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Localized Chemical Decontamination of Cattle Hides to Reduce Microbial Loads and Prevalence of Foodborne Pathogens

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ABSTRACT

Hides of cattle have been shown to be the principal source of *E. coli* O157:H7 at slaughter. This project was conducted to determine if localized interventions are a viable option for cattle hide decontamination. Localized decontaminant treatments consisting of negative control (CT), warm water (H_2O), 6.0% lactic acid (LA), 5.0% acetic acid (AA), and 2.7% sodium hydroxide (NaOH) were applied to the pattern lines of beef hides. Samples were collected before and after treatment application and from dressed carcass surfaces and were analyzed for aerobic bacterial plate counts (APC), total coliform count (TCC), biotype 1 *E. coli* count (ECC), *E. coli* O157:H7, and *Salmonella*. APCs on cattle hides were reduced by 0.11, 2.62, 2.30 and 1.66, TCCs were reduced by -0.09, 3.82, 3.76 and 3.63, and ECCs were reduced by -0.03, 3.77, 3.83 and 3.54 log CFU/400 cm^2 following application of H_2O, LA, AA and NaOH, respectively. Prevalence of *E. coli* O157:H7 was reduced by -6.0%, 46.0%, 28.0% and 53.2% and *Salmonella* was reduced by -20.0%, 0% (none detected), 24.0% and 17.1% following application of H_2O, LA, AA and NaOH, respectively. Use of localized chemical interventions on cattle hides is an effective mechanism for reducing incoming loads of bacteria on hide surfaces and reducing foodborne pathogen prevalence on dressed carcasses.

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INTRODUCTION

In spite of continued efforts and investments in research and application, food safety issues involving raw beef products continue to be an issue for the U.S. beef industry. A recent report from the Centers for Disease Control (CDC) indicated that the number of cases of human illness associated with *Escherichia coli* O157:H7 had declined to 0.99 cases per 100,000 population *(6)* by 2009. Despite marked progress in the numbers of recalls and of cases of human illnesses attributed to *E. coli* O157:H7 from beef products and a reduction in prevalence of *E. coli* O157:H7 in raw ground beef between 2002 and 2007, the number of beef recalls *(15)* associated with *E. coli* O157:H7 and *Salmonella* (21 in 2007; 17 in 2008; 18 in 2009 and 7 in 2010) and prevalence of *E. coli* O157:H7 in raw ground beef (0.30% in 2009) and 0.33% in 2010 *(16)* are still considered higher than desirable.

Tissues of healthy animals are essentially sterile *(13)*; however, the aforementioned statistics clearly indicate that contamination with pathogenic microorganisms does occur and persists throughout the meat production chain despite efforts to prevent, eliminate and reduce contamination via the use of trimming, washing/rinsing with water (hot and cold) and antimicrobial chemicals *(2, 9, 11)*. The source of this persisting contamination has been determined to be the hides of cattle entering the harvest floor *(1, 10, 12)*. Although efforts to improve the microbiological quality of incoming cattle hides (preharvest) have shown to be beneficial *(3, 4, 5, 8)*, the largest opportunity for uniform and effective hide decontamination strategies remains at the harvest facilities. Hide decontamination systems previously described *(12)* and commercially implemented may not be accessible to many U.S. beef processing plants for a variety of reasons, including, but not limited to, the requirements for large capital expenditures and space. The challenge is to modify the concepts represented by these decontamination systems to make them applicable to all U.S. beef processing plants.

This study was designed to evaluate the efficacy of localized hide pattern line (inside hind legs, midline and foreshanks) chemical decontamination of cattle hides, prior to dressing, in reducing or eliminating *E. coli* O157:H7 and *Salmonella* both on the hide surface and the subsequent dressed carcass surfaces and resulting trimmings in a commercial setting.

MATERIALS AND METHODS

Sampling locations

Carcass samples (N = 750) were collected with cellulose sponges hydrated with 25 ml of Dey/Engly (D/E) neutralizing broth, from carcasses at 3 locations along the harvest chain in a commercial beef processing facility that harvests primarily non-fed cattle. The first sample (Hide Before) was collected on the external hide surface from the ventral midline of the carcass (~ 400 cm^2), between the forelegs, immediately following exsanguination as carcasses were being fed onto the harvest chain. The second sample (Hide After) was collected on the external hide surface, approximately 3 minutes following the application of a chemical intervention to the hide pattern line, from the ventral midline of the carcass (~ 400 cm^2). Hide surface samples collected before and after intervention application alternated between an upper and lower ventral midline position to avoid sampling from the same area of the hide surface. The third sample (Carcass) was taken following the removal of the hide from an ~ 8,000 cm^2 area of the round and forequarter as outlined by Arthur et al. *(1),* using 2 sponges that were subsequently combined into a single sample. It should be noted that all carcass samples were collected prior to carcass pathogen intervention applications in this facility and that these results do not represent the microbiological quality of inspected and passed carcasses produced in this facility. Carcasses were followed through the chilling and fabrication process and the resulting trimmings were sampled (in combo-bins) with the IEH N60 Plus™ sampling device (IEH Laboratories and Consulting Group, Lake Forest Park, WA).

Application of treatments

Localized chemical decontamination treatments were applied to the pattern lines (medial aspect of the hind legs and ventral midline) of beef hides, using a syringe pump mixing system (Hydro Blend, Boise, ID) and hand held spray wand (Birko, Corp, Henderson, CO) fitted with a TP8004E-SS spray nozzle (Teejet, Wheaton, IL). All treatments were mixed with potable tap water provided by the plant at the referenced temperature. Treatments (n = 50/treatment) included negative control (CT), no intervention application; warm water (60°C) (H_2O); 6.0% lactic acid (30°C) (LA); 5.0% acetic acid (30°C) (AA); and 2.7% sodium hydroxide (10°C) (NaOH). Application of all treatments (excluding the negative control) was done using a handheld wand with direct application to the hide pattern line of the carcass at a pressure of 2.0 atm. The total elapsed duration of intervention application was ~15 s (time taken to apply localized treatment to the entire hide pattern areas), with 0.33 l of solution applied.

Microbiological analyses

Following collection, all samples were transported to IEH Laboratories and Consulting Group for analysis. Sponge samples collected from the hide both before and after the application of chemical interventions, dressed carcass samples and trim samples were analyzed for aerobic bacterial plate counts (APC), total coliform count (TCC) and biotype I *E. coli* count (ECC) and for the presence of *E. coli* O157:H7 and *Salmonella*. Samples were pummeled using an IUL Masticator (Neutec Group Inc, Plainview, NY) for 1 to 2 min and serial dilutions of 1 ml of the diluent were prepared in 0.1% sterile buffered peptone water (BPW, International BioProducts, Bothwell, WA). One ml of the diluted solution was then placed on a 3M™ Petrifilm™ Aerobic Count Plate (APC) and a 3M™ Petrifilm™ E. coli/Coliform Count Plate (3M Microbiology Products, St. Paul, MN) and incubated for 48 h at 32°C. Following incubation, APCs were determined by counting all colonies recovered from the Petrifilm APC plates. The TCC and ECC results were derived by counting colonies recovered from the Petrifilm *E. coli* coliform plates. TCCs were determined by counting both red and blue colonies associated with a gas bubble, and ECCs were determined by counting only the blue colonies associated with a gas bubble. All counts were log transformed prior to statistical analysis and are reported as log CFU/400 cm^2.

Presence or absence of *E. coli* O157:H7 and *Salmonella* were determined using the IEH *E. coli*, Stx-producing *E. coli* (STEC) with the Intimin and *Salmonella* Test System (AOAC 100701) as outlined by Stopforth et al. *(14)*.

TABLE 1. Least squares means ± standard error for microbial populations and prevalence of *E. coli* O157:H7 and *Salmonella* from the surface of hides and dressed carcasses treated with various interventions

	N	APC[b] log CFU/ 400 cm^2	TCC log CFU/ 400 cm^2	ECC log CFU/ 400 cm^2	EC O157:H7 Prevalence (%)	*Salmonella* spp. Prevalence (%)
			Negative Control[a]			
Hide Before[b]	50	9.23^{y} ± 0.05	6.81^{x} ± 0.05	6.62^{x} ± 0.05	60.0^{x}	0
Hide After	49	8.56^{z} ± 0.07	6.80^{x} ± 0.08	6.68^{x} ± 0.08	100.0^{xy}	0
Carcass	48	8.44^{z} ± 0.24	5.56^{y} ± 0.10	5.39^{y} ± 0.10	47.9^{y}	0
			Water[a]			
Hide Before	50	9.35 ± 0.04	7.49^{x} ± 0.11	7.38^{x} ± 0.11	78.0	68.0^{y}
Hide After	50	9.24 ± 0.05	7.58^{x} ± 0.06	7.41^{x} ± 0.06	84.0	88.0^{x}
Carcass	50	9.22 ± 0.23	5.68^{y} ± 0.09	5.48^{y} ± 0.09	72.0	72.0^{y}
			Acetic acid[a]			
Hide Before	50	9.46^{x} ± 0.06	7.56^{x} ± 0.06	7.47^{x} ± 0.06	76.0^{x}	0
Hide After	50	6.85^{z} ± 0.21	3.81^{z} ± 0.36	3.77^{z} ± 0.36	30.0^{y}	0
Carcass	49	8.31^{y} ± 0.23	5.68^{y} ± 0.13	5.60^{y} ± 0.14	36.7^{y}	0
			Lactic acid[a]			
Hide Before	50	9.40^{y} ± 0.05	7.53^{x} ± 0.06	7.37^{x} ± 0.06	84.0^{x}	74.0^{x}
Hide After	50	7.11^{z} ± 0.22	3.88^{z} ± 0.44	3.63^{z} ± 0.42	56.0^{y}	50.0^{y}
Carcass	49	9.35^{y} ± 0.16	5.74^{y} ± 0.23	5.28^{y} ± 0.22	48.0^{y}	26.0^{z}
			NaOH[a]			
Hide Before	50	9.61^{x} ± 0.04	7.51^{x} ± 0.09	7.31^{x} ± 0.09	94.0^{x}	60.0^{x}
Hide After	50	7.97^{z} ± 0.14	3.97^{y} ± 0.37	3.84^{y} ± 0.36	40.8^{y}	42.9^{x}
Carcass	49	8.91^{y} ± 0.11	4.13^{y} ± 0.13	3.96^{y} ± 0.13	20.8^{y}	0^{y}

[a]Cattle hides were treated with nothing (Negative Control), water (60°C), acetic acid (4.8%, 30°C), lactic acid (6.4%, 30°C) and NaOH (2.68%, 12°C). All solutions were applied for ~ 15 s at 2.0 atm.

[b]Samples were collected from 3 harvest process locations (Hide Before, Hide After and Carcass), with intervention application occurring between the Hide Before and Hide After sampling locations.

[x,y,z]Least squares means, within column and intervention (e.g., NaOH), that lack a common superscript letter differ ($P < 0.05$).

Statistical analysis

Plate count results were analyzed by use of the Proc GLM procedure of SAS 9.1. Least squares means for plate counts were generated for the main effect of sample location within intervention treatments and least squares means for reductions in plate counts were generated for the main effect of treatment. Differences in least squares means were determined by use of the PDIFF option with an alpha level of 0.05. Categorical responses (presence or absence of pathogens) were analyzed by means of the Proc GLIMMIX procedure of SAS 9.1.

RESULTS AND DISCUSSION

Results for APC, TCC, and ECC, and prevalence of *E. coli* O157:H7 and *Salmonella*, from samples collected prior to localized intervention application (Hide Before), following the application of a localized intervention (Hide After) and from dressed carcass surfaces are reported in Table 1. Samples were collected from adjacent external hide surfaces for the purpose of quantifying the direct effect of each intervention on hide microbial loads and pathogen prevalence. Arthur et al. *(1)* identified a strong positive correlation between hide surface bacterial levels and carcass surface bacterial levels and between hide *E. coli* O157:H7 prevalence and carcass surface *E. coli* O157:H7 prevalence when samples were collected from the plate region of the hide and from an ~ 8,000 cm^2 area of the round and forequarter. Surface samples collected from the hide prior to intervention application had APC, TCC and ECC loads ranging from 9.23 to 9.61, 6.81 to 7.56 and 6.62 to 7.47 log CFU/400 cm^2, respectively, and pathogen prevalence ranging from 60% to 94% and 0 to 74% for *E. coli* O157:H7 and *Salmonella*, respectively. A negative control sample set (n = 50) was evaluated

TABLE 2. Least squares means ± standard error for reductions in microbial populations and prevalence of *E. coli* O157:H7 and *Salmonella* on cattle hides following the application of localized interventions

	N	APC[b] log CFU/ 400 cm^2	TCC log CFU/ 400 cm^2	ECC log CFU/ 400 cm^2	EC O157:H7 Prevalence (%)	*Salmonella* spp. Prevalence (%)
Negative Control[a]	48	0.68^z ± 0.08	0.02^z ± 0.09	-0.05^z ± 0.09	-40.0	0
Water Control	50	0.11^z ± 0.07	-0.09^z ± 0.00	-0.03^z ± 0.09	-6.0	-20.0
Acetic Acid	49	2.62^x ± 0.23	3.82^y ± 0.37	3.77^y ± 0.37	46.0	0
Lactic Acid	49	2.30^x ± 0.24	3.76^y ± 0.43	3.83^y ± 0.42	28.0	24.0
NaOH	48	1.66^y ± 0.15	3.63^y ± 0.39	3.54^y ± 0.38	53.2	17.1

[a]Cattle hides were treated with nothing (Negative Control), water (60°C), acetic acid (4.8%, 30°C), lactic acid (6.4%, 30°C) and NaOH (2.68%, 12°C). All solutions were applied for ~ 15 s at 2.0 atm.

[b]Reductions were calculated as the difference between hide microbial populations prior to intervention application (Hide Before) and the hide microbial populations following treatment application and an ~3 minute dwell time (Hide After).

[x,y,z]Least squares means, within column, that lack a common superscript letter, differ ($P < 0.05$).

to quantify changes in microbial indicators and pathogen loads as carcasses moved through the dressing process in this facility. Aerobic plate counts at the Hide Before location (prior to localized intervention application) and at the Hide After location (~3 minutes following localized intervention application) differed ($P < 0.05$); however, no differences ($P > 0.05$) were observed in TCC or ECC in the negative control sample population (Table 1). The prevalence of *E. coli* O157:H7 on hides did not differ ($P > 0.05$) between the Hide Before and Hide After sampling locations, but was lower ($P < 0.05$) on dressed carcass surfaces, indicating that no substantial reductions in microbial populations occurred in the absence of a hide intervention. Further, APC loads were similar on hides sampled at the Hide After location when compared to carcass surface APC loads ($P > 0.05$). It should be noted that all carcass samples were collected prior to pathogen intervention application in this facility and that these results do not represent the microbiological quality of inspected and passed carcasses produced in this facility. Total Coliform Counts and ECC were all lower ($P < 0.05$) on negative control carcass surfaces following hide removal, compared with hide surface samples obtained from the "after" location (Table 1). These results indicate that sufficient contamination was present on hides to permit evaluation of differences due to treatment and that, although differences in APC loads were found between samples collected at the "before" and "after" sampling locations, no substantial changes in microbial loads or pathogen prevalence occurred in this facility in the absence of a microbial intervention.

In addition to the negative control, a warm (60°C) water control was evaluated to establish the expected reductions in microbial loads and pathogen prevalence attributable to the washing/rinsing action of the applied interventions. The localized application of warm water to the pattern line of the hide had no impact ($P > 0.05$) on APC, TCC, ECC and *E. coli* O157:H7 prevalence on the treated hide surfaces (Table 1). The prevalence of *Salmonella* increased ($P < 0.05$) following the localized application of water to the hide pattern line (Table 1). This could have been a result of mobilization of hide contamination, as the temperature of the water utilized was not sufficient to result in microbial death. These results support the findings of Carlson et al. *(8)* and suggest that intervention strategies that attempt to remove contamination through low volume, low pressure washing do not effectively reduce hide contamination loads and that an antimicrobial compound is required to obtain microbial and pathogen load reductions *(7)*.

Acetic acid (AA) at a 10% concentration has been shown to be effective at reducing microbial loads and pathogens on hide surfaces *(7, 8)*. In this study, a 30°C AA solution was applied (2.0 atm) at a target concentration of 5.0% (actual measured concentration of 4.8%). This level was the maximum achievable concentration with readily available equipment. Localized application of 4.8% AA resulted in a multiple log CFU/400 cm^2 reduction ($P < 0.05$) in APC, TCC and ECC, as well as a reduction ($P < 0.05$) in *E. coli* O157:H7 prevalence on hide surfaces (Table 1). Microbial loads on the dressed carcass surfaces were higher ($P < 0.05$) than levels observed on the hides following the localized application of AA; however, the prevalence of *E. coli* O157:H7 was reduced from the observed incoming prevalence level (Table 1).

Lactic acid (LA) at a 10% concentration has been shown to be effective at reducing microbial loads and pathogens on hide surfaces *(7, 8)*. In this study, a 30°C LA solution was applied (2.0 atm) at a target concentration of 6.0% (actual measured concentration of 6.40%). This level was the maximum achievable concentration with the readily available substrate and equipment. Localized application of 6.4% LA resulted in a multiple log reduction ($P < 0.05$) in all microbial indicators and in *E. coli* O157:H7 and *Salmonella* prevalence on the treated hide (Table 1).

Sodium hydroxide (NaOH) at a 3% concentration has been shown to be effective at reducing microbial loads and pathogens on hide surfaces *(7, 8)*. In this study, a 10°C NaOH solution was applied (2.0 atm) at a target concentration of 3.0% (actual measured concen-

TABLE 3. Least squares means ± standard error for reductions in microbial populations, and prevalence of *E. coli* O157:H7 and *Salmonella* on dressed carcass surfaces following the application of various interventions to incoming cattle hides

	N	APC[b] log CFU/ 400 cm^2	TCC log CFU/ 400 cm^2	ECC log CFU/ 400 cm^2	EC O157:H7 Prevalence (%)	*Salmonella* spp. Prevalence (%)
Negative Control[a]	48	0.78^{y} ± 0.25	1.26^{z} ± 0.11	1.23^{z} ± 0.11	12.1	0
Water Control	50	0.13^{z} ± 0.24	1.81^{y} ± 0.14	1.90^{y} ± 0.14	6.0	-4.0
Acetic Acid	49	1.22^{y} ± 0.23	1.87^{y} ± 0.15	1.86^{y} ± 0.16	39.3	0
Lactic Acid	49	0.04^{z} ± 0.17	1.80^{y} ± 0.24	2.11^{y} ± 0.24	36.0	48.0
NaOH	48	0.70^{y} ± 0.12	3.38^{x} ± 0.15	3.35^{x} ± 0.15	73.2	60.0

[a]Cattle hides were treated with nothing (Negative Control), water (60°C), acetic acid (4.8%, 30°C), lactic acid (6.4%, 30° C) and NaOH (2.68%, 12°C). All solutions were applied for ~ 15 s at 2.0 atm.

[b]Reductions were calculated as the difference between hide microbial populations prior to intervention application (Hide Before) and the carcass microbial populations following treatment application and hide removal (Carcass), but prior to carcass intervention applications.

[x,y,z]Least squares means, within column, that lack a common superscript letter differ ($P < 0.05$).

tration of 2.7%). Localized application of 3.0% NaOH resulted in a reduction ($P < 0.05$) in all microbial indicators on the hide surface and in pathogen prevalence on the resulting carcasses (Table 1).

To compare the efficacy of intervention treatments, reductions in APC, TCC, and ECC were computed for the localized intervention (Table 2) application (hide before – hide after) and for the treatment and dressing process (hide before – dressed carcass) (Table 3). For the localized interventions, reductions in APC were AA = LA > NaOH > CT = H_2O; reductions in TCC were AA = LA = NaOH > CT = H_2O; and reductions in ECC were AA = LA = NaOH > H_2O = CT (Table 2). These data indicate that utilization of any of these chemical interventions results in microbial reductions on the hide surface. Evaluating the treatment and dressing process, with and without localized chemical interventions, reductions in APC were AA = CT = NaOH >H_2O = LA; reductions in TCC were NaOH > AA = H_2O = LA > CT; and reductions in ECC were NaOH > LA = H_2O = AA > CT (Table 3). These data suggest that NaOH was more effective at controlling coliform bacteria (TCC and ECC) than the other treatments were, as application of this localized chemical intervention to incoming hides resulted in the largest reductions in coliform indicator organisms on dressed carcass surfaces.

Trimmings generated from each treatment group of carcasses were segregated and sampled. Trimmings from all treatment groups were negative for the presence of both *E. coli* O157:H7 and *Salmonella* and had similar ($P > 0.05$) levels of APC, TCC and ECC (data not shown) following exposure to the food safety interventions system employed in this processing facility

These findings confirm the importance of sanitary dressing practices, as initial reductions in indicator organism levels were diminished during the dressing process, with dressed carcasses having equal or higher indicator loads than localized hide areas treated with chemical interventions. Additionally, these findings support the application and effectiveness, under commercial conditions, of using localized chemical decontamination strategies on hide surfaces, as *E. coli* O157:H7 and *Salmonella* prevalence on carcass surfaces were reduced, when these organisms were present initially.

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