



Knowledge to Go Places

FINAL REPORT

Use of warm (55°C) 2.5% or 5.0% lactic acid for: (a) reducing microbial counts on beef subprimal cuts and beef trimmings following fabrication, and (b) reducing incidence of *E. coli* O157:H7 in combo-bins of beef trimmings and inside (in the interior) beef cuts subjected to blade/needle or moisture-enhancement tenderization

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American Meat Institute Foundation

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III. Project Title: Use of warm (55°C) 2.5% or 5.0% lactic acid for: (a) reducing microbial counts on beef subprimal cuts and beef trimmings following fabrication, and (b) reducing incidence of *E. coli* O157:H7 in combo-bins of beef trimmings and inside (in the interior) beef cuts subjected to blade/needle or moisture-enhancement tenderization.

IV. Stated Objectives: The goals of the proposed research study are: (a) to identify a microbiological intervention (as a “processing aid”) for use during fabrication of beef carcasses that will limit or reduce microbial counts on beef cuts and trimmings, as well as reduce the incidence of *E. coli* O157:H7-positive combo-bins of beef trimmings, and, (b) to identify a protocol for decontamination of beef cuts, intended for blade/needle or moisture-enhancement tenderization, and reduction of risk of finding *E. coli* O157:H7 inside the beef cuts following blade/needle or moisture-enhancement tenderization.

V. Background Information About the Need for This Research:

In October 1994, in response to an outbreak of *Escherichia coli* O157:H7 (*E. coli* O157:H7) that resulted in several deaths from the consumption of undercooked ground beef, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) declared *E. Coli* O157:H7 to be an adulterant under the Federal Meat Inspection Act (FMIA) and implemented a sampling program for raw ground beef prepared in federally inspected plants and in retail stores (FSIS policy, January 1999). The beef industry remains highly concerned about the possibility that meatborne pathogens, specifically *E. coli* O157:H7 will be present on beef cuts, in ground beef,

and—potentially—in mechanically tenderized (blade-tenderized and moisture-enhanced) beef products. Much has been done, using multiple-hurdle microbiological decontamination interventions during slaughtering/dressing, to reduce the risk that pathogens will be present on beef carcasses following chilling (Ransom *et al.*, 2002). But, more remains to be done to further reduce risk and to minimize cross-contamination of beef subprimal cuts and trimmings during fabrication.

Psychotropic and mesophilic bacterial populations can readily proliferate in conditions approximating mild to moderate temperature abuse, and primal cuts, subprimal cuts and trimmings can be further contaminated during fabrication (Phebus *et al.*, 1997; Samelis *et al.*, 2001). Cross-contamination occurs directly, between carcasses, primal cuts and trimmings, or indirectly, from contact with hands/knives of employees or meat-contact surfaces. Gill *et al.* (1999) reported that *E. coli* counts increased on beef surfaces during fabrication of carcasses into subprimal cuts because debris containing large populations of aerobic bacteria was obscurely located on equipment and, when bacteria-harboring equipment was running, even before product passage, bacteria were transferred to meat-contact surfaces, ultimately resulting in product contamination. Kain *et al.* (2003) followed beef through fabrication, transportation/distribution and retail preparation, to supermarkets in five states; APC, TCC, ECC, *Listeria* spp. and *Listeria monocytogenes* increased ($P < .05$) from carcasses to subprimal cuts, at the packing plant, but not thereafter. Those researchers attributed the dramatic increases in bacterial counts/incidences to cross-contamination of product, primarily originating from conveyor belts. Gill *et al.* (2001) reported that TCC and ECC on primal cut surfaces were approximately 2 logs higher than those recovered from carcasses and were comparable to those recovered from fabrication tables; contamination of primal cuts from table surfaces was implicated.

Various treatments have been designed to reduce bacterial levels, specifically *Escherichia coli* O157:H7, from beef carcasses pre- and post-chill. These treatments include the use of sanitizing products, such as lactic acid and hot water. The use of lactic acid for such purposes was approved by the USDA, as an antimicrobial step, on beef carcasses prior to chilling and fabrication nine years ago (FSIS, 1996) and more recently,

5.0% lactic acid. These practices have not been approved for use on beef subprimals or trimmings, but if the proposed study yields positive results, FSIS-USDA might consider such approval. Bacon *et al.* (2002) studied efficacy of an ambient-temperature 1.5% to 2.5% lactic acid solution applied to carcasses, fabrication table surfaces and/or subprimal cuts, and reported results indicating only minimal effects in reducing bacterial contamination. Ellebracht *et al.*(1999) found that dipping beef trimmings into hot water (95°C) reduced the level of *E. coli* O157:H7 by 0.5 log₁₀ CFU/g, and by dipping beef trimmings into hot water followed by 2% lactic acid reduced *e. coli* O157:H7 levels by 1.6 log₁₀ CFU/g. Following a similar protocol, Ellebracht *et al.* (2005) dipped beef trimmings in a warm (55°C) 2% lactic acid solution following a water dip and found a 1.3 log₁₀ CFU/cm² reduction *E. coli* O157:H7. Castillo *et al.* (1998) utilized a spray cabinet to apply a water wash, hot water wash and warm (55°C) lactic acid wash and found a 4.5, 5.0 and 4.48 log reduction of *E. coli* O157:H7 from outside round, brisket and clod surfaces, respectively (initial levels were approximately 5.0 log CFU/cm²). Spraying, rather than misting or dipping the lactic acid solutions will better simulate practical in-plant application. These results indicate that lactic acid at concentrations of at least 2% when heated to a warm (55°C) temperature may increase the efficacy of lactic acid, and in a multiple hurdle system, be especially useful in reducing bacterial levels on beef primals and trimmings.

FSIS-USDA expanded the *E. coli* O157:H7 adulteration policy to include non-intact products (blade/needle tenderized primals, restructured meat, chemically injected/enhanced meat) because bacteria can be translocated to the interior of the finished product (Krizner, 1999) and survive the cooking process (Ortega-Valenzuela *et al.*, 2001). Dolezal *et al.* (2002) expressed industry concerns about use of these technologies fearing that *E. coli* O157:H7 might be transferred from outside-surfaces to the interior of meat cuts and thus present a public health threat. Seven months later that fear materialized when FSIS announced that Stampede Meat, Inc. (Chicago, IL) had voluntarily recalled 739,000 pounds of frozen beef products that might have been contaminated with *E. coli* O157:H7 (www.fsis.usda.gov/OA/recalls/rec_actv.htm; June 29, 2003). Steaks are not generally considered a high-risk source of *E. coli* O157:H7, the

products subject to recall were injected with tenderizing and flavor-enhancing solutions, and that process may have transferred the bacteria from the surface to the inside of the product where it could have survived cooking if the internal temperature of the meat did not reach 160°F (www.meatnews.com/index.cfm?fuseaction=Particle&artNum=565; July 1, 2003).

A study of incidence of *E. coli* O157:H7 on surfaces of beef cuts intended for blade/needle tenderization by Warren-Serna *et al.* (2002) revealed a 0.2% occurrence on 1,014 cuts from six packing plants or purveyors geographically dispersed throughout the U.S. The fact that *E. coli* O157:H7 does (albeit rarely) occur on beef primal/subprimal cuts generates risk of its entry into cuts when blade/needle or moisture-enhancement tenderization technologies are used, and its probability of occurrence is dramatically increased if improper cleaning/sanitizing of equipment is practiced. Gill and McGinnis (2004) collected 25 samples from four grocery stores, two of 100 samples, both from the same store, had detectable levels of *E. coli* on the internal surface of the product, further indicating that the risk of transferring *E. coli* O157:H7 to the interior of muscle samples is low and often dependent on site specific cleaning and sanitation programs.

Blade tenderization has been found to transfer 3 to 4% (Hajmeer *et al.*, 2000; Phebus *et al.*, 2000) or 1 to 7% (Lambert *et al.*, 2001) of surface contamination to the interior of the muscle; needle injection (during enhancement) results in 4 to 8% translocation of surface contamination to the center of the cut (Lambert *et al.*, 2001). To minimize risk of transferring *E. coli* O157:H7 from the exterior, to the interior, of a solid-muscle cut, primals/subprimals intended for blade/needle or moisture-enhancement tenderization should be decontaminated prior to use of either of these invasive technologies. Gill and McGinnis (2005) indicate that contamination of deep tissue in mechanically tenderized meat can be minimized when machines and blades are designed to minimize the number of bacteria carried below the incised surface.

The packer, purveyor and food-service operator sectors need decontamination protocols and/or microbiological interventions for use on beef primal/subprimal cuts that will

subsequently be blade/needle tenderized or moisture-enhanced. Some companies (e.g., Excel/Cargill) have not produced moisture-enhanced beef, partially because of fears of recalls, litigation and endangering public health. By utilizing microbial interventions that result in adequate bacteriological reductions prior to tenderizing/pumping beef primal/subprimal cuts, these important palatability-improvement technologies can continue to be used commercially with substantially mitigated risk of a foodborne illness incident or outbreak. The most promising technology for this purpose involves use of warm (55°C) 2.5% or 5.0% lactic acid.

VI. Achievement of the Specific Objective Stated in the Proposal:

Samples were collected during June 2004. A total of 120 outside round pieces were inoculated and subjected to three antimicrobial interventions and subsequent blade tenderization or moisture enhancement. At the beginning of the study, 72 outside round pieces were treated and processed. The prevalence of *E. coli* 0157:H7 within the interior of treated and processed outside round pieces was 98.6% (71 positives).

As these results were obtained, the research team redesigned the experiment to include the enumeration of *E. coli* 0157:H7 at 3 locations during the procedure to isolate the effect of the antimicrobial intervention and the transference of *E. coli* 0157:H7 to the interior of cuts. From this point forward, an additional 96 outside round pieces were treated with an antimicrobial intervention and either blade tenderized or moisture enhanced. During this process, surface samples were taken prior to application of an intervention, following application of an intervention, and from the internal surface of blade tenderized or moisture enhanced products. This resulted in a total of 288 *E. coli* 0157:H7 enumerations from the external and internal surfaces of inoculated beef outside rounds treated with one of three antimicrobial interventions and subjected to either blade tenderization or moisture enhancement.

The in-plant portion of the study has not been completed; USDA-FSIS approval is required to continue as planned. A pilot study has been submitted to USDA-FSIS in support of the continuation of this study and a response is be awaited.

VII. **Materials and Methods:**

Inoculation Cultures

Three strains of *Escherichia coli* O157:H7 (ATCC 35150, FSNS 4312-4, and FSIS EC465-97) were grown and a cocktail was prepared by streaking frozen stocks onto Tryptic Soy Agar (TSA - Difco 236950) and incubating for 24 hours at 35°C. Cultures were transferred to 100 ml of Tryptic Soy Broth (TSB - Difco 211822) and incubated an additional 18-24 hours at 35°C. Liquid cultures were combined in a cocktail, centrifuged, washed three times with Butterfield's Phosphate Buffer (BPB) and resuspended in fresh TSB. The cocktail was diluted and streaked onto the surface of the outside-round pieces for a final target concentration of 10^2 colony forming units (CFU) of *E. coli* O157:H7/100 cm² of sample surface).

Inoculation of Outside-Round Pieces

Outside rounds were obtained from a commercial packing company and cut into equal halves. Product delivery was spread over a five-week period in order to allow time for experiments to be performed. Each shipment of product was inoculated at Food Safety Net Services (San Antonio, Texas) with a target of 10^2 CFU/cm² (inoculation populations were determined on 12, 100 cm² surface samples; 3 samples/product shipment), application levels averaged 2.17 log CFU/cm². Inoculated outside-round pieces were individually vacuum packaged and stored for 10 to 18 days at 2 - 4°C.

Intervention Application

Samples were suspended from a sterilized meat hook and one of three pathogen interventions was applied to each outside round piece: (1) 20 s hot (82°C) water by spray application (HW), (2) 20 s warm (55°C) 2.5% lactic acid by spray application (2.5% LA), (3) 5.0% lactic acid by spray application (5% LA). Interventions were applied to each outside-round piece using a handheld sprayer (RL FLO-MASTER®, Root-Lowell Manufacturing, Lowell, MI). Outside-round pieces were allowed to sit for five minutes

before undergoing blade tenderization or moisture enhancement to simulate in-plant line speed.

Study A

Outside round pieces (N = 72) were inoculated with a target of 10^2 CFU/cm² of *E. coli* O157:H7 as previously described. Following storage, individual round pieces were subjected to one of five bacterial interventions (n = 24/intervention) and subjected to either blade tenderization (N = 36; n = 12/intervention/process) using a Honeywell blade tenderizer (Kansas City, MO) or needle-injected enhancement (N = 36; n = 12/intervention/process) using an Inject Star® (“NT”-“BI-52/72”, Brookfield, CT) and a solution of salt, phosphate and sodium lactate as described by Vote et al. (2000). Products were enhanced such that they reached 112% of their green weight. Following processing: (a) round pieces were suspended from a sanitized hook and the external surface was thoroughly seared with a propane torch, (b) the segment of the piece where the hook had been inserted was cut off and discarded, and (c) the remainder of each piece was ground once in a small, sanitized commercial meat grinder, placed in a whirl-pak bag and stored at 2 - 4°C. Ground samples were analyzed and the prevalence of *E. coli* O157:H7 was determined using PCR-Bax (Ransom et al., 2003a). Between samples, tables, cutting boards, the blade tenderizer and the injector were rinsed with cold water at a high pressure, and then sanitized with hot (82 - 90° C) water. Meat grinders were disassembled and thoroughly sanitized using soap and hot (82 - 90° C) water.

Study B

Outside round pieces (N = 96) were inoculated and interventions were applied as previously described. Individual round pieces were subjected to one of three bacterial interventions (n = 32/intervention) and subjected to either blade tenderization (N = 48; n = 16/intervention/process) using a Ross Tenderizer (Model TC700M, Elkwood, Virginia) or needle enhancement (N = 48; n = 16/intervention/process) using an Inject Star® (“NT”-“BI-52/72”, Brookfield, CT) and a solution of salt, phosphate and sodium lactate as described by Vote et al. (2000). Products were enhanced to a weight that was 112% of their green weight.

Samples (100 cm² surface samples) were collected upon removal from vacuum packages (pre-treatment), following the application of interventions (post-intervention) and following blade tenderization (BT) or moisture enhancement (ME) (post-processing). Following processing: (a) round pieces were suspended from a sanitized hook, the inoculated surface was carefully trimmed away with a sterilized knife, and the entire outside-round piece was thoroughly seared with a propane torch, (b) the segment of the piece where the hook had been inserted was cut off with a sterilized knife and discarded, (c) the remainder of each piece was placed on a sanitized cutting surface (inoculated side up) and, using a sanitized knife (80° C for > 1 minute), a 5 cm slice was removed from the geometric center of the cut, and (d) each slice was aseptically placed into a Whirl-pak™ sample bag. Samples were transported to the laboratory where they were removed from the sample bag and, using a sterile scalpel and aseptic technique, each slice was split into two, 2.5 cm thick, slices. A 100 cm² surface sponge sample was then collected from the newly exposed internal surface of one slice.

Between samples, all tables and all cutting boards were rinsed with cold water at a high pressure, scrubbed with soap and water, rinsed with cold water, sprayed with bleach (5 ppm) and then rinsed with hot (82 - 90° C) water. The tenderizer and injector were comparably sanitized, excluding the use of bleach (to prevent rusting and corrosion of the machinery). Environmental samples were collected each day of sampling from the blade tenderizer (1 positive out of 9 samples) and needle injector (0 positive out of 9 samples). Surface samples were analyzed to quantitatively determine the surface populations of *E. coli* 0157:H7 by direct plating on CT-SMAC and counting morphologically typical colonies. All results are reported in log CFU/cm².

Uninoculated (N = 40) outside round pieces were subjected to the interventions and processing treatments as previously described (n = 4/intervention/process).

Data Analysis

Mixed models procedures and General Linear Models procedures of SAS (Cary, NC) were used to compute least squares means and standard errors for *E. coli* 0157:H7 counts and reductions due to intervention or processing.

VIII. Results and Discussion

Study A

The prevalence of *E. coli* O157:H7 found within outside round pieces, inoculated with 2.17 log CFU/cm² of a *E. coli* O157:H7 cocktail, subjected to one of four antimicrobial interventions and further processed using a blade tenderizer or needle-injector was 97.2% (70 out of 72 samples). One sample sanitized with warm 2.5% LA (55° C) water and blade-tenderized did not return a positive PCR-BAX result (Table 1). These results can be attributed to the fact that the inoculation levels used in this study are far higher than levels of *E. coli* O157:H7 one would expect to find on uninoculated beef surfaces. Comparatively, Warren-Serna et al. (2002) reported levels <0.375 CFU/cm² (395 times lower than the inoculation level used in this study) on the surface of 2 of 1,014 cuts that were found to be positive for the pathogen.

Table 1. Number of positive samples/number of samples tested (% positive) for prevalence of *E. coli* O157:H7, as determined by PCR-BAX, after inoculated outside round pieces were subjected to one of four antimicrobial interventions and blade-tenderized (BT) or moisture-enhanced (ME). Inoculated, untreated outside-rounds served as Positive Controls.

	Positive Control^a	Hot Water (82° C)^b	Warm 2.5% LA (55° C)^c	Warm 5% LA (55° C)^d
BT	12/12 (100)	12/12 (100)	11/12 (91.7)	12/12 (100)
ME	12/12 (100)	11/12 (91.7)	12/12 (100)	12/12 (100)

^aPositive controls were inoculated and not subjected to an antimicrobial intervention.

^bHot water (82° C) was sprayed on the surface of each cut for 20 s.

^c2.5% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^d5.0% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

Study B

Product Storage

Inoculated outside rounds were vacuum packaged and stored (2-4° C) for 10 to 18 days, post-inoculation. Samples collected at the time of inoculation indicated that surface levels of a three-strain (ATCC 35150, FSNS 4312-4 and FSIS EC465-97) cocktail of *E. coli* O157:H7 were 2.17 log CFU/cm². Following vacuum packaging and refrigerated storage, surface samples were collected prior to application of any antimicrobial treatments or further processing. During storage, inoculated populations increased from an average of 2.17 log CFU/cm² to 3.4 to 3.7 log CFU/cm² indicating that *E. coli* O157:H7, when present in high levels, can survive and grow in vacuum packaged at refrigerated temperatures (Table 2).

Table 2. Least squares means \pm standard error of *E. coli* O157:H7 (log CFU/cm²) from the surface (minimum detection level = 1.0 CFU/cm²) of inoculated inside rounds prior to intervention application (PRE), from the surface of inoculated outside round pieces following antimicrobial intervention application (POST), reduction due to antimicrobial interventions (RED) and percent survival (SUR). Inoculated, untreated outside-rounds served as Positive Control (n=32 for each intervention treatment).

	Positive Control ^a	Hot Water (82° C) ^b	Warm 2.5% LA (55° C) ^c	Warm 5% LA (55° C) ^d
PRE	-	3.6 \pm 0.06 ^{xm}	3.6 \pm 0.06 ^{xm}	3.5 \pm 0.06 ^{xm}
POST	3.5 \pm 0.06 ^{xm}	2.6 \pm 0.06 ^{yn}	2.6 \pm 0.06 ^{yn}	2.4 \pm 0.06 ^{yn}
RED	N/A	1.0 \pm 0.06 ^{zm}	1.0 \pm 0.06 ^{zm}	1.1 \pm 0.06 ^{zm}
SUR	100	72.2	72.2	68.6

^aPositive controls were inoculated and not subjected to an antimicrobial intervention.

^bHot water (82° C) was sprayed on the surface of each cut for 20 s.

^c2.5% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^d5.0% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^{x,y,z}Least squares means, within columns, lacking common superscript letters, differ (P < .05).

^{mn}Least squares means, within rows, lacking common superscript letters, differ (P < .05).

Antimicrobial Interventions

Following inoculation and storage, outside round pieces were removed from their vacuum package and suspended on a sanitized hook. Each cut was then subjected to one of three antimicrobial interventions: (1) Hot (82° C) Water (HW), (2) 2.5% (55° C) Lactic acid (2.5% LA), and (3) 5% (55° C) Lactic acid (5% LA). Intervention treatments resulted in a 1.0, 1.0 and 1.1 log CFU/cm² reduction, respectively (Table 2). Survival of *E. coli* O157:H7 ranged from 68.6 to 100 % of pre-intervention surface levels and all interventions equally ($P > .05$) reduced surface levels with numerical reductions resulting from antimicrobial interventions being 5%LA>2.5% LA=HW.

Blade Tenderization and Needle-Injection/Enhancement

Inoculated outside round pieces were surface (100 cm²) sampled prior to the application of an antimicrobial intervention, following one of three antimicrobial interventions and following blade tenderization. As previously described, antimicrobial interventions resulted in a 1.0, 0.9 and 1.0 log CFU/cm² reduction ($P < .05$) of *E. coli* O157:H7 on the surface of inoculated outside rounds treated with HW, 2.5% LA and 5% LA prior to blade tenderization (Table 3). Internal surface samples from decontaminated outside rounds subjected to blade tenderization resulted in lower ($P < .05$) *E. coli* O157:H7 counts compared to post-intervention and pre-intervention surface samples and all samples subjected to an antimicrobial intervention resulted in *E. coli* O157:H7 counts below detectable limits of the analysis (Table 3).

Table 3. Least squares means \pm standard errors for *E. Coli* O157:H7 (log CFU/cm²) recovered (minimum detection level = 1.0 CFU/cm²) from the external surface of inoculated outside round pieces prior to application of an antimicrobial intervention (PRE) and following an antimicrobial intervention (POST), and internal surface levels of *E. coli* O157:H7 (cfu/cm²) following blade tenderization (BT). Inoculated, untreated outside-rounds served as Positive Control (n = 16 for each intervention treatment).

	Positive Control ^a	Hot Water (82° C) ^b	Warm 2.5% LA (55° C) ^c	Warm 5% LA (55° C) ^d
PRE	-	3.6 ± 0.08 ^x	3.5 ± 0.08 ^x	3.4 ± 0.08 ^x
POST	3.4 ± 0.08 ^x	2.6 ± 0.08 ^y	2.6 ± 0.08 ^y	2.4 ± 0.08 ^y
BT	1.0 ± 0.08 ^y	0.9 ± 0.08 ^z	0.9 ± 0.08 ^z	0.9 ± 0.08 ^z

^aPositive controls were inoculated and not subjected to an antimicrobial intervention.

^bHot water (82° C) was sprayed on the surface of each cut for 20 s.

^c2.5% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^d5% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^{x,y,z}Least squares means, within columns, lacking common superscript letters, differ (P < .05).

Needle-injection/enhancement of inoculated outside round pieces, following treatment with an antimicrobial intervention resulted in similar levels of transference of *E. coli* O157:H7 compared to blade tenderization (Table 4). The combination of an antimicrobial intervention and further processing resulted in less than 1.0% transference of inoculated pathogen loads to the internal surface of outside round pieces when subjected to one of three antimicrobial interventions (Table 5). Inoculated, untreated controls, once again demonstrate that needle-injection/enhancement results in numerically higher internalization rates of surface pathogens compared to blade tenderization. This indicates that the needle-injection/enhancement process more efficiently internalizes surface bacteria, especially when elevated levels of organisms are present.

Table 4. Least squares means ± standard error (% transference from post-intervention surface loads) for reductions in *E. coli* O157:H7 (log CFU/cm²) recovered from internal surface samples (100 cm²) of inoculated, outside round pieces compared to external surface levels of *E. coli* O157:H7 following the application of one of four antibacterial interventions and blade tenderization (BT) or moisture enhancement (ME). Inoculated, untreated outside-rounds served as Positive Controls (n = 16/intervention/process).

	Positive Control ^a	Hot Water (82° C) ^b	Warm 2.5% LA (55° C) ^c	Warm 5% LA (55° C) ^d
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BT	2.4 ± 0.08 (0.82) ^y	1.7 ± 0.08 (3.63) ^z	1.7 ± 0.08 (3.71) ^z	1.5 ± 0.08 (4.52) ^z
ME	1.5 ± 0.08 (3.88) ^z	1.3 ± 0.08 (5.26) ^z	1.3 ± 0.08 (5.54) ^z	1.3 ± 0.08 (6.08) ^z

^aPositive controls were inoculated and not subjected to an antimicrobial intervention.

^bHot water (82° C) was sprayed on the surface of each cut for 20 s.

^c5.0% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^d5.0% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^{yz}Least squares means, lacking common superscript letters, differ (P < .05).

Table 5. Least squares means ± standard error (% transference from pre-intervention surface loads) for reductions in *E. coli* O157:H7 (log CFU/cm²) recovered from internal surface samples (100 cm²) of inoculated, outside round pieces compared to external surface levels of *E. coli* O157:H7 prior to the application of one of four antibacterial interventions and blade tenderization (BT) or moisture enhancement (ME). Inoculated, untreated outside-rounds served as Positive Controls (n = 16/intervention/process).

	Positive Control ^a	Hot Water (82° C) ^b	Warm 2.55% LA (55° C) ^c	Warm 5% LA (55° C) ^d
BT	2.4 ± 0.06	2.7 ± 0.06	2.7 ± 0.06	2.5 ± 0.06

	(0.82) ^y	(0.41) ^y	(0.40) ^y	(0.51) ^y
ME	1.5 ± 0.06 (3.88) ^z	2.4 ± 0.06 (0.59) ^y	2.3 ± 0.06 (0.82) ^{xy}	2.5 ± 0.06 (0.53) ^x

^aPositive controls were inoculated and not subjected to an antimicrobial intervention.

^bHot water (82° C) was sprayed on the surface of each cut for 20 s.

^c2.5% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^d5.0% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^{x,y,z}Least squares means, within row, lacking common superscript letters, differ (P < .05).

Similar to results observed when inoculated outside rounds were subjected to an antimicrobial intervention followed by blade tenderization, surface levels of *E. coli* O157:H7 on inoculated outside rounds subjected to an antimicrobial intervention decreased (P < .05, Table 3). Levels of *E. coli* O157:H7 on the internal surfaces of needle-injected/enhanced products were numerically higher than those found on internal surfaces of blade tenderized products, but again, surface levels of *E. coli* O157:H7 on inoculated outside rounds subjected to an antimicrobial intervention decreased (P < .05, Table 6). These results indicate that surface interventions effectively reduce pathogen levels on the external surface of beef cuts and that the risk of internalizing pathogens from the surface of products can be reduced through the application of antimicrobial interventions prior to further processing.

Table 6. Least squares means ± standard errors for *E. Coli* O157:H7 (log CFU/cm²) recovered from the external surface (minimum detection level = 1.0 CFU/cm²) of inoculated outside round pieces prior to application of an antimicrobial intervention (PRE) and following an antimicrobial intervention (POST), and internal surface levels of *E. coli* O157:H7 (cfu/cm²) following needle-injection/enhancement (ME). Inoculated, untreated outside-rounds served as Positive Control (n = 16 for each intervention treatment).

	Positive Control ^a	Hot Water (82° C) ^b	Warm 2.5% LA (55° C) ^c	Warm 5% LA (55° C) ^d
PRE	-	3.6 ± 0.08 ^{mx}	3.7 ± 0.08 ^{mx}	3.6 ± 0.08 ^{mx}
POST	3.6 ± 0.08 ^{ox}	2.5 ± 0.08 ^{my}	2.7 ± 0.08 ^{my}	2.4 ± 0.08 ^{ny}

ME 2.1 ± 0.08^{oy} 1.2 ± 0.0^{mnz} 1.4 ± 0.08^{mz} 1.2 ± 0.08^{nz}

^aPositive controls were inoculated and not subjected to an antimicrobial intervention.

^bHot water (82° C) was sprayed on the surface of each cut for 20 s.

^c2.5% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^d5% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^{x,y,z}Least squares means, within columns, lacking common superscript letters, differ (P < .05).

^{mno}Least squares means, within rows, lacking common superscript letters, differ (P < .05).

Uninoculated Outside-Round Pieces

In order to more closely simulate conditions likely to be encountered in U.S. beef processing facilities, uninoculated outside rounds (N = 40) were subjected to one of three antimicrobial interventions prior to either blade tenderization (Table 7) or needle-injection/enhancement. Prior to antimicrobial interventions, 23 of 24 uninoculated outside rounds (12 subjected to blade tenderization and 12 subjected to needle-injection/enhancement) had undetectable levels of *E. coli* O157:H7. The uninoculated sample positive for *E. coli* O157:H7 may have been cross-contaminated while in the research facility, though extensive measures were taken to sterilize all work surfaces and equipment. Following application of one of three antimicrobial interventions neither the subsequent external nor internal surface sample was found to have detectable levels of *E. coli* O157:H7 following blade tenderization (Table 7). These results support those reported by Warren-Serna et al. (2002) that the incidence of *E. coli* O157:H7 on the surface of beef subprimals is low and that the application of an antimicrobial intervention prior to blade tenderization or needle-injection/enhancement will reduce these low levels to non-detectable levels.

Table 7. Means of *E. Coli* O157:H7 (log CFU/cm²) recovered from the external surface (minimum detection level = 1.0 CFU/cm²) of uninoculated outside round pieces prior to application of an antimicrobial intervention (PRE) and following an antimicrobial intervention (POST), and internal surface levels of *E. coli* O157:H7 (cfu/cm²) following blade tenderization (BT). Inoculated, untreated outside-rounds served as Positive Control (n = 4 for each intervention treatment).

	Control ^a	Hot Water (82° C) ^b	Warm 2.5% LA (55° C) ^c	Warm 5% LA (55° C) ^d
PRE	-	< 1.0	< 1.0	1.0

POST	< 1.0	< 1.0	< 1.0	< 1.0
BT	<1.0	< 1.0	< 1.0	< 1.0

^aControls were not subjected to an antimicrobial intervention.

^bHot water (82° C) was sprayed on the surface of each cut for 20 s.

^c2.5% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

^d5% lactic acid (55° C) was sprayed on the surface of each cut for 20 s.

Conclusions

Results of this study indicate that when surface levels of *E. coli* 0157:H7 are several hundred fold higher than those reported in national surveys, application of antimicrobial interventions of hot (82° C) water, warm (55° C) 2.5% lactic acid, or warm (55° C) 5% lactic acid can reduce pathogen loads on the surface of subprimal cuts and, for those subjected to further processing, can reduce the internalization of surface pathogens. Both blade tenderization and needle-injection/enhancement resulted in the transmission of pathogen from external surfaces into internal surfaces of inoculated subprimals. Needle-injection/enhancement resulted in greater transmission rates compared to blade tenderization. Implementation of a surface intervention, prior to further processing, would reduce the risk of pathogenic organisms being internalized as well as reducing the risk of encountering foodborne illness from non-intact, blade-tenderized or needle-injection/enhanced beef products.

IX. Publications, Abstracts, Manuscripts in Progress, Thesis or Presentations that Resulted from this Research:

A manuscript will be prepared for submission to a refereed scientific journal and an abstract has been submitted to the International Association for Food Protection for presentation at their annual scientific meeting, August 14-17, 2005 in Baltimore, MD.

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