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EVALUATION OF ANTIMICROBIAL INTERVENTIONS APPLIED TO BOB VEAL CARCASSES INOCULATED WITH SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) SURROGATES BEFORE AND AFTER CHILLING

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Introduction

- The United States Department of Agriculture Food Safety Inspection Service reported greater prevalence of Shiga toxin producing *E. coli* (STEC) on veal carcasses and ground veal.
- Ruminants are natural reservoir of STEC and the microorganisms can easily be transferred to carcasses during the conversion of animals to meat, especially for veal.
- Literature on efficacy of antimicrobial interventions in reducing STEC populations on veal carcasses is limited.

Objectives

- Three experiments were conducted to evaluate lactic acid (4%; LA), peroxyacetic acid (300 ppm; PAA), and hot water (80˚C; HW), for their individual and/or combined ability to reduce STEC surrogates on bob veal carcasses pre- and post-chill, and through subprimal fabrication.

Materials and Methods

Experiment 1:

- Hot carcasses (n=9) were inoculated with a 5-strain cocktail (ca. 8 log CFU/ml) containing rifampicin-resistant surrogate *Escherichia coli* (*E. coli*; BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431) to simulate carcass contamination during slaughter and then treated with HW, LA, or PAA.
- Carcasses were chilled (0±1°C) for 24 h, split in halves and each side was treated with LA or PAA.

Experiment 2:

- Hot carcasses (n=3) were inoculated with the 5-strain cocktail and chilled for 24 h.
- After chilling, carcasses were split and each side was treated with either LA or PAA.

Experiment 3:

- Carcasses (n=3) where chilled for 24 h, split, and then inoculated (simulating post-slaughter contamination) and treated with either LA or PAA.
- Inoculated carcasses were allowed to rest for 15 min for attachment.

Statistical analysis

Experiment 1 was designed as a randomized split-plot with carcass as the whole plot and side as the sub-plot. Experiments 2 and 3 were completely randomized designs with side as the experimental unit. For each experiment, *E. coli* population (log CFU/cm^2) was analyzed using PROC GLM (SAS V.9.4) for the main effects of antimicrobial treatment, sampling time point, and their interaction, when applicable. Means were considered different at $\alpha \leq 0.05$.

Results

- Experiment one: Collective reductions achieved from; HW+LA, and HW+PAA were 2.88 and 2.07 log CFU/cm^2, respectively; LA+LA and LA+PAA were 3.48 and 2.20 log CFU/cm^2, respectively; and PAA+LA and PAA+PAA were 1.32 and 0.99 log CFU/cm^2, respectively.
- For experiment 2: Application of LA and PAA on the chilled carcasses resulted in 0.91 and 0.24 log CFU/cm^2 reductions.
- In experiment 3: There was no difference ($P > 0.05$) between LA and PAA in reducing surrogate *E. coli* when applied to veal carcasses.
- Measurements on cut surfaces for translocation during fabrication showed that all antimicrobial treatments resulted in undetectable levels (<0.3 log CFU/cm2) of surrogate E. coli for experiment 1 and 2, and low levels (1.66 and 0.97 log CFU/cm2 for LA and PAA, respectively) for experiment 3.

Conclusion

- Of the antimicrobial interventions utilized, lactic acid was more effective in reducing STEC surrogate populations on veal carcasses, pre- and/or post-chill.

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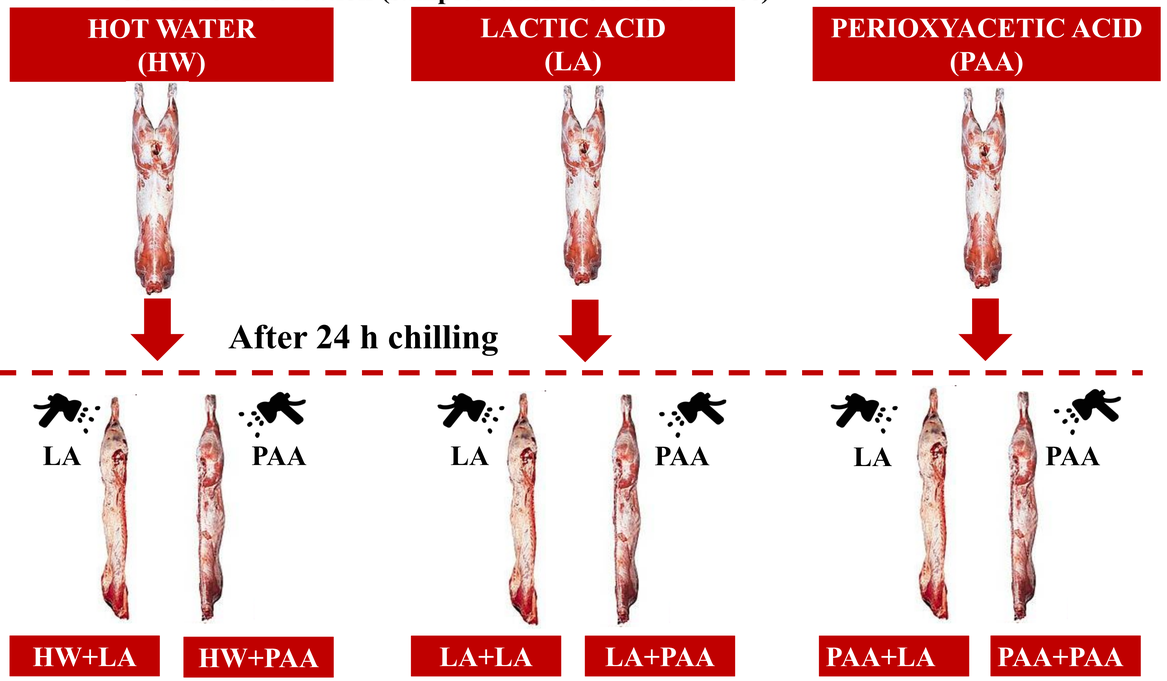
Experiment 1

Microbial Sampling

- The exterior of each carcass was evenly inoculated with a surrogate *E. coli* cocktail and left undisturbed for 15-min (25°C) to achieve ≥ 5 log CFU/cm² attachment.

- After 15-min, carcasses were subjected to a water wash (24°C), and a pre-chill antimicrobial treatment with either HW, LA, or PAA and chilled for 24 h. After 24 h chill, each carcass was split in half and each side was treated with LA or PAA and then fabricated.

- Each carcass was sampled at six different points during processing:
  1. After inoculation
  2. After water wash
  3. After the pre-chill carcass antimicrobial spray application
  4. Post-24 h chilling
  5. After the 24 h post-chill carcass antimicrobial spray application
  6. After fabrication (samples taken from cut surface)



Results

Table 1. Least squares means and standard deviation of rifampicin resistant surrogate *E. coli* (log CFU/cm²) found on bob veal carcasses treated with different combinations of antimicrobial interventions

Antimicrobial Combinations[a]	Inoculation	Water wash[b]	Pre-chill antimicrobial	Post 24 h chill	Post-chill antimicrobial	Fabrication[c]
HW+LA	5.28 ± 0.29 ^{AX}	4.90 ± 0.31 ^{ABX}	4.10 ± 0.44 ^{BCX}	3.31 ± 0.99 ^{CX}	1.23 ± 0.18 ^{DX}	≤ 0.30 ^{EX}
HW+PAA	5.28 ± 0.29 ^{AX}	4.90 ± 0.31 ^{ABX}	4.10 ± 0.44 ^{BCX}	3.31 ± 0.99 ^{CX}	2.04 ± 0.15 ^{DXZ}	≤ 0.30 ^{EX}
LA+LA	5.26 ± 0.18 ^{AX}	4.70 ± 0.06 ^{AX}	1.97 ± 0.60 ^{BY}	0.86 ± 0.99 ^{CDY}	≤ 0.30 ^{DY}	≤ 0.30 ^{DX}
LA+PAA	5.26 ± 0.18 ^{AX}	4.70 ± 0.06 ^{AX}	1.97 ± 0.60 ^{BY}	0.86 ± 0.99 ^{CY}	1.39 ± 1.39 ^{CX}	≤ 0.30 ^{DX}
PAA+LA	5.12 ± 0.02 ^{AX}	4.78 ± 0.10 ^{AX}	3.13 ± 0.81 ^{BZ}	1.83 ± 1.31 ^{CZ}	2.16 ± 0.63 ^{BCXZ}	≤ 0.30 ^{DX}
PAA+PAA	5.12 ± 0.02 ^{AX}	4.78 ± 0.10 ^{AX}	3.13 ± 0.81 ^{BZ}	1.83 ± 1.31 ^{CZ}	2.49 ± 0.16 ^{BCZ}	≤ 0.30 ^{DX}

^{A,B,C,D,E}Means within rows that do not share a common letter are different ($P \leq 0.05$); ^{X,Y,Z}Means within columns) that do not share a common letter are different ($P \leq 0.05$).
[a]Antimicrobial interventions: Hot water (80°C; HW), lactic acid (4%; LA), peroxyacetic acid (300 ppm; PAA).
[b]All carcasses were subjected to a standard water wash (24°C) prior to antimicrobial application.
[c]Fabrication samples collected from all antimicrobial treatment combinations were below the detection limit (<0.3 log CFU/cm²).

Summary

- The collective reductions achieved from; HW+LA, and HW+PAA were 2.88 and 2.07 log CFU/cm², respectively; LA+LA and LA+PAA were 3.48 and 2.20 log CFU/cm², respectively; and PAA+LA and PAA+PAA were 1.32 and 0.99 log CFU/cm², respectively.

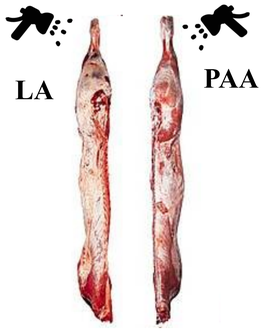
- Of the six combinations, LA+LA was the most effective ($P \leq 0.05$) treatment for reducing surrogate *E. coli* on veal.

- Fabrication results showed that all combination of antimicrobial treatments resulted in undetectable levels (<0.3 log CFU/cm²) of surrogate *E. coli*.

Experiment 2

Microbial Sampling

- The exterior of each carcass was evenly inoculated with a surrogate *E. coli* cocktail to achieve ≥ 5 log CFU/cm^2 attachment.
- Carcasses were held on the slaughter line for 15 min (25°C) for attachment, followed by a water wash (24°C), and then chilled for 24 h. After 24 h chill, carcasses were spilt and each side sprayed with either LA or PAA prior to fabrication.
- Each carcass was sampled at five different points during processing:
    1. After inoculation
    2. After water wash (24°C)
    3. Post-24 h chilling
    4. After the 24 h post-chill carcass antimicrobial spray application
    5. After fabrication (samples taken from cut surface)



Results

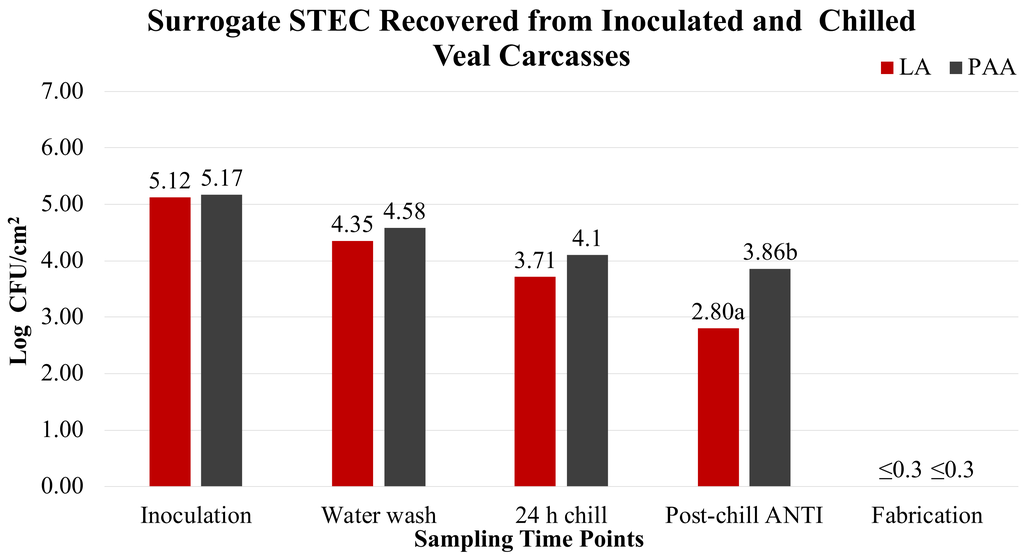


Figure 1. Surrogate *E. coli* populations recovered from bob veal carcasses that were inoculated and subsequently chilled for 24 h before antimicrobial interventions lactic acid (4%; LA), or peroxyacetic acid (300 ppm; PAA) were applied. Means within sampling time point that do not share a common letter are statistically different ($P \leq 0.05$). Fabrication samples were below the detection limit (<0.3 log CFU/cm^2) for both treatment groups.

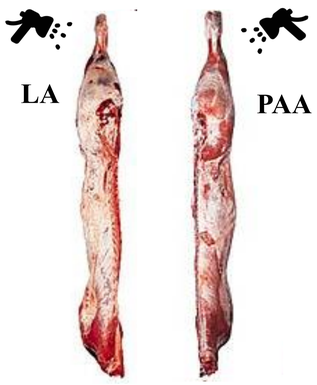
Summary

- Within sampling time points, recovered population of surrogate *E. coli* were all similar ($P > 0.05$) except for post-chill antimicrobial spray application.
- Application of LA and PAA on the chilled carcasses resulted in 0.91 and 0.24 log CFU/cm^2 reductions of surrogate *E. coli* when compared to sampling after 24 h chill. Lactic acid resulted in greater ($P \leq 0.05$) reductions compared to PAA.
- Fabrication samples taken from cut subprimal surfaces showed that both antimicrobial treatments resulted in undetectable levels (<0.3 log CFU/cm^2) of surrogate *E. coli*.

Experiment 3

Microbial Sampling

- After 24 h chilling, the exterior of each carcass was evenly inoculated with surrogate *E. coli* cocktail (simulating pre-fabrication contamination) to achieve ≥ 5 log CFU/cm^2 attachment.
- Inoculated carcasses were allowed to rest for 15 min (25°C) for attachment and then treated with either LA or PAA.
- Each carcass was sampled at three different points during processing:
    1. After inoculation
    2. After the 24 h post-chill carcass antimicrobial spray application
    3. After fabrication (samples taken from cut surface)



Results

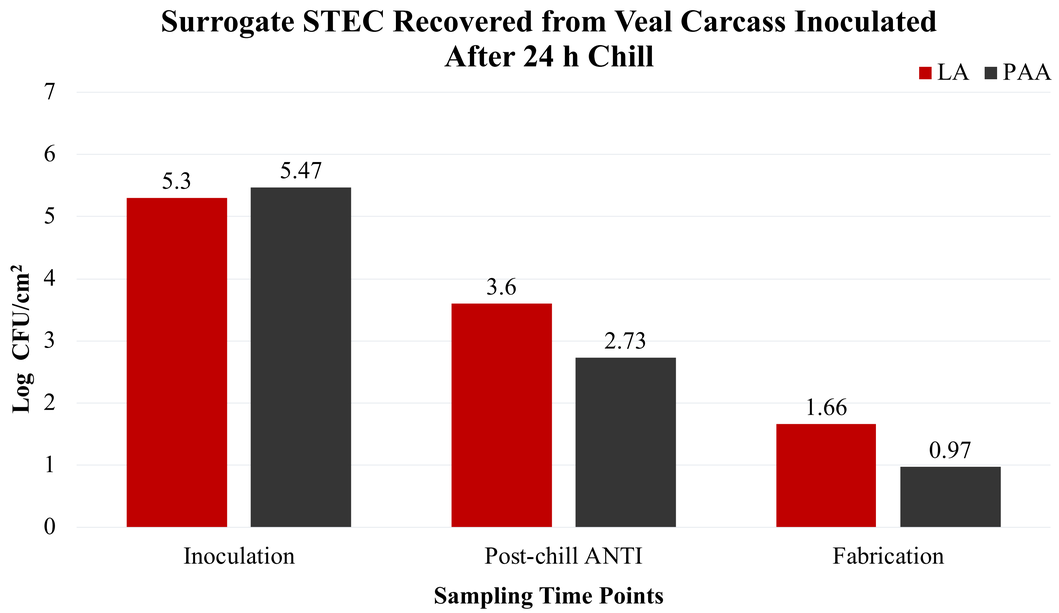


Figure 2. Surrogate *E. coli* populations recovered from bob veal carcasses that were inoculated and treated with antimicrobial interventions lactic acid (4%; LA), or peroxyacetic acid (300 ppm; PAA) after 24 h chill.

Summary

- There was no difference ($P > 0.05$) between LA and PAA for reducing surrogate *E. coli* when applied to chilled veal carcasses.
- Measurements on subprimal cut surfaces for translocation during fabrication showed low levels (1.66 and 0.97 log CFU/cm^2 for LA and PAA, respectively) surrogate *E. coli.*