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**Project Title: Characterizing Differences in Shiga Toxin-Producing *Escherichia coli* (STEC)
Attachment to Pre-Rigor and Chilled Beef Carcass Surfaces**

Presenter: Carla Schwan, Gary Acuff, Harshavardhan Thippareddi, Donka Milke, Minto Michael, Nicholas Severt, Matthew Krug, Lisa Lucia, Jessie Hudson, Christopher I. Vahl, Elizabeth Holmgren, Amanda Wilder, Sarah Schuetze, and **Randall Phebus**

Presenters email address: phebus@ksu.edu

Presenters mailing address: 1530 Mid-Campus Drive North, Manhattan, KS 66506

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Objective: This study evaluated the efficiency of a mixed STEC inoculum to attach to raw beef carcass surfaces (lean or fat), and efficacy of 4.5% lactic acid (LA) or water (W) spray to reduce STEC populations, under four scenarios: (A) pre-rigor surface STEC inoculated (ca. 4 log cfu/cm²), 30-min ambient temperature attachment, spray with LA or W; (B) pre-rigor inoculated, 24-h chilled attachment, spray; (C) tissue chilled 24 h, inoculated, 30-min attachment, spray; and (D) tissue chilled 24 h, rewarmed to 37°C, inoculated, 30-min attachment, spray.

Experimental Design & Analysis:

Lean and fat tissues (230 cm²) were collected from two fed cattle at harvest and assigned to the four scenarios, followed by post-inoculation ambient temperature W or LA spray. Tissue samples were collected pre- and post-treatment to enumerate STEC populations. The experiment was a completely randomized design with four replicates.

Key Results:

STEC attachment levels to lean and fat tissues were similar across scenarios ($p > 0.05$). LA reduced STEC levels 1.0-1.8 log cfu/cm² greater ($p \leq 0.05$) than the W spray across the four inoculation scenarios. A significant treatment by scenario interaction was observed for STEC reductions, with LA being more effective in scenarios A and B (pre-rigor inoculation) than C and D (post-rigor inoculation). The STEC reduction observed in scenario D (chilled then re-warmed to inoculate and treat with the antimicrobial spray, as might occur in some laboratory pathogen

validation studies) was similar to scenarios A and B indicating validity for use in inoculated validation studies where STEC contamination occurs pre-rigor.

How can this information be applied in the industry:

To validate beef antimicrobial technologies, inoculation of tissue surfaces with pathogens and/or surrogate cultures is often required. The literature demonstrates a range of approaches utilized for inoculation (pre-rigor, post-rigor chilled, post-rigor but rewarmed, etc), which may impact the results obtained from each intervention. This research provides guidance on best practices for carcass inoculation when conducting antimicrobial validation studies. Further, it demonstrates that an ambient temperature 4.5% LA spray has little effect when applied to chilled beef products, but is effective when used on freshly slaughtered pre-rigor carcass surfaces.

Figure 1. STEC log reductions on 15x15 cm² beef surfaces under different treatment scenarios after antimicrobial application. Antimicrobial treatments followed by a different letter within scenarios were significantly different (p ≤ 0.05).

