillo, Dickson, Clayton, Lucia, & Acuff, 1998a). Van Donkersgoed, Jericho, Grogan, & Thorlakson (1997) note that tag (defined as mud, bedding, or manure), whether wet or dry, hard or soft, large or small, can stick in clumps to the hide on the legs, belly, and sides of cattle. As reported by Beach, Murano, & Acuff (2002), factors such as transport stress, feed withdrawal, and animal commingling can influence the number of cattle contaminated with pathogens, such as *Salmonella*, before slaughter. Feedyards can be a likely source of enterohemorrhagic *E. coli* O157 and *Salmonella* because cattle are co-mingled from multiple sources, and cattle are kept in high-density pens (Barham et al., 2002). The presence of foodborne pathogens on cattle hides can significantly increase during the time period between the farm and slaughter, especially during transport and holding at the slaughter facility (Collis et al., 2004; Barham et al., 2002). During these situations, cattle experience a stress-induced shedding of pathogens (Collis et al., 2004).

In a study to determine the prevalence of cattle shedding *E. coli* O157:H7, Smith et al. (2001) reported that this organism should be considered common to cattle grouped together in feedlot pens, and that pen floor condition can influence the prevalence of pathogen shedding. This effect is combined with an increase in the prevalence of pathogens on hides caused by direct (animal to animal) or indirect (animal to environment to animal) contact during transport or holding (Collis et al., 2004). Collis et al. (2004) determined that both the livestock market process and the unloading-to-

skinning process at abattoirs can potentially allow for the spread of contamination on hides not only within, but also between, lots of cattle. Collis et al. (2004) and Small, Reid, Avery, Karabasil, Crowley, and Buncic (2002) reported that the prevalence of *E. coli* O157 on some environmental surfaces in the unloading-to-slaughter area in cattle abattoirs could be up to 50%, especially on pen floors and in stunning boxes. Beach et al. (2002) listed potential sources of *Salmonella* contamination throughout the transport and slaughter process, including transport vehicles, holding pens, knocking boxes, workers, and equipment.

The degree of visible contamination on the hide surface has been shown to directly affect the degree of resultant contamination of the carcass (Reid et al., 2002; McEvoy et al., 2000). Skeletal muscle from healthy animals is considered sterile prior to slaughter with the exception of the lymph nodes (Romans, Costello, Carlson, Greaser, & Jones, 1994). Bacteria present on hides can eventually be transferred to underlying “sterile” carcass tissue surface during the hide removal process. A large portion of beef carcass contamination begins with dirt, dust, and fecal matter associated with the hide and occurs when the hide is removed (Ellebracht et al., 2005; Ayers, 1955; Elder et al., 2000). Contamination can occur when manure on the hide surface that has not been washed away before slaughter is carried onto the underlying carcass tissue (Delazari, Iaria, Riemann, Cliver, & Jothikumar, 1998; Ransom, Belk, Bacon, Sofos, Scanga, & Smith, 2002). During the hide removal process, pathogens such as *E. coli* O157:H7 and *Salmonella* can be transferred from the hide where they are in high prevalence, to the carcass (Bosilevac et al., 2004b). As a result, any increase in hide contamination with

pathogens before slaughter will also increase the risk of contamination of the carcass tissue (Collis et al., 2004).

A study by Chapman, Siddons, Wright, Norman, Fox, & Crick (1993) reported that approximately 30% of carcasses from animals whose hides tested positive for *E. coli* O157:H7 were contaminated with the pathogen after dehidng. In contrast, Bolton, Byrne, & Sheridan (1998) reported that 100% of carcasses from cattle with hides inoculated with *E. coli* O157:H7 were contaminated with the pathogen following dehidng. This leads to the conclusion that interventions designed to reduce or eliminate pathogens from cattle hides should be identified as critical control points to reduce the incidence of *E. coli* O157:H7 on beef carcasses (Nou et al., 2003).

Previous research conducted by Texas A&M University's Food Microbiology Laboratory investigated pre-harvest cattle washing systems, but reported limited effectiveness (Mies, Covington, Harris, Lucia, Acuff, & Savell, 2004). It was determined that hide washes (single water wash, double water wash, water wash with 0.5% L-lactic acid, or water wash with 50 ppm chlorine) released pathogenic bacteria present in fixed locations on hide surfaces, enabling the migration of pathogens within the wash from areas of heavy contamination on the hide to all areas of the hide. This is of particular concern along the mid-line where the initial openings through the hide are made and are most prone to contamination. This study also noted that application of high concentrations of organic acids or ethanol to live cattle can lead to animal welfare issues, such as animal stress and irritation to the eyes and nose (Mies et al., 2004).

Occasional failures occur in the slaughter and dressing process that lead to a level of contamination that is greater than what can be removed effectively with current

carcass interventions (Bosilevac et al., 2004b). Because errors in slaughter and dressing have been implicated as the primary vehicles for contamination of beef carcasses (Bacon, Belk, Sofos, Clayton, Regan, & Smith, 2000), many processors have incorporated carcass wash cabinets in their slaughter and processing lines to reduce levels of microbial contamination (Delazari et al., 1998). However, the most effective method of eliminating beef carcass contamination would be to prevent it by cleaning the hide before its removal (Bosilevac et al., 2004a; Nou et al., 2003). To strengthen the food safety system, the beef industry is in search of preventive procedures that can reduce levels of pathogenic bacteria found on hides before those bacteria have a chance of reaching the carcass.

Dehairing technology was developed in the 1990s in an effort to remove hair, dirt, and feces from the carcass surface during the beginning stages of the slaughter and dressing process. Upon further examination, while dehairing did reduce visual contamination of the beef carcass, it did not decrease the overall bacteria load (Schnell et al., 1995). Major beef processors have also investigated post-exsanguination hide washing systems; however, these systems often do not reduce the solubilization and migration of pathogenic bacteria on hide surfaces.

The most common methods used to decontaminate the carcass are application of hot water, organic acids, or steam (Castillo et al., 1998a). Targeting decontamination of the hide, rather than direct decontamination of the carcass surface, has an advantage because the hide is considered an inedible by-product. This allows for use of a larger selection of antimicrobials, as using non-food grade chemicals on hides does not carry implications for residues in, or diminished sensory qualities of, the underlying carcass tissue (Small, Wells-Burr, & Buncic, 2005).

The current availability of water will likely ensure its continuation as the most widely used intervention in beef slaughter facilities (Ransom, Belk, Sofos, Stopforth, Scanga, & Smith, 2003). The effectiveness of water as a decontaminant is determined by the temperature, pressure, and time at which it is applied (Graves-Delmore, Sofos, Reagan, & Smith, 1997). Davey and Smith (1989) determined that the use of spray washing with water at 83.5°C for 10 to 20 sec resulted in 2.2- and 3.0-log bacterial count reductions, respectively. Smith (1992) reported greater than 3.0-log reductions in inoculated *E. coli*, *Salmonella*, *E. coli* O157, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Pseudomonas fragi* and *Listeria monocytogenes* from the surface of beef tissue after application of 80°C water for 10 to 20 sec.

Isopropyl alcohol is most active at 70% concentration and retains some activity down to 10% (Jeffrey, 1995). Alcohol has the advantage of evaporating quickly and leaving no residues, and has therefore been used as a spray disinfectant in the food industry (Jeffrey, 1995). Hydrogen peroxide has good antibacterial properties, and has been used in formulations at 5 to 20% (Jeffrey, 1995). This compound is very reactive, but is not very stable, and is destroyed by alkalis. Hydrogen peroxide is used in sterilizing cardboard packaging materials for milk because its breakdown products are water and oxygen (Jeffrey, 1995). A study conducted by Small et al. (2005) reported significant microbial reduction at 50°C when hides were treated with a disinfecting solution containing hydrogen peroxide and peracetic acid. Although iodine is one of the most active disinfectants known, this compound is not very soluble and can be considered too corrosive and staining to use as a microbiocidal (Jeffrey, 1995).

The microbiocidal properties of acids exist due to their low pH. All acids are slow acting and have a low concentration exponent (Jeffrey, 1995). Interventions such as organic acid and hot water rinses have been somewhat effective at reducing the microbial load on hot carcasses prior to chilling (Ellebracht et al., 2005; Castillo, Lucia, Goodson, Savell, & Acuff, 1998b). Spraying carcass surfaces with organic acids has been found effective in reducing microbial contamination (King, Lucia, Castillo, Acuff, Harris, & Savell, 2005; Castillo et al, 1998b; Dickson, 1992; Hardin, Acuff, Lucia, Oman, & Savell, 1995). Hardin et al. (1995) reported that organic acid treatments were more effective methods of removing *S. Typhimurium* and *E. coli* O157:H7 contamination than trimming or water washing alone.

Snijders, van Logtestijn, Mossel, and Smulders (1985) determined that lactic acid results in immediate and delayed effects that help extend the shelf life of meat. Snijders et al. (1985) reported an immediate bactericidal effect of lactic acid decontamination of beef, veal, and pork carcasses as lactic acid reduced aerobic plate counts (APCs) by 1.5 \log_{10}/cm^2 . Time and temperature of application has some effect on the efficacy of lactic acid. Snijders et al. (1985) determined that spraying hot carcasses at 45 min postmortem with 1% lactic acid resulted in greater bacterial reduction than spraying chilled carcasses.

Cetylpyridinium chloride (CPC) is a water-soluble compound that has been used for more than 50 years in oral hygiene products such as toothpaste and mouthwash (Cutter et al., 2000). This compound has a low surface tension with hydrophilic and lipophilic properties that allow it to work well to hydrate and penetrate tissue (Cutter et al., 2000); however, CPC is a quaternary ammonium compound and, as such, is rapidly neutralized by organic matter (Bosilevac et al., 2004b; McDonnell & Russell, 1999). In

addition, quaternary ammonium compounds have a relatively narrow range of activity, and are not very effective against Gram-negative bacteria; however, their efficacy can be improved through the addition of a surfactant such as polymeric biguanide hydrochloride (Small et al., 2005; Sprenger, 1997). The action of quaternary ammonium compounds is fairly rapid and can be increased with an increase in temperature (Jeffrey, 1995).

Cetylpyridinium chloride is reported as being efficacious for reducing *Salmonella* contamination of poultry carcasses (Kim & Slavik, 1996; Xiong, Li, Slavik, & Walker, 1998; Yang, Li, & Slavik, 1998), as well as for preventing cross-contamination during poultry slaughter (Cutter et al., 2000). An effective concentration of CPC on poultry carcasses has been reported at 0.5% (Bosilevac et al., 2004b; Kim & Slavik, 1996; Xiong et al., 1998; Yang et al., 1998). Bosilevac et al. (2004a) determined that a CPC concentration of 1% demonstrated sufficient activity to reduce beef hide contamination with *E. coli* O157, and that using concentrations above this level can result in sample processing problems, while using concentrations below this level does not provide sufficient decontamination.

The activity of CPC has been shown to begin as soon as 30 s after application, and last as long as 4 h after application (Bosilevac et al., 2004a). CPC can be applied to live cattle before these animals enter the slaughter facility; however, this can increase the level of stress and bruising introduced to these animals having a resultant effect on product quality (Bosilevac et al., 2004a). Bosilevac et al. (2004a) reported that the percentage of bruised beef carcasses in their study that required trimming was greater for CPC treated carcasses than for controls. In a study by Cutter et al. (2000) examining spray-washing (862 kPa, 15 s, 35°C) lean beef surfaces with 1% CPC, this compound

was able to reduce 5 to 6 log₁₀ CFU/cm² of inoculated *E. coli* O157:H7 and S. Typhimurium to practically undetectable levels (0 log₁₀ CFU/cm²). This same study on adipose beef surfaces also reduced 5 log₁₀ CFU/cm² of inoculated *E. coli* O157:H7 and S. Typhimurium immediately (>2.5 log₁₀ CFU/cm²) (Cutter et al., 2000).

There is some concern that CPC left on a sampling sponge must be neutralized before sample plating. Bosilevac et al. (2004b) observed an overestimation of CPC activity because of inadequate neutralization of absorbed residual CPC in the sponge sample. As a result, Bosilevac et al. (2004b) elected to use 2 × Dey and Engley (DE) as the sampling buffer because this compound has broad effectiveness, and because it can neutralize quaternary ammonium compounds, phenols, iodines, and aldehydes. Bosilevac et al. (2004b) also included a centrifugation and resuspension step to effectively remove any residual CPC left in the samples.

One potential method of achieving hide cleanliness would be to first closely trim the hair from the area where a knife will be used for opening to remove any attached dirt or fecal material. In a study investigating multiple methods of hide surface preparation before antimicrobial agent application, Small et al. (2005) determined that clipping in combination with singeing was the most effective treatment examined achieving average microbial reductions greater than 2 log₁₀ CFU/cm². There was, however, a technical problem associated with singeing as this process resulted in loose ash from charred hairs that could potentially lead to airborne contamination of skinned carcasses. Small et al. (2005) also noted that large quantities of this resultant ash could lead to a significant occupational health issue for workers.

CHAPTER II

MATERIALS AND METHODS

2.1. Trial I

Fresh beef hides (n = 12; 4 per rep) were cut into 900-cm² sections with a minimum of 12 sections removed from each. Half of these sections were blown dry (Air Express Blow Dryer III, Sullivan's Supply, Inc., Dunlap, IA) and clipped (hair removed) using Oster ClipMaster[®] clippers (Sunbeam Products, Inc., Boca Raton, FL) while the other half remained non-clipped. The following day, hide sections were stretched over plastic clipboards and inoculated over a 400-cm² area with a bovine fecal slurry (10 g bovine feces mixed with 10 mL 0.1% sterile peptone water, Difco Laboratories, Detroit, MI) that was determined to contain approximately 10⁶ CFU/g. The inoculum was allowed a 20 min attachment period before gross fecal material was washed away using a handheld, compressed-air sprayer (Model 1002 BH; Better Homes and Gardens, marketed by Wal-Mart, Inc., Bentonville, AK) standardized to deliver approximately 1 L of water over 90 sec. The sprayer nozzle was kept approximately 6 to 8 in away from the hide section in order to maintain consistent water delivery.

Microbiological samples were collected from each untreated hide section following water wash using a sterile sponge (BioPro Sampling System; BioTrace International, Bothell, WA) to determine pre-treatment counts on hide surfaces. Prior to sampling, a sponge was moistened with 25 mL of sterile 0.1% peptone water, and sample collection then was achieved by firmly rubbing the damp sponge over a 100-cm² area of the hide section. The sponge then was transferred to a plastic bag for subsequent analysis. Following pre-treatment sampling, sections were assigned to receive one of six

antimicrobial agents that were applied using saturated (50 mL), sterile sponges: distilled water, isopropyl alcohol, 3% hydrogen peroxide (Aaron Industries, Inc., Clinton, SC), 2% L-Lactic acid (Purac[®], Rotra International, Wood Dale, IL), 10% Povidone-iodine (Vetadine, Vedco, Inc., St. Joseph, MO), and 1% (wt/vol) cetylpyridinium chloride (Zeeland Chemicals, Zeeland, MI). Sponge application consisted of ten passes vertically, ten passes horizontally, and ten passes diagonally, with even pressure applied throughout all passes. All treatments were applied at room temperature with the exception of 2% L-lactic acid, which was applied at 55°C following common industry practice.

Following treatment, hide sections were sampled as described previously for pre-treatment sampling. Sodium thiosulfate (4 g/L; EM Science, Gibbstown, NJ) was added to the diluent and placed into each bag containing sponges with 10% Povidone-iodine to neutralize the iodine and ensure that any bactericidal effect that occurred on the hide surface did not continue to occur during sample transport and processing (Lacey, 1979; Papageorgiou, Mocé-Llivina, & Jofre, 2001). Each sponge sample then was hand-massaged inside its plastic bag for 1 min before examination for aerobic plate counts (APCs) and coliform and *E. coli* counts. Coliform and *E. coli* counts were generated by plating appropriate dilutions of the sponge sample onto Petrifilm *E. coli*/Coliform count plates (3M Microbiology & Products, St. Paul, MN). Samples were incubated for 24 ± 2 h at 35°C before colonies were counted. *E. coli* counts were achieved by counting colonies that appeared blue with a gas bubble, while total coliform counts were achieved by counting both blue and red colonies with a gas bubble. Aerobic plate counts were determined by plating appropriate dilutions of the sponge sample onto Petrifilm aerobic

count plates (3M), incubating at room temperature (25°C) for 48 h, and then counting all colonies.

2.2. Trial II

Beef carcasses (n = 9; 3 per rep) were selected for sampling at the Rosenthal Meat Science and Technology Center at Texas A&M University. Cattle were exsanguinated, clipped (hair removed) in the brisket area, and inoculated in the brisket area with a nonpathogenic indicator bacteria designed to represent possible contamination with fecal material containing enteric pathogens such as *Salmonella* or *E.coli* O157:H7. The indicator consisted of a non-pathogenic *E. coli* Type I strain that was transformed to produce a green fluorescing protein (GFP) and express ampicillin resistance properties (100 µg/L). A bovine fecal slurry (10g bovine feces mixed with 10 mL 0.1% sterile peptone water, Difco Laboratories, Detroit, MI) was generated with the GFP indicator bacteria to produce feces containing approximately 10⁶ CFU/g, and 10 g was used to inoculate 400 cm² of the clipped hide. The brisket area was selected for sampling because it is traditionally considered a region of the hide surface that is visibly contaminated with feces and dirt at slaughter. The incidence of *E. coli* O157 on the brisket area of cattle hides at slaughter can be as high as 11% (Elder et al., 2000) or 22% (Reid et al, 2002).

Immediately following inoculation, gross fecal material was washed off using a handheld, compressed-air sprayer standardized to deliver approximately 1 L of water over 90 sec. The sprayer nozzle was kept approximately 6 to 8 in away from the hide in order to maintain consistent water delivery. Following washing, a 100-cm² area was

sampled using a pre-moistened sterile sponge, as described previously, to determine initial counts. Cattle then were assigned to receive one of three antimicrobial agents (2% L-lactic acid, 3% hydrogen peroxide, and 1% CPC). These agents were chosen because they were deemed most effective in Trial I. Sponge application consisted of ten passes vertically, ten passes horizontally, and ten passes diagonally, with even pressure applied throughout all passes. All treatments were applied at room temperature with the exception of 2% L-lactic acid, which was applied at 55°C following common industry practice.

After application of antimicrobial agents, hides were sampled, as described previously, to determine post-treatment counts. Following hide removal, a 100-cm² area of the carcass in the brisket region was sampled for the indicator bacteria using a pre-moistened sterile sponge as described previously. Each sponge sample then was hand-massaged inside its plastic bag for 1 min and plated using appropriate serial dilutions on tryptic soy agar (TSA) supplemented with ampicillin (100µg/L). Plates were incubated for 24 h at 36°C before fluorescent colonies were counted under a UV light.

2.3. Trial III

Beef carcasses (n = 18) with hides attached were selected from a small commercial processor for use in Trial III. Following exsanguination, approximately 100-cm² of the hide in the brisket area was sampled with a pre-moistened, sterile sponge as described in Trial I to determine pre-treatment counts on hide surfaces. Following sampling, hides were clipped in approximately a 400-cm² area in the brisket region of the carcass. Cattle then were assigned to receive one of three antimicrobial treatments (2%

L-lactic acid, 3% hydrogen peroxide, and 1% CPC) deemed effective in Trials I and II, and treatments were applied using saturated (50 mL), sterile sponges. Sponge application consisted of ten passes vertically, ten passes horizontally, and ten passes diagonally, with even pressure applied throughout all passes. All treatments were applied at room temperature with the exception of 2% L-lactic acid, which was applied at 55°C following common industry practice.

After application of the designated treatment, hides were sampled again as described previously to determine initial post-treatment counts. Following plant procedures, the brisket and foreshanks of each carcass were rinsed with water before opening the hide for removal. This procedure also likely removed loose hair left behind due to clipping and antimicrobials remaining on the hide after application. Following hide removal, 100 cm² of the carcass surface in the brisket area was sampled using a pre-moistened sterile sponge, as described previously, to determine carcass counts. Sponge samples were placed in an insulated shipping container with refrigerant to keep them cool for transport to Texas A&M University's Food Microbiology Laboratory (College Station, TX). The following day, sponge samples were hand-massaged inside their plastic bags for 1 min before examination for APCs and coliform and *E. coli* counts, as described for Trial I.

2.4. Trial IV

Fresh beef hides were cut into 900-cm² sections (n = 18). Half of these sections were blown dry and clipped while the other half remained non-clipped. The following day, hide sections were stretched over plastic clipboards. As described in Trial II, a

bovine fecal slurry was inoculated with the nonpathogenic GFP indicator bacteria to produce feces containing approximately 10^6 CFU/g, and 10 g was used to inoculate 400 cm^2 of the hide section. Gross fecal material then was washed off using a handheld, compressed-air sprayer standardized to deliver approximately 1 L of water over 90 sec. The sprayer nozzle was kept approximately 6 to 8 in away from the hide section in order to maintain consistent water delivery.

Microbiological samples were collected from each untreated hide following water wash using a sterile sponge to determine pre-treatment counts on hide surfaces, as described in Trial I. The sponge then was transferred to a plastic bag for subsequent analysis. Following pre-treatment sampling, sections were subjected to one of three antimicrobial treatments (3% hydrogen peroxide, 2% L-Lactic acid, and 1% CPC), and treatments were applied using saturated (50 mL), sterile sponges. Sponge application consisted of ten passes vertically, ten passes horizontally, and ten passes diagonally, with even pressure applied throughout all passes. All treatments were applied at room temperature with the exception of 2% L-lactic acid, which was applied at 55°C following common industry practice.

After application of antimicrobials, hides were sampled as described previously to determine post-treatment counts. As described in Trial II, each sponge sample then was hand-massaged inside its plastic bag for 1 min and plated using appropriate serial dilutions on tryptic soy agar (TSA) supplemented with ampicillin (100 $\mu\text{g/L}$). Plates were incubated for 24 h at 36°C before fluorescent colonies were counted.

2.5. *Statistical analysis*

Data were analyzed using PROC GLM of SAS (SAS Institute, Cary, NC).

Microbial reductions were tested for significance ($P < 0.05$) by analysis of variance using PROC GLM. Least squares means were generated for each main effect and separated using the PDIFF option when appropriate.

CHAPTER III

RESULTS AND DISCUSSION

3.1. Trial I

Least squares means for the interaction of clipping × antimicrobial agent on APC reduction for hide sections inoculated with 10^6 CFU/g fresh bovine feces are reported in Table 1. Within non-clipped samples, the greatest reductions were associated with 1% CPC and 2% L-lactic acid at 4.1 and 2.7 \log_{10} CFU/100 cm^2 , respectively. Within clipped samples, 1% CPC, 3% hydrogen peroxide, and 2% L-lactic acid produced the greatest reductions at 4.6, 4.4, and 4.1 \log_{10} CFU/100 cm^2 , respectively.

Table 1
Least squares means for the interaction of clipping × antimicrobial agent on reductions in aerobic plate count (APC)

Antimicrobial	\log_{10} CFU/100 cm^2 reduction ^a	
	Non-clipped	Clipped
Water	0.9c	0.6c
Alcohol	0.5c	1.8bc
1% CPC	4.1a	4.6a
10% Povidone-iodine	1.3c	1.8bc
2% L-lactic acid	2.7b	4.1a
3% Hydrogen peroxide	1.5bc	4.4a
^b SEM	0.48	0.48

LS means lacking common letters differ ($P < 0.05$).

^a \log_{10} CFU/100 cm^2 reduction = (\log_{10} CFU/100 cm^2 on untreated hide area) – (\log_{10} CFU/100 cm^2 on treated hide area).

^bSEM is the standard error of the least squares means.

Least squares means for the interaction of clipping × antimicrobial agent on coliform reduction for hide sections inoculated with 10^6 CFU/g fresh bovine feces are reported in Table 2. Within non-clipped samples, 1% CPC produced the greatest reduction at 5.3 \log_{10} CFU/100- cm^2 , followed by 2% L-lactic acid, iodine, and 3%

hydrogen peroxide. Within clipped samples, 1% CPC, 2% L-lactic acid, and 3% hydrogen peroxide produced the greatest reductions with 4.5, 4.1, and 3.9 log₁₀ CFU/100-cm² reported, respectively.

Table 2

Least squares means for the interaction of clipping × antimicrobial agent on reduction of coliform bacteria

Antimicrobial	log ₁₀ CFU/100 cm ² reduction ^a	
	Non-clipped	Clipped
Water	-0.1d	0.5d
Alcohol	0.2d	1.8c
1% CPC	5.3a	4.5ab
10% Povidone-iodine	2.4c	2.5c
2% L-lactic acid	2.8c	4.1b
3% Hydrogen peroxide	2.2c	3.9bc
^b SEM	0.43	0.43

LS means lacking common letters differ ($P < 0.05$).

^aLog₁₀ CFU/100 cm² reduction = (log₁₀ CFU/100 cm² on untreated hide area) – (log₁₀ CFU/100 cm² on treated hide area).

^bSEM is the standard error of the least squares means.

Least squares means for the treatment effect of antimicrobial agent on *E. coli* reductions are reported in Table 3. Least squares means for the treatment effect of hair removal (clipped vs. non-clipped) on the effectiveness of antimicrobial agents on *E. coli* reduction are reported in Figure 1. Non-clipped samples had a mean reduction of 2.0 log₁₀ CFU/100 cm² and clipped samples had a mean reduction of 2.8 log₁₀ CFU/100 cm². Within the antimicrobials tested, 1% CPC produced the greatest reduction at 4.5 log₁₀ CFU/100 cm², followed by 2% L-lactic acid (3.3 log₁₀ CFU/100 cm²) and 3% hydrogen peroxide (2.9 log₁₀ CFU/100 cm²).

Table 3

Least squares means for the treatment effect of antimicrobial agent on *E. coli* reduction

Antimicrobial agent	\log_{10} CFU/100 cm ² reduction ^a
Water	0.2d
Alcohol	0.9d
1% CPC	4.5a
10% Povidone-iodine	2.4c
2% L-lactic acid	3.3b
3% Hydrogen peroxide	2.9bc
^b SEM	0.30

LS means within treatment effects lacking common letters differ ($P < 0.05$).

^a \log_{10} CFU/100 cm² reduction = (\log_{10} CFU/100 cm² on untreated hide area) – (\log_{10} CFU/100 cm² on treated hide area).

^bSEM is the standard error of the least squares means.

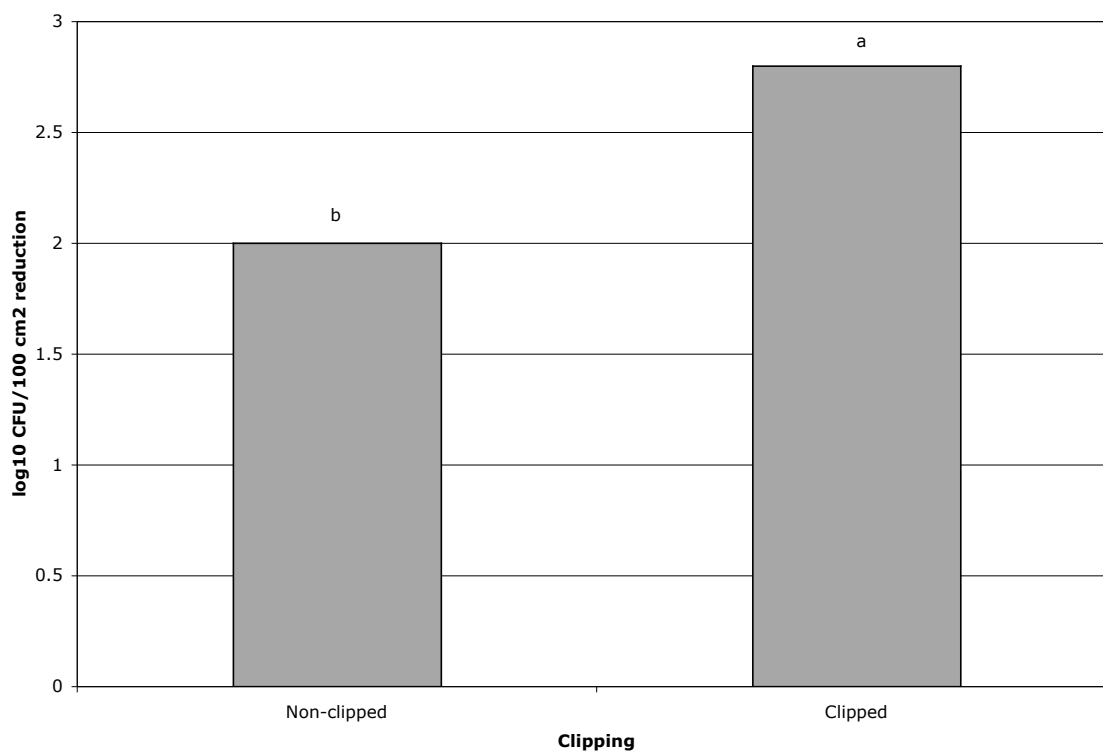


Fig. 1. Least squares means for the treatment effect of hair removal (clipped vs. non-clipped) on the effectiveness of antimicrobial agents on *E. coli* reduction. Standard error of the least squares means (SEM) = 0.17.

Across all treatments, clipping appeared to be more effective than non-clipping when applying antimicrobial agents to reduce bacterial counts on the hide surface. After completion of Trial I, clipping together with 1% CPC, 2% L-lactic acid, and 3% hydrogen peroxide were determined to be the three most effective clipping/antimicrobial combinations, and were selected for further evaluation in Trials II, III, and IV.

3.2. Trial II

Least squares means for GFP indicator bacteria reductions on inoculated (10^6 CFU/100 cm² fresh bovine feces) brisket areas of hides clipped and treated with antimicrobial agents are reported in Table 4. There were no ($P > 0.05$) differences among treatments for hide reductions. Though few differences existed between antimicrobial treatments, all three (1% CPC, 2% L-lactic acid, and 3% hydrogen peroxide) resulted in an approximate 2- \log_{10} CFU/100 cm² reduction when applied to clipped hide surfaces in the brisket region of the carcass. There were no differences ($P > 0.05$) between antimicrobial treatments for carcass counts in GFP reduction.

Table 4

Least-squares means for green fluorescing protein (GFP) indicator bacteria reductions on inoculated (10^6 CFU/g fresh bovine feces) brisket areas of hides clipped and treated with antimicrobial agents

Treatment effects	\log_{10} CFU/100 cm ² reduction ^a
1% CPC	2.1a
2% L-lactic Acid	2.6a
3% Hydrogen peroxide	2.0a
^b SEM	0.63

^a \log_{10} CFU/100 cm² reduction = (\log_{10} CFU/100 cm² on untreated hide area) – (\log_{10} CFU/100 cm² on treated hide area).

^bSEM is the standard error of the least squares means.

Table 5

Least squares means for APCs, coliform, and *E. coli* counts and log reductions on brisket area of before and after clipping and treatment with antimicrobial agents

Indicator organism	Treatment	log ₁₀ CFU/100 cm ²		
		Before	After	Reduction ^a
<i>APC</i>	1% CPC	8.2a	4.4c	3.9a
	2% L-lactic acid	7.5b	5.2b	2.3b
	3% Hydrogen peroxide	8.7a	6.5a	2.2b
	^b SEM	0.22	0.21	0.28
<i>Coliforms</i>	1% CPC	4.6b	1.3b	3.2a
	2% L-lactic acid	3.7c	1.1c	2.6a
	3% Hydrogen peroxide	5.2a	2.5a	2.7a
	^b SEM	0.20	0.27	0.29
<i>E. coli</i>	1% CPC	4.3b	1.3a	2.9a
	2% L-lactic acid	3.2c	1.1c	2.1a
	3% Hydrogen peroxide	5.1a	2.1a	3.0a
	^b SEM	0.24	0.29	0.33

LS means within a column and within an indicator organism lacking common letters differ ($P < 0.05$).

^aReduction = (log₁₀ CFU/100 cm² on untreated hide area) – (log₁₀ CFU/100 cm² on treated hide area).

^bSEM is the standard error of the least squares means.

3.3. Trial III

The average initial hide counts before treatment application were 8.1 log₁₀ CFU/100 cm² for APC, 4.2 log₁₀ CFU/100 cm² for coliforms, and 4.5 log₁₀ CFU/100 cm² for *E. coli*. Least squares means for APCs, coliform, and *E. coli* counts and log reductions on brisket areas of hides before and after treatment are reported in Table 5. For APCs, 1% CPC produced the greatest reduction on the hide of 3.9 log₁₀ CFU/100 cm² reported. For coliforms and *E. coli*, there were no ($P > 0.05$) differences among treatments for hide reductions. Though few differences existed between antimicrobial treatments, all three resulted in approximately a 3-log₁₀ CFU/100 cm² reduction when applied to clipped hide surfaces in the brisket region of the carcass. There were no

differences ($P > 0.05$) between antimicrobial treatments for carcass counts on APC and coliform reduction. For *E. coli* reduction, 3% hydrogen peroxide exhibited slightly higher carcass counts ($1.9 \log_{10}$ CFU/100 cm²) when compared to 1% CPC ($1.2 \log_{10}$ CFU/100 cm²) and 2% L-lactic acid ($1.2 \log_{10}$ CFU/100 cm²).

3.4. Trial IV

Least squares means for the treatment effect of antimicrobial agent on GFP indicator bacteria reduction on hide sections inoculated with 10^6 CFU/g fresh bovine feces are reported in Table 6. Within the antimicrobials tested, 1% CPC and 3% hydrogen peroxide produced the greatest reductions at 3.2 and 3.3 \log_{10} CFU/100 cm², respectively, followed by 2% L-lactic acid at 1.0 \log_{10} CFU/100 cm². Least squares means for the treatment effect of hair removal (clipped vs. non-clipped) on the effectiveness of antimicrobial agents on GFP reduction are reported in Figure 2. Non-clipped samples had a mean reduction of 2.8 \log_{10} CFU/100 cm², and clipped samples had a mean reduction of 2.2 \log_{10} CFU/100 cm².

Table 6

Least squares means for the treatment effect of antimicrobial agent on GFP indicator bacteria reduction on hide sections inoculated with 10^6 CFU/g fresh bovine feces

Antimicrobial agent	\log_{10} CFU/100-cm ² reduction ^a
1% CPC	3.2a
2% L-lactic acid	1.0b
3% Hydrogen peroxide	3.3a
^b SEM	0.29

^a \log_{10} CFU/100 cm² reduction = (\log_{10} CFU/100 cm² on untreated hide area) – (\log_{10} CFU/100 cm² on treated hide area).

^bSEM is the standard error of the least squares means.

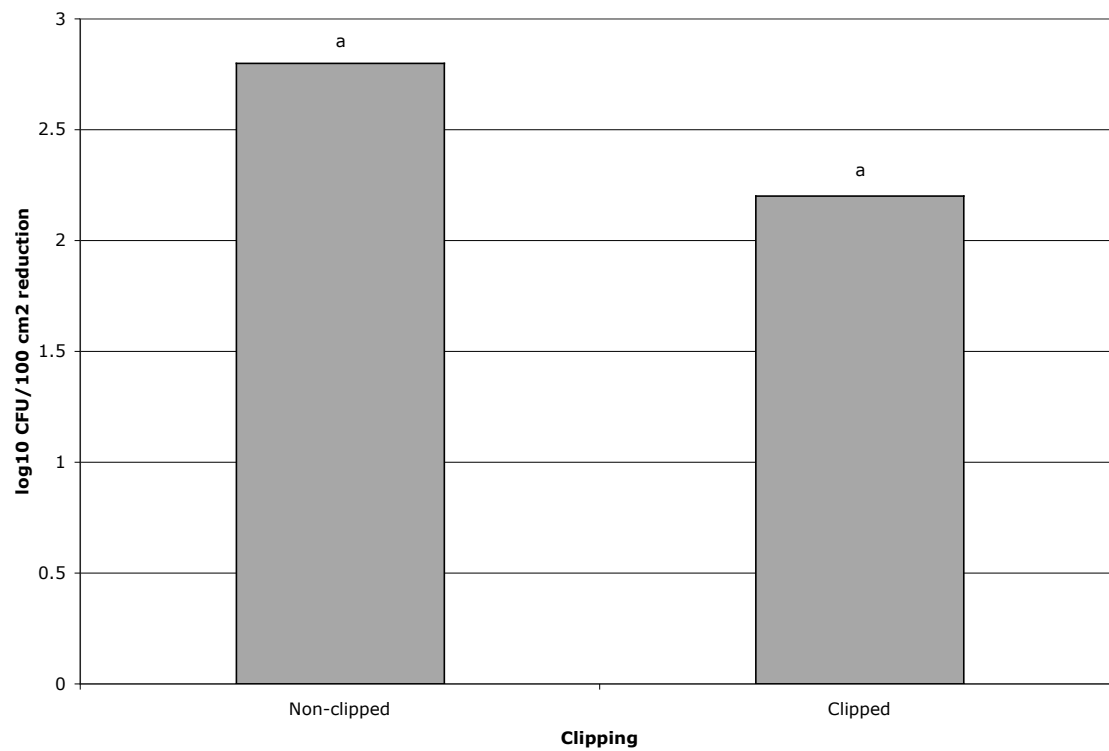


Fig. 2. Least squares means for the treatment effect of hair removal (clipped vs. non-clipped) on the effectiveness of antimicrobial agents on GFP reduction. Standard error of the least squares means (SEM) = 0.24.

CHAPTER IV

SUMMARY AND CONCLUSIONS

To strengthen a food safety system, processors should investigate proactive, preventive procedures that can reduce levels of pathogenic bacteria found on hides before those bacteria have a chance of contaminating the carcass surface. Clipping the hair and applying an antimicrobial agent directly to the hide opening area in the brisket region resulted in a reduction in bacterial counts on hide surfaces. The three most effective antimicrobial agents were 1% cetylpyridinium chloride (CPC), 2% L-lactic acid, and 3% hydrogen peroxide. This method targets a specific area on the hide that is very susceptible to fecal contamination, making it a critical source of contamination when opening up the hide for removal. Selective application of these antimicrobials to clipped hide opening sites can reduce bacterial counts on hide surfaces and, therefore, potentially reduce final carcass counts in these areas by reducing the potential for bacterial contamination during opening. Further research should be conducted to determine effectiveness on additional areas of the hide surface, and to evaluate the practicality of this process in a commercial setting.

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