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Parameters Affecting the Efficacy of Spray Washes against *Escherichia coli* O157:H7 and Fecal Contamination on Beef†

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ABSTRACT

A series of progressive experiments was conducted with a model carcass washer using tap water and 2% acetic acid sprays to determine if tissue type, inoculation menstruum, bacterial level, or spray temperature affect removal of bacteria from beef carcass tissue during spray washing. For the first experiment, prerigor (15 min postexsanguination), postrigor (24 h postexsanguination), or postrigor frozen (−20°C, 7 days), thawed, lean beef carcass tissue (BCT) was inoculated with bovine feces and subjected to spray washing (15 s, 56°C) with water or acetic acid. Spray washing with either compound resulted in bacterial populations that were similar for prerigor and postrigor BCT; however, remaining bacterial populations from spray-treated postrigor, frozen BCT were significantly ($P \leq 0.05$) less than for the other two tissue types. For the second experiment, prerigor, lean BCT was inoculated with *Escherichia coli* O157:H7 suspended in bovine feces or physiological saline and spray washed (15 s, 56°C) with water or acetic acid. Bacterial populations were reduced to similar levels with acid sprays, regardless of menstruum. For the third experiment, *E. coli* O157:H7 in feces was used to contaminate prerigor lean BCT to obtain different initial bacterial levels (7, 5, 3, and 1 log CFU/cm^2). Spray washes (15 s, 56°C) with acetic acid reduced the level of the pathogen to 2.51 and 0.30 log CFU/cm^2 when initial bacterial levels were 7 and 5 log CFU/cm^2, and to undetectable levels when initial bacterial levels were 3 and 1 log CFU/cm^2. In a fourth experiment, water or acetic acid (15 s), ranging from 30 to 70°C was applied to beef tissue contaminated with *E. coli* O157:H7 in feces. Remaining bacterial populations were not different between the water treatments or between the acid treatments at any temperature. While variables such as bacterial level and inoculation menstruum may affect the efficacy of spray washing with organic acids, these results indicate that tissue type or spray temperature do not.

Key words: Beef, acetic acid, *E. coli* O157:H7, decontamination

Spray washing of meat animal carcasses with organic acids or water during the slaughter process is currently approved by the U.S. Food Safety Inspection Service *(26)* to reduce bacteria on meat animal carcasses. Numerous studies have investigated the effects of spray washing with water, chlorine, and organic acids to reduce undesirable bacteria on meat animal carcasses *(11, 25)*. Previously, we demonstrated that when *Escherichia coli* O157:H7 was attached to postrigor, previously frozen, lean beef carcass tissue (BCT), spray washed (ca. 15 s, 5.52 bar, 24°C) with three types of organic acids at three different concentrations, and incubated for 24 h under refrigerated conditions, bacterial populations were not reduced to zero levels *(9)*. Specifically, spray treatments with organic acids at concentrations of 5% resulted in population reductions of *E. coli* O157:H7 ranging from 1.80 to 2.60 log CFU/cm^2 *(9)* from an initial inoculum of ca. 5 log CFU/cm^2. However, in another recent study, it was demonstrated that ca. 5 log CFU/cm^2 *E. coli* O157:H7 attached to prerigor lean BCT could be reduced by more than 3 log CFU/cm^2 immediately after spray treatments with 3% acetic acid and that bacterial populations also could be effectively suppressed during refrigerated storage up to 21 days *(17)*. Because of the inherent differences between the protocols of these two studies and other published studies *(4, 20, 22, 24)*, the present study attempted to determine whether variables such as tissue type, inoculation menstruum, inoculum amount, or temperature of spray wash affect the outcome of spray washing procedures for reducing fecal contamination or *E. coli* O157:H7 on beef.

MATERIALS AND METHODS

Feces preparation and bacterial strain

Fresh bovine feces were obtained immediately after defecation from cows fed a corn-silage ration and located at the Roman L. Hruska U.S. Meat Animal Research Center (RLHUSMARC). For experiment 1, feces were diluted 1:2 in sterile distilled water (pH 7.0). For experiments 2, 3, and 4, feces were diluted 1:2 in sterile distilled water, sterilized by autoclaving (121°C, 15 min), and resuspended to the original volume with sterile distilled water prior to inoculation with the test organism.

For experiments 2, 3, and 4, a streptomycin-resistant strain of *Escherichia coli* O157:H7 (Str^r) was obtained from the RLHUSMARC culture collection *(18)*, maintained in 75% glycerol at −20°C, and grown statically in tryptic soy broth (Troy Biologicals, Troy, MI) containing 250 μg/ml of streptomycin sulfate (Sigma

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† Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Chemical Company, St. Louis, MO) at 37°C for 18 h. *E. coli* O157:H7 cells were pelleted by centrifugation (1,400 × *g*, 15 min, 5°C) and resuspended to the original volume with sterile physiological saline (PS) or buffered peptone water (BPW), depending upon the experiment.

Experiment 1. Tissue type

Seven days prior to the experiment, lean beef carcass tissue (BCT) from the cutaneous trunci muscle of postrigor beef carcasses was obtained from a local packing plant, placed into plastic bags, stored in an insulated container, and transported back to the laboratory within 1 h postexsanguination. Samples were vacuum packaged (equipment from Hollymatic, Omaha, NE) according to the manufacturer's instructions and stored at −20°C. The day before the experiment, the postrigor frozen tissue was thawed (18 h, 5°C) and brought to room temperature (25°C). On the day of the experiment, the cutaneous trunci of prerigor (15 min postexsanguination) and postrigor (24 h, 5°C) beef carcasses was obtained from a local packing plant and transported back to the laboratory as described above. Six pieces of each type of BCT with intact fascia were aseptically cut to 10 by 10 by 0.5 cm pieces and spoon-inoculated with 1 g of feces (diluted 1:2 in sterile distilled water). After inoculation, tissues remained undisturbed for 15 min at 25°C and were left untreated or subjected to spray washes with water or acetic acid with the model carcass washer (MCW).

Experiment 2. Menstruum types

A culture of *E. coli* O157:H7 Str^r was pelleted and resuspended to original volume in physiological saline or inoculated directly into sterile feces to obtain ca. 6 log CFU/ml or CFU/g, respectively. Six pieces of the 100-cm^2 prerigor lean BCT were spoon-inoculated on the surface with 1 ml of cells in physiological saline or 1 g of inoculated feces to obtain ca. 4.50 log CFU/cm^2 or 4.80 log CFU/cm^2, respectively. After inoculation, tissues remained undisturbed for 15 min at 25°C and were left untreated or subjected to spray washes with water or acetic acid with the MCW.

Experiment 3. Inoculum level

E. coli O157:H7 Str^r was pelleted by centrifugation as described above and resuspended to original volume with BPW and inoculated into sterile feces to obtain bacterial populations of 8, 6, 4, and 2 log CFU/g. Five pieces of prerigor lean BCT were inoculated with 1 g of the inoculated feces to obtain bacterial populations of each of the following inoculum levels: ca. 7, 5, 3, or 1 log CFU/cm^2. After inoculation, tissues remained undisturbed for 15 min at 25°C and were left untreated or subjected to spray washes with water or acetic acid with the MCW.

Experiment 4. Spray temperature

An overnight culture of *E. coli* O157:H7 Str^r was pelleted by centrifugation as described above and resuspended to original volume with BPW and inoculated into sterile feces to obtain bacterial populations of 6 log CFU/g. Six pieces of prerigor lean BCT were inoculated with 1 g of the feces to obtain ca. 5 log CFU/cm^2. After inoculation, tissues remained undisturbed for 15 min at 25°C and were left untreated or subjected to spray washes in the MCW with heated water or heated acetic acid.

Spray washing procedures

A MCW, modified for use in a biological safety hood *(17)* and located at RLHUSMARC, was used to apply the water or organic acid to inoculated tissues in all experiments. Tap water or 2% acetic acid (pH = 3.03, wt/vol) (Fisher, Scientific Co., Pittsbugh, PA) was applied at 56°C for experiments 1, 2, and 3, to obtain ca. 52°C at the meat surface. The temperature was monitored at the meat surface with a mercury thermometer (Germonow Simon, Co., Rochester, NY). For experiment 4, tap water or acetic acid spray treatments were applied to obtain ca. 30, 40, 50, 60, or 70°C (±2°C) respectively, at the meat surface. Individual pieces of inoculated lean BCT were attached by alligator-jaw clips to a sanitized plastic board for mounting within the MCW. Operation parameters for the MCW in all four experiments were as follows: spray nozzle angle, 25° flat spray at 80 psi (nozzle 25/10, Spraying Systems, Co., Wheaton, IL); droplet size, 900 μm; oscillation speed, 60 cycles per min; exposure to spray, 15 s; nozzle pressure, 5.52 bar; flow rate, 4.2 liters/min; and distance from sample, 17 cm.

Bacterial enumeration procedures

From the untreated or spray-treated BCT, a 5 by 5 by 0.5 cm (25 cm^2 total surface area) piece was aseptically excised, homogenized in a Stomacher 400 (Tekmar, Inc., Cincinnati, OH) for 2 min in 25 ml of a stomaching buffer (BPW) (Difco Laboratories, Detroit, MI) and 0.1% Tween 20 (Fisher) and samples serially diluted in BPW. Samples from experiment 1 were spiral plated in duplicate on Trypticase soy agar (TSA) (Difco) using a Model D Spiral Plater (Spiral Biotech, Columbia, MD). Samples from experiment 2 containing *E. coli* O157:H7 were spiral plated in duplicate on sorbitol-McConkey agar (SMAC) (Difco) supplemented with 250 μg of streptomycin sulfate per ml (Sigma). The lowest level of detection of aerobic plate counts (APC) or the pathogen was 1.30 log CFU/cm^2 using spiral plating procedures. Therefore, samples from experiments 3 and 4 containing *E. coli* O157:H7 were spread plated in quadruplicate (250 μl per plate) on SMAC plates with the antibiotic to detect total number of CFU/ml. Plates from all experiments were enumerated after incubation at 35°C for 48 h.

Calculations and statistical analyses

Bacterial counts were converted from CFU/ml to log CFU/cm^2 prior to data analyses. Least squares means (LSM) of all bacterial populations were calculated from the experimental replications (experiment 1, $n = 6$; experiment 2, $n = 10$; experiment 3, $n = 5$; experiment 4, $n = 6$). Log reductions were calculated as differences between log CFU/cm^2 of untreated populations and log CFU/cm^2 of water- or acid-treated populations. An analysis of variance (ANOVA) was performed on the population data using the general linear models procedure of SAS Statistical Institute, Cary, NC. The probability level was $P \leq 0.05$, unless otherwise noted.

RESULTS

Experiment 1. Tissue type

To some degree, tissue type did affect immediate removal of fecal bacteria from beef tissue following treatments with tap water or 2% acetic acid (Figure 1). While the bacterial population of untreated postrigor frozen BCT was numerically lower (5.02 log CFU/cm^2), this population was not statistically different from untreated pre- (5.26 log CFU/cm^2) or postrigor (5.37 log CFU/cm^2) BCT, which were not different from each other. Following spray treatments with tap water, the remaining bacterial populations of postrigor frozen BCT were significantly lower (2.79 log CFU/cm^2) than populations remaining on the pre- (3.52 log CFU/cm^2), or postrigor (3.28 log CFU/cm^2) BCT. Similar results were observed for spray treatments with 2% acetic

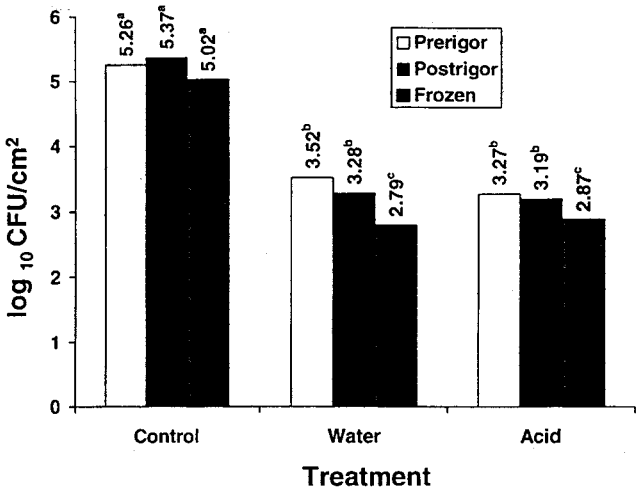

FIGURE 1. *The effect of tissue type on removal of fecal bacteria from beef following spray treatments (52°C, 15 s, 5.52 bar) with tap water or 2% acetic acid.*

acid. Bacterial populations of pre- (3.27 log CFU/cm^2) and postrigor (3.19 log CFU/cm^2) BCT were significantly different than frozen postrigor BCT (2.87 log CFU/cm^2).

Experiment 2. Menstruum types

Menstruum types did affect immediate removal of *E. coli* O157:H7 from beef tissue following spray treatments with water, but not with acid (Figure 2). Using similar culture and inoculation procedures, *E. coli* O157:H7 cells attached to prerigor beef at a statistically higher rate when suspended in sterile feces (SF) (4.87 log CFU/cm^2) than in physiological saline (PS) (4.53 log CFU/cm^2). Additionally, fewer *E. coli* O157:H7 cells in either PS or SF remained on prerigor BCT following spray treatments with 52°C acid (<1.8 log CFU/cm^2), compared with bacteria remaining after 52°C water sprays (>2 log CFU/cm^2). However, greater bacterial populations remained on BCT inoculated with the pathogen suspended in PS (2.83 log CFU/cm^2) and subjected to water washes than when the pathogen was suspended in SF (2.07 log CFU/cm^2) and subjected to similar treatments.

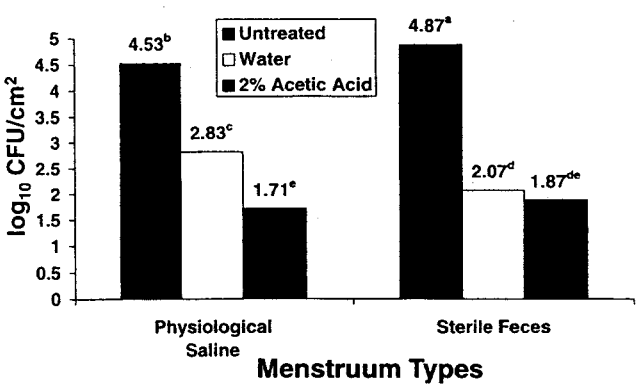

FIGURE 2. *The effect of menstruum types on removal of* E. coli *O157:H7 suspended in physiological saline or feces following spray treatments (52°C, 15 s, 5.52 bar) with tap water or 2% acetic acid.*

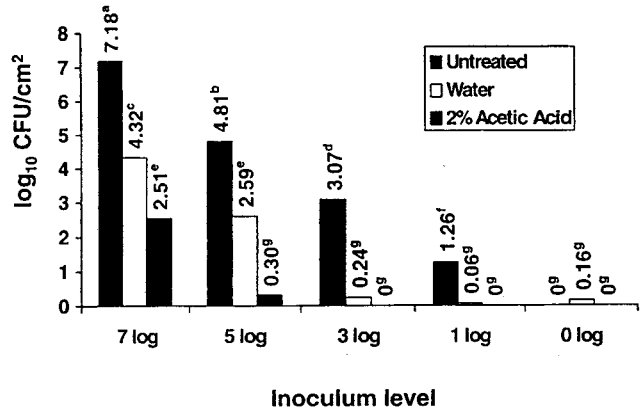

FIGURE 3. *The effect of inoculum level on removal of* E. coli *O157:H7 suspended in feces following spray treatments (52°C, 15 s, 5.52 bar) with tap water or 2% acetic acid.*

Experiment 3. Inoculum level

The efficacy of spray washes against *E. coli* O157:H7 was the most remarkable at different inoculum levels (Figure 3). When *E. coli* O157:H7 cells were inoculated at levels of 7.18, 4.81, 3.07, and 1.26 log CFU/cm^2 and subjected to spray washing with water, reductions of 2.86, 2.22, 2.83, and 1.26 log CFU/cm^2 respectively, were observed. Similar levels of *E. coli* O157:H7 cells subjected to 2% acetic acid spray washes afforded reductions of 4.67, 4.51, ≥3.07, and ≥1.26 log CFU/cm^2. More importantly, when *E. coli* O157:H7 cells were inoculated at levels of 3 and 1 log CFU/cm^2 and subjected to spray washes with 2% acetic acid, the pathogen was not detected by using the plating procedures described in this study. It should be noted that injured cells were not enumerated using the protocols described.

Experiment 4. Spray temperature

While acid spray treatments effected the greatest reductions of the pathogen in this experiment, acids applied at different temperatures were statistically similar at all temperatures (Figure 4). Similar results were observed with all the

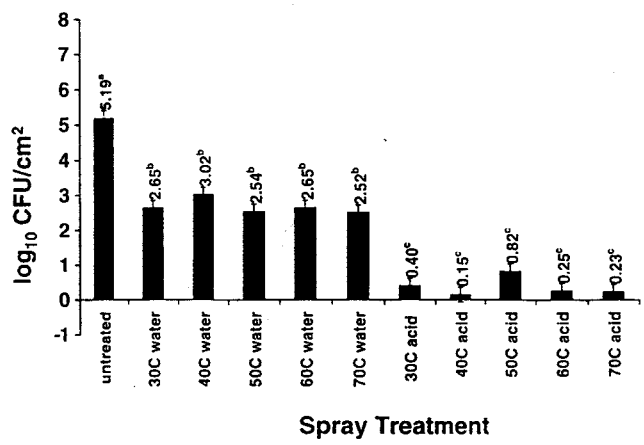

FIGURE 4. *The effect of spray wash temperature (ca. 30, 40, 50, 60, and 70°C, 15 s, 5.52 bar) on removal of* E. coli *O157:H7 suspended in feces following treatments with tap water or 2% acetic acid.*

water spray treatments. Overall, water spray treatments at any temperature reduced the pathogen <2.67 log CFU/cm^2 while any of the acid spray treatments reduced *E. coli* O157:H7 by >4.30 log CFU/cm^2.

DISCUSSION

Many studies have addressed the use of organic acids to reduce specific bacterial populations on meat animal carcasses. However, there appear to be inherent differences among the studies with regard to any one of the following parameters: acid type, acid concentration, methods for acid delivery, menstruum, temperature of acid, number of sprays, acid combinations, inoculum level, contact time, tissue type, organism, and sampling or enumeration techniques used by the researchers. These parameters ultimately may affect the results that are obtained during such experiments. Given the different protocols used by researchers, we carried out a series of experiments to determine what effects four such parameters would have on spray washing results for removal of fecal bacteria and *E. coli* O157:H7 from beef. No attempt was made to determine the impact that other variables, such as spray pressure or strain differences, would have for removal of *E. coli* O157:H7 from beef.

Of the studies carried out in this area, several researchers have utilized bacteria attached to postrigor beef tissue (in the form of beef cuts or surface tissue) to determine if organic acids are effective for removal of undesirable bacteria from beef *(1, 3–7, 9, 10, 13, 14)*. Similarly, spray washing has been investigated on prerigor beef carcasses or tissues *(2, 15–21)*. Postrigor tissue may not represent normal processing; however, this may be the only tissue that scientists are able to access in laboratory settings or under pilot-plant conditions. In the present study, when the same inoculum was applied to the surfaces of three beef carcass tissue types, bacterial populations attached to a lesser extent on the postrigor frozen and thawed tissue than on the pre- or postrigor tissues, although none of the populations were statistically different. While spray washing results were not different between the pre- and postrigor tissues, resulting populations on postrigor frozen and thawed tissue were lower than the other two tissue types. Dickson *(12)* found that irradiated frozen postrigor beef was not different than flash-frozen prerigor beef when examining bacterial attachment or efficacy of spray washing. In this study, it is possible that the surface of the postrigor frozen beef carcass tissue was altered slightly by freezing or thawing such that bacteria did not attach sufficiently and/or were removed readily by spray washing procedures. Based on our findings, pre- or postrigor tissue from surfaces of beef carcasses are suitable tissues and are more realistic for conducting spray washing experiments than experiments in which postrigor frozen tissue is used.

Our results did not encompass the effect of cut surfaces nor were any experiments conducted exclusively with adipose tissue. While cut surfaces and exposure to internal muscle do occur during processing, research has indicated that organic acids can effectively remove bacterial populations from these surfaces as well *(11, 12, 14)*. Similarly, the use of lean tissue from the cutaneous trunci during spray washing procedures in this study may represent a worst-case scenario. Research conducted to compare the effect of tissue types has indicated that bacteria are removed to a greater extent on adipose than on lean tissue *(9, 11, 12, 14)*. If organic acid washing procedures are effective on lean tissue, when the surfaces of beef carcasses are composed primarily of adipose tissue, it would appear that organic acid spray washing should remove bacteria more effectively from adipose surfaces.

Several previously published studies also have examined spray washing of organisms in a buffer menstruum *(7, 9, 20)*, rather than in feces *(3–5, 12, 13, 17–19, 22)*. Specifically, Dickson *(12)* demonstrated that fewer bacterial cells attached to beef tissue when *S. typhimurium* cells were suspended in feces and that cells were more resistant to the effects of spray washing. According to the author, feces may have provided a physical barrier for the pathogen or feces neutralized the acid during spray washing *(12)*. In the present study, we did not see any differences between types of menstruum when *E. coli* O157:H7 cells were suspended in physiological saline or sterile bovine feces and subjected to a 2% acetic acid spray wash. However, there was greater removal of the pathogen suspended in sterile feces than in physiological saline following spray washes with water.

In other published studies, as with this study, bacterial cells may be subjected to a centrifugation step in order to remove media from the cells. It was not determined if culturing or centrifugation procedures, as described in this study, removed cellular appendages involved in motility or attachment of *E. coli* O157:H7 cells to the beef surface. However, the authors believe that the centrifugation procedures were rigorous enough to pellet the cells, but not severe enough to remove appendages *(23)*.

It also should be noted that we did not address the effects of sterile and nonsterile feces on spray washing results. However, the resulting bacterial populations of *E. coli* O157:H7 following inoculation in sterile feces and spray washes with 2% acetic acid in experiments 2, 3, or 4 in this study were in close agreement with the results of Hardin et al. *(22)* and Dorsa et al. *(18)* in which antibiotic-resistant *E. coli* O157:H7 cells were suspended in nonsterile feces. Based on these limited observations, nonsterile or sterile feces are acceptable menstruum types and should be considered in the design of spray washing experiments.

Given that bacterial populations were slightly higher in sterile feces than in physiological saline in the present study, it is possible that these differences were related to initial inoculum levels rather than to spray washing effects. As demonstrated in experiment 3, reductions and resulting bacterial populations following spray treatments with 2% acetic acid or water were influenced by initial inoculum level of *E. coli* O157:H7 cells. Specifically, at initial populations of 7.18, 4.81, 3.07, and 1.26 log CFU/cm^2, reductions ranging from 4.67 to 1.26 log CFU/cm^2 were observed. Our results are not in agreement with two other publications. Brackett et al. *(7)* demonstrated that when *E. coli* O157:H7 was inoculated at 3.68 or 6.84 log CFU/g on beef and subjected to a low-pressure 1.5% acetic acid spray (55°C), no statistically different bacterial reductions were observed. Similarly, Greer and Dilts *(20)* observed reductions of <0.56, 0.54, and 0.46 log cycles when the initial

inoculum level of *E. coli* on beef was 3.1, 3.90, and 5 log CFU/cm^2 and the beef was subjected to immersion in 3% acetic acid (55°C). The limited reductions observed in these studies may be attributable to the method of acid application and/or tissue type. However, the results from the present study are in agreement with two other recent experiments. Hardin et al. *(22)* started with ca. 5 log CFU/cm^2 of *E. coli* O157:H7 on prerigor beef carcasses and observed populations of ≤0.50 log CFU/cm^2 after a dual spray wash with a commercial washer with water (35°C) and 2% acetic acid (55°C). Dorsa et al. *(18)* also demonstrated that *E. coli* O157:H7, at an initial level of ca. 4.2 log CFU/cm^2, could be reduced to ≤0.50 log CFU/cm^2 immediately after single spray washes with 3% acetic acid. In another separate study, Dorsa et al. *(19)* also demonstrated that initial inoculum level affected the magnitude of reductions and resulting bacterial populations when fecally contaminated beef was subjected to ambient (15.6°C) or hot water (54.4°C) spray washes. In all instances, prerigor beef and a spray washer were used to apply the organic acid or water, which are parameters normally encountered during the slaughter process. While pathogen inoculation levels >3 log CFU/cm^2 used in the present and other published studies may represent a worst-case scenario, it is possible that even higher levels of *E. coli* O157:H7 could be shed in bovine feces *(8)*.

To our knowledge, few studies have addressed the effect of spray wash temperature on reducing bacteria on beef. Anderson and Marshall *(4)* and Greer and Dilts *(20)* demonstrated that an increase in acid temperature resulted in concurrent reductions in bacterial populations. Contrary to these studies, our results indicate that increasing water or acid temperatures did not significantly alter populations of *E. coli* O157:H7 following spray washes. Since heating increases operational costs of the spray washing process, application of 2% acetic acid near ambient temperature and applied as described in this study can be used to effectively reduce *E. coli* O157:H7 population's to below detectable levels on beef when initial levels of the pathogen are ≤5 log CFU/cm^2.

We have demonstrated that of the four variables we examined, bacterial level significantly affects the efficacy of spray washing. Tissue type and menstruum also may affect the efficacy of spray washing to a much lesser extent, but temperature had no measurable effect on this process.

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