

**MICROBIAL TRANSLOCATION OF NEEDLE-FREE INJECTION ENHANCED
BEEF STRIP LOINS AS COMPARED WITH TRADITIONAL NEEDLE INJECTION**

by

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Abstract

The objective was to determine the effects of needle-free injection (NF) compared with traditional needle injection (N) on microbial translocation of generic *E. coli* in beef strip loins. *Longissimus* muscles (LM) (n=5) from USDA Select carcasses were used in preliminary research to determine the optimal injection pressure required for NF injections. Seven treatments with sterile colored saline solution were administered: 1) 90 psi ; 2) 55 psi ; 3) 50 psi ; 4) 45 psi ; 5) 30 psi ; 6) 25 psi ; or 7) 20 psi . For the second portion of the experiment 15 LM were obtained and halved; the surfaces were inoculated with generic *E. coli* at a level of 10^6 CFU/cm² (three replications of five loins). Matching halves were allocated to NF or N injection treatments with a phosphate, salt solution. Immediately after injection, two cores, 23 cm² in area, were taken aseptically from each half. A 2-mm thick cross-sectional slice was removed from the inoculated surface of the core and labeled “surface”. Using sterile technique, the two cores from each half were sliced into cross-sectional strips at depths of 1, 3, and 5 cm. Corresponding depth measurements were combined in stomacher bags with 99 ml of peptone water and stomached. Serial dilutions were then plated. From the preliminary study, it was determined that 25 psi was the optimal pressure for NF injection based on dispersion, visual appraisal, and solution retention. Samples taken from the surface of N injected LM had lower ($P < 0.05$) microbial counts than NF-injected muscles (2.79 versus 3.23 log CFU/g, respectively). The 3 and 5 cm depth samples from N injection had the least ($P < 0.05$) microbial contamination (1.69 and 2.12 log CFU/g) compared to NF injections. Samples from 1 cm deep of N injected LM had lower ($P < 0.05$) (2.53 log CFU/g) microbial counts than the 1 cm samples of NF injected LM (3.04 log CFU/g). Traditional N injection resulted in approximately 0.5 log CFU/g less microbial

contamination at all depths. N injection posed fewer microbial risks when compared with NF injection using these defined application settings.

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CHAPTER 1 - Literature Review

Palatability of a meat product is a combination of tenderness, juiciness, and flavor.

Tenderness is a large contributor to consumer satisfaction. Tenderness is the most predominate and important organoleptic characteristic of meat (Lawrie, 1979). According to Savell and Shackelford (1992), tenderness has the greatest influence on beef palatability. It is the sensory trait that most affects consumer acceptance (Morgan et al., 1991). In recent years, improving meat tenderness through injection enhancement, needle enhancement, or mechanical tenderization has become customary throughout the red meat industry.

Non-Intact Beef Products

Beef that is mechanically tenderized, either with hollow or solid needles, is classified as “non-intact beef”. The Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA) defines non-intact beef as any meat product, whole muscle or reconstructed, that has been enhanced with a solution, or by needle, cubing, frenching, or pounding (American Meat Institute et al., 2006). In addition, beef that is intended for ground beef manufacturing is also deemed a non-intact beef product.

Non-intact beef cuts that are not cooked to an appropriate degree of doneness could be hazardous to consumers (USDA, 2005). However, the National Cattlemen’s Beef Association and Cattlemen’s Beef Board (2005) stated that non-intact meats pose no greater risk of *Escherichia coli* O157:H7 than intact meat products. Engeljohn et al. (2005) summarized various methods that the FSIS recommends in order to prevent occurrences of *E. coli* O157:H7 outbreaks when processing non-intact meats. Examples of these procedures include sanitation of

equipment, antimicrobial applications, and testing of pre-eviscerated carcasses. The FSIS published a notice in the 2005 Federal Register that mandated establishments producing non-intact beef products to reassess their HACCP plans. This was in response to three *E. coli* O157:H7 outbreaks associated with non-intact beef occurring from 2000-2004 (National Cattlemen's Beef Association and Cattlemen's Beef Board, 2005). The Federal Register states that particular attention should be paid to interventions, purchase specifications, antimicrobial use, number of passes through the tenderizer, sanitation procedures, and cooking instructions (USDA-FSIS, 2005). There is a market for non-intact meat sales, but care must be taken with equipment sanitation and new technologies should be investigated to control the risks associated with translocation of pathogens into the interior muscle.

Tenderness

Injection Enhancement

One common way of producing non-intact beef is by injecting muscles with an enhancement solution, which has proved to be useful. Numerous researchers have investigated various other methods for improving the value of meats in conjunction with injection enhancement. To determine the affect of aging in combination with enhancement on tenderness of beef strip loins, Wicklund et al. (2005) conducted a study in which they randomly assigned 40 paired strip loins to one of two treatment groups. Paired strip loins were sectioned into fourths, one loin was used as a control and the other was injected with 0.3% salt and 0.4% sodium tripolyphosphate (STP). For a second study, paired strip loins were divided in two and either aged followed by enhancement or just aged (control), and then vacuum packaged. Strip loins from both treatment groups were aged for 7, 14, 21, or 28 d. Sensory panel scores indicate that tenderness increased when samples were enhanced at all aging periods, despite treatment group.

Enhanced samples were more tender than non-enhanced samples at d 7 and d 28 of aging. Results of Warner Bratzler shear force (WBSF) showed that there was no difference in tenderness between enhanced and control steaks at d 7; for all other days the enhanced steaks were more tender than control steaks. However, enhancement after aging did not result in a difference in WBSF values, even after 28 d of aging compared to control samples. These researchers concluded that enhancement before aging for at least 14 d was the most effective at improving tenderness.

Some researchers have been interested in the effects of enhancement treatments on a variety of muscles. Molina et al. (2005) fabricated 12 USDA Select beef chucks into the *complexus*, *latissimus dorsi*, *rhomboideus*, *serratus ventralis*, *splenius*, *subscapularis*, *supraspinatus*, and *triceps brachii* muscles. Muscles were randomly assigned to one of four treatments; control, marinated, needle pumped (injected), or vacuum tumbled. The brine contained 0.50% NaCl and 0.40% STP. Needle pumping was the most effective method for improving WBSF values in both the *complexus* and *triceps brachii*. All enhancement treatments decreased WBSF values in the *subscapularis*. Sensory panel scores showed an increase in tenderness of *subscapularis* when the muscle was needle-pumped but not when marinated or vacuum tumbled. All enhancement treatments increased tenderness scores when compared to the control in the *serratus ventralis*, *supraspinatus*, and *triceps brachii*. The *serratus ventralis*, *subscapularis*, *supraspinatus* and *triceps brachii* were the most responsive to treatments. Needle pumping was slightly more effective than other treatments, indicating that enhancement, especially needle pumping could be applied to specific low-quality muscles to increase their value.

Pietrasik and Shand (2005) wanted to determine how the *semimembranosus* would be affected by injection in combination with tumbling and blade tenderization. The *semimembranosus* muscle from 24 beef inside rounds was divided into six roasts and assigned to one of these treatments: tenderized (T or NT), tumbling time (0, 2, and 16 h) and injection (20 and 40% above green weight). Those roasts assigned to blade tenderization were tenderized and then all roasts were needle-enhanced. Roasts from each treatment group were then tumbled for the designated time. Injecting *semimembranosus* muscles following blade tenderization significantly decreased WBSF values. Blade tenderization in combination with tumbling at 16 h did not affect WBSF when compared to the control. Needle-injecting roasts at 20% in combination with 2 h tumbling improved tenderization. Roasts injected to 40% above green weight did not show a significant improvement in tenderness until 16 h tumbling. Tumbling in combination with needle-injection improved overall palatability; this was because of improved tenderness and increased water holding capacity which increased juiciness. This can be attributed to the disruption of the connective tissue within the muscle by the injection needles.

In a study conducted by Mueller et al. (2006), 67 *semimembranosus* and *adductor* muscles, and 66 *vastus lateralis* and *rectus femoris* muscles were either blade tenderized, enhanced with a phosphate and salt solution, or served as a control. Muscles were treated, cut into steaks, packaged, and two steaks of each treatment were sent to consumers to be cooked at home. Consumers rated overall palatability, and its components of tenderness, juiciness and flavor. The *semimembranosus* muscles that were enhanced were the most tender of all steaks. *Rectus femoris* and *vastus lateralis* muscles were more tender when enhanced than when blade tenderized. In addition, overall palatability (tenderness, juiciness, and flavor) of injection enhanced *vastus lateralis* steaks was rated the highest compared to blade-tenderized and non-

enhanced steaks. These researchers concluded that enhancing muscles with a phosphate and salt solution was more effective for tenderizing muscles of the beef round than blade tenderization.

Kerth et al. (1995) sectioned 24 strip loins into thirds at 48 h postmortem and assigned them to one of three treatments: non-injected control and enhanced with either 200 mM or 250 mM calcium chloride (CaCl₂). Steaks were cut 7 and 14 d postmortem. They found that injection with both solutions at 48 hr postmortem improved tenderness when compared to the control and there was no difference in tenderness of steaks from the two enhancement solutions. These results are in agreement with those of Wheeler et al. (1993), who established that *longissimus* muscles injected with CaCl₂ at a level of 200 mM, 24 hr postmortem were more tender than the non-injected loins. However, it was concluded in this same research that the improvement in tenderness was attributed to the CaCl₂ and not the injection itself.

In addition to the previous research, Morgan et al. (1991) investigated the effect of CaCl₂ injection on beef from mature cows. The top round, top sirloin, and strip loin were removed from alternating sides of 10 cow carcasses 30 min after exsanguination. These subprimals were immediately needle enhanced with a 0.3 mM CaCl₂ solution; they were then tumbled, vacuum packaged, and chilled for 24 hrs. The remaining carcasses were cold-boned at 24 hrs postmortem and the top round, top sirloin, and strip loin were removed. Strip loins injected with CaCl₂ had significantly lower WBSF values than the control strip loins. The treated top sirloin at d 1 had lower WBSF values than control top sirloins at d 14 of aging. Sensory panel scores of overall tenderness were lower (less tender) for the control muscles than the treated muscles. They concluded that needle enhancement with CaCl₂ improves tenderness at 24 and 48 hr postmortem for mature cows.

In contrast to mechanical tenderization, which will be discussed at a later point, injection enhancement has the added benefit of including a product in the brine that can extend shelf life and improve moisture retention. Sutton et al. (1997) included both sodium lactate (SL) and sodium tripolyphosphate (STP) in a solution that was pumped into six boneless pork loins at levels of 0, 1, and 2 % SL and 0, 0.2, and 0.4 % STP in combination with one another. Neither STP nor SL had an effect on WBSF values. As STP increased the amount of purge decreased. Shelf life increased with the addition of SL; however, there was no improvement in tenderness. The increased moisture retention and improved shelf life made the combination of SL and STP viable for the meat industry to implement.

Needle injection of phosphate solutions increases palatability and shelf life of meat products (Teicher, 1990; Farr and May, 1970; Shults and Wierbicki, 1974; Young and Lyon, 1986). For this reason, Detienne et al. (1999) studied the effects of varying levels of NaCl and STP on physical and textural characteristics of pork. Twenty-four boneless pork loins were quartered and assigned to be injected with a combination of NaCl (0.5, 1.0, or 1.5%) and STP (0, 0.15, 0.3, or 0.45%). WBSF values were the lowest at injection levels of 0.15% STP with 0.5% salt and 0.45% STP with either 0% or 1% salt. These results were in disagreement with those of Sutton et al. (1997) who reported no improvement in WBSF values at levels up to 0.40% STP; however, Sutton's research did not include salt. Based on Detienne's research the best results seemed to be obtained with STP at a level of 0.15% in combination with 0.5% salt. This combination worked as well as higher levels but would be more cost effective than other combinations because there are less amounts of either ingredient.

Mechanical Tenderization

Mechanical tenderization is one of the most effective ways to ensure that consumers consistently receive a tender product. Loucks et al. (1984) removed *semimembranosus* muscles from paired carcasses either 1hr post-exsanguination or 48 hr postmortem. All cuts were mechanically tenderized and evaluated for WBSF values. Mechanical tenderization had no effect on hot boned muscles, but significantly reduced WBSF values in cold-boned muscles. Beef roasts that were mechanically tenderized had lower WBSF values than non-tenderized roasts when applied to cold-boned cuts. According to Loucks in 1984, mechanical tenderization would be an effective method of tenderization in the beef industry.

Differences in tenderness of beef can be attributed to multiple factors, such as age, maturity, sex, USDA quality grade, marbling, and muscle characteristics. Brahman cattle, for instance, have less tender meat when compared to other purebred cattle. In a study conducted by Wheeler et al. (1990), 10 longissimus muscles were removed from carcasses from the following breed types: Brahman, Brahman x Hereford, Hereford x Brahman, and purebred Hereford. Muscles were then sectioned transversely into fourths and vacuum packaged for either 7, 14, 21, or 28 d. At the end of each aging period muscles were cut in half transversely. One half of each section was blade tenderized. As expected, results indicated that tenderness increased as days of aging increased. Tenderness of crossbred animals was not different than tenderness of purebred Herefords prior to or following blade tenderization. Prior to blade tenderization, muscles from Brahman steers were less tender than purebred Herefords, but following blade tenderization, tenderness increased so that WBSF values were similar to that of purebred Hereford and crossbred Brahman cattle (Wheeler et al., 1990).

Seidman et al. (1977) passed *Psoas major* muscles through a mechanical tenderizer 0 or 1 times (1X); *semitendinosus* muscles were passed through 0, 1 (1X), 2 (2X), or 3 (3X) times.

Sensory panel evaluations were conducted and WBSF values were obtained. Blade tenderization decreased WBSF of the *psoas major* by 10.5% in comparison to the control and decreased WBSF of the *semitendinosus* by 14.6 (1X), 29.2 (2X), and 33.3 (3X) % when compared to the control. Passing the *semitendinosus* muscle twice through a blade tenderizer could reduce WBSF values to the level of the *psoas major*. Although, WBSF values suggest that the *semitendinosus* can be made as tender as the *psoas major*, sensory panels did not concur, even when *semitendinosus* muscles were passed through the tenderizer three times.

Glover et al. (1977) chose to compare the effect of needle tenderization on muscles from three different regions of the carcass. Chuck roasts, top round roasts, and loin steaks were either needle tenderized or left as controls. WBSF values of treated round roasts and loin steaks were significantly decreased when compared with controls. There was a tendency for treated chuck roasts to have improved tenderness. However, sensory panel results showed only a significant difference between the treated and control treatments only the round roast and a trend for scores of the loin steaks to be higher (more tender) for treated steaks than the controls. There was no difference in sensory panel scores for chuck roasts. Round roasts and loin steaks showed a significant improvement in WBSF after being mechanically tenderized. Mechanically tenderizing top round roasts and loin steaks was recommended.

Petersohn et al. (1979) selected eight paired USDA Utility grade longissimus muscles which were blade tenderized at levels of 1X with the belt advancing 7.62 cm per plunge, 2X at a rate of 5.08 cm per plunge, 3X with the belt advancing 2.54 cm per plunge, and 6X in which loins were passed through the tenderizer twice at a rate of 2.54 cm per plunge. When comparing the effect of mechanical tenderization on USDA Choice vs. USDA Utility loins there was an increase in tenderness for those low grading cuts during sensory panel evaluation and

improvement in tenderness for Choice and Utility grade steaks when measured by WBSF. These researchers concluded that mechanical tenderization is an effective method for increasing tenderness of low grading beef.

A significant improvement in tenderness can be attributed to mechanical tenderization (Bowling et al., 1976; Boyd et al., 1978; Campbell et al., 1976; Glover et al., 1977; Hinnergardt et al., 1975; Mandigo and Olson, 1982; Miller, 1975; Neer et al., 1978; Savell et al., 1977; Schwartz and Mandigo, 1974). Substantial research has been conducted concerning the effectiveness of mechanical tenderization.

Bacterial Translocation of Blade or Needle-Injection

With improved tenderness from mechanical tenderization comes the potential for bacteria to contaminate equipment and, in turn, contaminate meat products. In a study to determine the amount of pathogenic bacteria that is translocated from the surface to the interior of meat, Phebus et al. (2000), inoculated the surface of top butt subprimals with *E. coli* O157:H7 and then passed them through a blade tenderizer. This resulted in approximately 3 logs of *E. coli* O157:H7 being transferred to the interior of the muscle when a high level (10^6 CFU/cm²) of inoculum was used and 1.8 logs translocated when a low level (10^3 CFU/cm²) was used. Ultimately 3-4% of surface contamination was translocated to the center of the product. They concluded that non-intact beef products have the potential for pathogens to be relocated to the interior.

For the first part of the experiment 32 top butt beef subprimals were inoculated with 10 ml of a 5-cocktail strain of *E. coli* O157:H7 to obtain target levels of 0.5, 1.5, 2.5, or 3.5 log CFU/cm². The lean sides of all top butts were inoculated, but only 16 were passed through the

blade tenderizer, the other 16 served as the controls. In the second phase of the experiment microbiological contamination of subprimals that were injected lean vs. fat side up, as well as, single and double-passes through the injector were compared. Ten cores were taken from each subprimal for both phases of the experiment. Each core was then sliced into 6 segments. Segments 1 through 4 were taken from the lean side moving toward the fat side at intervals of 1 cm. Segments 5 and 6 were 2 cm each. Serial dilutions were completed. Results from the first phase of the experiment indicated that the pathogen could be detected in tenderized subprimals at segments 1 through 3 for the target inoculum levels of 0.5, 1.5, and 2.5 CFU/cm²; whereas at an inoculum level of 3.5 CFU/cm² the pathogen was detected in all 6 segments. As an average 90-100% of the pathogens were recovered from segment 1, 55-98% from segment 2, and 0-5% from segments 3-5. There was no statistical difference between segments 3 through 6 regardless of inoculum level. In phase 2, 52% of pathogens were recovered at segment 1 for lean tenderized, single pass subprimals. 33%, 13%, and 25% log CFU of the pathogen was transferred to segment 1 for those cores obtained from lean tenderized, double pass subprimals, fat tenderized, single pass subprimals, and fat tenderized, double pass subprimals, respectively. There was a linear decrease in the number of pathogens recovered from each segment with increasing distance from the surface of the sample. There were no statistical differences between fat- or lean-side tenderization or between single- and double-passes through the tenderizer. In general, segment 1 had the greatest number of pathogens present regardless of treatment. In addition, the number of passes through the tenderizer and the direction from which the meat was tenderized was irrelevant.

A solution that is pumped into a whole muscle product is likely to be re-circulated, by the injector equipment adding to the microbial risk associated with needle injection (American Meat

Institute et al., 2006). Gill et al. (2005) stated that injecting meat significantly increases the concentration of aerobic bacteria in the interior of the products. Pork loins were injected with recirculating brine containing sodium polyphosphate and salt. Aerobic bacteria were the only organisms recovered from brine samples after 30 min of circulating, possibly contributing to the contamination of the interior of the product. No aerobic bacteria were recovered from the interior of non-injected meat. Thippareddi et al. (2000) halved pork loins then inoculated them with either a high (4.67 log CFU/g) or low (2.95 log CFU/g) level of *Salmonella typhimurium*. Loins were then blade tenderized and allowed to sit for 3 h. Samples were taken using a coring device. Cores were then cut into four cross-sectional strips of 1 cm thick. These samples were then diluted and plated. Blade tenderization caused the *Salmonella* to be translocated to the interior at a depth of 4 cm (the entire length of the core). The low and high inoculum levels resulted in 0.98 and 1.40 log CFU/g, respectively, of *Salmonella* being transferred to the sample taken at 4 cm in depth. They found that translocation of bacteria from the surface to the interior of meat occurs when muscles are blade tenderized.

Petersohn et al. (1979) used eight loins to compare tenderized and non-tenderized beef. Four of the loins were left as the control and four loins were mechanically tenderized at a level of 6X; once through the tenderizer fat side down and then once more fat side up, at a rate of 2.54 cm per plunge. Four steaks were sampled from both tenderized and non-tenderized loins on d 0, 1, 2, 5, and 10. Aerobic, psychrotrophic, and anaerobic bacterial counts were generally higher for tenderized steaks than for non-tenderized steaks, although not significantly different.

Hajmeer et al. (2000) passed beef striploins through a tenderizer either once, twice or not at all. Aerobic bacteria were translocated to the interior of tenderized steaks; however, *E. coli* counts were similar for all three treatments. The authors found that the translocation of

pathogens on the surface of meat to the interior of blade-tenderized *longissimus dorsi* was comparable to muscles that were not blade-tenderized. This is likely explained by the fact that the aerobic plate counts, coliform, and *E. coli* counts were minimal on the surface before injection.

Other researchers have also found no differences in interior bacterial counts when comparing needle tenderization with non-tenderized steaks. In a study conducted by Petersohn et al. (1979), beef loins were tenderized and microbial sampling was completed on days 0, 1, 2, 5 and 10 of retail display. Aerobic bacteria were present in slightly higher numbers in tenderized steaks, although the number was not significant. Anaerobic and psychrotrophic bacteria counts were similar for both the control and the injected loins.

Based on findings in agreement with those of Petersohn et al. (1979), participants at the *Non-Intact Products Processing Workshop* in Dallas, Texas concluded that translocation of *E. coli* O157:H7 to the interior of meat due to moisture enhancement or needle-tenderization was not reasonably likely to occur *when* proper controls are in place (National Cattlemen's Beef Association and Cattlemen's Beef Board, 2005).

Vaccinating Livestock with Needle-Free Injector to Prevent Needle Fragments in Meat

The livestock industry has already benefited from needle-free injection technology for vaccinations and antibiotic application. This change in industry practices has the potential to decrease the occurrence of needle fragments and carcass defects associated with injection site lesions (Houser et al., 2004). In their study, pigs were given three injections each: control, hypodermic needle injection, and needle-free transdermal injection. Needle-free injection was equally as effective as the traditional needle injection in preventing pseudorabies.

There was no significant difference in the number of lesion site occurrences. They found that the elimination of needle fragments and the effectiveness of needle-free injection warrant further research.

Although lesion sites are undesirable, the greatest obstacle associated with live production is the occurrence of needle-fragments in meat products, which are usually attributed to broken needles that have been straightened and reused (Hoff and Sundberg, 1999). This practice contributes a great deal to carcass defects. According to Morgan et al. (1993), 11.3% of pork carcasses slaughtered in 1993 were discounted due to improper injections.

Summary

Based on my review of literature, I would recommend that muscles be injection enhanced with a brine to improve palatability of meat products. Although, some researchers believe that bacteria will not be translocated to the interior of meat, I think the research by Phebus et al. (2000) and Thippareddi et al. (2000) proves that the possibility still exists and every measure should be taken to prevent the occurrence of a pathogenic outbreak. Furthermore, with the knowledge that needle-free injection is as effective as traditional needle injection at delivering vaccines into muscle while increasing the safety of meat products by removing the risk associated with needle fragments, it seems logical to consider needle-free injection treatments for other situations where needles are used. This leads me to believe that needle-free injection become a viable alternative to needle injection in normal meat animal production. Additionally, this could reduce the risks of sequential contamination to beef cuts since physical contact of needles to meat surfaces would be eliminated.

Literature Review

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CHAPTER 2 - Effects of Needle-Free Injections on the Microbial Translocation of Generic *E. coli* to the Interior of Beef Muscle when compared with Traditional Needle Injection

The objective of this study was to determine the effects of needle-free injection (NF) compared with traditional needle injection (N) of salt and phosphate solutions on microbial translocation of generic *E. coli* from the surface to the interior of beef strip loins. *Longissimus* muscles (LM) (n=5) from USDA Select carcasses were used in preliminary research to determine the optimal injection pressure required for NF injections. Seven treatments with sterile colored saline solution were administered: 1) 90 pounds per square inch (psi) ; 2) 55 psi ; 3) 50 psi ; 4) 45 psi ; 5) 30 psi ; 6) 25 psi ; or 7) 20 psi . From the preliminary study, it was determined that 25 psi was the optimal pressure to use based on dispersion, visual appraisal, and enhancement solution retention. For the second portion of the experiment, 15 LM were obtained and halved and the surfaces were inoculated with generic *E. coli* to achieve of 10^6 CFU/cm² (three replications of five loins). Matching halves were randomly allocated to NF or N injection treatments with a phosphate and salt solution. Immediately after injection, two cores, 23 cm² in area, were taken from each half. A 2 mm thick cross-sectional slice was removed from the external, inoculated surface of the core and labeled “surface”. The two cores from each half were sliced into cross-sectional strips at depths of 1, 3, and 5 cm. The two core samples for each depth measurement of each half were combined in stomacher bags with 99 ml of peptone water

and stomached. Serial dilutions were then plated. Samples taken from the surface of N injected LM had lower ($P < 0.05$) microbial counts than NF-injected muscles (2.79 log CFU/g versus 3.23 log CFU/g, respectively). The 3 and 5 cm depth samples from N injection had the least ($P < 0.05$) microbial contamination (1.69 and 2.12 log CFU/g) compared to NF injection. Samples from 1 cm deep of N injected LM had lower ($P < 0.05$) (2.53 log CFU/g) microbial counts than the 1 cm samples of NF injected LM (3.04 log CFU/g). Traditional N injection had approximately 0.5 log CFU/g less microbial contamination at all depths on each day of the experiment. N injection posed fewer microbial risks when compared with NF injection using the defined application parameters of the study.

Key words: beef, needle injection, needle free injection, microbiology

Introduction

Palatability of a meat product is a combination of tenderness, juiciness, and flavor. Tenderness is a large contributor to consumer satisfaction. For a number of years, tenderness has been the most predominate and important organoleptic characteristic of meat (Lawrie, 1979). According to Savell and Shackelford (1992), tenderness has the greatest influence on beef palatability and is the sensory trait that most affects consumer acceptance (Morgan et al., 1991). In recent years, improving meat tenderness through injection enhancement, needle enhancement, or mechanical tenderization has become customary. With the improvement of one problem, another one has arisen—microbial translocation from the surface to the interior of meat.

Needle-free technology has been used in the livestock industry for a number of years. Its use for vaccinating and applying antibiotics has proven to benefit the meat industry

inadvertently; the occurrence of needle fragments would be eliminated with the implementation of needle-free injections (Houser et al., 2004).

It is thought that the lack of contamination on needles could contribute to a decrease in bacterial translocation. Therefore, the objective of this study was to determine if the use of needle-free injection enhancement would decrease the amount of microbial translocation from the surface to the interior of beef longissimus muscle.

Materials and Methods

Preliminary Study

In order to determine the optimal injection pressure (pounds per square inch (psi)) to be used for needle-free injection, preliminary research was conducted. Longissimus muscles (n=5) from USDA Select carcasses were obtained from a commercial abattoir at 4-5 days postmortem and then transported to Kansas State University where they were stored at 2°C for an additional 9-10 days. Muscles were divided into four sections and assigned to one of seven treatments with sterile colored saline solution: 1) 90 psi; 2) 55 psi; 3) 50 psi; 4) 45 psi; 5) 30 psi; 6) 25 psi; or 7) 20 psi. The sterile saline solution was mixed with blue food-grade coloring so that the dispersion of the solution could be tracked throughout the product after injection. Slices approximately 0.6 cm thick were made through the muscles for visual evaluation of depth, uniformity and extensiveness of the injection enhancement solution. The optimal psi was selected based on dispersion, visual appraisal, and penetration level. We determined that 25 psi was the best choice due to the greatest distribution, the absence of injection ‘channels’ (which were found in all psi levels above 30), and the deepest penetration (Figure 1 contains examples of injection distributions).

Needle-free injection enhancements were made 0.805 cm apart in a grid pattern, using a plexiglass template, at a psi of 25. It was stated by the manufacturer of the NF injector that each injection dispenses 2 ml of liquid. 3785.41 ml (1 gallon of water) was used as a standard measurement to determine the amount of liquid needed to increase the green weight by 10%. I concluded that the average strip loin weighed 5.45 kg; as a result, the goal for weight increase was 0.545 kg. Therefore, spacing of injection sites for the template was selected based on this equation: $3785.41 \text{ ml} / 2 \text{ ml} = 1.9 \text{ g}$ of liquid; $0.545 \text{ kg} / 1.9 \text{ g} = 283.91$ injections. This was then applied in the equation to determine template spacing: 228.6 cm^2 (the typical surface area of a striploin) / 283.906 injections = 0.805 cm apart on the template.

Figure 2.1 [Photographs illustrating penetration and distribution of the sterile dye solution](#)



25 psi

45 psi



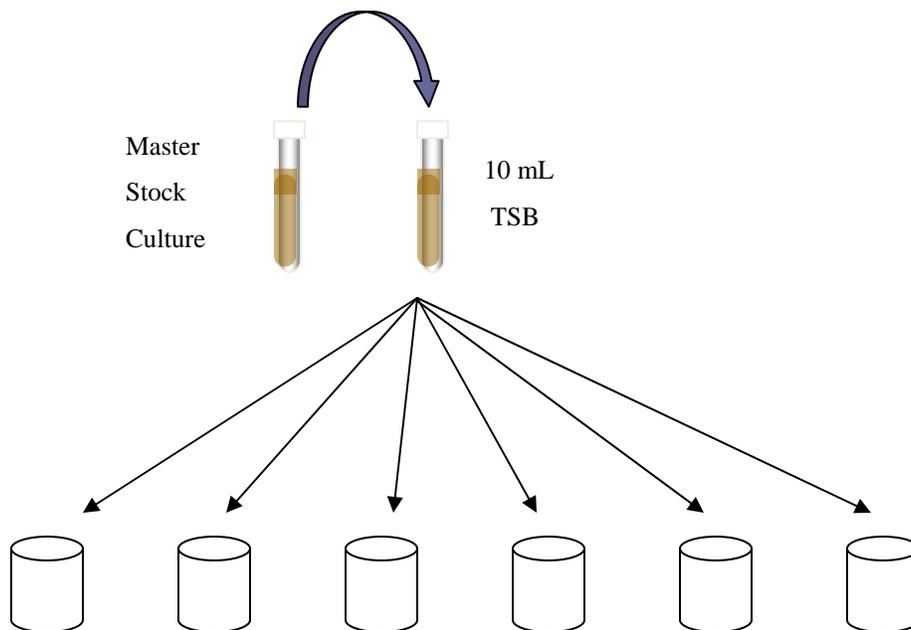
90 psi

Microbiology

This study was replicated three times. For each replicate, beef *longissimus* muscles (n=5) from USDA Select, A maturity carcasses were obtained from a commercial abattoir at 2 d postmortem and held to 7 d postmortem. Fat was trimmed to 1/8" and the loins were halved and randomly assigned to one of two treatments: needle-injection (Model N30, Wolftec Inc., Werther, Germany) or needle-free injection (Pulse NeedleFree Systems, Lenexa, KS). The spacing of needles in the needle injector was 1.77 cm x 2.54 cm apart in a staggered pattern.

Non-pathogenic generic *E. coli* (ATCC number 25922, MicroBiologics, St. Cloud, MN) was grown in 10 mL of Tryptic Soy Broth (TSB, Difco Co., Sparks, MD) for 24 h at 35°C. 0.1 mL of this stationary phase culture was transferred into six bottles of 250 mL TSB and incubated for 24 h at 35°C (Figure 2). The bottles were then centrifuged at 6000 x g for 10 min at 4°C.

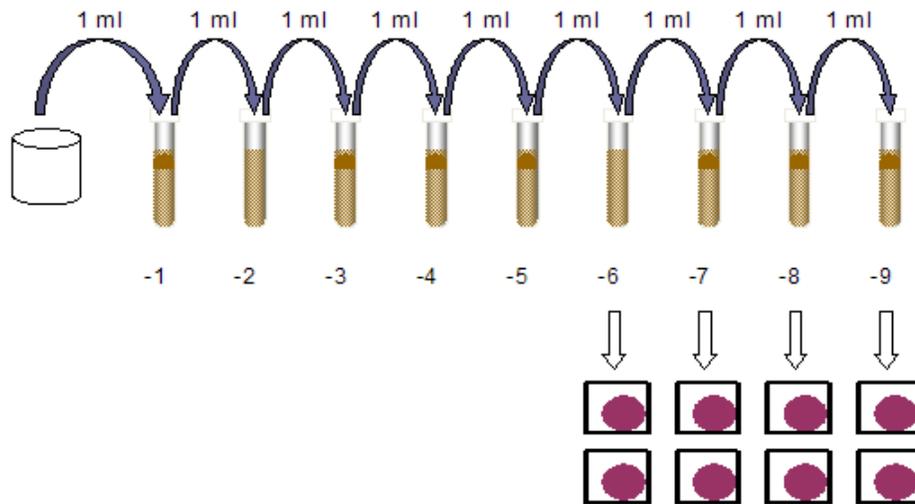
Figure 2.2 [Inoculum preparation](#)



The supernatant from each bottle was decanted and the *E. coli* cell pellets were removed from centrifuge bottles by suspending in 100 mL of 0.1% peptone water. This resulted in an inoculum level of 10^9 CFU/mL. All re-suspended pellets were then combined into a single sterile bottle referred to as the master inoculum (MI; 600 mL). The MI was enumerated by completing serial dilutions and plating on *E. coli* / coliform (ECC) Petrifilms (3M Corporation, St. Paul, MN) (Figure 3). The MI was then transferred to a sterile misting bottle.

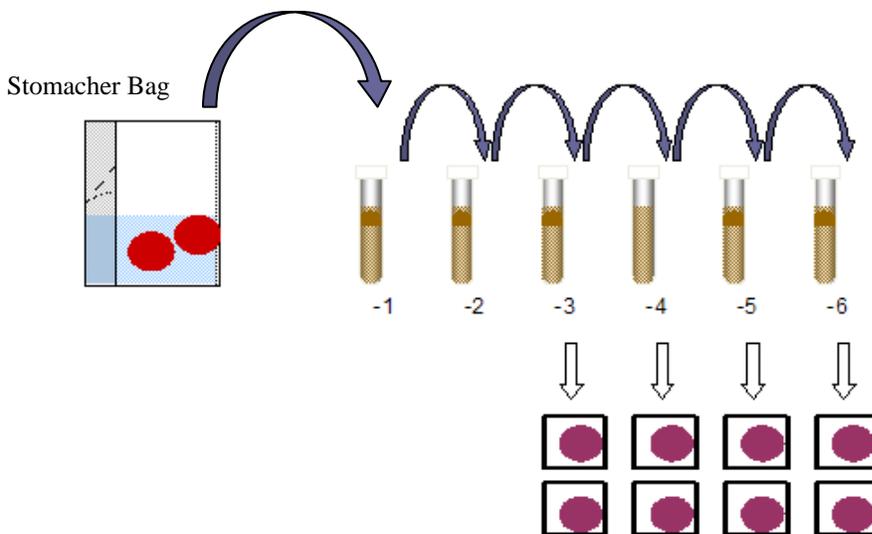
Each of the two matching loin halves were placed on a sanitized tray with white butcher paper and set inside of a sealed inoculation chamber. Ten pumps of the MI were then sprayed onto the surface of the meat approximately 30 cm above the surface. Only the top surface (fat-side) was inoculated. Loins were allowed to sit inside the chamber for 10 min, removed, and *E. coli* was allowed to attach for 1 h at 7-10°C.

Figure 2.3 [Dilution Scheme to Enumerate *E. coli* concentration of the Master Inoculum](#)



Once *E. coli* had attached, surface samples were taken to enumerate the actual number of bacteria present. This was done by excising two samples, 5.2 cm in diameter by 0.30 cm deep, from the inoculated surface on opposite ends of the loin halves. The two samples were combined in a single stomacher bag with 99 mL of 0.1% peptone water. The samples were stomached for 2 min and serial dilutions were plated on duplicate ECC films (Figure 4).

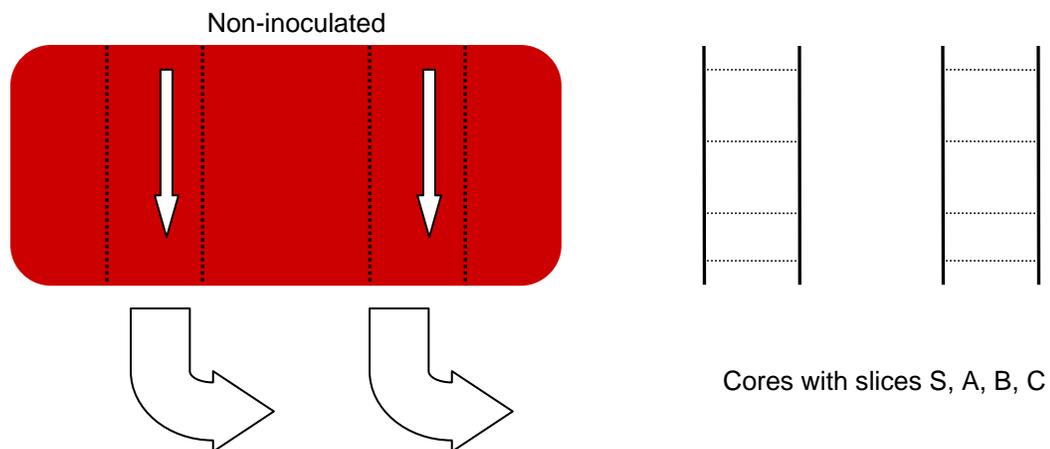
Figure 2.4 [Dilution scheme to enumerate the population of *E. coli* on inoculated loin surfaces](#)



The matching inoculated loin halves were then randomly assigned to either the needle injection (N) or needle-free injection (NF) treatments. Loins were injected one at a time; those that were not being injected at that moment were refrigerated at 2-3°C. Loin halves were injected from the inoculated side with brine containing 4.4% phosphate (Brifisol 85 Instant, BK Giulini Corp., Simi Valley, CA) and 2.2% salt. Both the needle injector and the needle-free

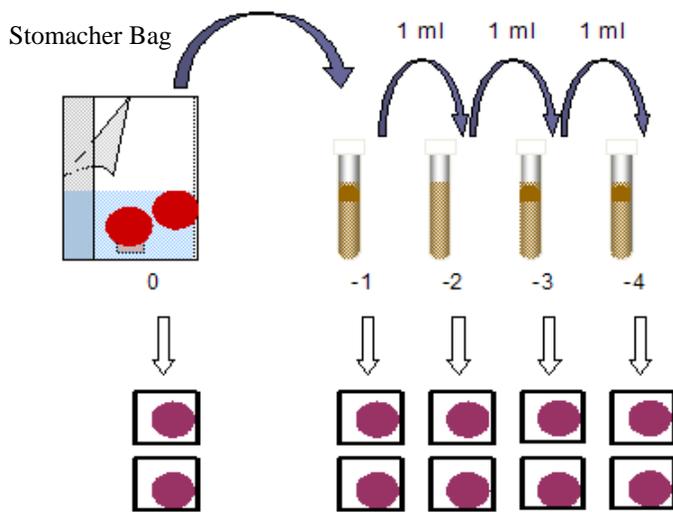
injector were cleaned after each loin injection with a solution of bleach and water to sterilize the machine. Ten gallons of the solution was pumped through the needle injector; due to its size only 1 gallon of the solution was pumped through the needle-free injector. The template used for the needle-free injections was soaked in a bleach water solution between injections as well. After being injected, loins were placed on drain-top trays for 1 h. Loins were then placed non-inoculated side up on sanitized trays and two 5.2 cm diameter cores were taken. A sterile glass rod was used to push the core out of the coring device from the opposite side that the core was taken to prevent artificial contamination. Both cores were placed onto a sanitized tray, with the original inoculated surface on the tray surface and placed in the freezer at -4°C for 1 h (to facilitate uniform slicing of each core). Cores were then removed from the freezer and slices were taken across the muscle fibers beginning at the inoculated side using the sterile technique defined by Phebus et al (2000). The inoculated surface was removed (0.3 cm) and labeled “surface”. Slice A was then cut to 1 cm in depth; slices B and C were cut 2 cm thick each (Figure 5). All slices were cut using aseptic techniques and then weighed.

Figure 2.5 [Removal of cores and slices A, B, C, and D](#)



The respective slices from the two cores (total of 10.4 cm² surface area) of the same loin half were then placed into the same stomacher bag with 99 mL of 0.1% peptone water and stomached for 2 min. Serial dilutions were plated. ECC Petrifilms were then incubated at 35°C for 24 h and enumerated (Figure 6).

Figure 2.6 [Serial dilutions for depth slices S, A, B, and C](#)



Statistical Analysis

The microbiological data were analyzed as a split-plot design using the MIXED-procedure of SAS. Fisher's least significant difference was used to determine differences among bacterial populations at the different depths. The fixed effects were day, loin (day), treatment, day*treatment, treatment*loin (day), depth, day*depth, treatment*depth, and day*treatment*depth. Significance was determined at probability values of $P < 0.05$.

Results and Discussion

Translocation of E. coli into Muscle Interior

There was a difference in post-treatment *E. coli* levels among all depths ($P < 0.0001$) in both treatments. The mean log CFU/g of *E. coli* found on the surface of the sample was the highest of any depth, as expected because it was the inoculated surface. At depth A, microbial contamination decreased from ~ 5 log CFU/g on the surface to ~ 3 log CFU/g. At depth B, the microbial contamination continued to decrease significantly to ___ log CFU/g; however, microbial contamination at depth C increased significantly compared to depth B, although *E. coli* counts were still lower than observed on both surface and depth A. Figure 7 shows the distinct difference between surface, depth A, B, and C. Phebus et al. (2000) demonstrated in previous research that showed 3-4% of surface inoculated bacteria were transferred to the interior of the muscle at the geometric center. The tendency of coliform counts to decrease from surface to depths A and B, but then increase in depth C, is likely due to contamination introduced when the brine pooled on the table top surface during injections.

E. coli counts were higher ($P < 0.001$) for NF injections than N injections (Figure 8). After treatments, microbial contamination of needle-free injected muscles was approximately 0.8 log CFU/g higher at all depths, not including surface contamination. The closer spacing between injection sites provided for a greater number of penetrations in NF-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 and dispersed more evenly.

Figure 2.7 Mean log CFU/g values of microbial contamination at depths S, A, B, and C (average of 3 replications) between N and NF injection

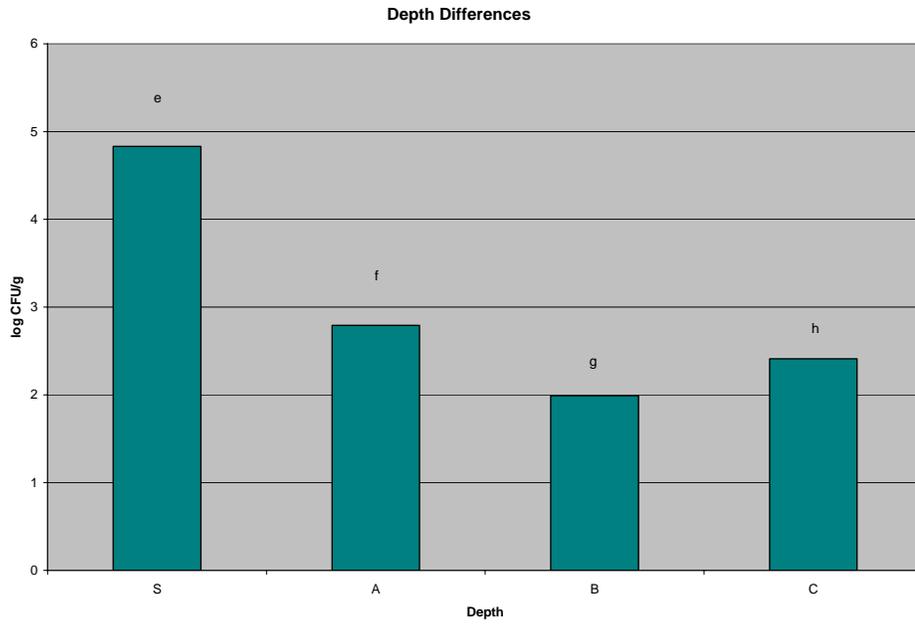


Figure 2.8 Mean log CFU/g values for needle vs. needle-free injections averaged over all depths

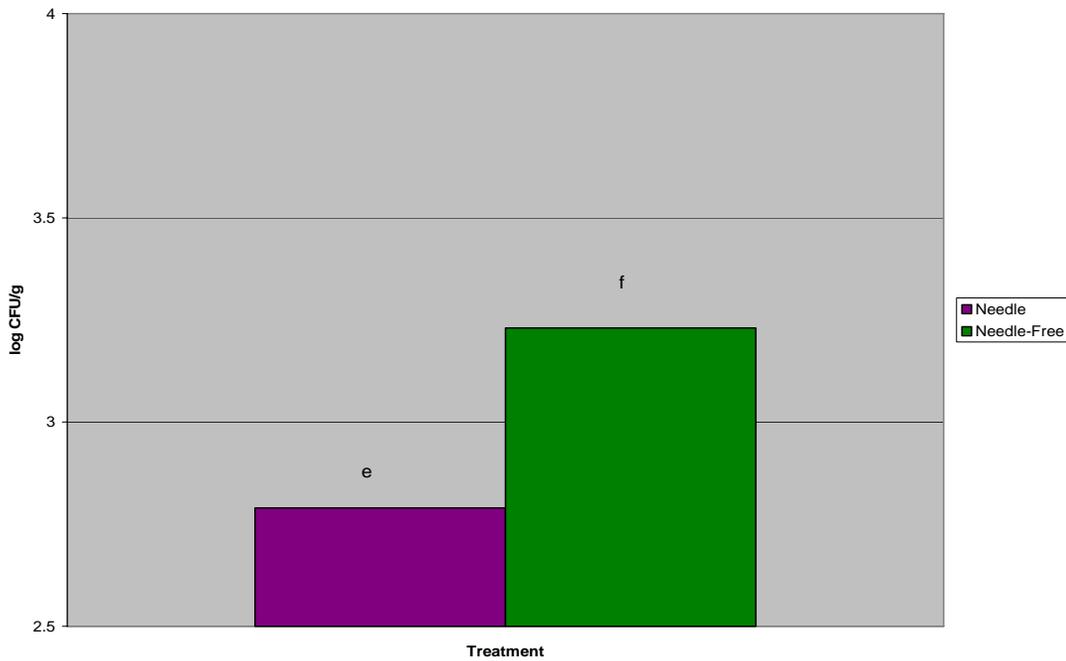
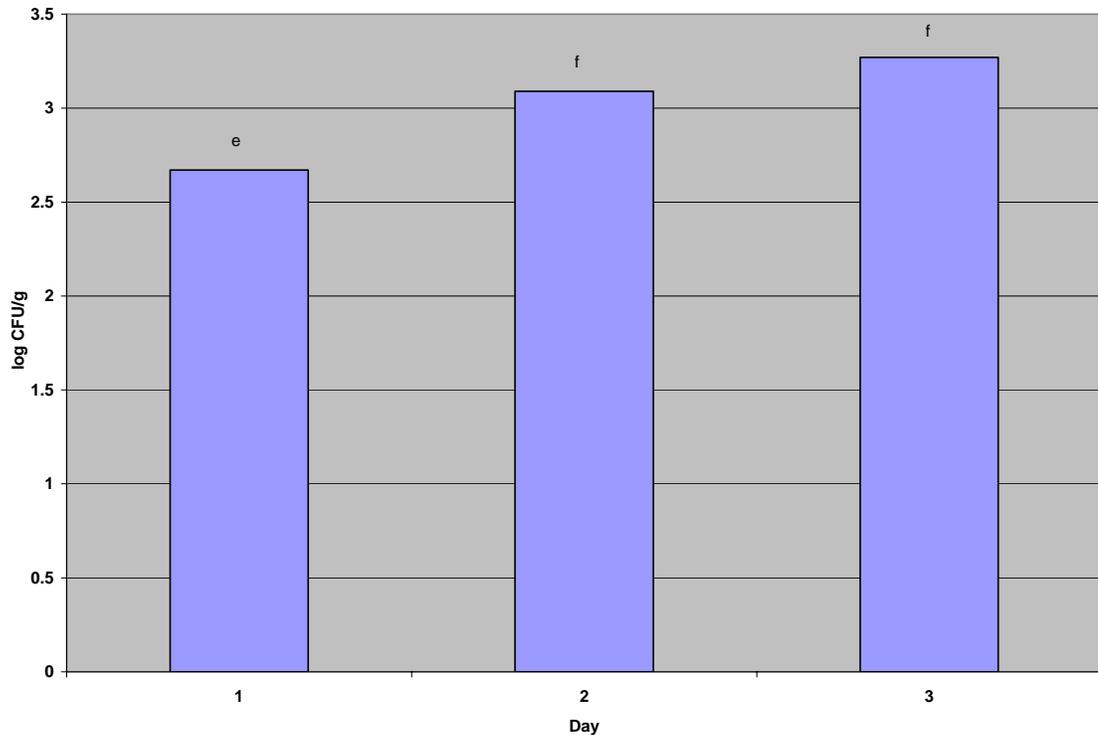


Figure 2.9 Mean log CFU/g of microbial contamination for both N and NF injections across day (replication)

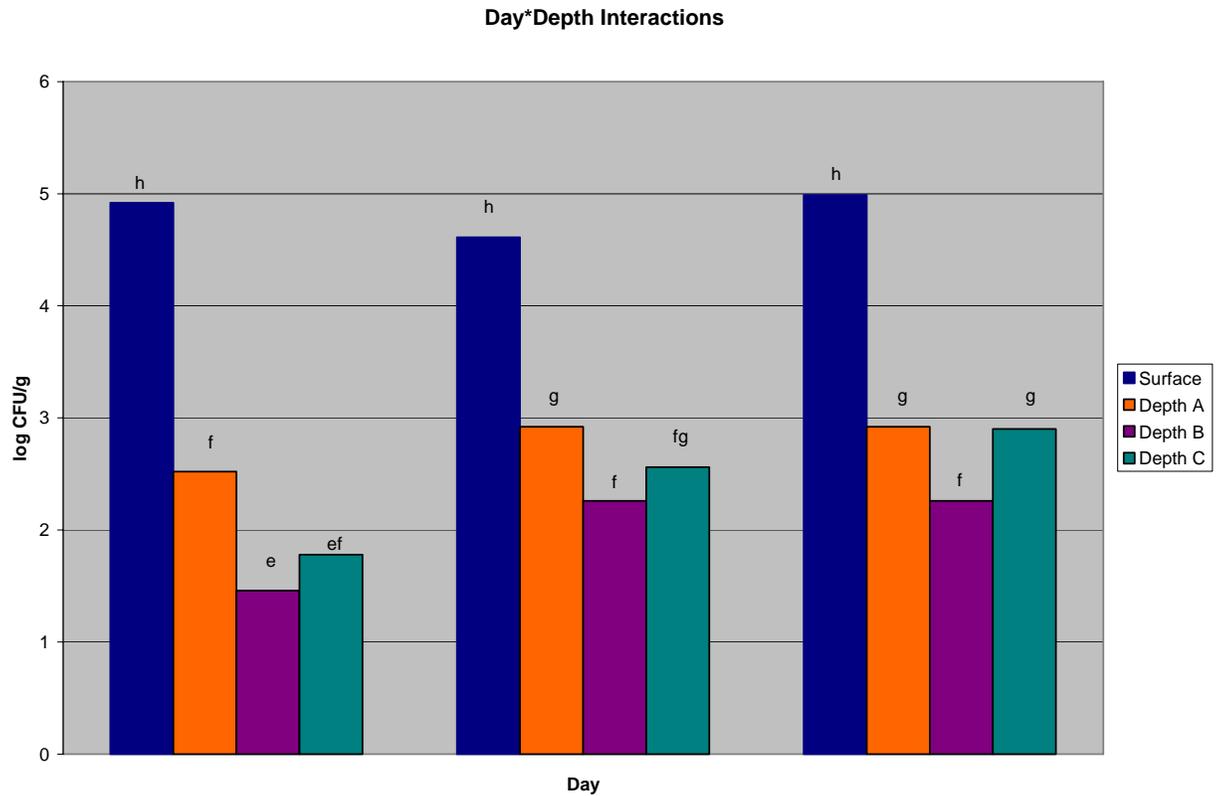


Day (replication) one was different than both days two and three ($P < 0.01$) (Figure 9) with less microbial contamination on d 1. This difference could be attributed to experimental error.

There was a significant ($P < 0.0006$) day*depth interaction for log CFU/g of microbial contamination for N and NF injection combined. As expected, the surface sample had the highest microbial counts for all 3 d (replications). Samples at Depth A had higher ($P < 0.05$) microbial counts than samples at depth B on all days, but were not higher than samples from depth C on any day. Samples from depth B were lower than samples from depths A and C on d 3. The higher microbial counts at depth A compared to depth B was expected due to the proximity to the surface. Although depth C counts were expected to be lower due to its distance

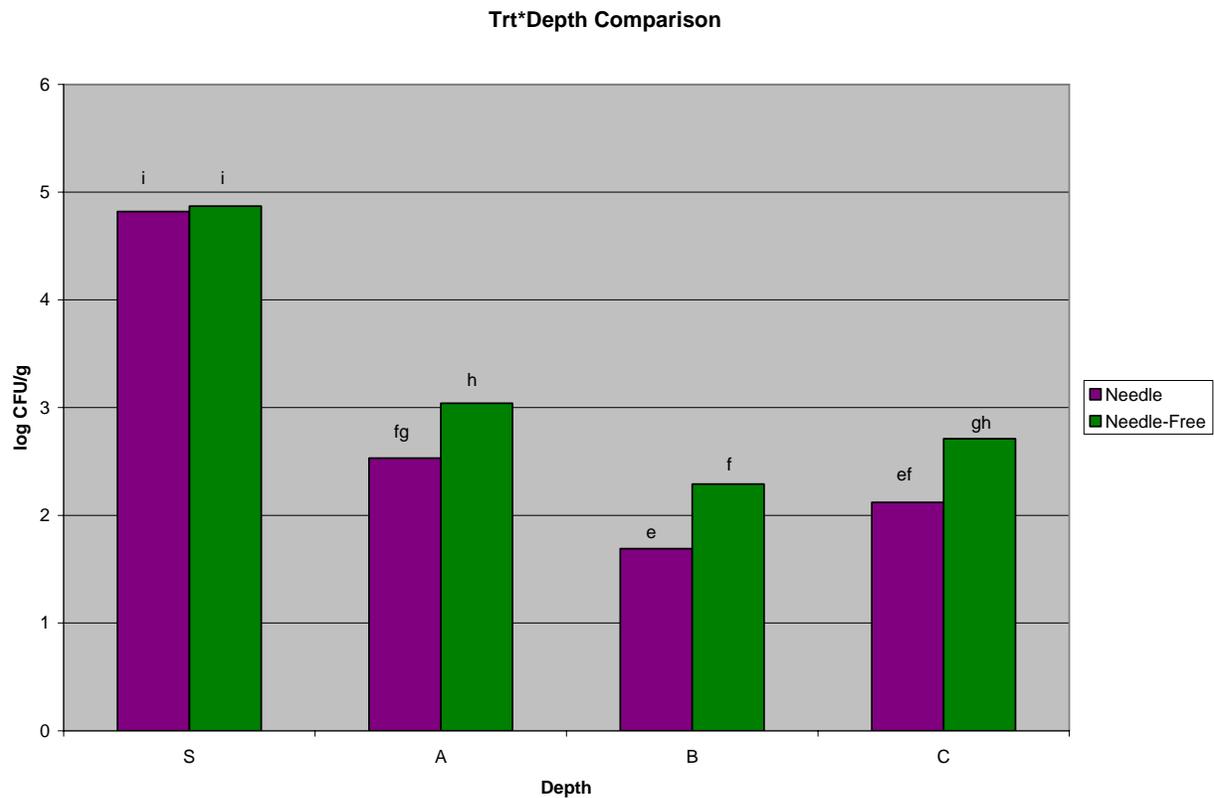
from the inoculated surface, the higher observed counts can be explained by the contact with the pooled brine on the surface of the table-top.

Figure 2.10 Mean log CFU/g depth x day (replication) interaction for N and NF injection combined.



There was a treatment*depth interaction trend ($P < 0.06$) for N and NF injection samples. Lower microbial counts were found at depths A, B, and C with N injection than for NF injection (Figure 11). However, microbial counts for NF injection at depths B, and C were similar ($P > 0.05$) to N injection at depth A. As expected, depth A had the highest ($P < 0.05$) microbial counts.

Figure 2.11 Mean log CFU/g values for depth x treatment interaction for N and NF injection samples



Conclusion

Prior to this experiment, I hypothesized that microbial contamination of NF injected muscles would be lower than that of tradition needle injected muscles. However, results of this study indicate that microbial translocation to the interior of post-rigor beef muscle was greater using needle-free injection. This difference can be attributed to the dispersion capabilities of the needle-free injector and the increased number of penetrations.

In addition, it was assumed that microbial contamination would decrease as depth into the meat increased. Again, my hypothesis was not supported by experimental observations, as an increase in microbial contamination at depths B and C. This increase may be attributed to the

relation of the non-inoculated surface of the meat to the pooled brine from the injections. In order to prevent the “pooling” of brine it would be best to have a template with a screen that allows for draining on the bottom surface.

The level of inoculation was extremely high in order that we might develop a “worst case” scenario and also so the magnitude of *E. coli* translocation could be quantified. The difference between N and NF injection was approximately 0.8 log CFU/g which could arguably be insignificant in microbiological terms.

I feel these results merit further research with needle-free injection if inoculation levels were reduced and spacing of both treatment injection sites were the same. Sensory characteristics should be evaluated further to determine if needle-free injection would tenderize meat adequately.