COMPARISON OF THE BIO-BULLET VERSUS TRADITIONAL INJECTION TECHNIQUES ON TISSUE DAMAGE AND TENDERNESS IN BEEF SUBPRIMALS

By

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COMPARISON OF THE BIO-BULLET VERSUS

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ON TISSUE DAMAGE AND

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CHAPTER I

INTRODUCTION

Injection-site lesions in beef occur at every level of production. When an injection is administered tissue damage occurs in the muscle. The severity of this damage is dependent upon route of administration, type of pharmaceutical used and cleanliness of conditions. In the past, there was a significant problem with injection-site lesions in the top butt. Through extensive education of cow/calf, stocker, and feedyard producers there has been a decrease in lesions found in this location. Now it is recommended that all subcutaneous and intramuscular injections be given anterior to the scapula.

The traditional type of administration is a needle and syringe, but in recent years there have been new technologies developed using a pneumatic, needle-less injection, and a ballistic, air powered delivery system that utilizes bio-bullets for pharmaceutical administration. The notable difference between these three routes of administration is that cattle do not have to be restrained in order for the bio-bullet to be utilized. From a producer standpoint, there is a considerable amount of labor and animal stress involved with gathering cattle for vaccination, especially if the vaccinations must be repeated after several weeks.

After an injection occurs the integrity of the muscle can be affected up to three inches away from the site. Injection-site lesions do not shrink in size as they grow older, but instead grow with the animal. When the wound from an injection is in the healing process there is a deposition of fat and connective tissue where the muscle tissue was disrupted. This deposition increases the shear force taken to sever the muscle up to three

inches away from the site of the injection. As a result, the usable portion of muscle is directly affected from a lean tissue standpoint and consumer acceptability from a tenderness standpoint. It is with the beef purveyors, who lose profit due to trim loss, and beef consumers, who do not have a pleasurable eating experience that the beef industry loses money and consumer confidence.

CHAPTER II

REVIEW OF LITERATURE

Occurrence of Injection Site Lesions in Today's Beef Industry

Vaccines, antimicrobials, and vitamins injected into the muscle have been shown to cause injection-site lesions and create tough beef, with the extent of damage depending on the calf's age at injection, the volume of the product injected per site, the anatomical site of the injection, the route of injection, and the product administered (Van Donkersgoed et al., 2000). Injection-site lesions are caused by several factors including the animal's sensitivity to the vaccine, the vaccination injury itself, the adjuvant used to enhance the immune response, and contamination of the needle and hide at the time of vaccination (Straw et al., 1986).

Age of Animal. Pharmaceuticals are commonly administered to cattle at various stages of their lives (Taylor and Field, 1999). Clostridial diseases can affect beef cattle of all ages, but are a primary concern in cattle between 6 months and 2 years of age. Because feeder cattle are marketed by the time they reach 2 years of age, vaccinating for clostridial disease is an important matter for cow-calf producers, stocker cattle operators, and feedlot managers (Troxel et al., 2001). Since feedlot managers do not know the injection history of the cattle they have on feed, administering another clostridial vaccination is very inexpensive insurance (Galyean and Eng, 1998). It has been reported that between 93.2 to 99.9% of lesions found at the retail/purveyor level were chronically aged lesions that occurred early in life (George et al., 1995a).

Tissue damage. Damaged beef muscle tissue resulting from intramuscular injections of animal health products represents a "quality control" problem and an economic loss to the beef industry (Roeber et al., 2001). This loss is not taken by the producer or the packing plant, but by the retailer and inevitably the consumer. According to Roeber (2002) "injection site lesions are seldom detected at packing plants because damage is concealed within the muscles and below the subcutaneous fat." This means that the injection-site damage will be mainly discovered at the retail establishment where primal cuts are fabricated into retail cuts (Roeber, 2002). As a result, the purveyor or retailer will be responsible for removal of the usually affected area and the consumer's odds of getting a tough steak increase. According to George et al. (1995a) tenderness is affected whether the injection site lesion is visible within a steak or not. In beef rounds, it was found that cores sampled as far as 7.6 cm from the center of the injection-site lesion had average shear force values of 5.8 kg, thereby being considered tough by retailers and purveyors (George et al., 1995b). According to the National Beef Quality Audit -1995 30% of beef packers, purveyors, and retailers indicated that the incidence of injection-site lesions had declined since the 1991 NBQA (Smith et al., 1995); however, these same packers, purveyors and retailers still named injection-site lesions among the top ten challenges of fed cattle.

Roeber et al. (2001) found that between November 1995 and July 2000, the incidence of lesions classified as 'nodular' (lesion with nodules, the central foci of necrosis, surrounded by granulomatous inflammation) and 'mineralized' (lesion containing

mineralized remnants of muscle cells) decreased significantly, but the occurrence of 'clear' lesions (older lesions that contain clear connective tissue) increased significantly. During this same time frame, 84% of the lesions were classified as 'older' lesions (Roeber et al. 2001). A change in lesion type could be due to a more critical selection of vaccine types, lower-dose vaccines, adjuvant changes, and the introduction of new products.

Reductions in lesion incidence in top sirloin butts from U.S. fed steer and heifers for the period of November 1995 through July 2000 (11.4% to 2.1%) generated an approximate net savings of $2.15 per calf slaughtered. This equated to an industry-wide savings of $7,078,100, based on the projected numbers of cattle to be harvested in 2000 (USDA, 2000).

Routes of Administration

Needle Injection. Needle injection, the traditional injection administration technique, is listed in many studies involving lesions and their impact on tenderness. While the abscesses and muscle damage that can develop at injection sites are clearly undesirable, a potentially more serious problem for the meat industry is the presence of broken needles and needle fragments in carcasses. Breakage of needles typically occurs when needles are bent, re-straightened and continued in use for injections (Hoff and Sundberg, 1999). Because the detection of needle fragments in muscle and meat is limited, a significant number of needle fragments are likely to be found in finished, processed meat products. The potential for consumer dissatisfaction is obvious. Lawsuits for several millions of US

dollars have been filed by consumers who have encountered needle fragments in processed meats (Murphy, 2001).

In the mid 1990's, cattle feeders received and responded to messages regarding ways to decrease the incidence of injection site lesions, but the message was not being received by producers in other segments of the beef industry who have control of the cattle at earlier stages in life. Additionally, some educational efforts to reduce lesion occurrence in the top sirloin butt resulted in producers giving injections in the inside and outside round. Producers also argued, at this point, that injection-site issues were a "feedlot problem". As a result, a study was conducted by Colorado State University to determine if age of the animal was influential in the development of injection site lesions. This study indicated that lesions occurred at harvest in both cattle that were intermuscularly injected at 2 months and 6 months of age. This provides irrefutable evidence that the location of the injection and the timing (age when given) of injection are most important determinants in resulting injection-site lesions (George et al., 1995a).

Troxel et al. (2001) reported that 28 days after injection, 64.9% of cattle developed injection-site lesions after being administered a clostridial vaccination with needle and syringe. The same study reported that 45% of cattle had smaller, but still detectable lesions 112 d after injection (Troxel et al., 2001). Futhermore, it was reported that following the often required second injection, the percentages of injection-site lesions and injection-site swelling were greater than those occurring after the first injection. Troxel et al. (2001) also documented that titers against clostridial diseases are enhanced when injection site lesions develop.

Some recommendations for avoiding injection-site lesions that have been provided to cattle producers and veterinarians include: (1) administer all clostridial bacterins subcutaneously in the neck regions, preferably using the 'tented' technique, (2) avoid multiple or repeat injections of clostridial bacterins, especially late in the feeding period, (3) avoid intramuscular injections of all products when other routes of administration are listed in the label recommendations, (4) use acceptable intramuscular and subcutaneous injection locations, (5) encourage the use of subcutaneous routes whenever possible, (6) inject no more than 10 mL per injection site, (7) change needles every 15 injections (more frequently if cattle are dirty), (8) immediately discard bent needles, do not straighten and reuse them, (9) do not use products that damage edible tissue at any time during production, (10) choose products that are approved for subcutaneous, intravenous or oral administration whenever possible, and (11) choose products that have low-volume doses whenever possible (George et al., 1997).

The location of the site of injection of clostridial vaccines on beef cattle may be important not only because of the seroconversion and damage to the carcass evident at slaughter, but also because of the decrease in performance during the early part of the finishing program. Subcutaneous vaccination in the ear produced performance that was not different from vaccination anterior to the scapula. Vaccination in the ear did produce a greater proportion of lesions at the base of the ear than prescapular vaccination, but it was observed that there was little effect on performance (Chirase et al., 2001).

Bio-bullet Injection. Most prior investigations have only included needle and syringe types of injection. SolidTech Animal Health Inc., Newcastle, OK, has created a method that uses an air powered delivery system and biodegradable projectiles such as freeze

dried ceftiofur sodium. Utilization of the bio-bullet technology is increasing because it can save producers time and decrease labor because producers do not have to gather cattle again for re-vaccination. Producers are often reluctant to revaccinate due to stress, shrink, as well as possible injury and increased labor cost. The bio-bullet biodegradable implant is fired with the help of compressed air. The 1.59 cm long, .64 cm diameter bio-bullet travels at 274.2 feet per second. The bio-bullet which is fired contains the bacterins or active ingredient and the capsule in which the ingredient is placed. The active ingredient is freeze dried and the capsule casing assimilates natural components of the animals body (Hansen, 2001). The bio-bullet pierces the skin and inserts 1 to 3 cm into the muscle (Haley, 2000). When the product penetrates animal tissue, the bacterin releases within three hours and the casing liquefies within 24 hours. The animal's body assimilates the casing ingredients (Hansen, 2001).

Morgan et al. (2004) reported that cattle receiving bio-bullet treatments more than 21d before slaughter had no detectable injection site lesions in the biceps femoris muscle. Furthermore, only steers treated with the bio-bullet implant at 7 and 14 d before slaughter displayed presence of injection-site lesions in the biceps femoris; thus, no detrimental effects on beef tenderness would likely be realized with a bio-bullet treatment of 21 d or more before slaughter (Morgan et al., 2004).

Needle-less Injection. The needle-less route of administration, while infrequently used, holds some advantages to the needle and syringe. This alternative would unequivocally remove all risk of needle fragments in pork products if used for delivery of vaccines, sera, and antibiotics. These devices have been observed to have several advantages in

human applications, including faster delivery of injected compounds to the circulatory system than traditional subcutaneous injections (Henry, 2000).

Product Quality

Wound Healing. Tenderness or shear force is dependent upon many factors, but decreasing tenderness issues due to injection-site lesions can be controlled. Once an animal has been administered an injection, a wound will be created and it will take time to heal that wound. As mentioned above, most lesions that were identified came from old wounds (George et al., 1995a). Similar to a fire brand applied to the hide of a calf, as the calf grows, the injection-site lesion will also grow. When a wound heals, there are architectural changes that occur in the collagen matrix due to the intricate process of remodeling. In addition to the deposition and maturation of collagen, a rise in tensile strength occurs (Harkness, 1968). The ratio of wound collagen to mucopolysaccharide is the best determinant of the gain in tensile strength. The alterations in the cohesive forces between collagen microstructures are directly related to this ratio (Bryant and Weeks, 1967). It was also observed that the number of effective network chains, per unit volume had a direct impact on load-bearing structure, not the total number of chains (Milch, 1965).

Warner-Bratzler Shear Force Values. It has been observed that when using the bio-bullet administration technique, the cooked beef tenderness was negatively affected only when administration resulted in the development of injection-site lesions, and this associated toughness was only observed in the direct location of the lesion core (Morgan et al.,

2004). Morgan et al. (2004) reported that samples isolated from the lesion core location of bio-bullet steaks and control samples located 5.08 cm from core locations had similar Warner-Bratzler shear force values compared with other treated and control samples. In George et al., (1995), cores were taken from the center of the injection lesion sites and from sites located 2.54, 5.08, and 7.62 cm away from the lesion center for Warner-Bratzler Shear Force. The Warner-Bratzler Shear Force values for lesioned steaks were 13.87, 10.00, 7.6, and 5.8 kg at the center of the lesion, 2.54 cm, 5.08 cm, and 7.62 cm, respectively, which was different from control steak (no lesions present) measurements of 4.11 kg at 2.54 cm, 4.30 kg at 5.08 cm, and 3.90 kg at 7.62 cm. According to Savell et al., 2006 in the overview of the National Beef Tenderness Study, Warner-Bratzler Shear Force Values for all grades of shoulder clod steaks combined to be 2.81kg, and 3.4 kg for all grades of eye of round steaks and 3.67 kg for all grades of bottom rounds steaks, respectively.

Collagen Determination. Light et al. (1985) observed a correlation between toughness and collagen content of muscles. It was reported that in connective-tissue reactions to injury in wound healing, or in a fibroproliferative process, there is initially neosynthesis of collagens of pericellular type V and basement membrane type IV. Eventually, synthesis and deposition occurs of fine, fibrillar type III collagen, which is followed closely by the formation of a matrix composed of interstitial type I collagen resembling scar tissue (Sherman et al., 1980). Type I collagen is reported to have a larger diameter than type III collagen that is correlated with decreased muscle tenderness (Gay, 1983). To go along with this increase in the concentration of collagen and the increase in diameter of collagen fibrils is the loss of solubility of collagen; collagen solubility

decreases progressively with age due to the development of heat-stable covalent interchains (Bailey, 1972).

Morgan et al. (2004) found that increases in total, soluble, and insoluble collagen concentrations at the lesion center decreased in concentration as the radius from the lesion center increased. It was also suggested that this result would imply that a fibroproliferative process occurred subsequent to intramuscular injection of a pharmacological agent, forming a lesion core and resulting in cooked meat toughness.

George et al. (1995b) reported that core samples taken at a distance of 5.08 cm from the center of the lesion required greater shear-force to sever than cores from corresponding control steaks, but that the amount of insoluble (heat stable) collagen was not significantly different, and was actually numerically lower than in control steak samples. This could be explained as the collagen assay that was employed measures hydroxyproline residues, and the percentage of residues differs for type I collagens and type III collagens. Regardless, the significant correlations ($r = 0$ .67 and 0.50; $P < 0.001$) between Warner-Bratzler shear values and insoluble as well as soluble collagen concentrations, respectively, indicate that the increase in these parameters accounts for the majority of the differences in shear force values (George et al., 1995b).

Summary

There were few studies that investigated different types of injection administration routes in beef chuck and round muscles. Additionally, the number of studies evaluating the decrease in beef tenderness due to type of pharmaceutical administered and route of administration was limited. However, in the studies that were

similar to the study at hand, there were contrasts in the findings regarding tenderness and lesion-site injections. The findings of George et al. (1995b) suggested that tissues as much as 5.08 cm from the lesion core were negatively altered in terms of tenderness, collagen amount and tissue proportions. These findings were contradicted by Morgan et al. (2004) who reported that only minor tissue alterations were evident in the biceps femoris from steers treated with the bio-bullet implant procedure immediately before slaughter. Prior to the start of the project it was hypothesized that there would be visible tissue damage in some cases, and that tissue damage would decrease tenderness values in steaks from the lesion core and some distance away from the lesion core.

CHAPTER III

COMPARISON OF THE BIO-BULLET VERSUS TRADITIONAL INJECTION TECHNIQUES ON TISSUE DAMAGE AND TENDERNESS IN BEEF SUBPRIMALS

ABSTRACT

The incidence and severity of injection-site lesions has decreased since the start of the Beef Quality Assurance program. This investigation evaluated route of administration and its effect on the tenderness, collagen concentration, and histological evaluations on beef chucks and rounds. One hundred ninety two yearling steers (initial BW = 383 ± 29.4 kg) were sorted from a group of 454 steer calves received from central Oklahoma auction markets between January 16 and February 2, 2006. Based on initial BW, steers were blocked into 2 groups of 96 each and randomly allocated within block into pens of 6 head each (16 pens per block). Each pen was randomly assigned an injection treatment protocol. On May 19, 2006 (d 0), steers were administered treatment injections of one of the following: standard BioBullet containing 100 mg of Naxcel; a traditional needle and syringe dose of Naxcel; a standard Biobullet containing Titanium 5; a traditional needle and syringe dose of Titanium 5; a needle-less injection of Vista 5; a traditional needle and syringe dose of Vira Shield 5; a standard BioBullet containing no pharmaceutical product; and a traditional needle and syringe dose of sterile water. Additionally, steers were implanted with estradiol and trenbalone acetate (Revalor-S, Intervet, Millsboro, DE) and sorted into home pens. Final individual BW was collected the day prior to shipment (d 101 for steers in block 1 and 122 for steers in block 2).

The majority of the lesions identified were clear scars (n = 108 out of 121) in the chuck and round. In all steaks evaluated, there were mature fibrous tissue and collagen fibers within adipose tissue. Warner-Bratzler Shear Force values of lesion center cores in chucks were found to be significantly different (P = 0.07) than cores from the control steaks, and at 2.54 and 5.08 cm away from the lesion core. Lesion cores from the BioBullet * Titanium 5 had a Warner-Bratzler Shear Force value of 7.01 kg, which were greater (P < 0.05) than lesion center cores from chucks injected with a BioBullet * H_20 (6.27 kg) or a Needle * Naxcel (5.08 kg). There were no significant differences (P > 0.10) observed in the total collagenous connective tissue (B) in samples extracted from the chuck or round. The comparison between the lesion site and control (no lesion site) samples for lipid concentration showed no significant difference (P > 0.10) for route and product in the round.

It was concluded that the BioBullet did not create a greater incidence of lesions in the chuck or round. Additionally, it was observed that the BioBullet injection did not create more tissue damage than intramuscular injections with a needle route of administration. With this in mind, it is still not appropriate to use the BioBullet in the round of animals as it does causes similar damage to a needle. The BioBullet can, however, effectively be used in prescapular applications without additional negative effects on tenderness.

INTRODUCTION

The National Cattlemen's Beef Association has worked for more than 15 years on developing the Beef Quality Assurance program to resolve quality challenges such as tissue damage and tenderness complications created by injection-site damage in the top

sirloin butt and in muscles of the round. In the late 1990's George et al. (1997) demonstrated that subprimals that contained lesions (visible or non-visible) had higher ($P < 0.01$) shear force values and greater tenderness variation than non-injected control subprimals. As a result of this and similar research, greater influence was placed on moving injections to the neck region for all routes of administration of pharmaceutical products.

More recently, SolidTech Animal Health Inc., Newcastle, OK has devised a method for injectable administration that uses an air-powered delivery system and biodegradable projectiles containing products such as freeze-dried ceftiofur sodium. These bio-bullets penetrate into the animal's muscle and begin to be absorbed. Morgan et al. (2004) conducted a preliminary study on the impact of these bio-bullets on tissue damage and tenderness in beef rounds. Morgan et al. (2004) documented that visible tissue damage was limited in cattle that were treated with bio-bullets 21, 28, and 35 days prior to slaughter.

While the research conducted by Morgan et al. (2004) indicates that the bio-bullet administration method of Naxcel, when used at least 30 days prior to harvest, led to no detectable increase in tissue damage or tenderness, no comparisons between the bio-bullet and traditional administration techniques have been made.

The current study was conducted to evaluate lesion occurrence, tenderness, and collagen content between different routes of injection administration. If there was little difference between the injection techniques in these areas, the utilization of new injection technologies could save producer time and livestock stress.

MATERIALS AND METHODS

Cattle. Steers (n = 192) of known treatment history were selected and transported to the Willard Sparks Beef Cattle Research Center at Oklahoma State University. One hundred ninety two yearling steers (initial BW = 383 ± 29.4 kg) were sorted from a group of 454 steer calves received from central Oklahoma auction markets in between January 16 and February 2, 2006. Cattle had no previous injections in the neck or round muscles on the animal's right side before the initiation of the trial. The steers had previously been used in receiving and growing experiments at the Willard Sparks Beef Research Center (WSBRC). Steers were considered for inclusion in the study based on no prior treatments for bovine respiratory disease, British x continental phenotype, and body weight. Beginning May 17, 2006, steers were individually weighed on two consecutive days for allocation. All steers had been program-fed a 94% concentrate diet for at least 30d prior to initial weighing. Based on initial BW, steers were blocked into 2 groups of 96 head each and randomly allocated within block into 32 pens of 6 head each (16 pens per block). Each pen was randomly assigned an injection treatment protocol. On May 19, 2006 (d 0), steers were administered the appropriate treatment injection: standard BioBullet containing 100 mg of Naxcel (1 BioBullet administered on right side, 2 BioBullets administered on left side); a traditional needle and syringe dose of Naxcel (3.5 mL using a 2.54 cm, 16 gauge needle); a standard Biobullet containing Titanium 5 (1 BioBullet administered on right side, 2 BioBullets administered on left side); a traditional needle and syringe dose of Titanium 5 (2 mL using a 2.54 cm, 16 gauge needle); a needle-less injection of Vista 5 (2 mL); a traditional needle and syringe dose of Vira

Shield 5 (5 mL subcutaneous, 1.59 cm, 16 gauge needle); a standard BioBullet containing no pharmaceutical product (1 BioBullet administered on right side, 2 BioBullets administered on left side); and a traditional needle and syringe dose of sterile water (5 mL, 2.54 cm, 16 gauge needle). Steers were also implanted with estradiol and trenbalone acetate (Revalor-S, Intervet, Millsboro, DE); and sorted into home pens. Steers were fed in 4.6 m x 15.2 m partially covered (by a 4.6 m metal awning) feedlot pens. Water was offered ad libitum in fence-line basins and 0.8 m of bunk space was available per steer. An 84% concentrate ration was initially offered at 2% of initial BW and gradually increased over 7 days. For the remainder of the trial, steers were twice daily fed ad libitum a 95% concentrate finishing diet. Orts were weighed at the end of each weigh period and when feed became old or wet.

Steers were weighed on a pen basis on d 56. Final individual BW was collected the day prior to shipment (d 101 for steers in block 2 and d 122 for steers in block 1). Steers in block 1 were also weighed on a pen basis on d 101. All weights except for individual BW were given a pencil shrink of 4% for ADG and G:F calculations. Carcass adjusted final weight was also calculated by dividing individual hot carcass weight by average dress for each block (64.47% for block 1 and 64.78% for block 2). One steer died of bloat during the trial.

Treatment. In treatments including Naxcel, Titanium 5, and sterile water Bio-Bullet and traditional needle comparisons, cattle were administered the dosage intramuscularly in either the neck (prescapular) or round (lower quarter) region. The Vira Shield 5 treatment group was injected in the neck region subcutaneously. A trained Solid Tech Animal Health representative administered all Bio-Bullet dosages at a distance of 6.1

meters, while trained Oklahoma State University Staff administered all other injections. Biobullet and traditional injections were placed in the same location either in the neck or in the round. Administration of products followed protocol approved by the Institutional Animal Care and Use Committee at Oklahoma State University (IACUC # AG0666).

Harvest and Meat Samples. After completion of the finishing period, (n = 191) steers were transported to Emporia, KS for harvest at a commercial abbatoir. Cattle were tracked through the facility from the immobilization box to the rapid chilling cooler to maintain animal identity. After harvest and chilling, trained Oklahoma State University personnel collected and recorded carcass data and identified the chuck or round in order to maintain carcass identity through fabrication. After the completion of carcass data collection, rounds or chucks were tagged and inked for identity and carcasses were fabricated according to Institutional Meat Purchasing Specifications (IMPS; USDA, 1996). Outside round flats (bicep femoris muscle, IMPS #171a) and 2-piece boneless chucks (IMPS #115) from the right side were collected for the trial. Subprimals were vacuum packaged and transported back to the Oklahoma State University Food and Agriculture Products Center where they were aged 14 days at 3°C ± 1°C.

After the aging period, each subprimal (n = 129) was fabricated into 1.27 cm steaks on a sanitized commercial band saw. After fabrication, each steak was observed and palpated for the presence of injection-site lesions by trained Oklahoma State University personnel. When a lesion was identified, the lesion was verbally described using the 5-point classification system as described by Dexter et al. (1994), which categorizes lesions as cystic, scar with nodules, mineralized scar, clear scar, or woody callus. If a lesion was present, steaks were identified to represent the center or core of the

lesion, and steaks representing areas that were 2.54, 5.08 and 7.62 away from the lesion core. A steak was taken to represent the same muscle as far from the core as possible and additional control and lesioned steaks were also taken for proximate and hydroxyproline analysis. Furthermore, if large enough, a portion of the lesion was excised for histological examination to verify that tissue damage was the result of an injection. If no lesion was found in the subprimal, steaks were taken from the region where the lesion should have occurred (i.e., where the injection was given) along with a control steak for Warner-Bratzler Shear Force testing, proximate analysis and collagen determination.

Warner-Bratzler Shear Force. Steaks were randomly assigned to cooking order across treatment group. Steaks were allowed to temper for 24 h at 4°C prior to cooking and were then broiled on an impingement oven (model 11132-00-A; Lincoln Impinger, Fort Wayne, IN) at 180°C to an internal temperature of 70°C. Internal steak temperatures were monitored using copper constantan thermocouples (model OM-202; Omega Engineering, Inc., Stamford, CT). Steaks were allowed to cool for 2 h to 25°C before coring. The Warner Bratzler Shear Force (WBSF) at the lesion site and the average of the WBSF for the four cores at each distance of 2.54, 5.08, and 7.62 cm from the lesion location was calculated and recorded for each steak. Cores 1.27 cm in diameter, were removed parallel to the muscle fiber orientation from each steak. Following the procedure outlined by George et al. (1995), a core was removed from the immediate area near the lesion core/product administration location, and four additional cores were removed at a radial distance of 2.54, 5.08, and 7.62 cm from the lesion/administration location. Each core was sheared once by a Warner-Bratzler head attached to an Instron Universal Testing Machine (model 4502; Instron Corp., Canton, MA) at a crosshead

speed of 200 mm/min. Peak force (kg) of cores as recorded by an IBM PS2 (Model 55 SX) using software provided by the Instron Corp. (Canton, MA).

Histological Examination. Histopathological examinations of muscle samples were performed by the Oklahoma Animal Disease Diagnostic Laboratory at Oklahoma State University to verify that tissue damage was a result of an injection. Tissue samples were placed in 10% formaldehyde solution for fixation and coded for submission so that the product administered, or the distance of the sample from the lesion center was unknown to the pathologist evaluating the muscle sections. Slides were prepared using Masson's trichrome connective tissue stain (Luna, 1968).

Proximate Analysis. Proximate analysis of the samples was performed in duplicate and averaged according to the procedures outlined by AOAC (1990). Each sample was frozen individually in liquid nitrogen and pulverized to a powder in a Waring blender (Dynamics Co. of America, New Hartford, CT). Three grams of the powdered sample was placed in filter paper, dried at 100°C for 24 h, desiccated for 1 h, and reweighed to determine moisture. Following moisture determination, each sample was placed in a soxhlet for 24 h for ether extraction of lipid, followed by drying at 100°C for no more than 12 h. Each sample was desiccated and re-weighed to calculate lipid content.

Collagen Determination. Hydroxyproline is quantitatively determined as a measure of collagenous material in meat and meat products. Collagenous connective tissue contains 12.5% hydroxyproline when a collagen-protein factor of 6.25 is used (Kolar, 1990). To determine the collagen content, a sample was freeze dried using liquid nitrogen and powdered using a blender as described above. A 4 g sample was then hydrolyzed using

sulfuric acid at 105ºC for approximately 16 h. Upon completion of heating, solution was filtered and diluted using protocol as listed by Kolar (1990). The hydroxyproline was oxidized by using chloramine –T. The reddish purple color that developed after the 60ºC water bath was a result of the addition of 4-dimethylaminobenzaldehyde. After arriving at this step, the sample is measured photometrically at 560 nm. Upon retrieving absorption data from spectrophotometer, the calculation of hydroxyproline content (H) was as follows: H,g/100 g = (h X 2.5) / (m X V), where h = hydroxyproline, µg/2 mL filtrate, read from calibration curve; m = weight of sample, g; and V = volume, mL, of filtrate taken for dilution to 100 mL for the hydrolysis step. The result from this calculation was an arithmetic mean of two calculated values for each sample. In calculating the collagenous connective tissue content (B), the following formula was utilized: B,g /100 g = H X 8. It should be noted that collagenous connective tissue contains 12.5% hydroxyproline if the nitrogen-to-protein factor is 6.25.

Statistical Analysis. All post harvest results were analyzed using General Linear Model (PROC MIXED, SAS Inst., Inc., Cary, NC). Feedlot performance (BW, DMI, ADG, G:F) was analyzed using the Mixed Procedure of SAS with pen as the experimental unit. Drug administered, injection technique, and drug x injection method were initially included in the model. However, the interaction was not significant ($P > 0.2$) for all variables and it was removed from the model. Because all steers were administered Naxcel and a viral vaccine, only injection technique was included in the final model. Data were analyzed to determine the effect of pharmaceutical, route of administration, and pharmaceutical by route of administration on lesion occurrence, Warner-Bratzler Shear

Force, and fat and collagen content. Means were separated when a significant F test (α = 0.05) was observed. Means were separated using a pair-wise t-test.

CHAPTER IV

RESULTS AND DISCUSSION

Yield and Quality Grade Data. Quality and yield grade data were collected from carcasses before fabrication. There was little difference in yield grade and quality grade between treatment groups. However, it was observed that the Needle* Titanium 5 group harvested with the lowest quality grade (Slight 65) and yield grade (2.54), while the treatment group with the highest quality grade was Needle*Vira Shield 5 (Small 20). The treatment group with the highest yield grade was BioBullet* Naxcel (2.94).

Lesion Presence. Results of visual palpation and inspection of the 69 rounds and 60 chucks that were evaluated identified a visual lesion in 71.83% of all Control (H_20) rounds, which was similar to rounds injected with Naxcel, which had a 70.83% visual lesion presence (Table 1). Rounds injected with Titanium 5 had a visual lesion present in 77.83%, which was the highest percentage of all rounds and chucks. The control chucks and chucks injected with Titanium 5 had a visual lesion incidence percentage of 52.60% and 52.36%, respectively. The highest lesion percentage occurred in chucks injected with Naxcel; 56.84% of chucks had a visible lesion.

Although lesion occurrence was not significantly different ($P > 0.10$) between the two routes of administration, 83.33% of rounds injected with a Bio-Bullet had a visible lesion as compared to 63.66% of rounds injected using a needle, 56.25% of chucks injected with a BioBullet, and 57.08% of chucks injected with a needle. This contradicts findings by Morgan et al. (2004) that indicated cattle receiving a BioBullet injection at least 21 days prior to harvest had no ($P = 0.88$) detectable injection site lesion in the

biceps femoris. Rounds and chucks injected with Vira Shield 5 with a needle-less route of administration showed no significant difference ($P > 0.05$) in visible lesion presence compared to rounds and chucks injected with H_20.

The types of lesions found in the chucks and rounds included clear scars and woody calluses, as well as metallic and nodular lesions. The majority of the lesions identified were clear scars (n = 108 out of 129; 83.7%) in the chuck and round. In the 2-piece chucks that were evaluated, there were 14 lesions that were found in the clod as compared to the 57 lesions that were identified in the chuck roll. Lesions found in the chuck roll and clod were commonly found in seam fat between the muscles, whereas the lesions found in the round were generally found in lean muscle tissue. In several instances, clear lesions found in the eye of round were long and narrow white tracks going across the grain of the muscle fiber. These results indicated variable lesion type and occurrence in beef sub-primals from injection route and product type. As most cattle receive at least one injection in their lifetime, it is of utmost importance that those injections are placed in the proper location.

Histology. Histological examination of all samples confirmed the diagnosis of injection site lesions as described by George et al. (1995b). It was noted that lesions within sections of all eight specimens revealed variable evidence of chronic fibrosing inflammation involving skeletal muscle and adipose tissue. In all steaks evaluated there were mature fibrous tissue and collagen fibers within adipose tissue. George et al. (1995b) reported that the lesion center contains dense sheets of fibroblasts with extensive collagen deposition, which is gradually replaced by adipose tissue as the distance from the lesion increases. Additional George et al. (1995a) reported finding dense, mature

connective tissue with a few trapped muscle fibers and sheets of adipose tissue, resulting from the intramuscular injection of commercially available biological and pharmacological preparations into branding- and weaning-age calves.

Tenderness. Warner-Bratzler Shear Force values for USDA Select Infraspinatus and Serratus ventralis muscles located in the chuck (aged 2 d) were 4.75 kg and 4.68 kg, respectively (Savell et al., 2006). For USDA Select Biceps femoris muscle located in the round, the Warner-Bratzler Shear Force Value was 5.86 kg (aged 2 d) (Savell et al., 2006). An interaction between route of administration and product injected was observed in beef chuck lesion core samples and samples from 7.62 cm away from the lesion core. Warner-Bratzler Shear Force values of lesion site center in chucks tended to be significantly different (P=0.0662) than cores from the control and 2.54 and 5.08 cm away from the core. In a similar study, George et al. (1995 b) reported that WBSF values from cores taken from the lesion site and steaks 2.54, 5.08, and 7.62 cm from the lesion were significantly different ($P < 0.001$) from the corresponding measurements in control steaks.

Lesion cores from the BioBullet*Titanium 5 had a WBSF value of 7.01 kg, which were significantly different ($P < 0.05$) from lesion center cores from chucks injected with a BioBullet H_20 or a Needle Naxcel where WBSF values were 6.27 kg and 5.08 kg, respectively. However, this contradicts research conducted by Morgan et al. (2004) in which only steers treated with a BioBullet injection at 7 or 14 days before harvest displayed the presence of injection lesions in the biceps femoris; thus, no detrimental effects on beef tenderness would likely be realized with BioBullet treatment 21 days or more before slaughter. The steak for the same interactions of BioBullet*

Titanium 5, BioBullet*H_20, Needle*Naxcel were 4.78 kg, 4.73 kg, and 4.61 kg, respectively. WBSF values for samples 7.62 cm away from the lesion center were significantly different in shear force values, with the toughest samples resulting from the needle*H_20 interaction group (6.35 kg).

Warner-Bratzler Shear Force values for chuck steaks taken at 2.54 cm and 5.08 cm away from the lesion core were not significant, although in all instances by product or by route, those samples required more force to shear than those of the control chuck steaks. Cores from Vista 5 * Needleless injections showed no significant difference from control steak cores. These data support findings by George et al., (1995) that concludes that when an injection is administered in beef cattle, the tenderness of affected tissues is significantly reduced at, and an area up to 7.62 cm away from the lesion center. Tenderness is a key factor in satisfaction for beef consumers. If there are injection lesions present, it will likely affect a large portion of the cut of meat and consequently increase the odds of an unpleasant eating experience and a dissatisfied customer.

Collagen Content. Tenderness is impacted by the amount of collagen and connective tissue that occurs in the muscle. When a wound or injury occurs, the healing process involves the deposition of connective tissue and collagen in and around that wound. There was no significant differences ($P > 0.05$) found in the total collagenous connective tissue in samples extracted from the chuck or round. Although there were differences found in WBSF values between treatments in chucks, this does help to explain the similarities in WBSF values for rounds. Total collagenous connective tissue content for Vista 5* needle-less steaks was significantly different ($P = 0.03$) from control steaks.

Lipid Content. Lipid concentrations also vary with tenderness and muscle damage, as increased amounts of fat create more tender beef in post mortem muscle. In addition, when damage occurs in living muscle, fat deposition increases. The comparison between the lesion site and control (no lesion site) samples for lipid concentration showed no significant difference for route or product in the round. However, for lipid concentrations in the chuck, values calculated from steaks from the Titanium 5 treatment group were shown to be significantly different ($P < 0.05$) from steaks from the Naxcel treatment group. However, H_20 steaks were not different from Naxcel steaks ($P = 0.10$) or the Titanium 5 vaccine steaks ($P = 0.29$). This is supported by the findings of Morgan et al. (2004) and George et al. (1995b) that lipid content is higher at the lesion site than in the control steak. Lipid content findings in steaks from Vira Shield 5 * needle-less were significantly higher ($P < 0.01$) when compared to lipid content of control steaks.

IMPLICATIONS

Although injection-site lesions are decreasing in prevalence, new technologies have given a new twist to the traditional needle and syringe. Utilizing these new routes of administration may ease the stress of handling livestock several times for repeated vaccination, but concern must be raised in that the emerging technology causes similar amounts of tissue damage in valuable muscle. From a production standpoint, the results indicate that it is still best to administer vaccines to cattle anterior to the scapula to decrease the chance of lean tissue being damage, resulting in trim loss and tenderness. Moreover, we can estimate losses due to extra handling of animals, trim loss, etc, but we cannot calculate the cost of a lost consumer due to poor beef palatability as a result of a injection lesion.

Table 1. Lesion presence percentage in beef subprimals (n = 129)[1] stratified by product type.

	Product Type				
	Control (H_20)	Naxcel	Titanium 5	SEM	P > F[2]
Round	71.83%	70.83%	77.83%	3.79	0.29
Chuck	52.60%	65.04%	52.36%	7.23	084

[1]N = 129: n = 69 for round by product type;; n = 60 for chuck by product type
[2]Probability of overall F test

Table 2. Lesion presence percentage in beef subprimals (n = 129)[1] stratified by product

	Bio-Bullet	Needle	SEM	P > F[2]
Round	83.33%	63.66%	13.88	0.29
Chuck	56.25%	57.08%	0.59	0.84

[1]N = 129: n = 69 for round by product type; n = 60 for chuck by product type
[2]Probability of overall F test

3. Warner-Bratzler Shear Force values (kg) in beef rounds (n = 51)[1] stratified by product

	Product Type				
Sample Location	H_20[2]	Naxcel[3]	Titanium 4[4]	SEM	P > F[5]
Control[6]	5.12	5.17	5.27	0.07	0.53
Lesion Core	4.53	4.66	6.66	1.20	0.19
2.45 cm from Core	4.93	4.61	5.25	0.32	0.33
5.08 cm from Core	5.06	4.68	5.78	0.56	0.23
7.62 cm from Core	4.54	5.05	4.86	0.25	0.61

[1]N = 51: n = 16 for H_20; n = 17 for Naxcel; n = 18 for MLV vaccine
[2]Represents rounds injected with control (saline) solution.
[3]Represents rounds injected with Naxcel, a ceftiofur sodium product
[4]Represents rounds injected with a Titanium 5, a modified live vaccine for IBR, BVD, BRSV, and PI_3
[5]Probability of F Test
[6] Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion

Table 4. Warner-Bratzler Shear Force values (kg) in beef rounds (n = 51)[1] stratified by route of administration

	Route of Administration			
Sample Location	Bio-Bullet	Needle	SEM	P > F[2]
Control[3]	5.36	5.02	0.24	0.53
Lesion Core	5.16	5.41	0.17	0.19
2.45 cm from Core	4.69	5.17	0.33	0.33
5.08 cm from Core	4.88	5.47	0.41	0.23
7.62 cm from Core	4.73	4.90	0.11	0.61

[1]N = 51: n = 30 for Bio-Bullet; n = 21 for Needle.
[2]Probability of F Test
[3]Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion

Table 5. Warner-Bratzler Shear Force valued (kg) in beef chuck lesion site cores stratified by product * route interaction.

Route * Product	Control[1]	Lesion Center
Bio-Bullet * Titanium 5[2]	4.78	7.01[a]
Bio-Bullet * H[2]0[3]	4.73	6.27[ab]
Needle * Naxcel[4]	4.61	5.08[abc]
Needle * Titanium 5[2]	4.44	4.66[bc]
Needle * H_20[3]	4.29	4.61[bc]
Bio-Bullet * Naxcel[4]	4.83	3.81[c]
SEM	0.31	0.15
P > F[5]	0.93	0.06

[a,b,c] Within a column, means without a common superscript letter differ ($P < 0.05$)
[1]Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion
[2]Represents rounds injected with Titanium 5, a modified live vaccine for IBR, BVD, BRSV, and PI_3
[3]Represents rounds injected with control (saline) solution.
[4]Represents rounds injected with Naxcel, a ceftiofur sodium product
[5]Probability of F Test

Table 6. Warner-Bratzler Shear Force values (kg) in beef chuck lesion site cores (n = 34)[1] extracted 2.54 cm and 5.08 cm from lesion center in relation to product

Sample Location	Product Type			SEM	P > F[5]
	H_20[2]	Naxcel[3]	Titanium 5[4]		
Control[6]	4.65	4.50	4.56	0.07	0.92
2.54 cm from Core	5.28	4.61	4.81	0.33	0.62
5.08 cm from Core	5.16	4.75	4.89	0.20	0.90

[1]N = 34: n = 10 for Control Product; n = 13 for Ceftiofur Sodium; n = 11 for MLV vaccine
[2]Represents rounds injected with control (saline) solution.
[3]Represents rounds injected with Naxcel, a ceftiofur sodium product
[4]Represents rounds injected with a Titanium 5, a modified live vaccine for IBR, BVD, BRSV, and PI_3
[5]Probability of F Test
[6]Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion

Table 7. Warner-Bratzler Shear Force values (kg) in beef chuck lesion site cores (n = 34)[1] extracted 2.54 cm and 5.08 cm from lesion center in relation to route of administration

	Route of Administration			
	Bio-Bullet	Needle	SEM	P > F[2]
Control[3]	4.67	4.48	0.12	0.92
2.54 cm from Core	4.91	4.89	0.01	0.62
5.08 cm from Core	4.87	5.00	0.09	0.90

[1]N = 34: n = 17 for Bio-Bullet; n = 17 for Needle.
[2]Probability of F Test
[3]Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion

Table 8. Warner-Bratzler Shear Force values (kg) in beef chuck lesion site cores extracted 7.62 cm from lesion center with interaction to product and route

Route * Product	Control[1]	7.62 cm from lesion core
Needle * H_20[2]	4.78	6.35[ab]
Bio-Bullet * H_20[2]	4.73	4.84[b]
Needle * Naxcel[3]	4.61	4.77[b]
Bio-Bullet * Naxcel[3]	4.44	4.28[b]
Needle * Titanium 5[4]	4.29	4.27[b]
Bio-Bullet* Titanium 5[4]	4.83	3.67[b]
SEM	0.31	0.33
P> F[5]	0.93	0.02

[a,b,c] Within a column, means without a common superscript letter differ ($P < 0.05$)
[1]Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion
[2]Represents rounds injected with control (saline) solution.
[3]Represents rounds injected with Naxcel, a ceftiofur sodium product
[4]Represents rounds injected with Titanium 5, a modified live vaccine for IBR, BVD, BRSV, and PI_3
[5]Probability of F Test

Table 9. Collagenous connective tissue content (mg/g) (n = 64)[1] of lesion stratified by product type

	Product Type				
	H_20[2]	Naxcel[3]	Titanium5[4]	SEM	P > F[5]
Round					
Control[6]	0.71	0.75	0.85	0.07	0.23
Lesion	2.04	0.78	0.76	0.73	0.42
Chuck					
Control[6]	2.02	0.90	1.06	0.59	0.39
Lesion	1.20	1.79	1.30	0.30	0.20

[1]N = 64: n = 41 for Round by Product; n = 23 for Chuck by Product
[2]Represents rounds injected with control (saline) solution.
[3]Represents rounds injected with Naxcel, a ceftiofur sodium product
[4]Represents rounds injected with a Titanium 5, a modified live vaccine for IBR, BVD, BRSV, and PI_3
[5]Probability of F Test
[6]Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion

Table 10. Collagenous connective tissue content (mg/g) (n = 64)[1] of lesion stratified by route of administration.

	Bio-Bullet®	Needle	SEM	P > F[2]
Round				
Control[3]	0.74	0.80	0.45	0.23
Lesion.	1.62	0.76	0.58	0.42
Chuck				
Control[3]	0.98	1.67	0.48	0.39
Lesion	1.58	1.28	0.19	0.20

[1]N = 64: n = 41 for Round by Route; n = 23 for Chuck by Route.
[2]Probability of F Test
[3]Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion

Table 11. Lipid concentration percentage (n = 69)[1] of lesion stratified by product type

	Product Type				
	H_20[2]	Naxcel[3]	Titanium 5[4]	SEM	P > F[5]
Round					
Control[6]	4.69	5.52	5.04	0.44	0.35
Lesion	3.70	3.83	3.33	0.25	0.85
Chuck					
Control[6]	6.34[ab]	8.22[a]	5.14[bc]	1.57	0.02
Lesion	6.45	8.05	4.01	1.94	0.25

[a,b,c] Within a column, means without a common superscript letter differ (P < 0.05)
[1]N = 69: n = 45 for Round by Product; n = 24 for Chuck by Product
[2]Represents rounds injected with control (saline) solution.
[3]Represents rounds injected with Titanium 5, a modified live vaccine for IBR, BVD, BRSV, and PI_3
[4]Represents rounds injected with Naxcel, a ceftiofur sodium product
[5]Probability of F Test
[6]Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion

Table 12. Lipid concentration percentage (n = 69)[1] of lesion stratified by route of administration.

	Route of Administration			
	Bio-Bullet	Needle	SEM	P > F[2]
Round				
Control[3]	4.93	5.23	0.17	0.35
Lesion	3.42	3.82	0.27	0.85
Chuck				
Control[3]	7.22	5.91	0.93	0.02
Lesion	7.08	5.26	1.21	0.25

[a,b,c] Within a column, means without a common superscript letter differ (P < 0.05)
[1]N = 69: n = 45 for Round by Route, n = 24 for Chuck by Route
[*]Probability of F Test
[3]Sample from same muscle in same round or chuck with lesion, but excised on opposite end of muscle from lesion

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VITA

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Candidate for the Degree of

Master of Science

Thesis: COMPARISON OF THE BIO-BULLET VERSUS TRADITIONAL INJECTION TECHNIQUES ON TISSUE DAMAGE AND TENDERNESS IN BEEF SUB-PRIMALS

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Major Field: Animal Science

Scope and Method of Study:

The objective of this study was to evaluate the effects of route of injection administration on lesion occurrence, tenderness, and collagen concentration in beef chucks and rounds. Steers (n=192) were blocked by BW and randomly allocated to treatment groups: BioBullet/Naxcel; needle/Naxcel; Biobullet/Titanium5; needle/Titanium5; needle-less/Vista5; a needle/ViraShield5; BioBullet/water; and needle/water.

Findings and Conclusions:

Chuck and round lesions were 83.9% clear scars. Warner-Bratzler Shear Force values of chuck lesion cores were tougher (P=0.07) than control tissue cores and at points 2.54 and 5.08cm from the core. BioBullet/Titanium5 cores were 0.74kg higher than lesion cores from chucks injected with BioBullet/H_20 or Needle/Naxcel treatments. The BioBullet did not create greater incidence of lesions in the chuck or round, nor did more tissue damage than needle injections. BioBullet is not recommended in the round as it does not meet quality assurance guidelines. The BioBullet can effectively be used in prescapular applications without additional negative effects on tenderness.

ADVISER'S APPROVAL: _____