# Comparison of Intervention Technologies for Reducing *Escherichia coli* O157:H7 On Fresh Beef Carcass Adipose Tissue

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**SUMMARY**

This study compared the efficacy of lactoferricin B (LB), peroxyacetic acid (PAA), acidified sodium chlorite (ASC), cetylpyridinium chloride (CPC), acetic acid (AA), lactic acid (LA), acidified chlorite (AC) and water when analyzed as dipping solutions for fresh beef carcass tissue (BCT) surfaces for reducing populations of *Escherichia coli* O157:H7. In this study, CPC reduced *E. coli* O157:H7 populations by 4.8 log CFU/cm$^2$ when applied to BCT. Heated (55$^o$C) LA was the most efficacious approved chemical intervention in decontaminating BCT, as it reduced *E. coli* O157:H7 counts by as much as 3.3 log CFU/cm$^2$. Additionally, both ASC and AA effectively lowered *E. coli* O157:H7 counts by 2.06 and 1.61 log CFU/cm$^2$, respectively. Water (25$^o$C) reduced the microbial load by as much as 1.00 log CFU/cm$^2$. As used in this study, AC and LB reduced *E. coli* O157:H7 on BCT by only 0.7 log CFU/cm$^2$.

**Key Words:** *Escherichia coli* O157:H7, Chemical Interventions, Beef

**INTRODUCTION**

According to the Centers for Disease Control and Prevention there are approximately 76 million cases of food-borne illness in the United States annually, with 14 million of those cases attributed to known pathogens (Mead *et al*., 1999). As a part of the Pathogen Reduction; HACCP Systems Final Rule (Federal Register, 1996) Food Safety Inspection Service (FSIS) regulation, FSIS recommended that all beef, pork and lamb slaughter establishments apply at least one antimicrobial treatment to carcasses before chilling. Any antimicrobial compounds previously approved by FDA and FSIS could be used for carcass decontamination (Federal Register, 1996). Numerous carcass decontamination (microbiological interventions) strategies have been researched to determine which are most effective against bacterial pathogens such as *E. coli* O157:H7.

Van Donkersgoed *et al*. (1999) reported that less than 10% of slaughter cattle carried *E. coli* O157:H7 in their feces or on their hide when entering the abattoir. Ransom *et al*.(2001) found that 36.7% of lots of cattle contained positive *E. coli* O157:H7 hide samples and 13.3% had positive fecal samples. Elder *et al.* (2000) showed that 43% of beef carcasses prior to evisceration, were positive for *E. coli* O157:H7; but because of carcass decontamination strategies in place in study plants, only 18% and 2% respectively, of carcasses sampled postevisceration and postprocessing were positive for *E. coli* O157:H7.

Carcass decontamination technologies that have previously been studied include: (a) sanitizing solutions (e.g., water plus chlorine, organic acids, hydrogen peroxide, trisodium phosphate, ozone, activated ozone, etc.), (b) spray-washing with water, (c) thermal (hot water) pasteurization, (d) steam pasteurization, (e) hot-water or steam vacuuming, and (e) conventional knife-trimming, with or without subsequent washing. Studies of these microbiological intervention technologies have determined their efficacy in decontamination of carcasses, cuts and/or trimmings. In general, washing and sanitizing agents have been effective in reducing bacterial counts by 1-3 logs and in decreasing the occurrence of pathogens on beef carcasses and cuts (Smith *et al.*, 2000).

There are new additives/chemicals that may, singularly or sequentially, be more effective against *Escherichia coli* O157:H7 than those previously studied. Interventions presently used by industry for decontamination of beef carcasses/cuts are thermal (hot-water) pasteurization, steam/hot-water vacuuming, steam pasteurization and organic acid solution rinsing. Lactoferricin B (for preventing attachment and growth of pathogens on carcass surfaces), as well as peroxyacetic acid, acidified chlorine, acidified sodium chlorite and cetylpyridinium chloride (for wounding or killing bacteria) are microbiological intervention technologies that have recently received attention for their antimicrobial properties.

The objective of this study was to compare the effectiveness of decontamination technologies that are presently in use with new chemicals that are not presently used in microbiological intervention systems to determine their effectiveness in reducing *Escherichia coli* O157:H7 counts on beef tissue.

**MATERIALS AND METHODS**

***Experimental Design***

Fresh beef carcass tissue (BCT) was obtained from a local abattoir and then transported to the microbiology laboratory at Colorado State University. The BCT was portioned to obtain 100 pieces that were approximately 5 cm x 2.5 cm x 1 cm (total surface area of 40 cm$^2$).

***Preparation of Cultures***

A five-strain composite culture of *E. coli* O157:H7 was prepared for use in this study.

***Control and Treatment Groups***

Pieces of BCT were inoculated with adequate quantities of *Escherichia coli* O157:H7 to recover either 3 to 4 $\log_{10}$ CFU/cm$^2$ (low dose) or 5 to 6 $\log_{10}$ CFU/cm$^2$ (high dose).Following inoculation, the BCT were stored at 4$^o$C for 30 minutes, to allow adequate time for bacterial attachment. The inoculated pieces of BCT were assigned to 1 of 10 control or treatment groups: (1) Uninoculated/Untreated (negative control), (2) Inoculated/Untreated (positive control), (3) Water (at 25°C) (4) Acidified Chloride (10 ppm), (5) Acetic Acid (2% at 25°C), (6) Lactic Acid (2% at 55°C), (7) Lactoferricin B (1%), (8) Peroxyacetic Acid (5%), (9) Acidified (with 2% lactic acid) Sodium Chlorite (7%) and (10) Cetylpyridinium Chloride (0.5%). The inoculated BCT pieces (with exception of those in the two control groups) were dipped into 500 ml of either ambient temperature or heated solutions for 30 s. Control and treated BCT samples were placed into stomacher bags with 0.1% Buffered Peptone Water (BPW, Difco Laboratories). Aliquots (0.1 ml) were plated on tryptic soy agar containing 0.6% added yeast extract (TSAYE) for the enumeration of all aerobic bacteria including *E. coli* O157:H7, and on sorbitol MacConkey agar (SMAC) for enumeration of *E. coli* O157:H7. Plates were incubated at 37°C for 48 h.

## RESULTS

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound that resulted in a reduction ($P < 0.05$) of 4.83 log CFU/cm$^2$ (Table 1) for the high-dose. The *E. coli* O157:H7 count on the low-dose inoculated BCT was reduced to almost undetectable levels at 0.54 log CFU/cm$^2$. Unfortunately, CPC is not currently permitted for direct application to fresh meat products due to FDA regulations.

Lactic acid (LA) is one of the most widely studied of the organic acids currently used in the beef industry. The effects of the use of LA differ among studies, but generally suggest the achievement of a 1 to 2 log CFU/cm$^2$ reduction. In this study, LA (2%) (at 55°C) was the second most effective decontamination agent studied, significantly reducing the presence of *E. coli* O157:H7 on BCT. Lactic acid reduced the *E. coli* O157:H7 counts of high- dose inoculated product from the initial 5.78 log CFU/cm$^2$ to the final 2.50 CFU/cm$^2$. *E. coli* O157:H7 counts on BCT administered the low-dose inoculation were reduced ($P < 0.05$) by of 2.60 log CFU/cm$^2$.

Acetic acid (AA) is another commonly used organic acid that, in the present study, achieved lower reduction in *E. coli* O157:H7 counts than did LA. In this study, 2% AA was applied at room temperature; a 1.61 log CFU/cm$^2$ reduction ($P < 0.05$) in *E. coli* O157:H7 counts was observed on the high-dose inoculated BCT. Acetic acid was slightly more effective in reducing the pathogen load on the low-dose inoculated BCT as shown by a 2.04 log CFU/cm$^2$ decrease in *E. coli* O157:H7 counts. The use of either lactic acid or acetic acid would be feasible and effective for pathogen reduction of sufficient magnitude to aid in increasing the safety of beef.

Acidified Sodium Chloride reduced ($P < 0.05$) *E. coli* O157:H7 counts on high-dose inoculated BCT by 1.93 log CFU/cm$^2$ and on the low-dose inoculated BCT by 1.99 logs. On the TSAYE plates (Table 2), ASC reduced the counts by 2.06 and 1.28 on high-dose and low-dose inoculated BCT, respectively.

In previous research, Farrell *et al.* (1998) determined the use of peroxyacetic acid as a sanitizer for meat contact surfaces. Peroxyacetic acid was effective in reducing the bacterial load, but total elimination of *E. coli* O157:H7 was not achieved. The 15% solution of PA used, significantly reduced (1.38 and 1.40 log CFU/cm$^2$) counts of *E. coli* O157:H7 on high-dose and low-dose inoculated BCT, respectively. A slightly higher reduction of *E. coli* O157:H7 counts was observed when counts were obtained from use of TSAYE plates. In this study, peroxyacetic acid is just as effective as acetic acid; however, it does not currently have federal approval for use as a food sanitizer.

The cheapest, easiest and oldest microbial intervention strategy is the use of water. Like lactic and acetic acid, the use of water as a decontamination technology has been studied by many researchers. The effectiveness of water as a decontamination technology is related to the water and product temperature, as well as the pressure at which it is applied; increasing the temperature and pressure will enhance water's effectiveness as a pathogen intervention (Graves-Delmore et al., 1997). In the present study, water was applied at room temperature (25°C) and its use reduced ($P < 0.05$) the presence of *E. coli* O157:H7 by 1.17 and 0.58 log CFU/cm$^2$ on high-dose and low-dose levels of inoculation, respectively. Similar data were observed when TSAYE plates were used to obtain *E. coli* O157:H7 counts (Table 2). Water will likely continue to be the most widely used intervention in beef slaughtering facilities.

The effectiveness of Acidified Chlorine was minimal, as it only reduced the pathogen level by 0.68 and 0.60 CFU/cm$^2$ for high-dose and low-dose levels of inoculation, respectively, as indicated on SMAC plates (Table 1). Increasing the ppm used during application would likely enhance the effectiveness of this sanitizer.

In previous research, Venkitanarayanan *et al.* (1999) demonstrated that use of 50 and 100 µg/ml of lactoferricin B reduced *E. coli* O157:H7 by 0.7 and 2.0 log CFU/ml, respectively. As applied in the present study, lactoferricin B (10 µg/ml) reduced the *E. coli* O157:H7 count on high-dose inoculated product by 0.66 log CFU/cm$^2$ and did not reduce the level of the pathogen on the low-dose inoculated product when counts were enumerated from the TSAYE plates (Table 2). Reductions of 0.70 and 0.55 log CFU/cm$^2$ in *E. coli* O157:H7 counts were observed on SMAC plates (Table 1) at high-dose and low-dose inoculation levels, respectively, when lactoferricin B was used.


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Table 1. Least-squares means (standard error) for the survival and reduction of *Esherichia coli* O157:H7 (log CFU/$cm^2$), plated on sorbitol MacConkey agar (SMAC), on inoculated beef carcass tissue.

| **Group** | **High Inoculation** | | **Low Inoculation** | |
|---|---|---|---|---|
| (Control or Treated) | Survival (log CFU/$cm^2$) | Reduction (log CFU/$cm^2$) | Survival (log CFU/$cm^2$) | Reduction (log CFU/$cm^2$) |
| Uninoculated/Untreated* | 1.58 (0.16) | ----- | 1.50 (0.09) | ----- |
| Inoculated/Untreated** | 5.78 (0.05)[a] | ----- | 4.12 (0.07)[a] | ----- |
| Water (at 25°C) | 4.61 (0.16)[d] | 1.17 | 3.54 (0.14)[b] | 0.58 |
| Acidified Chloride (10 ppm) | 5.42 (0.04)[b] | 0.36 | 3.36 (0.05)[b] | 0.76 |
| Acetic Acid (2%) | 4.17 (0.12)[d] | 1.61 | 2.04 (0.09)[d] | 2.08 |
| Lactic Acid (2% at 55°C) | 2.50 (0.16)[e] | 3.28 | 1.07 (0.14)[e] | 3.05 |
| Lactoferricin B (1%) | 5.08 (0.10)[c] | 0.70 | 3.57 (0.06)[b] | 0.55 |
| Peroxyacetic Acid (5%) | 4.40 (0.06)[d] | 1.38 | 2.72 (0.11)[c] | 1.40 |
| Acidified Sodium Chlorite (7%) | 3.85 (0.16)[d] | 1.93 | 2.13 (0.22)[d] | 1.99 |
| Cetylpyridinium Chloride (0.5%) | 0.95 (0.23)[f] | 4.83 | 0.54 (0.15)[f] | 3.58 |

* Negative control
** Positive control
a,b,c,de,f Means within each survival column bearing common superscript letter are not different ($P > 0.05$).
Means in reduction columns were not tested for statistical significance.

Table 2. Least-squares means (standard error) for the survival and reduction of *Esherichia coli* O157:H7 (log CFU/$cm^2$), plated on tryptic soy agar with 0.6% yeast extract (TSAYE), on inoculated beef carcass tissue.

| **Group** | **High Inoculation** | | **Low Inoculation** | |
|---|---|---|---|---|
| (Control or Treated) | Survival (log CFU/$cm^2$) | Reduction (log CFU/$cm^2$) | Survival (log CFU/$cm^2$) | Reduction (log CFU/$cm^2$) |
| Uninoculated/Untreated* | 1.52 (0.14) | ----- | 1.52 (0.14) | ----- |
| Inoculated/Untreated** | 6.36 (0.04)[a] | ----- | 4.28 (0.05)[a] | ----- |
| Water (at 25°C) | 4.80 (0.16)[c] | 1.56 | 3.92 (0.09)[b] | 0.36 |
| Acidified Chloride (10 ppm) | 5.68 (0.03)[b] | 0.68 | 3.68 (0.15)[b] | 0.60 |
| Acetic Acid (2%) | 4.92 (0.10)[c] | 1.44 | 2.53 (0.18)[d] | 1.75 |
| Lactic Acid (2% at 55°C) | 3.68 (0.10)[e] | 2.68 | 1.68 (0.11)[e] | 2.60 |
| Lactoferricin B (1%) | 5.70 (0.06)[b] | 0.66 | 4.40 (0.12)[a] | -0.12 |
| Peroxyacetic Acid (5%) | 4.83 (0.10)[c] | 1.53 | 3.21 (0.07)[c] | 1.07 |
| Acidified Sodium Chlorite (7%) | 4.30 (0.16)[d] | 2.06 | 3.00 (0.19)[c] | 1.28 |
| Cetylpyridinium Chloride (0.5%) | 1.53 (0.28)[f] | 4.83 | 0.81 (0.24)[f] | 3.47 |

* Negative control
** Positive control
a,b,c,de,f Means within each survival column bearing common superscript letter are not different ($P > 0.05$).
Means in reduction columns were not tested for statistical significance.