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Reduction of shiga toxin-producing E. coli and salmonella typhimurium on cattle hides by spray treatment with "Fit-L" (Levulinic acid plus sodium dodecyl sulfate)

Abstract

The efficacy of levulinic acid plus sodium dodecyl sulfate (SDS) at different concentration to inactivate STEC and Salmonella Typhimurium on cattle hides in vitro and in vivo as a surface spray treatment was determined. A mixture of six isolates of STEC, including serovars O26, O45, O103, O111, O121, and O157 (108CFU/ml) and a mixture of 5strains of S. Typhimurium (108CFU/ml) were sprayed on the surface of 10x10-cm sections of cattle hide. The inoculated hides were dried under a hood at 21˚C for 72h or held 4˚C for 24 h. The hides were treated by surface spray with a commercial microbicide (Fit-L) at different concentrations at 45 psi for 15s. Water only applied at 45 psi for 15sec was used as the negative control. Sponge samples of the hides were collected at 1, 3, and 5min after treatment and enumerated for STEC and Salmonella. For STEC-contaminated hides, treatment of Fit-L diluted at 1:11(v/v) in tap water (4% levulinic acid plus 0.4% SDS for 5min reduced STEC populations by 3.7logCFU/cm2, when compared with the water only treatment. For S. Typhimurium-contaminated hides, treatment of Fit-L reduced the Salmonella population by 4.6logCFU/cm2. Scrubbing hides with a brush for 30s followed by Fit-L spray treatment further reduced Salmonella contamination by 0.5log/cm2. Fit-L product diluted in tap water at 1:22 (v/v, 2% levulinic acid plus 0.2% SDS) was used as a surface wash for live beef cattle. Results revealed that surface spray of cattle with Fit-L reduced the E. coli population by 3.4logCFU/cm2 at 5min when compared with the tap water wash only control. No adverse effects for the cattle were observed after the spray treatment.

Keywords: levulinic acid, sodium dodecyl sulfate, e. coli, salmonella, stec, cattle hides

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Introduction

Epidemiological investigations have revealed that undercooked ground beef and, less frequently, unpasteurized milk are vehicles of outbreaks of *E. coli* O157:H7 infection, with cattle being a major reservoir of this pathogen.[1–18] The prevalence of *E. coli* O157:H7 in individual cattle ranged from 5 to 20%, at levels of<100 to>10^4CFU/g of feces.[1–4] *E. coli* O157:H7 can be excreted through feces at cell numbers of 10^6CFU/g, of *E. coli* O157:H7 at levels ≥10^4CFU/g of feces are termed "super shedders."[3,11]" *E. coli* O157:H7 can survive on hides, in drinking water troughs, in pens and bedding, on tools, and in the farm environment for several months.[14]

While *E. coli* O157:H7 is the most important Shiga toxin-producing *E. coli* (STEC) to be recognized as a major food safety threat, the other "non O157:H7 STEC" have been increasingly implicated in human illness outbreaks.[13] Because of this linkage, a group of six non-O157 serogroups (O26, O45, O103, O111, O121, and O145) have joined O157:H7 as being classified as adulterants in beef (USDA/FSIS, 2012).

Animal hides are an important source of zoonotic pathogens which contaminate carcasses at beef slaughter. Studies have revealed the hide of cattle hide is the primary source of carcass contamination by STEC during slaughter.[19] STEC on the hide can be transferred to the carcass during the hide opening and removal process. In the United States, multiple hurdle intervention strategies are applied to reduce STEC and other pathogens during beef processing. Examples include trimming, steam vacuuming, steam pasteurization, water washes, and organic acid washes in combination to achieve large reductions in carcass contamination. Studies of *E. coli* O157:H7 and *Salmonella* in U.S. abattoirs that process fewer than 1,000 head of cattle per day have revealed that *E. coli* O157:H7 is on 76% of animal hides coming into slaughter houses, but not on carcasses leaving the cooler. However, pre-evisceration carcass prevalence of *E. coli* O157:H7 and other STEC serotypes varied greatly, ranging from 0 to 93% for *E. coli* O157:H7 on different days at different plants.[3]

These strategies generally use different bactericidal approaches to mitigate pathogen contamination and studies have revealed these strategies are effective in substantially reducing STEC contamination of finished products. However, the presence of individual super-shedder cattle which horizontally spread the pathogen during transport and at lairage, can produce a high level of STEC.[10] Cattle hide heavily contaminated with STEC will reduce the efficacy of the multiple interventions that are applied.

Although considerable progress has been made to reduce *E. coli* O157:H7 and *Salmonella* contamination and in cattle at pre-harvest,

an effective hide treatment for pathogen reduction is still needed. We reported previously favorable results of a food-grade microbicide (levulinic acid plus SDS) that is non-chlorine-based, has a broad bactericidal effect within a short contact time and it efficacious in killing bacteria in the presence of organic materials.[20,21] Furthermore, this microbicide did not adversely affect the mucous membranes of the oral cavity of mice.[22] The objective of this study is to determine efficacy of different concentrations of levulinic acid plus SDS at different contact times on reducing STEC and *Salmonella* populations on cattle hides, with the goal of identifying the best concentration and contact time for use of this treatment as a hide spray for live cattle.

Materials and methods

Hide

Whole beef hides were freshly collected from a local slaughter house. The hides were held at 5˚C and transported to the Center for Food Safety within 3h. The hides were held at -30˚C and then at 5˚C for 48hours prior to use in a trial. The hides were cut into 10x10cm sections before use.

Bacterial isolates and inoculum mixture preparation

For laboratory assays, a 6-strain mixture of STEC, including serotype O26:H11 (cattle isolate), O45:H2 (human isolate), O103:H2 (beef isolate), O111:H8 (human isolate), O121:H7 (beef isolate), O157:H7 (human isolate) and a 5-strain mixture of *Salmonella Typhimurium*, including H2662 (cattle isolate), 11942A (cattle isolate), 13068A (cattle isolate), 152N17-1 (dairy isolate), and H3279 (human isolate) were used. Each strain of STEC and *Salmonella* was grown individually at 37°C for 18h in tryptic soy broth (TSB, Becton Dickinson, Sparks, MD) and transferred at least three times at 24-h intervals before use. The bacterial cells were three times sedimented by centrifugation at 4,000rpm for 20min and re-suspended in 0.1% peptone solution. An equal volume of each isolate were combined and used as a mixture. The cell number of each isolate was individually confirmed by the method as we described previously.[21]

Inoculation of hides

Bacterial suspension (ca. 2ml) was applied to each 10x10cm hide sample by a hand sprayer (250-ml, Decon laboratories, King of Prussia, PA). Depending on the experiment plan, some were used in a trial immediately after inoculation, whereas others were dried for 24-72h in a Biosafety-2 hood, or without drying and then used in a trial.

Chemicals

Levulinic acid (Sigma-Aldrich, St. Louis, MO) and sodium dodecyl sulfate (SDS, Sigma-Aldrich) were freshly prepared solutions and "Fit-L" (HealthPro Brands, Mason, OH) was diluted with tap water immediately before each trial.

Sample collection from inoculated hide

An area of ca. 20cm at each sampling location was wiped with a Whirl-Pak sponge probe (Nasco, Fort Atkinson, WI) with sterile gloves. Each sponge was soaked with 10ml of 0.1M phosphate buffer, pH 7.2 (PBS) in a 710ml (24-oz) Whirl-Pak bag prior to use.

Isolation of inoculated STEC or Salmonella

After sampling, each sponge probe in a Whirl-Pak bag with 10ml of PBS solution was pummeled in a Stomacher blender at 230rpm for 1min (Seward Medical, London, UK). The fluid was serially (1:10) diluted in 0.1% peptone solution (Becton Dickinson) and 0.1ml from each dilution tube was plated in duplicate on MacConkey agar (Becton Dickinson), Sorbitol MacConkey agar (SMA, Becton Dickinson), and XLD agar (XLD, Becton Dickinson) plates. The plates were incubated at 37°C for 48h, then colonies typical of *E. coli* (dark red), *E. coli* O157:H7 (colorless) and *Salmonella* (black) were counted as presumptive *E. coli*, *E. coli* O157:H7, and black as presumptive *Salmonella*. Up to five colonies were randomly selected from plates with the highest dilution for confirmation of *E. coli* or *Salmonella* by biochemical tests (API 20E assay, bioMérieux, Hazelwood, MO) and also by latex agglutination assay (Oxoid) for confirmation of *E. coli* O157 or *Salmonella* and by PCR for STEC.[21,23–25]

Hide spray treatment of live cattle

Beef cattle (12-months of age) were used for this study. The study was conducted at the Lambert-Power Meat Laboratory and the experimental protocol was approved by the Institutional Animal Care and Use Committee of Auburn University (PRN 2014-2602). Two sides of the animal were selected for treatment, with the right side receiving the microbicide wash and left side receiving a water wash, i.e., serving as the negative control. Each side was further divided into front and rear part for sample collection. A similar approach was used for the face spray study with eye open. Whole head, including ear, nose, and eye of cattle was sprayed with microbicide as the treatment and the cattle sprayed with tap water only were used as the negative control.

Sample collection from live cattle

An 18-oz. sterile "speci-sponge" (3.8x7.6cm, Nasco, Fort Atkinson, WI) was used for wiping with sterile gloves a 10x10-cm area at each sampling location on the cattle hide (one of sample site per hide). Samples were collected with sterile gloves at pre-wash, and at 5 and 10min following the spray treatment after 10min. Each sponge was mixed immediately with 10ml of 0.1M phosphate buffer, pH 7.2 (PBS), then held at 5˚C and transported to the Center for Food Safety for microbiological analysis within 2h.

Enumeration of Escherichia coli

Each sponge in the Whirl-Pak bag with 10ml of PBS solution was pummeled in a Stomacher blender at 230rpm for 1min (Seward Medical). The fluid was serially (1:10) diluted in 0.1% peptone and 0.1ml from each dilution tube was plated in duplicate on MacConkey agar (MSA, Becton Dickinson), Sorbitol MacConkey agar (SMA), and XLD agar (XLD) plates in duplicate. The plates were incubated at 37°C for 48h and typical *E. coli* colonies (dark red) were counted as presumptive *E. coli*. Up to 5 colonies from the highest dilution were randomly selected and confirmed as *E. coli* by biochemical assays (API 20E assay, bioMérieux). The final *E. coli* counts were adjusted based on the confirmation assays.

Eye safety

A filter-sterilized (0.2µm Millex 25mm, Millipore) solution (0.5-1.5ml per eye) containing 0.2% levulinic acid and 0.02% SDS was applied to each eyes of 6 cattle. Any stimulus systems including the blink speed, redness, and pain response were recorded for up to 20min from the time of application, then again at 24h. A 0.85% saline solution was used as the negative control and was applied to 12 eyes of 6 cattle.

Statistical analysis

For the hide inoculation studies, each trial was repeated twice with duplicate plates. The mean population of pathogens per ml or cm^2 was converted to log CFU/cm^2. For the live cattle wash studies each trial included multiple samplings at different locations with duplicate plates. The mean population of *Escherichia coli* at each location was converted to log CFU/cm^2. The effects between the microbicide spray on inactivation of *E. coli* and the water only control were analyzed for analysis of variance (ANOVA) by SAS software (SAS 9.3, SAS Institute, Cary, NC) to determine least significant differences ($P<0.05$) among the treatments.

Results

Inactivation on contaminated hides

Treatment of STEC *in vitro* contaminated hides with 3% levulinic acid plus 0.5% SDS for 5min reduced STEC populations by 4.7logCFU/cm^2, compared to the water-only treatment. Treatment of S. Typhimurium *in vitro* contaminated hides with 2% levulinic acid plus 0.2% SDS for 5min reduced the *Salmonella* population by 2.9logCFU/cm^2 (Table 1). Scrubbing hide sections with a brush for 30s followed by the microbicide spray treatment further reduced *Salmonella* contamination by 0.5log/cm^2. However, for wet hides on which *Salmonella* are more resistant, a spray treatment with 4% levulinic acid plus 2% SDS for 5min reduced by only 1.3logCFU/cm^2 when compared to the water-only treatment (data not shown).

Concentrations and contact times

Four concentrations of levulinic acid (i.e., 0.5, 1, 2, and 3%), and SDS (i.e., 0.05, 0.1, 0.2 and 0.5%, and five contact times (i.e., 1, 2, 3, 5, and 10min were evaluated on hides that were dried for 72 h after inoculation with either STEC or *Salmonella*. Results revealed that the inactivation of STEC and *Salmonella* was directly related to the concentration of levulinic acid plus SDS applied. A concentration of 0.5% levulinic acid plus 0.05% SDS was least effective, whereas 3% levulinic acid plus 0.5% SDS was most effective. The contact time of 1, 2, 3, 5, and 10min yielded similar results for all of the treatment, suggesting that contact time of 3min may be sufficient for effective inactivation of STEC and *Salmonella* on hides (Table 1). The bactericidal effect on wet hides was significantly less (only 1.3logCFU/cm^2) compared in vitro with dry hides. Perhaps this wet condition produce a barrier to prevent the microbicide from penetrating into the hide.

"Fit-L" evaluation

For STEC-contaminated *in vitro* hides, treatment for 5min with "Fit-L" diluted at 1:11(v/v) in tap water reduced STEC populations by 3.7logCFU/cm^2, when compared with the water-only treatment. For S. Typhimurium-contaminated hides, treatment with "Fit-L" diluted at 1:11(v/v) reduced the *Salmonella* population by 4.6logCFU/cm^2 (Table 2). Scrubbing hides with a brush processing for 30s followed by a "Fit-L" spray treatment reduced *Salmonella* contamination by an additional 0.5log/cm^2.

Efficacy determination in live cattle

Based on results obtained from the *in vitro* hide inoculation studies, "Fit-L," was used for an in vivo hide spray study on live cattle. "Fit-L" was diluted in tap water at a ratio of 1:22 (v/v; equal to the concentration of 2% levulinic acid plus 0.2% SDS) and was applied to the hide of live cattle for 5 or 10min. Seven beef cattle (12-months of age) were selected for the study. The head of each animal was mechanically immobilized for ease of operation.

Results revealed that the average *E. coli* count before washing (26 samples from 7 cattle) was 6.6logCFU±1.0/cm^2. For tap water-only washed cattle (28 samples from 7 cattle) the average *E. coli* count was 6.0logCFU±1.1/cm^2 at 5min and 6.1logCFU±1.5/cm^2 at 10min (Table 3). For "Fit-L"-washed cattle (28 samples from 7 cattle) the average *E. coli* count was 2.6logCFU±1.0/cm^2 at 5min and 2.3logCFU±0.9CFU/cm^2 at 10min. Following the "Fit-L" washing with tap water washing, the *E. coli* count was 2.3logCFU±0.8/cm^2 (Table 3). These data revealed that a simple "Fit-L" spray with a 5- or 10min exposure time could reduce the *E. coli* population by 3.4log and 3.8log on the surface of cattle hides, respectively, when compared with a tap water-wash only. A tap water-only wash reduced the *E. coli* population by 0.5logCFU/cm^2 compared with samples collected before the wash. Following the "Fit-L" washing with a tap water wash did not further reduce *E. coli* on the surface of the cattle hides. Similar results were also observed with cattle face wash (Table 4). These results suggest a "Fit-L" spray immediately before cattle entered the slaughter facility will substantially reduce the population of *E. coli* on cattle hides.

Table 1 STEC and *S.Typhimurium* counts on hides (10x10cm, dried for 72h) treated by LV+SDS by spray application at 21°C

Concentration of lvulinic acid+SDS	STEC counts (log CFU/cm^2)[a,b] on hides at min on sample number					
	1		3		5	
	#1	#2	#1	#2	#1	#2
Water only	6.9±1.2	6.8±0.6	6.4±1.0	6.6±0.5	6.6±1.1	6.4±0.7
0.5% LV+0.05% SDS	6.0±1.4[d]	5.0±0.3[d]	4.2±0.7[d]	2.3±0.5[d]	3.8±0.4[d]	5.1±0.5[d]
3% LV+0.5% SDS	2.2±0.4[d]	2.2±0.3[d]	<1.7[bd]	2.0±0.1[d]	2.0±0.2[d]	<1.7[d]
Water only	6.5±0.6	6.4±0.5	6.5±0.3	6.4±0.2	6.4±0.2	6.5±0.5
1% LV+0.1% SDS	4.9±1.1[d]	4.5±0.6[d]	4.1±0.2[d]	4.1±0.5[d]	3.7±0.7[d]	3.2±0.4[d]
2% LV+0.2% SDS	5.2±1.6[d]	4.5±1.0[d]	4.0±0.5[d]	2.4±0.2[d]	2.0±0.4[d]	2.5±0.3[d]
*S.*Typhimurium counts (log CFU/cm^2)[c] on hides at min on sample number						
Water only	5.6±1.0	5.8±0.8	4.8±0.3	5.6±0.7	5.0±0.4	5.6±0.5
2% LV+0.2% SDS	2.4±0.5[d]	3.1±1.1[d]	3.0±1.1[d]	2.6±0.3[d]	2.6±0.5[d]	2.1±0.3[d]

[a]A 6-strain mixture of STEC, including O26, O45, O103, O111, O121, O157 was used.
bMinimum detection level by direct plating method log 1.7 CFU/cm2.
cA 5-strin mixture of S. Typhimurium, including H2662 (cattle isolate), 11942A (cattle isolate), 13068A (cattle isolate), 152N17-1 (dairy isolate), and H3279 (human isolate) was used.
dResults were significant different (P<0.05) when the LV+SDS treatment was compared with the water-only treatment.

Citation: Zhao T, Wang L, Zhao P, et al. Reduction of shiga toxin-producing *E. coli* and *salmonella typhimurium* on cattle hides by spray treatment with "Fit-L" (Levulinic acid plus sodium dodecyl sulfate). *MOJ Food Process Technol.* 2016;2(5):157–162. DOI: 10.15406/mojfpt.2016.02.00049

Table 2 STEC and S. Typhimurium counts on hides (10x10cm, dried for 72h following inoculation) treated by Fit-L (1:11, v/v in tap water) spray treatment with brush at 21°C

Treatment	STEC counts (log CFU/cm²)[a,b] on hides at min on sample number							
	1		2		3		5	
	#1	#2	#1	#2	#1	#2	#1	#2
Water only	7.5±0.4	7.6±0.9	7.1±0.2	6.9±0.7	6.9±0.3	6.9±0.8	7.0±0.1	6.7±0.6
Fit bactericide (1:11)	<1.7[bd]	2.7±0.2[d]	4.3±0.5[d]	2.4±0.2[d]	<1.7[d]	3.2±0.4[d]	2.4±0.0[d]	3.9±0.8[d]
Salmonella Typhimurium counts (log CFU/cm²)[c] on hides at min								
Water only	7.5±0.8	7.2±0.1	6.7±0.2	7.1±0.2	6.8±0.5	7.1±0.7	7.3±0.4	6.7±0.1
Fit bactericide (1:11)	<1.7[d]	<1.7[d]	<1.7[d]	<1.7[d]	<1.7[d]	2.5±0.1[d]	<1.7[d]	3.2±0.3[d]

[a]A 6-strain mixture of STEC, including O26, O45, O103, O111, O121, O157 were used.
[b]Minimum detection level by direct plating method is log 1.7 CFU/cm2.
[c]A 5-strin mixture of S. Typhimurium, including H2662 (cattle isolate), 11942A (cattle isolate), 13068A (cattle isolate), 152N17-1 (dairy isolate), and H3279 (human isolate) were used.
[d]Statistical analysis was significant (P<0.05) when compared LV+SDS treatment with water treatment only.

Table 3 Escherichia coli counts of cattle hides on one side washed with "Fit-L" (1:22, v/v) and the other side washed with tap water with both applied at 45-55 psi

Wash method	Sample location	*Escherichia coli* count (log CFU/cm²)[a] at			
		Pre-wash	5 min	10 min	Water wash
Tap water wash	Front 1	7.5±0.5	5.4±0.4	5.9±0.6	1.9±0.1[c]
	Front 2	6.8±0.8	5.7±0.9	5.7±0.5	2.0±0.1
	Rear 1	7.2±0.2	6.5±0.6	6.3±0.7	
	Rear 2	7.1±0.3	6.4±0.4	6.3±0.3	
"Fit-L" wash (1:22, v/v)	Front 1		2.0±0.2[c]	1.7±0.1[b,c]	
	Front 2		2.3±0.2[c]	1.7±0.1[c]	
	Rear 1	6.0±0.6[c]	2.8±0.6[c]	2.8±0.7[c]	2.7±1.1[c]
	Rear 2	6.4±0.3[c]	3.1±0.4[c]	2.8±0.7[c]	2.8±0.9[c]

[a]Average±SD of 7 cattle.
[b]Minimum detection level is log 1.7/cm2.
[c]Results were significantly different (P<0.05) when the LV+SDS treatment was compared with the water-only treatment.

Table 4 Escherichia coli count of cattle heads washed with "Fit-L" product (1:22, v/v) in tap water

Cattle No.	Wash method	Sample location	*Escherichia coli* count (log/cm²)[a] at		
			Pre-wash	5 min	10 min
1	"Fit-L" wash	Left head	3.7±0.4	3.4±0.9[c]	2.7±0.3[c]
		Right head	4.9±1.1	<1.7[b,c]	1.7±0.1[c]
2	"Fit-L" wash	Left head	3.6±0.7	<1.7[c]	<1.7[c]
		Right head	3.4±0.2	<1.7[c]	<1.7[c]
3	"Fit-L" wash	Left head	3.6±0.9	<1.7[c]	<1.7[c]
		Right head	3.6±0.3	<1.7[c]	<1.7[c]
4	Water only	Left head	3.6±0.5	4.7±1.0	5.2±1.5
		Right head	4.4±0.6	4.1±1.1	5.8±1.2
5	Water only	Left head	4.0±0.2	3.9±0.1	3.7±0.3
		Right head	4.7±0.4	4.1±0.6	4.5±0.7
6	Water only	Left head	4.7±0.2	4.0±0.3	4.4±0.2
		Right head	4.8±0.1	4.7±0.9	4.6±0.9

[a]Average±SD of 7 cattle.
[b]Minimum detection level was log 1.7/cm2.
[c]Results was significantly different (P<0.05) when the LV+SDS treatment was compared with the water-only treatment.

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Safety assays

Safety evaluation of the microbicide as an eye-drop application on cattle eyes revealed that the application was not irritating. There was no difference in blink speed (5/min versus 5/min) for animals receiving an eye-drop with 0.2% levulinic acid plus 0.02% SDS and eye-drop compared with those receiving an eye-drop with 0.85% saline. Neither eye redness, nor pain response was observed before or after application for any cattle within 24h (Figure 1).

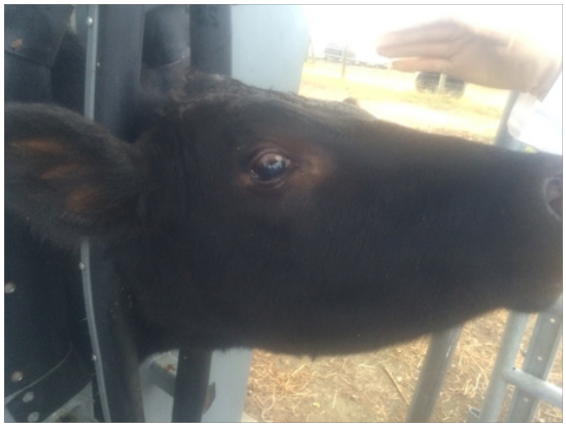

Figure 1 Eye response following application of ca. 1.0-ml eye-drop with 2% levulinic acid plus 0.2% SDS.

Discussion

Animal hides are a significant source of zoonotic pathogens which contaminate carcasses at beef slaughter.[4,9,10,19] Survey data from cattle at slaughter facilities have revealed that those parts of the cattle surface are covered with feces and dirt and have the greatest population of bacteria when the animal enters the slaughter facility. Generally, the population of *E. coli* is 10^{7-8}CFU/cm^2 and the population of *E. coli* O157:H7 can be as high as 10^{5-6} CFU/cm^2.[1–5,7,18] Hence, cattle hide surface can be a major source of *E. coli* and *E. coli* O157:H7 of meat in processing facilities during slaughter operations.[4,19]

Various chemicals, including lactic acid (2, 4, and 6%), acetic acid (2, 4, and 6%), chlorine (100, 200, and 400 ppm), alcohol (70, 80, and 90%), and Oxy-Sept 333 (0.5, 2, and 4%) were evaluated for their efficacy in killing rifampicin-resistant *Salmonella Typhimurium* inoculated on fresh beef hides.[19] Results revealed that alcohols at all concentrations were effective (≥5log/cm^2 reduction) and acetic and lactic acids at high concentrations (4 and 6%) were effective (≥3log/cm^2). However spray wash treatments with chlorine (100, 200, and 400ppm) and Oxy-Sep 333 had no effect (P>0.05) in reducing *S. Typhimurium* population when compared with a water-only control.[19] Previously cattle washing studies to determine the efficacy of various pre-harvest treatments (e.g., 0.5% lactic acid and 50ppm chlorine) in reducing microbiological contamination on living animals revealed that there was no statistical difference (P>0.05) between water wash groups and chemical treatment wash groups in aerobic plate counts, and coliform, and *E. coli* levels.

Baird et al.[4] evaluated the antimicrobial efficacy of several chemicals, including 70-90% isopropyl alcohol, 3% hydrogen peroxide, 2% lactic acid, 10% povidone-iodine, and 1% cetylpyridinium chloride on the fresh beef hides contaminated with a bovine fecal slurry. Results revealed that 1% cetylpyridinium chloride, 2% lactic acid, and 3% hydrogen peroxide treatment yielded the greatest reduction in coliform counts (4.5, 4.1, and 3.9 log CFU/100cm^2, respectively).

Water Management Resources (Overton, Nevada) have installed at a Cargill beef plant, a hide-on-carcass wash machine, as a "car wash for cattle", in which the hides of animals are scrubbed with spinning bristles and a mild bromine solution that reduced bacteria contamination at the beginning of the slaughter process. This scrubbing helps to better remove dirt and debris, thereby reducing bacteria contamination.

Study results for prewashing cattle before they enter the abattoir are open to discussion. Cattle are alert at this stage and they must be treated humanely, hence any chemicals that irritate the eyes should not be applied. Furthermore, the cattle hide often contain fecal materials that negate the antimicrobial activity of chlorine-based chemical. Previous studies revealed that treating beef with levulinic acid plus sodium dodecyl sulfate-based "Fit-L" substantially reduced STEC and *Salmonella Typhimurium* populations while retaining the quality traits (tenderness, juiciness, and beef flavor) of the meat.[6,22] For the present study, "Fit-L" was diluted in tap water at 1:22 (v/v, 2% levulinic acid plus 0.2% SDS) and applied as a surface spray for live beef cattle. Results revealed that surface spray of cattle with "Fit-L" with an exposure time of 5 min reduced the *E. coli* population by 3.4 log. Interestingly, the efficacy of "Fit-L" was reduced when hides were wet, but scrubbing the hide with scrubbing the treated hides with a brush increase pathogen reduction.

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Conflict of interest

The author declares no conflict of interest.

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