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Antimicrobial Efficacy of a Lactic Acid and Citric Acid Blend against Shiga Toxin–Producing *Escherichia coli, Salmonella,* and Nonpathogenic *Escherichia coli* Biotype I on Inoculated Prerigor Beef Carcass Surface Tissue

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ABSTRACT

Studies were conducted to (i) determine whether inoculants of nonpathogenic *Escherichia coli* biotype I effectively served as surrogates for *E. coli* O157:H7, non-O157 Shiga toxin–producing *E. coli,* and *Salmonella* when prerigor beef carcass tissue was treated with a commercially available blend of lactic acid and citric acid (LCA) at a range of industry conditions of concentration, temperature, and pressure; (ii) determine the antimicrobial efficacy of LCA; and (iii) investigate the use of surrogates to validate a hot water and LCA sequential treatment as a carcass spray intervention in a commercial beef harvest plant. In an initial laboratory study, beef brisket tissue samples were left uninoculated or were inoculated (~6 log CFU/cm^2) on the adipose side with *E. coli* O157:H7 (5-strain mixture), non-O157 Shiga toxin–producing *E. coli* (12-strain mixture), *Salmonella* (6-strain mixture), or nonpathogenic *E. coli* (5-strain mixture). Samples were left untreated (control) or were treated with LCA, in a spray cabinet, at one of eight combinations of solution concentration (1.9 and 2.5%), solution temperature (43 and 60°C), and application pressure (15 and 30 lb/in^2). In a second study, the *E. coli* surrogates were inoculated (~6 log CFU/cm^2) on beef carcasses in a commercial facility to validate the use of a hot water treatment (92.2 to 92.8°C, 13 to 15 lb/in^2) followed by an LCA treatment (1.9%, 50 to 51.7°C, 13 to 15 lb/in^2, 10 s). In the in vitro study, surrogate and pathogen bacteria did not differ in their response to the tested LCA treatments. Treatment with LCA reduced ($P < 0.05$) inoculated populations by 0.9 to 1.5 log CFU/cm^2, irrespective of inoculum type. The hot water and LCA sequential treatments evaluated in the commercial facility reduced ($P < 0.05$) the inoculated nonpathogenic *E. coli* surrogates on carcasses by 3.7 log CFU/cm^2. This study therefore provides the meat industry with data for this sequential multiple hurdle system for the operation parameters described.

Considerable effort has been expended since the 1990s to control pathogens in fresh beef products *(1–3, 19, 23, 29, 30, 34)*. *Escherichia coli* O157:H7, non-O157 Shiga toxin–producing *E. coli* (STEC), and *Salmonella* are regarded as the pathogens of public health concern in these products *(1–3, 7, 8, 19, 23, 30, 33)*. The greatest progress for control of pathogens in beef began after *E. coli* O157:H7 was declared an adulterant in ground beef in 1993 and then again in 2012 when six non-O157 serogroups of STEC (O26, O45, O103, O111, O121, and O145) were also declared adulterants *(33, 34)*. Industry and government agencies have invested in research efforts to reduce pathogens on beef and have shown success. Results of the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) microbiological testing of raw ground beef and raw ground beef components for *E. coli* O157:H7 and non-O157 STEC have shown a decline in the number of positive samples between 2001 and 2012, from 0.77 to 0.24% *(34)*.

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Pathogens that contaminate beef products primarily originate from cattle hides *(4, 6–8, 9, 23)*. Multiple hurdle technology is the most common and most effective method for controlling risk of pathogens on meat *(17, 23, 29, 32)*. These systems include several sequential food safety interventions in a process to continually provide control through reduction of pathogens *(4, 17, 23, 29, 32)*. At present, physical and chemical interventions such as steam pasteurization, hot water treatments, and antimicrobial solution treatments are the most effective interventions used in a U.S. beef harvest floor *(14–16, 23, 30, 32)*. A variety of chemical interventions have been considered for use in the meat industry, with various efficacies and costs *(9, 14–16, 23, 30, 32)*.

The USDA-FSIS requires verification of process control by validating that critical control point food safety interventions are effective against prevalent pathogens (9 CFR 413, 417, 430). Using pathogen testing or natural meat microflora to verify process control may be difficult due to the low number of bacterial cells naturally present on products *(2)*. The use of high levels of nonpathogenic

TABLE 1. *Pathogenic strains used in the study*

Inoculum	Serotype/serogroup	Strain ID	Origin	Source
E. coli O157:H7	O157:H7	ATCC 43895	Raw hamburger	ATCC[a]
	O157:H7	C1-072	Bovine feces	Carlson et al. *(12)*
	O157:H7	C1-109	Bovine feces	Carlson et al. *(12)*
	O157:H7	C1-154	Bovine feces	Carlson et al. *(12)*
	O157:H7	C1-158	Bovine feces	Carlson et al. *(12)*
Non–O157 STEC	O26:H11	hSTEC_03	Human	USMARC[b]
	O26:H2	93.0494	Human	ERC[c]
	O45	99E_2750	Human	USMARC
	O45	O45-2	Human	USMARC
	O103	MDR0089	Beef	USMARC
	O103:H2	90.1764	Cow	ERC
	O111:H8	hSTEC_08	Human	USMARC
	O111	4.0522	Cow	ERC
	O121	10896	Human	USMARC
	O121	imp_450	Beef	USMARC
	O145:NM	hSTEC_22	Human	USMARC
	O145	MAY109	Beef	USMARC
Salmonella	Agona	Nonfed plant 1	Cattle hides	Bacon et al. *(5)*
	Anatum	Fed plant 3	Cattle hides	Bacon et al. *(5)*
	Saint-Paul	Fed plant 2	Cattle hides	Bacon et al. *(5)*
	Reading	Fed plant 1	Cattle hides	Bacon et al. *(5)*
	Newport	FSL S5-436	Bovine	Cornell University[d]
	Typhimurium DT104 var. Copenhagen	Fed plant 2	Cattle hides	Bacon et al. *(5)*

[a] American Type Culture Collection.
[b] Dr. Tommy Wheeler, U.S. Meat Animal Research Center, USDA Agricultural Research Service, Clay Center, NE.
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[d] Dr. Martin Wiedmann, Department of Food Science, Cornell University, Ithaca, NY.

surrogate microorganisms that mimic the behavior of specific pathogens may be a helpful mechanism to validate efficacy of a food safety intervention in a commercial beef harvest facility and to provide evidence that a system is working properly *(2, 11, 18, 21, 22, 25, 27)*. One antimicrobial that is composed of a combination of lactic acid and citric acid has not been adequately researched to provide validating evidence to beef processing facilities in regard to its effectiveness against pathogens.

Thus, the objectives of an initial laboratory-conducted study were to (i) determine whether inoculants of nonpathogenic *E. coli* biotype I effectively served as surrogates for *E. coli* O157:H7, non-O157 STEC, and *Salmonella* when prerigor beef carcass tissue was treated with a commercially available blend of lactic acid and citric acid (LCA) at a range of industry conditions of concentration, temperature, and application pressure; and (ii) determine the antimicrobial efficacy of LCA against the tested inocula. The objective of a second in-plant–conducted study was to use the surrogate organisms to validate that a hot water treatment, in sequence with an LCA spray, effectively reduces microbial contamination on carcass surfaces in a commercial beef harvest facility.

MATERIALS AND METHODS

Bacterial strains and preparation of inocula. Four inoculum mixtures were used in the laboratory study: (i) a 5-strain mixture of rifampin-resistant *E. coli* O157:H7 (Table 1), (ii) a 12-strain mixture of rifampin-resistant non-O157 STEC (two strains each of serogroups O26, O45, O103, O111, O121, and O145; Table 1), (iii) a 6-strain mixture of *Salmonella* (Table 1), and (iv) a 5-strain mixture of rifampin-resistant nonpathogenic *E. coli* biotype I (ATCC BAA-1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, and ATCC BAA-1431 *(21, 25, 27)*) that could serve as surrogates for *E. coli* O157:H7, non-O157 STEC, and *Salmonella* spp. Spontaneous rifampin-resistant mutants of the *E. coli* O157:H7, non–O157 STEC, and nonpathogenic *E. coli* strains were used for selection and differentiation of the inocula from natural meat–associated microbial populations. These strains have previously been used in various inoculated challenge studies conducted with beef products in our laboratory *(15, 28, 31)*. Xylose lysine deoxycholate (XLD; Acumedia, Neogen Corp., Lansing, MI) agar was used for selective enumeration of the *Salmonella* inoculum.

Stock cultures of the strains were maintained at −70°C in tryptic soy broth (TSB; Difco, BD, Sparks, MD) containing 15% glycerol (for *Salmonella* serotype strains) or in TSB supplemented with 100 μg/ml rifampin (Sigma, St. Louis, MO) and glycerol (15%) (for all rifampin-resistant *E. coli* strains). Working cultures of the *Salmonella* serotype strains were maintained at 4°C on XLD agar and those of the rifampin-resistant *E. coli* strains on tryptic soy agar (TSA; Acumedia, Neogen Corp.) supplemented with rifampin (100 μg/ml; TSA+rif). Before the start of each experiment, bacterial strains were individually cultured and subcultured (35°C, 24 ± 2 h) in 10 ml of TSB (for *Salmonella* serotype strains) or TSB supplemented with rifampin (100 μg/ml; for all rifampin-resistant *E. coli* strains). After subculturing, cultures of the strains belonging to the same inoculum type were combined, and cells were harvested by centrifugation (Eppendorf model 5810 R, Brinkmann Instruments Inc., Hamburg, Germany) at 3,220 × *g* for 20 min at 4°C. Cell pellets were washed with 10 ml of phosphate-buffered saline (PBS, pH 7.4; Sigma), recentrifuged,

TABLE 2. *For each treatment combination,* P *values express the direct comparison of each pathogen group to the nonpathogenic* E. coli *surrogates for plate count results obtained with tryptic soy agar from warm beef carcass surface tissue treated with a blend of lactic acid and citric acid (LCA) at various combinations of solution temperature, application pressure, and solution concentration*[a]

LCA treatment combination			*P* value		
Temp (°C)	Pressure (lb/in^2)	Concn (%)	*E. coli* O157:H7 vs surrogates	Non-O157 STEC vs surrogates	*Salmonella* vs surrogates
43	15	1.9	0.8316	0.3428	0.2396
43	30	1.9	0.1552	0.0751	0.2447
60	15	1.9	0.9770	0.3136	0.7929
60	30	1.9	0.3887	0.3300	0.6287
43	15	2.5	0.3412	0.2724	0.2499
43	30	2.5	0.4166	0.4652	0.0056
60	15	2.5	0.0830	0.1103	0.0591
60	30	2.5	0.9257	0.7481	0.3771

[a] *P* value $\geq$ 0.05 indicates no difference in behavior between the pathogenic group and the surrogate inoculum.

and resuspended in PBS to the original volume (*E. coli* O157:H7 [50 ml], non-O157 STEC [120 ml], *Salmonella* [60 ml], and surrogates [50 ml]). The cell concentrations of the inoculum suspensions were ~8 to 9 log CFU/ml.

The inoculum mixture used for inoculation of carcasses in the commercial beef plant consisted of the parental strains of the five nonpathogenic *E. coli* strains: ATCC BAA-1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, and ATCC BAA-1431. The strains were individually cultured and subcultured (35°C, 24 ± 2 h) in 10 ml of TSB. Broth cultures of all five strains were then combined, and the cells were harvested and washed as previously described. Resulting cell pellets were resuspended to the original volume in PBS to obtain a concentration of ~8 log CFU/ml.

Sample collection and inoculation of warm beef carcass surface tissue. For the initial laboratory study, 200 sections in total of prerigor, warm (i.e., not chilled) beef carcass surface brisket tissue were collected from carcasses after being subjected to a hot water treatment during harvest at a commercial beef processing facility in northern Colorado. The samples were transferred in insulated containers to the laboratory of the Center for Meat Safety & Quality, Department of Animal Sciences, at Colorado State University (Fort Collins) within 30 min of collection. Each of the warm (25 ± 5°C) beef brisket tissue sections was divided into two portions (10 by 10 cm^2) and randomly assigned to either an untreated (control) or an LCA treatment group. Beef tissue portions were either left uninoculated, for evaluation of the antimicrobial effects of LCA against natural microflora, or were spot inoculated on the external adipose side. Beef samples were spot inoculated with 0.2 ml of one of the four inoculum types for a target inoculation level of ~6 log CFU/cm^2. The drops of inoculum were randomly dispersed on the surface of the tissue and were not spread over the entire surface so as to more closely simulate a carcass contamination event occurring during beef harvesting procedures. Inoculated samples were allowed 15 min at room temperature for bacterial cell attachment; this time reflected the maximum amount of time that would be expected for bacterial cells to attach to the carcass surface under normal operating conditions at a commercial beef facility. After allowing time for cell attachment, control samples were immediately analyzed to obtain initial microbial plate counts, while the remaining samples were spray treated with LCA and then analyzed for numbers of surviving populations.

In the second study, conducted in a commercial beef harvest facility, the surrogate bacteria were used to validate the use of a hot water cabinet and an LCA spray cabinet, as a combined system, to control pathogenic *E. coli* and *Salmonella*. Carcass sides, after evisceration treatments, but before hot water treatment and acid treatment, were inoculated on the out-rail to allow ease of inoculation and to prevent carcasses from touching. The external carcass surface was inoculated within three separate zones (10 by 10 cm^2, with at least 5 cm between each zone), designated aseptically with carcass ink, on the chuck. Inoculation was performed using separate sterile sampling sponges (3M, St. Paul, MN) hydrated with 10 ml of the inoculum and then sponging (10 vertical and 10 horizontal passes) the carcass surface within the marked zones. Separate inoculum-hydrated sponges were used to inoculate each zone. The target inoculation level was ~6 log CFU/cm^2. After inoculation, carcasses were left stationary for ~10 min, for bacterial cell attachment, before exposure to the hot water and LCA treatment.

Antimicrobial treatment of beef samples. For the laboratory study, uninoculated and inoculated samples were randomly assigned to one of eight possible LCA (Beefxide, Birko Corp., Henderson, CO) treatments applied using a custom-built spray cabinet (Chad Co., Olathe, KS) designed to simulate a commercial beef carcass spray cabinet. Samples were aseptically placed on the spray cabinet hook to simulate a chain moving a carcass on the harvest floor; the chain speed was constant for all treatments (15-s duration, per sample, in cabinet; four samples per min). The LCA solution was applied, for ~5 s per sample, to uninoculated or inoculated beef tissue at a range of industry conditions of solution temperature (43 and 60°C), solution concentration (1.9 and 2.5%), and application pressure (15 and 30 lb/in^2) for eight treatments in total (Table 2). LCA-treated samples were left undisturbed for a 10-min dwell time before microbial analysis for numbers of surviving populations. Additional pieces of uninoculated beef (extra portion from each piece of beef brisket) were treated at each LCA application parameter and level for sample pH evaluation.

The in-plant study treatment parameters included a hot water cabinet (92.2 to 92.8°C water, ≥70°C carcass surface temperature measured with a multiprobe Datapaq temperature logger [Datapaq Food Tracker System, Datapaq, Inc., Derry, NH], 13 to 15 lb/in^2) followed by an LCA carcass spray intervention (1.9%, 50 to 51.7°C, 13 to 15 lb/in^2, 10 s), at a chain speed of ~165 head per h. Sampling of the carcass zones was conducted using a sterile sponge hydrated with 10 ml of Dey/Engley neutralizing broth (3M) via 10 horizontal passes, flipping the sponge over, and 10 vertical passes. Before entering the hot water cabinet, both an inoculated

and uninoculated (naturally contaminated) zone were sampled to serve as the "before treatment" sample to obtain initial plate counts for the whole system. The carcasses continued through the hot water cabinet followed by sampling of a second zone (the middle of the intervention system). The carcasses then continued through the LCA spray cabinet and the chain was stopped to sample a third set of inoculated and uninoculated treated zones to serve as the "after treatment" samples for the whole system.

Microbiological and pH analyses. Samples from the laboratory study were analyzed for total plate counts (for inoculated and uninoculated samples) and levels of inoculated populations (for inoculated samples). Each sample was placed in a Whirl-Pak bag (Nasco, Fort Atkinson, WI) to which 175 ml of Dey/Engley neutralizing broth (Difco, BD) was added, followed by pummeling (Masticator, IUL Industries, Barcelona, Spain) for 2 min. Sample rinsates were 10-fold serially diluted in 0.1% buffered peptone water (Difco, BD). Appropriate dilutions were then surface plated (0.1 or 1 ml) in duplicate onto TSA to determine total bacterial counts, onto TSA+rif for enumeration of inoculated rifampin-resistant *E. coli* populations, and onto XLD agar for inoculated *Salmonella* populations. Colonies were counted after incubation of plates at 35°C for 24 h (TSA+rif and XLD agar) or 25°C for 72 h (TSA).

To obtain sample pH values, uninoculated untreated (control) and LCA-treated samples were diluted (1:5 dilution) with deionized water followed by mechanical pummeling (2 min). The pH was measured with a calibrated pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO).

For the in-plant study, carcass sponge samples were sent to a commercial laboratory (Food Safety Net Services, Fresno, CA) for microbial analysis. Upon arrival, 15 ml of Butterfield's buffer (Hardy Diagnostics, Santa Maria, CA) was added to each sample for 25 ml of diluent in total (10 ml of Dey/Engley neutralizing broth and 15 ml of Butterfield's buffer). Samples were mechanically pummeled for 2 min and serially diluted (10-fold) in buffered peptone water. Appropriate dilutions were plated in duplicate to enumerate aerobic plate counts (Petrifilm aerobic count plates, 3M) and *Enterobacteriaceae* (EB) counts (Petrifilm EB count plates, 3M). Colonies on Petrifilm plates were enumerated after a 48-h incubation at 37°C.

Statistical analysis. The laboratory study was designed as a randomized complete block with a $2 \times 2 \times 2$ factorial. The study was repeated on 5 days, with $n = 5$ per treatment for each inoculum type. Bacterial populations were expressed as least-squares means for log CFU per square centimeter (standard error [SE]) calculated under an assumption of a lognormal distribution of plate counts. Data were evaluated using the Mixed Procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC), with independent variables including inoculum type, LCA concentration, LCA temperature, LCA application pressure, and the respective interactions. To compare surviving populations of the surrogates to those of each pathogen group (*E. coli* O157:H7, non-O157 STEC, and *Salmonella*) after application of LCA, data were analyzed using a Mixed Procedure such that the model included the plate counts of inoculated untreated (control) samples as a covariate to adjust least-squares means to a common pretreatment plate count. All differences were reported using a significance level of $\alpha = 0.05$. The study conducted in-plant was designed as a paired comparison replicated over 2 days, with $n = 40$ samples per treatment. Bacterial populations recovered were analyzed using the Mixed Procedure of SAS, and data are expressed as least-squares means. Data are presented as least-squares means with differences reported using a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Laboratory study: surrogate behavior. Based on counts recovered with TSA, the behavior of the surrogates was, in all but one treatment combination, not different ($P \geq 0.05$) from that of the pathogenic inocula in response to the treatment parameters of solution temperature (low = 43°C, high = 60°C), application pressure (low = 15 lb/in^2, high = 30 lb/in^2), and solution concentration (low = 1.9%, high = 2.5%) for application of LCA onto warm beef carcass surface tissue (Table 2). A difference ($P < 0.05$) of 0.3 log CFU/cm^2 was obtained between the surrogates and *Salmonella* inoculum for the treatment combination of low temperature (43°C), high pressure (30 lb/in^2), and high concentration (2.5%). Note that although this difference was found to be statistically significant ($P < 0.05$), a difference of 0.3 log units is not considered as microbiologically meaningful *(26)*. When plate counts recovered with the selective media (i.e., TSA+rif, XLD agar) were compared, no differences ($P \geq 0.05$) in behavior were detected between the surrogates and each of the tested pathogen inocula (data not shown).

Cabrera-Diaz et al. *(11)* evaluated fluorescent protein–marked nonpathogenic *E. coli* strains as potential surrogates for *Salmonella* and *E. coli* O157:H7 that could be used for validation purposes in commercial beef facilities. They compared growth kinetics, attachment properties, and acid and heat resistance of the nonpathogenic and pathogenic strains and found, overall, no difference ($P \geq 0.05$) in behavior between the groups. They determined *D*-values of the strains in heated PBS and found no difference ($P \geq 0.05$) in thermal resistance between the target pathogens and the surrogates *(11)*. Acid-resistant properties were compared using lactic acid–acidified PBS (pH 2.5 to 3.5), and it was determined that log reductions were the same ($P \geq 0.05$) for the surrogates, *E. coli* O157:H7, and most of the *Salmonella* strains. Specifically, some of the *Salmonella* serotype strains were less acid resistant than the surrogate bacteria *(11)*. Although the methods of the study by Cabrera-Diaz et al. *(11)* and the present study were different, the objectives were similar. The present study used a specific intervention to determine whether the surrogate organisms would appropriately represent the pathogens, whereas Cabrera-Diaz et al. *(11)* took a more general approach to determine whether the surrogate organisms behaved as the pathogens with respect to growth, attachment, and resistance properties.

Ingham et al. *(20)* used a more direct approach to compare the potential of a five-strain *E. coli* biotype I mixture, a five-strain non–*E. coli* coliform mixture, and a lactic acid bacteria starter culture to serve as surrogates for *E. coli* O157:H7 for use in evaluation of beef dry aging. Ingham et al. *(20)* considered the surrogates suitable if the intervention produced a reduction in surrogate populations that was not greater ($P \geq 0.05$) than that observed for *E. coli* O157:H7; a similar criterion was used to draw conclusions

TABLE 3. *Adjusted least-squares mean plate counts obtained from selective and nonselective media for inoculated warm beef carcass surface tissue that was left untreated or was spray treated with a blend of lactic acid and citric acid*[a]

	Adjusted least-square mean (SE) counts (log CFU/cm^2)							
	E. coli O157:H7		Non-O157 STEC		*Salmonella*		Surrogate	
Culture medium[b]	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
TSA+rif/XLD	6.2 A (0.02)	5.3 B (0.04)	6.2 A (0.02)	5.2 B (0.06)	6.0 A (0.02)	4.5 B (0.04)	6.3 A (0.02)	5.2 B (0.06)
TSA	6.2 A (0.03)	5.2 B (0.04)	6.2 A (0.02)	5.3 B (0.06)	6.2 A (0.03)	4.9 B (0.05)	6.3 A (0.02)	5.2 B (0.06)

[a] The inoculation level was 6 log CFU/cm^2. Within each row and inoculum type, least-squares means with different letters are different ($P < 0.05$).

[b] Selective media included tryptic soy agar with rifampin (TSA+rif) for *E. coli* O157:H7, non-O157 STEC, and surrogates, and xylose lysine deoxycholate (XLD) agar for *Salmonella*; the nonselective medium was TSA.

in the present study. The investigators *(20)* treated samples with either a 6-day dry aging period or one of three acids (acetic acid, lactic acid, or Fresh Bloom [a mix of erythorbic, ascorbic, and citric acids]) followed by 1-day dry aging. All three tested surrogate inocula were found suitable as surrogates for *E. coli* O157:H7 for use in dry aging and acid spray treatments of beef *(20)*. In the present study, surrogate and pathogen inocula responded similarly when LCA was applied at a range of industry conditions of concentration, temperature, and application pressure on prerigor, warm beef carcass tissue.

Laboratory study: antimicrobial effect of LCA treatments. No main effects ($P \geq 0.05$) of treatment parameters (temperature, pressure, and concentration) were detected; thus, the data were combined and are presented as counts of LCA-treated samples versus counts of the untreated controls, for each inoculum type (Table 3). Counts of the tested pathogen inocula and nonpathogenic *E. coli* surrogates on untreated samples were 6.0 to 6.3 log CFU/cm^2 (Table 3). All tested treatment combinations effectively ($P < 0.05$) reduced bacterial contamination on inoculated warm carcass surface tissue by 0.9 to 1.5 log CFU/cm^2, depending on the inoculum type and recovery medium (Table 3). For the uninoculated samples, the average natural microbial contamination level on untreated prerigor beef carcass surface tissue was $\leq$1.3 log CFU/cm^2, and all samples had lower counts after treatment with LCA; total plate counts of 32.5% of the LCA-treated samples were below the detection limit (0.2 log CFU/cm^2) (data not shown).

Laury et al. *(24)* evaluated the effects of 2.5% LCA, applied as a spray or immersion treatment, against inoculated (6.5 log CFU/ml of carcass rinse solution) *Salmonella* populations on whole broiler carcasses and against inoculated (5.5 log CFU/100 cm^2) *Salmonella* and *E. coli* O157:H7 populations on beef trim (beef tips). *Salmonella* populations on poultry carcasses were reduced ($P < 0.05$) by 1.3 log CFU/ml with a 5-s spray treatment and by 2.3 log CFU/ml with immersion treatments of 5, 10, or 20 s *(24)*. Furthermore, LCA, applied in a spray cabinet, reduced ($P < 0.05$) *E. coli* O157:H7 populations by 1.4 log CFU/100 cm^2 and *Salmonella* populations by 1.1 log CFU/100 cm^2 *(24)*. Therefore, the reductions of *E. coli* O157:H7 and *Salmonella* achieved by Laury et al. *(24)* for inoculated beef trim are similar to those obtained in the present study. Overall, results from the present study indicated that LCA effectively ($P < 0.05$) reduced the surrogate and pathogen inocula when applied within a range of industry conditions of acid concentration and spray temperature and pressure on warm beef carcass surface tissue and that it could be used in a beef slaughter plant as an addition to multiple hurdle intervention systems.

pH analysis. There were no main effects ($P \geq 0.05$) of LCA treatment on the outcome of sample pH of warm beef carcass surface tissue. The pH values of samples from all the LCA treatment combinations were lower ($P < 0.05$) than those of the untreated samples. The pH of untreated beef tissue samples was 5.99, whereas the pH of the treated samples ranged from 4.62 to 5.01 (data not shown). There are no previous reports on the effect of LCA treatment on meat pH; however, in a study conducted by Cutter and Siragusa *(13)*, pH measurements were taken from beef carcass adipose tissue treated with 1, 3, or 5% lactic acid or citric acid. It was reported that all samples treated with the individual lactic acid and citric acid treatments had a lower ($P < 0.05$) pH than that of the untreated tissue samples *(13)*. The pH values for adipose beef carcass tissue treated with 3% citric acid or lactic acid were 3.55 and 3.72, respectively *(13)*.

In-plant validation. Aerobic plate counts (APC) and EB counts of 6.2 and 5.8 log CFU/cm^2, respectively, were obtained for inoculated zones of beef carcasses before entering the hot water cabinet and LCA spray cabinet (Table 4). The hot water cabinet on its own reduced ($P < 0.05$) APC and EB counts by 3.5 and 3.2 log CFU/cm^2, respectively, whereas when the hot water cabinet and LCA spray cabinet were used in sequence, APC and EB counts were reduced ($P < 0.05$) by 3.9 and 3.7 log CFU/cm^2, respectively (Table 4). In a study conducted by Bosilevac et al. *(10)* in a commercial beef harvest facility, naturally contaminated (i.e., uninoculated) hot beef carcasses were

TABLE 4. *Adjusted least-squares mean aerobic plate counts (APC) and* Enterobacteriaceae *(EB) counts obtained from inoculated and uninoculated warm beef carcass surface tissue before and after a hot water treatment, alone or in sequence with a lactic acid and citric acid (LCA) spray treatment*[a,b]

	Adjusted least-square mean (SE) counts (log CFU/cm^2)											
	Inoculated sample						Uninoculated sample					
	APC			EB			APC			EB		
Treatment	Before	After	*P* value	Before	After	*P* value	Before	After	*P* value	Before	After	*P* value
After hot water treatment	6.2 (0.11)	2.7 (0.10)	<0.0001	5.8 (0.12)	2.6 (0.11)	<0.0001	<1.2 (0.14)	<−0.2 (0.13)	<0.0001	<0.6 (0.12)	<−0.4 (0.10)	<0.0001
After sequential hot water treatment and LCA spray treatment	6.2 (0.11)	2.3 (0.10)	<0.0001	5.8 (0.12)	2.1 (0.10)	<0.0001	<1.2 (0.14)	<0.4 (0.12)	<0.0001	<0.6 (0.12)	<−0.7 (0.12)	<0.0001

[a] Inoculation level was approximately 6 log CFU/cm^2 nonpathogenic *E. coli* surrogate bacteria; hot water treatment was 92.2 to 92.8°C water, ≥70°C carcass surface temperature, 13 to 15 lb/in^2; and spray treatment was 1.9% LCA, 50 to 51.7°C, 13 to 15 lb/in^2, 10 s.
[b] Least-squares means with a less than symbol (<) indicate one or more of the samples within the treatment had plate counts below the analysis detection limit (–0.9 log CFU/cm^2). *P* values <0.05 are significant.

treated with a preevisceration intervention composed of a 2% lactic acid spray (42°C), a hot water wash (74°C, 5.5 s), or both treatments in sequence starting with the hot water treatment. The hot water cabinet reduced carcass APC from 6.2 to 3.5 log CFU/100 cm^2 (2.7-log reduction), whereas the whole system (hot water followed by lactic acid) reduced APC from 6.4 to 4.2 log CFU/100 cm^2 (2.2-log reduction), which was no more effective than the hot water treatment alone. Intuitively, the sequential hot water and lactic acid treatments should have resulted in a greater reduction of APC; however, Bosilevac et al. *(10)* believed this finding probably occurred by chance and that the lactic acid possibly had a cooling effect, thus reducing the effectiveness of the hot water. Carcasses were not inoculated in their study, so it is possible that variation in levels of microflora among carcasses may have caused the unexpected results for the whole intervention system.

As previously described, uninoculated zones of carcasses were also sampled and analyzed for APC and EB counts in the present study. Levels of natural microbial contamination on the carcasses were low, with APC and EB counts of <1.2 and <0.6 log CFU/cm^2 obtained, respectively, before application of the hot water and LCA spray treatments (Table 4). In all cases, carcass contamination was reduced by the hot water and LCA spray cabinets (Table 4), and due to the low initial levels, counts for many of the samples were reduced to below the detection limit (–0.9 log CFU/cm^2). Of the analyzed samples from uninoculated zones of carcasses that received only the hot water treatment, 70% had undetectable (less than –0.9 log CFU/cm^2) APC (data not shown). In addition, of the analyzed samples from uninoculated zones of the carcasses that received the hot water treatment alone or in sequence with the LCA spray treatment, 30 and 40% of samples had undetectable (less than –0.9 log CFU/cm^2) EB counts, respectively.

Overall, both inoculated and uninoculated warm beef carcass surface tissue treated with a hot water cabinet and an LCA spray cabinet, at the operation parameters specified in this study, reduced bacterial contamination. A hot water cabinet with an LCA spray cabinet may be an effective postharvest carcass intervention to serve as a biological critical control point. In addition, the results of this study indicated that the *E. coli* biotype I surrogate organisms were viable surrogates (for *E. coli* O157:H7, non-O157 STEC, and *Salmonella*) for validation purposes in commercial beef plants for evaluation of LCA intervention treatments. Furthermore, the findings indicate that LCA, when applied at a range of industry conditions of acid concentration and spray temperature and pressure, to warm beef carcass tissue may be an effective antimicrobial intervention against pathogen and surrogate organisms that could be implemented into a beef harvest plant as an additional intervention for multiple hurdle systems.

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