Influence of ceftiofur sodium biobullet administration on tenderness and tissue damage in beef round muscle

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ABSTRACT: The effect of a biobullet (BB) containing freeze-dried ceftiofur sodium antibiotic on the presence of injection lesions, tissue damage, and histological properties, as well as Warner-Bratzler shear force (WBSF), of the biceps femoris was investigated. Steer calves (n = 25) were individually identified and assigned randomly to a product administration treatment date (7, 14, 21, 28, or 35 d before slaughter). At each preslaughter ceftiofur BB administration time, identified steers (n = 5) were humanely placed into a standard commercial restraining chute, where a BB implant was administered from a distance of 6.09 m. Following a standard finishing period (120 d), steers were transported to a commercial beef processing and humanely slaughtered. Following a 36-h postmortem chilling (1°C) period, carcasses were graded and fabricated according to industry-accepted procedures. Paired muscle samples were individually identified, collected, and aged for 14 d postmortem. Muscles were dissected into 1.27-cm strips, followed by observation and palpation for the presence of injection site lesions. Preslaughter administration times of 7 and 14 d resulted in the presence of injection lesions (80 and 20%, respectively). In addition to the control samples, no muscle damage was observed in cattle treated with BB implants 21, 28, or 35 d before slaughter. Warner-Bratzler shear force measurements taken near lesions of BB steaks and in areas 5.08 cm from lesions of control steaks tended to be higher ($P < 0.10$) than for other BB and control sample locations. Concentrations of insoluble and soluble collagen were higher ($P < 0.05$) at the site of the lesion center in lesion-afflicted vs. with control steaks. Histological determinations of the relative proportions of muscle, connective tissue, and fat were altered ($P < 0.05$) in BB lesion-afflicted steak cores; however, these differences were negated outside the core location of BB-treated and control steaks. It seems that using the ceftiofur BB implant system within 14 d of slaughter does create injection site lesions and increase WBSF; however, when the BB implant system, containing 100 mg of freeze-dried ceftiofur sodium, was used according to the recommended procedure ($\geq$ 30 d preslaughter), tissue damage, alterations in histological and collagen properties, and increased meat toughness were not observed.

Key Words: Beef, Incidence, Injection, Lesions, Tenderness


Introduction

Damaged beef muscle tissue resulting from i.m. injections of animal-health products represents a “quality control” problem and an economic loss to the beef industry. Results of the National Beef Quality Audit—2000 (McKenna et al., 2002) revealed that beef packers believed the greatest quality improvement since 1991 has been the decreased frequency of injection site lesions found in beef top sirloin subprimals. Although the incidence of injection site lesion defects in top sirloins is at a record low of 2.1% (Roeber et al., 2001), purveyors and retailers still ranked this as one of the greatest quality challenges facing the U.S. beef industry.

Pharmaceuticals are commonly administered to cattle at various stages of their lives (Taylor and Field, 1999), and if given i.m., tissue damage can occur (George et al., 1995). The National Cattlemen’s Beef Association has recommended that s.c. injections be administered when allowable; however, treating cattle in open-pasture situations lends to potential problems, including the stress of being held from the herd as well as unwanted restraint techniques.

Until recently, administering biological and pharmaceutical products to animals has meant that needles and syringes would be required. SolidTech Animal Health Inc., Newcastle, OK, has devised a method that uses an air-powered delivery system and biodegradable projectiles containing products such as freeze-dried ceftiofur sodium. “Biobullets” (BB) penetrate into the ani-
Главный мышечный орган и начинает усваиваться в организме, но нет ничего известного о влиянии этого деликатеса на вареное мясо. Поэтому, целью настоящего исследования было определить 1) насколько влияние BB технологий вызывает проблему качества при создании инъекционных зон, 2) влияние, если есть, BB на любые патологические изменения в круговом мышечном теле, и 3) насколько влияние BB администрации влияет на вареное мясо тендерность.

Материалы и методы

Экспериментальные процедуры

Стейр калves (n = 25), известного местоположения, находятся в Willard Sparks Beef Cattle Research Facility, Oklahoma State University, были выбраны для участия в этом исследовании. Стейры не имели предыдущих инъекций в любую из круговых мышц до начала эксперимента и были идентифицированы и случайно назначены к административному дельтат др. (7, 14, 21, 28 или 35 d до убоя).

Производственное заведение в эксперименте было стандартное BB, содержащее 100 мг замороженное сухое цефтифурана натрия (NaF; Pharmacia & Upjohn, Kalamazoo, MI). На каждом производственном заведении периода, пять стейр были идентифицированы и размещены по стандартной коммерческой стресс-штампу. После того, как была определена административная зона над мечевидным мышцами (BF), BB был администрирован в дистанции от 6.09 м, управляющий SolidTech Animal Health представитель. Это следует отметить, что все стейры получали свою BB от идентифицированной круговой зоны, и в среднем проникновение BB было 3.7 cm в направлении цели. Когда было видимым присутствие BB было замечено, ткань упакованная, а также BB в идентифицированной круговой зоне, и период BB в BB administration was exposed, the affected tissue was expressed and weighed (to the nearest 0.3 г), оставаясь с линией были визуально выражены с использованием пяти-штатной классификационной системы описана Dexter et al. (1994).

Стеиры были затем упакованы вакуумным способом и хранены при −28°С до определения Warner-Bratzler shear force (WBSF) определений можно было провести.

Warner-Bratzler shear force

Стейры были случайно размещены в порядке BB-администрации. Стейры были разрешены на 24 h at 4°С до убоя. Стейры были затем брошены в an impingement oven (model OM-202; Omega Engineering, Inc., Stamford, CT.) and aged 14 d postmortem at approximately 1°С. After the aging period, each BF sample was removed and sliced from all subcutaneous fat and evaluated for the presence of injection site lesions. After fat removal, each BB- treated muscle section was dissected into 1.27-cm steaks (n = 15), followed by observation and palpation for the presence of injection site lesions. If any muscle tissue damage was exposed, the affected tissue was excised and weighed (to the nearest 0.3 г), along with the lesion being verbally expressed using the five-point classification system described by Dexter et al. (1994). Steaks were subsequently vacuum-packaged and stored at −28°C until Warner-Bratzler shear force (WBSF) determinations could be conducted.

Histological Examination

Историко-патологическое исследование BB-процессов и курорт BB-мышечных образцов было проведено в Oklahoma Animal Disease Diagnostic Laboratory in Stillwater. Дополнительные тканные образцы (n = 8 контроль и n = 4 BB-треатед) были помещены в 10% формальдегидное соединение для фиксации и кодированы для подачи, чтобы проверить присутствие/отсутствие линии, время BB администрации.
before slaughter, and/or the distance of the sample from the real or counterpart lesion center was unknown to the pathologist evaluating the histological sections. In all, 24 slides were prepared using Masson's trichrome connective tissue stain (Luna, 1968).

**Chemical Analyses**

Laboratory analyses of BF samples were conducted in duplicate according to procedures outlined by AOAC (1990). Each sample was frozen individually in liquid N and pulverized in a Waring blender (Dynamics Co. of America, New Hartford, CT). Three grams of the powdered sample was placed in glass thimbles, 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. Samples were then desiccated and reweighed to calculate lipid content. Using a combustion analyzer (model FP-428; Leco Corp., St. Joseph, MI), N content was determined and recorded from a complete block design, the type of product administration and time of injection were examined as main effects, and lesion weight and WBSF value were the dependent variables. When the main effect was significant ($P < 0.05$), least squares means separation was accomplished by the PDIFF option of SAS (a pairwise t-test).

**Results and Discussion**

The average incidence of injection site lesions in the BF segmented by administration method and time are shown in Table 1. The average incidence of injection site lesions from carcasses of cattle administered the BB 7 d before slaughter was higher (80% frequency; $P < 0.05$) than muscle from 14 d preslaughter administered steerers (20%). Cattle receiving BB ≥ 21 d before slaughter had no ($P = 0.88$) detectable injection site lesions in BF. As expected, no injection site lesions were observed in the opposite side muscles (untreated controls). In the most recent national audit estimating the incidence of injection site lesions in beef top sirloin butts, Roeber et al. (2001) indicated that lesion incidence decreased from 11.40 to 2.06% from November 1995 to July 2000. It should be noted that this decrease in the incidence of injection site lesions in top sirloin butts was continuous, with each subsequent incidence lower than the preceding audit incidence. However, the incidence of injection site blemishes found in muscles of the round from fed cattle was 11.3% (Roeber et al., 2001). These changes have likely been in response to educational efforts, such as the quality assurance programs of the National Cattlemen’s Beef Association and similar state beef quality assurance programs. Even with such a decrease in injection site lesion incidence in the past years, the beef industry was encouraged to remain cautious. Education must continue, and new, innovative pharmaceutical delivery systems must be discovered and implemented.

Table 1. Least squares means (±SE) for injection site lesion incidence and quantity of trim loss associated with removal of lesions from the biceps femoris as affected by the preslaughter administration time of cefiofur sodium biobullet (BB)

<table>
<thead>
<tr>
<th>Administration time, d</th>
<th>Incidence, %</th>
<th>Weight of tissue removed, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB</td>
<td>Control</td>
</tr>
<tr>
<td>7</td>
<td>80.0 ± 43.17&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0&lt;sup&gt;ax&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>20.0 ± 17.87&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0&lt;sup&gt;ax&lt;/sup&gt;</td>
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<tr>
<td>21</td>
<td>0&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>0&lt;sup&gt;ax&lt;/sup&gt;</td>
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<td>35</td>
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<sup>a,b,c</sup>Within a row, least squares means that do not have a common superscript letter differ ($P < 0.05$).

<sup>x,y</sup>Within a column, least squares means that do not have a common superscript letter differ ($P < 0.05$).

The average incidence of injection site lesions in the BF was 7.52 and 7.25, respectively (Cross et al., 1966). Spectrophotometric determination of hydroxyproline in the soluble and insoluble fractions was performed (Bergmann and Loxley, 1963), and the conversion factors used for quantifying soluble and insoluble collagen were 7.52 and 7.25, respectively (Cross et al., 1973).

**Statistical Analyses**

Data representing injection site lesion presence were analyzed using the frequency procedure of SAS (SAS Inst., Inc., Cary, NC). Significant differences between incidence values as associated with product administration type and time of administration were determined by calculating the $\chi^2$ statistic. Means representing the lesion weights, concentrations of soluble and insoluble collagen, muscle, connective tissue, and fat proportions, and WBSF were computed, and ANOVA was determined using the GLM procedure of SAS. Each steer was used as an experimental unit within a randomized complete block design, the type of product administration and time of injection were examined as main effects, and lesion weight and WBSF value were the dependent variables. When the main effect was significant ($P < 0.05$), least squares means separation was accomplished by the PDIFF option of SAS (a pairwise t-test).
thus, no detrimental effects on beef tenderness would likely be realized with BB treatment 21 d or more before slaughter.

Total collagen concentrations (fresh tissue basis) of tissue samples taken from the site of the injection lesions, as well as 2.54, 5.08, and 7.62 cm away from the lesion, were 18.26, 9.73, 9.56, and 8.48 mg/g, respectively, for the lesioned steaks vs. 8.80 mg/g of collagen in muscle samples from the control steaks (results not shown). Concentrations of soluble (heat-labile) collagen were 2.68, 1.84, 1.78, and 1.59 mg/g for samples taken at the site of the injection site lesion, 2.54, 5.08 and 7.62 cm away, and control steaks, respectively (Figure 2). Moreover, concentrations of insoluble collagen from the site of the lesion and from sites 2.54, 5.08, and 7.62 cm away from the lesion were 15.58, 7.89, 7.78, and 6.89 mg/g, whereas that for control steaks was 7.02 mg/g.

In wound healing, architectural changes in the collagen matrix occur as a result of the intricate process of remodeling. Concomitant with the deposition and maturation of collagen is an increase in tensile strength (Harkness, 1968). Quantitative changes in acid mucopolysaccharides accompany the collagen and tensile strength changes (Dunphy and Udupa, 1955). Bryant and Weeks (1967) reported the best determinant of the increase in tensile strength is the ratio of wound collagen to mucopolysaccharides, and that alterations in the cohesive forces between collagen microstructures were directly related to this ratio. Milch (1965) reported that the number of effective network chains (per unit volume) has a great influence on load-bearing structure. Results of the previous studies have provided concrete evidence that when injections are administered i.m. into beef cattle, the tenderness of affected tissues is significantly decreased at and in an area up to 7.62 cm away from the lesion center (George et al., 1995). In the current investigation, cooked beef tenderness was negatively affected only when BB administration resulted in the development of injection lesions, and this associated toughness was only observed in the direct location of the lesion core.

In the current study, increases in total, soluble, and insoluble collagen concentrations at the lesion center decreased \( (P < 0.05) \) in concentration as the radius from the lesion center increased (Figure 2). This result would imply that a fibroproliferative process occurred subsequent to i.m. injection of a pharmacological agent, forming a lesion core and resulting in cooked meat toughness. Sherman et al. (1980) reported that with connective tissue reactions 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 of fine, fibrillar type III collagen occurs, which is followed by the formation of matrix composed of interstitial type I collagen that resembles scar tissue. Moreover, this type I collagen is reported to have a larger fiber diameter than type III collagen, which was correlated with decreased muscle tenderness (Gay, 1983). Concurrent

![Figure 1. Warner-Bratzler shear force (WBSF) for Control (normal) and biobullet-lesioned (injected) biceps femoris steaks. Bars that do not have a common letter differ \( (P < 0.05) \).](image-url)
with this increase in concentration of collagen and the increase in diameter of collagen fibrils, collagen solubility decreases, especially with progressing development of heat-stable covalent interchain bonds (Bailey, 1972). It should be noted that George et al. (1995) detected a very pronounced muscle toughening effect, as far as 7.62 cm away from the lesion core. In the current study, however, only the lesion core in 7- and 14-d samples displayed increased toughness as a result of treating cattle with a BB.

Histological examination of all samples confirmed the diagnosis of injection site lesions as described by George et al. (1995). From visual estimations, the relative percentages of connective tissue, muscle, and fat (Figure 3) were 33.25, 40.23, and 26.52%, respectively, at the site of the lesion core; 11.25, 73.25, and 15.50%, respectively, at sites 2.54 cm from the lesion center; 12.25, 79.68, and 8.07%, respectively, at sites 5.08 cm from the lesion center; and 11.25, 79.98, and 9.78%, respectively, in control steaks. This finding supports results
from the collagen assays, indicating that severe disruption of the relative tissue proportions had occurred in the injection lesion cores. Compared with the findings of George et al. (1995), which suggested that tissues as much as 5.08 cm from the lesion core were negatively altered in terms of tenderness, collagen amounts, and tissue proportions, the current findings suggest that only minor tissue alterations were evident in the BF from steers treated with the BB implant procedure immediately before slaughter.

Implications

Injection site lesions are a serious quality concern in the U.S. beef industry. Although a great deal of progress has been made in decreasing injection lesions in top sirloins, substantial product loss, decreased labor efficiency, and the potential for meat tenderness to be jeopardized still occur in other locations of the beef carcass as a result of improper medicament and vaccination injection techniques and/or locations. Findings from this study demonstrate that the ceftiofur sodium bio-bullet implant system, when used according to the recommended procedure (≥30 d pre slaughter), resulted in the absence of damage as related to unnoticeable alterations in histological and collagen properties in tissue and, in turn, led to no detectable increases in meat toughness.

Literature Cited