Kansas Agricultural Experiment Station Research Reports

Volume 0
Issue 1 *Cattleman's Day (1993-2014)*

Article 96

2009

Needle-free injection enhancement of beef improves tenderness but slightly increases microbial translocation

A. Sutterfield

B.A. Crow

J.P. Grobbel

Randall K. Phebus

See next page for additional authors

Needle-free injection enhancement of beef improves tenderness but slightly increases microbial translocation

Abstract
Blade tenderization has been used for decades to increase tenderness in beef cuts that are highly variable in tenderness or predicted to be "tough." Injection enhancement also is commonly used in industry to increase tenderness, juiciness, and flavor of some beef muscles. These processes have the potential to translocate microbial organisms on the exterior to interior portions of whole muscles. One research study reported that 3 to 4% of surface bacteria are transferred into the interior of muscles but only penetrate an average of ¼ inch deep into the surface. Even though the frequency of subprimal surfaces being contaminated with pathogens is low, translocation of these contaminants into the interior of subprimals by tenderization or injection procedures poses a public health risk. Microbial contamination on beef surfaces generally is eliminated during typical cooking; however, given the low infectious doses of pathogens such as Escherichia coli O157:H7, internalized contamination may survive if adequate temperatures are not reached at the center of cuts (i.e., rare and medium rare endpoints) and lead to illness. Industry groups have developed a guide, Best Practices: Pathogen Control During Tenderizing/Enhancing of Whole Muscle Cuts to minimize any hazard that may be present with such technologies. Although needle injection enhancement currently is common in beef processing, there may be alternative, safer, or more effective means to apply these technologies. One potential method involves utilizing an air-pressured needle-free injection system similar to an instrument currently being investigated for use in vaccinating cattle. In theory, eliminating the need for physical penetration of the muscle with a needle-free instrument using air-pressure fluid streams would reduce the translocation of surface microbial contamination to the interior and would additionally minimize carryover contamination from subprimal to subprimal during continuous injection operations. Therefore, we investigated use of needle-free injection enhancement as an alternative strategy to needle injection enhancement. Our objectives were to determine the safety and efficacy of using needle-free injection for enhancing beef muscles and the application of needle-free injection enhancement for improving beef quality.

Keywords
Cattlemen's Day, 2009; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 1010; Kansas Agricultural Experiment Station contribution ; no. 09-168-S; Beef; Cattle; Needle-free injection; Tenderness

Authors
A. Sutterfield, B.A. Crow, J.P. Grobbel, Randall K. Phebus, Michael E. Dikeman, and Larry C. Hollis

Needle-Free Injection Enhancement of Beef Improves Tenderness but Slightly Increases Microbial Translocation

A. Sutterfield, R. K. Phebus, B. A. Crow, M. E. Dikeman, J. P. Grobbel, and L. Hollis

Introduction

Blade tenderization has been used for decades to increase tenderness in beef cuts that are highly variable in tenderness or predicted to be "tough." Injection enhancement also is commonly used in industry to increase tenderness, juiciness, and flavor of some beef muscles. These processes have the potential to translocate microbial organisms on the exterior to interior portions of whole muscles. One research study reported that 3 to 4% of surface bacteria are transferred into the interior of muscles but only penetrate an average of ¼ inch deep into the surface. Even though the frequency of subprimal surfaces being contaminated with pathogens is low, translocation of these contaminants into the interior of subprimals by tenderization or injection procedures poses a public health risk. Microbial contamination on beef surfaces generally is eliminated during typical cooking; however, given the low infectious doses of pathogens such as *Escherichia coli* O157:H7, internalized contamination may survive if adequate temperatures are not reached at the center of cuts (i.e., rare and medium rare endpoints) and lead to illness. Industry groups have developed a guide, *Best Practices: Pathogen Control During Tenderizing/Enhancing of Whole Muscle Cuts* to minimize any hazard that may be present with such technologies.

Although needle injection enhancement currently is common in beef processing, there may be alternative, safer, or more effective means to apply these technologies. One potential method involves utilizing an air-pressured needle-free injection system similar to an instrument currently being investigated for use in vaccinating cattle. In theory, eliminating the need for physical penetration of the muscle with a needle-free instrument using air-pressure fluid streams would reduce the translocation of surface microbial contamination to the interior and would additionally minimize carryover contamination from subprimal to subprimal during continuous injection operations. Therefore, we investigated use of needle-free injection enhancement as an alternative strategy to needle injection enhancement. Our objectives were to determine the safety and efficacy of using needle-free injection for enhancing beef muscles and the application of needle-free injection enhancement for improving beef quality.

Experimental Procedures

We determined from a preliminary study that the optimal air pressure for needle-free injection enhancement was 25 lb/in.^{2} based on dispersion, visual acceptability, and penetration level. We also determined that needle-free injections should be made 0.32 in. apart in a grid pattern, by using a plexiglass template, to attain the same injection enhancement volume/weight retention as needle injection enhancement. Needles in the needle injector were spaced 0.7 × 1.0 in. apart in a staggered pattern.

Beef longissimus muscles (N = 15) from USDA Select, A-maturity carcasses were obtained from a commercial abattoir at 2 days postmortem and transported to the Kansas State University Meat Laboratory and stored at 34°F until 9 days postmortem. Fat was trimmed to ⅛ in., and each loin was halved and randomly assigned to one of two treatments: (1) needle injected (Model N30, Wolftec Inc., Werther, Germany) or (2) needle-free injected (Pulse Needle-Free Systems, Lenexa, KS).

A nonpathogenic generic *E. coli* strain (ATCC number 25922, MicroBiologics, St. Cloud, MN) grown in tryptic soy broth was used to make a master (test) inoculum providing 10^9 CFU/mL. Each of the two matching loin halves was inoculated to a target level of 10^{5-6} CFU/cm^2 on the fat side of the meat. Loins were allowed to sit inside the inoculation chamber for 10 minutes, removed, and *E. coli* was allowed to attach for 1 hour at 50°F. Once *E. coli* had attached, surface samples were taken by excising two samples, 2 in. in diameter by ⅛ in. deep, from the surface on opposite ends of each loin half and plated on *E. coli*/coliform (ECC) Petrifilm (3M Corporation). The plates were incubated at 95°F for 24 hours and then enumerated. The experiment was replicated on three separate days.

Matching loin halves were then injection enhanced on the inoculated (fat side) side with needle or needle-free injection. Injection was set to achieve a desired pump yield of 12%. A solution of water, salt (0.3%), phosphate (0.3%), and potassium lactate (1.5%) (Brifisol 85 Instant, BK Giulini Corp., Simi Valley, CA) was used for injection enhancement.

After being injected, loins were drained for 1 hour, and two 2-in. diameter cores were taken aseptically cross-sectionally from each loin half to represent the entire thickness of the loin. Both cores were set with the inoculated surface facing downward on a sanitized tray and placed in the freezer at 24°F for 1 hour. Cores were then removed from the freezer, and slices were taken beginning at the inoculated side of the surface and at the defined depths of 0.4, 1.2, and 2.0 in. across the muscle fibers by using sterile techniques.

Fifteen additional loins of similar quality were injection enhanced by using the same procedure described previously. Three steaks (1-in. thick) were cut from the anterior end of each muscle section. Two of these steaks were placed in separate foam trays and covered with polyvinyl chloride film for simulated retail color display. The remaining steak from each muscle section was vacuum packaged and stored at 35°F for 4 days until cooked and measured for slice shear force.

Steaks for visual color evaluation were displayed under continuous fluorescent lighting for 5 days at 35°F. Trained visual color panelists (n = 8) evaluated display color and surface discoloration daily. The color scale used by panelists was: 1 = very bright red, 2 = bright red, 3 = dull red, 4 = slightly dark red, 5 = slightly dark red or reddish tan, 6 = moderately dark red to tannish red, and 7 = tan to brown. Also on days 1 to 5, discoloration scores were considered as a percentage of surface metmyoglobin with the following scale: 1 = none (0%), 2 = slight discoloration (1-19%), 3 = small discoloration (20-39%), 4 = modest discoloration (40-59%), 5 = moderate discoloration (60-79%), 6 = extensive discoloration (80-99%), and 7 = total discoloration (100%).

On day 13 postmortem, steaks were taken from the 35°F storage environment and cooked in a forced-air convection oven at 320°F to an internal temperature of 158°F for longissimus slice shear force measurements. Within 2 minutes after cooking, a 0.40-in.-thick, 2-in.-long slice was removed from the lateral end of each steak parallel to the muscle fibers. The slice was sheared perpendicular to the muscle fibers by using an Instron Universal Testing Machine with a flat, blunt-end blade and a crosshead speed of 0.33 in./second. Peak shear force was recorded in pounds.

Statistical Analysis

Microbiology data were analyzed as a split-plot design by using the MIXED procedure of SAS. Fisher's least significant difference was used to determine differences among bacterial populations at the different depths. Significance was determined at probability values of $P<0.05$. Display color and slice shear force data were analyzed as a split-plot design by using the MIXED procedure. Fisher's least significant difference was used to determine differences between treatments. Significance was determined at probability values of $P<0.05$.

Results and Discussion

There was a difference in generic *E. coli* counts among depths ($P<0.001$) in both treatments. Figure 1 shows the distinct difference between surface, 0.4-in., 1.2-in. and 2.0-in. samples. The tendency of *E. coli* counts to increase from the depth of 0.4 and 1.2 in. to 2.0 in. could be due to artificial contamination introduced when brine pooled on the table surface during injections.

E. coli counts were higher ($P<0.001$) for needle-free injections than for needle injections (Figure 2). The closer spacing between injection sites provided for a greater number of penetrations in needle-free injected loins, which could account for this increase. Also, the use of air pressure could have caused the inoculum to be pushed further into the loin.

There was a significant ($P<0.001$) day by depth interaction in which the lowest microbial counts occurred for depths of 0.4, 1.2, and 2.0 in. on day 1 (Figure 3). The depth of 1.2 in. consistently had the lowest microbial counts on all three respective days. There were no differences between depths of 0.4 and 2.0 in. on days 2 and 3. The surface enumerations were at least double those of all depths on all days.

There was a treatment by depth interaction trend ($P<0.06$). Lowest microbial counts were found at depths of 1.2 and 2.0 in. with needle injection (Figure 4). The needle-free injection at 1.2 in. had similar microbial counts to needle injection at 2.0 in. There was no difference between 0.4 and 2.0 in. for both needle-free and needle injection. The surface was different than all depths for both treatments.

Relative to microbial data generated, the novel, first generation system that we used was not optimized to control microbial cross-contamination during sample preparation because an injection template was used to apply a series of single injections across the loin surface, and the number of penetrations per surface area of the needle-free and needle systems were not equivalent. Therefore, it is likely that additional development of

a needle-free injection system could be accomplished to reduce microbial contamination during operation.

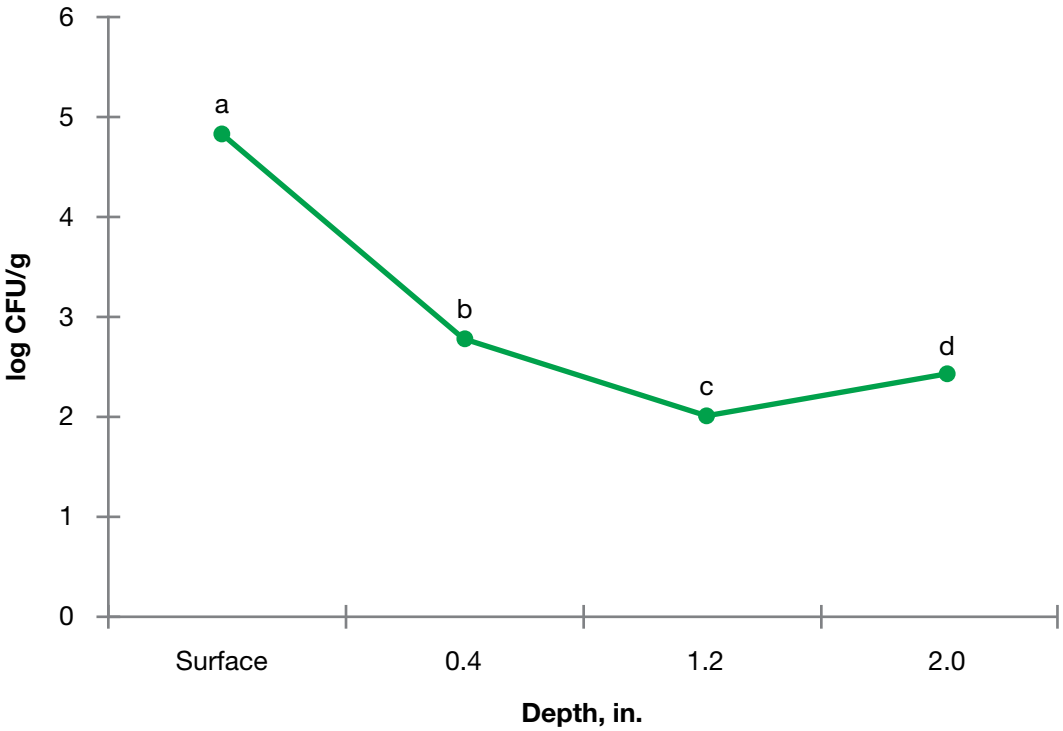
Pump yields were designed to be similar for both treatments so that differences in color and tenderness between treatments would not be a result of differing amounts of enhancement solution in the muscle. Average yields for needle-free and needle injection were 14.73 and 14.05%, respectively.

Steaks from both treatments became darker ($P<0.001$) as day of display increased, as expected. There was a treatment by day interaction ($P<0.05$) for visual color (Figure 5) in which needle injected steaks were darker on day 1 of display but not after day 1. There was no significant treatment or treatment by day interaction ($P>0.05$) effect for discoloration scores (data not shown). As expected, discoloration scores indicated that steaks from both treatments had increasing amounts of discoloration as day of display increased ($P<0.001$). Our results suggest that needle-free treatment improved visual color on day 1, but there were no differences between treatments for the remaining days of display.

Longissimus slice shear force values indicate that all steaks were tender, but steaks from loins that had been injected by using the needle-free technology were more tender ($P<0.05$) than those from loins that had been injected with the traditional needle injector (Table 1). Given the closer spacing of needle-free injection sites and application of injection from both sides, this increased mechanical tenderization is no surprise. However, the difference in appearance of muscle structure between steaks from the two treatments was virtually unnoticeable at the 25 psi setting that was used.

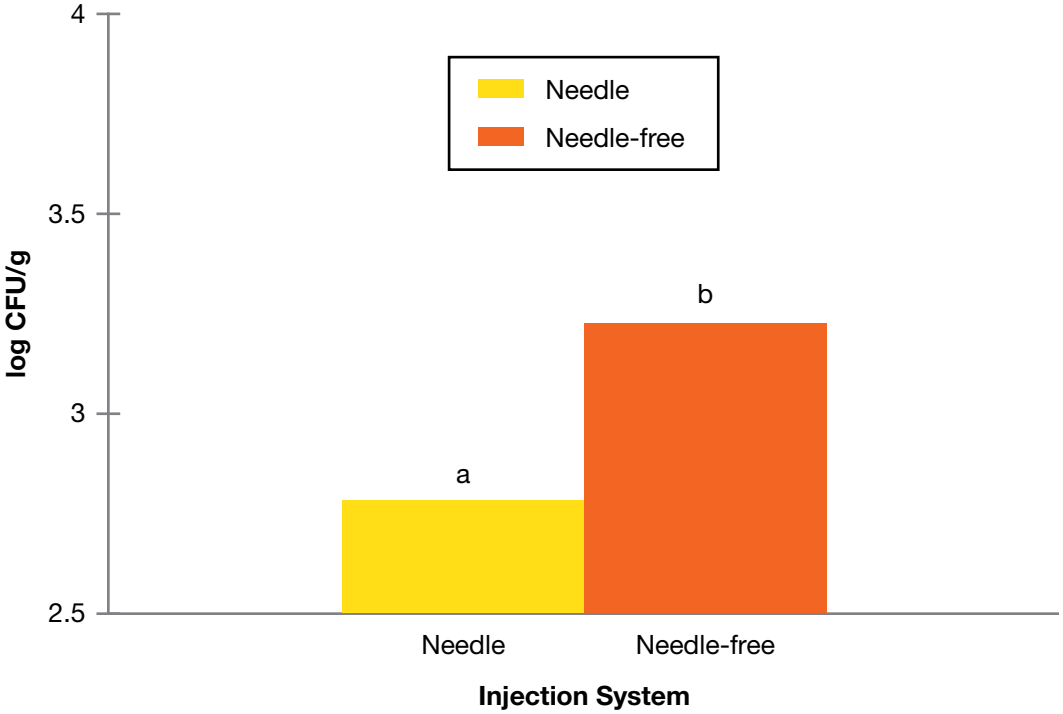
Implications

Our prototype needle-free injection enhancement system might be expected to slightly increase microbial translocation into the muscle interior by as much as 0.5 log10 CFU/g compared with needle injection but improve tenderness compared with needle controls and have no effect on color display life.



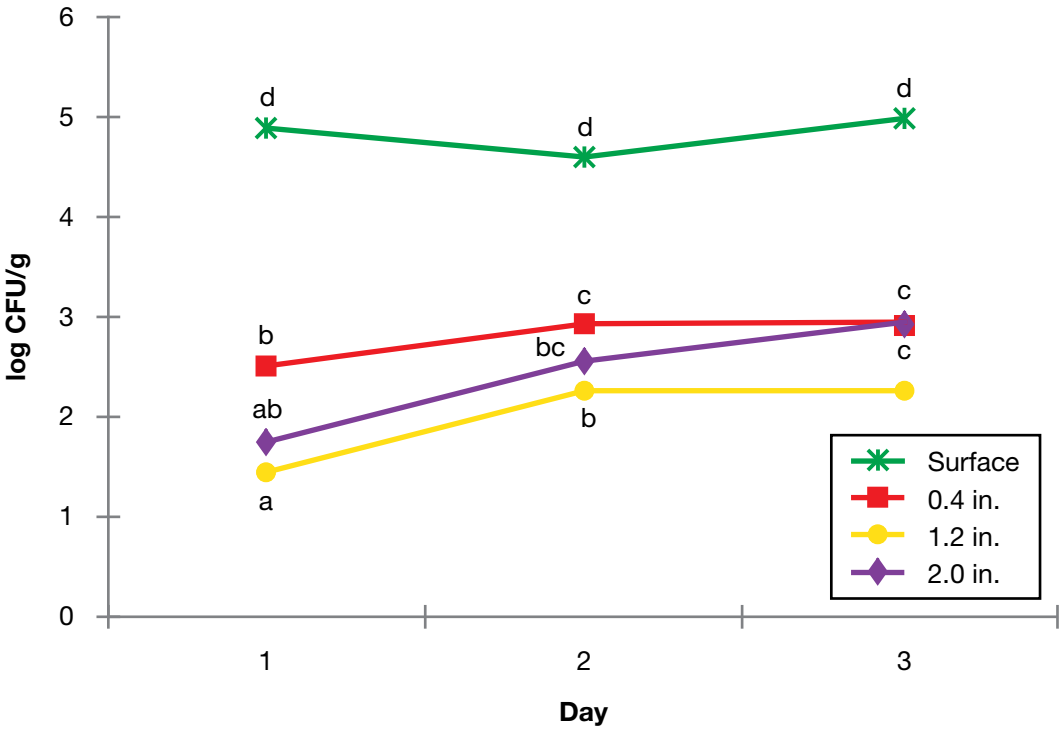
[abcd] Means with a different letter differ (P<0.05).

Figure 1. Average log CFU/g of generic *E. coli* on the inoculated surface and at depths of 0.40, 1.2, and 2.0 in. in beef longissimus enhanced by using needle and needle-free injection systems (pooled data).



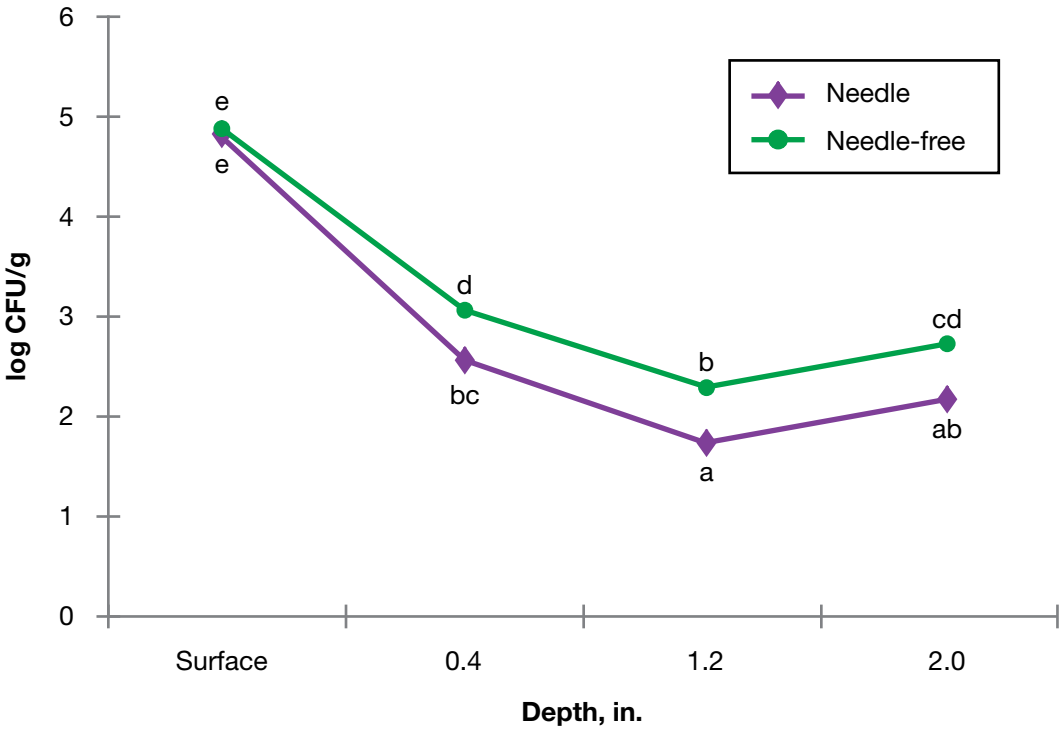
[ab] Means with a different letter differ (P<0.05).

Figure 2. Average log CFU/g of generic *E. coli* for needle or needle-free injection sytems used to enhance beef longissimus.



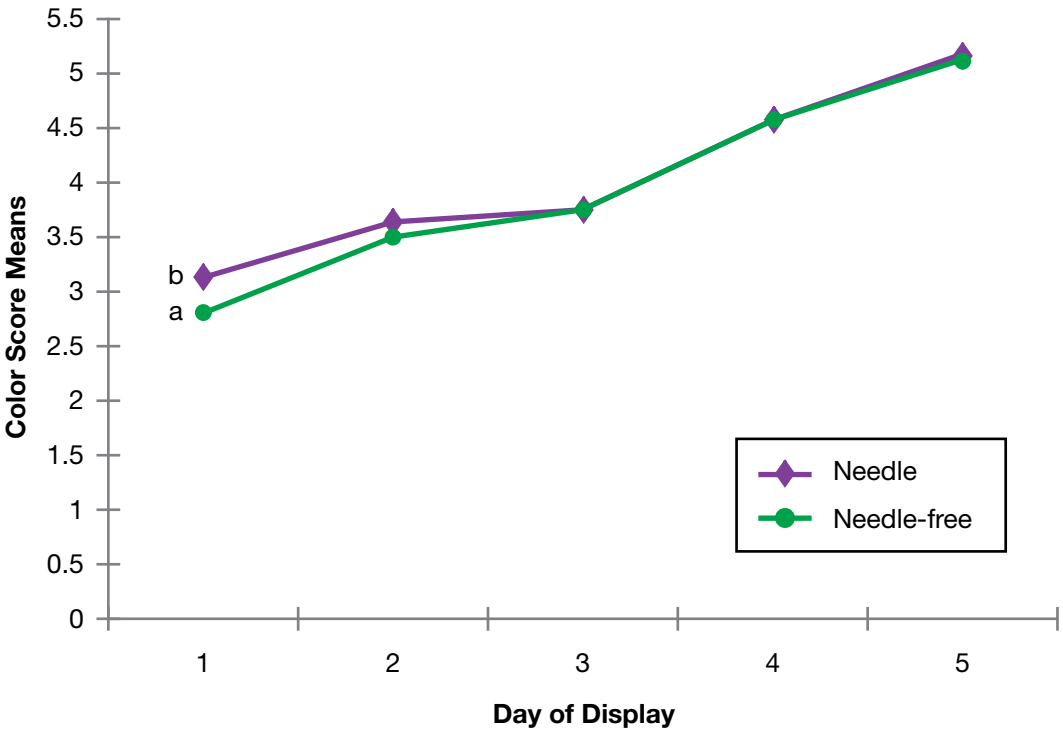
[abcd] Means with a different letter differ (P<0.05).

Figure 3. Mean log CFU/g depth by day (replication) interaction for needle or needle-free injection (pooled data) used to enhance beef longissimus.



[abcde] Means with a different letter differ (P<0.05).

Figure 4. Average log CFU/g of generic *E. coli* for needle and needle-free injection systems at the inoculated surface and at depths of 0.40, 1.2, and 2.0 in. in enhanced beef longissimus.



[ab] Means with a different letter differ (P<0.05).

Figure 5. Color score means for injection method by day of refrigerated display of beef longissimus enhanced by using needle or needle-free injection systems.
Display color score means scale: 1 = very bright red, 2 = bright red, 3 = dull red, 4 = slightly dark red, 5 = slightly dark red or reddish tan, 6 = moderately dark red to tannish red, 7 = tan to brown.

Table 1. Slice shear force values for beef longissimus enhanced by using needle or needle-free injection systems

Treatment	Shear values	SE
Needle-free	7.72[a]	0.70
Needle	10.08[b]	0.70

[ab] Within a column, means with a different superscript letter differ (P<0.05).