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Effects of Growth-Promoting Technologies on Muscle Structural Characteristics and Meat Tenderness

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Abstract
Skeletal muscle tissue consists of two main structures that elicit strong influences on cooked meat tenderness: myofibrillar and connective tissues. The myofibrillar component consists of contractile and cytoskeletal proteins that aid in muscle contraction and support. A large portion of meat science literature documents the effects that postmortem aging elicits in terms of weakening the myofibrillar component to improve tenderness. Connective tissue is primarily comprised of collagen, the most abundant protein within the body. The function of this tissue is to support the myofibrillar component and transfer the force of contraction. Collagen, characterized by its solubility, is most commonly identified as the muscle tissue structure that has a large influence on tenderness but is commonly characterized as unaffected by postmortem degradation.

Ractopamine hydrochloride (Optaflexx, Elanco Animal Health, Greenfield, IN) is a beta-adrenergic agonist that directs nutrients away from fat accretion to lean muscle production and is commonly fed during the finishing period of cattle production. Anabolic implants are another growth-promoting technology that increases muscle mass. Numerous studies indicate that both technologies increase muscle mass by increasing the cross-sectional area of muscle fibers. In addition, many studies have demonstrated that both growth promotants negatively affect tenderness, which can be improved by aging. Little is known about the effects of growth promotants or extended aging on collagen solubility. The purpose of this study was to examine the effects of growth-promoting technologies and extended aging on structural muscle characteristics and meat tenderness.

Keywords
growth-promoting technologies, implants, beta-adrenergic agonists, muscle structure, meat tenderness

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Introduction

Skeletal muscle tissue consists of two main structures that elicit strong influences on cooked meat tenderness: myofibrillar and connective tissues. The myofibrillar component consists of contractile and cytoskeletal proteins that aid in muscle contraction and support. A large portion of meat science literature documents the effects that postmortem aging elicits in terms of weakening the myofibrillar component to improve tenderness. Connective tissue is primarily composed of collagen, the most abundant protein within the body. The function of this tissue is to support the myofibrillar component and transfer the force of contraction. Collagen, characterized by its solubility, is most commonly identified as the muscle tissue structure that has a large influence on tenderness but is commonly characterized as unaffected by postmortem degradation.

Ractopamine hydrochloride (Optaflexx, Elanco Animal Health, Greenfield, IN) is a beta-adrenergic agonist that directs nutrients away from fat accretion to lean muscle production and is commonly fed during the finishing period of cattle production. Anabolic implants are another growth-promoting technology that increases muscle mass. Numerous studies indicate that both technologies increase muscle mass by increasing the cross-sectional area of muscle fibers. In addition, many studies have demonstrated that both growth promotants negatively affect tenderness, which can be improved by aging. Little is known about the effects of growth promotants or extended aging on collagen solubility. The purpose of this study was to examine the effects of growth-promoting technologies and extended aging on structural muscle characteristics and meat tenderness.

Experimental Procedures

Two groups of crossbred feedlot heifers were blocked by body weight (n = 33, initial body weight 946 ± 15 lb; n = 32, initial body weight 1,025 ± 15 lb) and assigned to one of three treatments: (1) control (no implant or Optaflexx supplementation); (2) implant (Component TE-200 implant [Elanco Animal Health] and no Optaflexx); (3) Optaflexx/implant (Component TE-200 implant and Optaflexx at 400 mg per head for the final 28 days for group 1 and 29 days for group 2). After the finishing period, animals were weighed and shipped to Creekstone Farms in Arkansas City, KS, for
harvest. At time of harvest, hot carcass weight was measured and carcasses were tagged for animal identification. Following a 48-hour chill period, boneless strip loins were collected and transported back to the Kansas State University Meat Laboratory.

Upon arrival, each loin was faced on the anterior end producing a steak that was used to analyze muscle fiber characteristics. Samples from this steak were embedded in a tissue freezing medium, sectioned onto frost-resistant slides, then subjected to antibody staining to determine the area of individual muscle fibers. The next five cuts were 2-inch roasts that were fabricated, vacuum-packaged, and then aged for 2, 7, 14, 21, or 35 days at 33°F. After aging, roasts were further fabricated into two, 1-inch steaks for Warner-Bratzler shear force and collagen solubility analysis, then stored at -4°F until further analysis was completed. Steaks collected for collagen solubility analysis were frozen in liquid nitrogen and pulverized using a Waring blender (Waring Products Division, Harford, CT). The pulverized tissue was weighed out into a glass tube and heated in a hot water bath at 158°F for 80 minutes. Samples were then cooled, centrifuged to separate insoluble and soluble collagen fractions, and hydrolyzed overnight with sulfuric acid. Afterward, samples were filtered and collagen concentration was determined using a colorimetric assay. Percentage soluble and insoluble collagen was calculated using the equation: 

\[ \frac{(soluble/insoluble\ collagen\ amount)}{(total\ collagen\ amount)} \times 100. \]

To determine meat tenderness, steaks aged to five different time periods were cooked and Warner-Bratzler shear force was measured. Prior to cooking, steaks were thawed at 33°F for 24 hours on a tray with a moisture-absorbing pad and plastic overwrap. The steaks were then cooked to an internal temperature of 152°F with a Cuisinart Griddler Deluxe (Cuisinart, East Windsor, NJ) set at 450°F. After cooking, steaks were chilled overnight at 33°F. Six cores, cut parallel to the muscle fibers, were sheared through the center using an Instron Universal Testing Machine (Instron, Canton, MA) with a Warner-Bratzler shear head. Peak force was recorded and averaged to determine objective tenderness of the steak.

**Results and Discussion**

Warner-Bratzler shear force analysis (Table 1) was conducted to obtain an objective measure of tenderness. As expected, the shear force values of all steaks from each treatment decreased \((P < 0.01)\) during aging, indicating an improvement in tenderness. For all steaks, shear force values decreased 42% from day 2 to day 35 of aging. No treatment \(\times\) day interaction \((P = 0.53)\) was detected, which indicates that all steaks aged at the same relative rate. Growth-promoting treatment did affect \((P < 0.01)\) Warner-Bratzler shear force values. During the entire aging study, steaks from the Optaflexx/implant group had 17% greater \((P < 0.01)\) shear force values compared with those from the control animals, and values tended to be 9% greater \((P = 0.07)\) compared with the implant-only steaks. The implant-only steaks’ shear force also tended to be 9% greater \((P = 0.09)\) than control steaks. The literature states that shear force values below 9.5 lb indicate a steak that a consumer would find tender. Control steaks reached this threshold on day 14 of aging, whereas the implant-only and Optaflexx/implant steaks reached the threshold on day 21 and 35, respectively. Therefore, utilization of growth-promoting technologies negatively affected tenderness, and the effects were not alleviated by extended aging.
The increase in shear force values for steaks subjected to growth-promoting technologies could be owing to modifications to the structural components of skeletal muscle. Muscle characteristics were analyzed (Table 2) to provide insight into how each fraction is influenced by growth promotants and how they affect meat tenderness. Treatment tended to increase cross-sectional area of type I and IIX muscle fibers ($P = 0.10$), whereas type IIA muscle fiber cross-sectional area was increased ($P < 0.01$). For the implant-only and Optaflexx/implant treatments, type IIA cross-sectional area was approximately 17% larger compared with the control treatment ($P < 0.01$), but they did not differ ($P = 0.76$) from each other. These findings indicate that increases in muscle fiber cross-sectional area, primarily type IIA muscle fibers, caused by growth-promoting treatments are a potential source of the negative effects these technologies have on meat tenderness. No treatment $\times$ day interaction or treatment effects for collagen solubility measures were detected ($P = 0.54$). Day of aging had an effect on both measures of collagen solubility ($P = 0.04$). Compared with day 7, collagen was 0.8 and 1.1% more soluble on day 21 and 35, respectively ($P < 0.01$). Also, solubility increased ($P < 0.01$) by 0.9% on day 35 compared with day 14. This change in solubility provides some explanation of the origin of the decrease in shear force for all steaks when subjected to extended postmortem aging. Research documents that most of the postmortem degradation of myofibrillar tissue is complete after 14 days of aging. Therefore, the improvement in tenderness past 14 days could be because of an increase in the solubility of collagen.

**Implications**

The addition of growth-promoting technologies decreased tenderness, which was potentially due to increases in muscle fiber cross-sectional area stimulated by growth promotants, because these technologies had no effect on collagen solubility. An extended postmortem aging program improved tenderness for all steaks, partly owing to increases in collagen solubility.
Table 1. Objective strip loin measurements from strip loin steaks of crossbred heifers' subjected to three growth-promoting programs, aged 2, 7, 14, 21, or 35 days, then subjected to cooking procedures2

<table>
<thead>
<tr>
<th>Objective measures</th>
<th>Treatment</th>
<th>P-value</th>
<th>Treatment</th>
<th>Day</th>
<th>Treatment × day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Implant</td>
<td>Optaflexx/ implant</td>
<td>SEM3</td>
<td>Treatment</td>
</tr>
<tr>
<td>Warner-Bratzler shear force, lb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>11.01</td>
<td>12.03</td>
<td>13.31</td>
<td>0.47</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Day 7</td>
<td>9.09</td>
<td>10.57</td>
<td>11.07</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>7.84</td>
<td>8.50</td>
<td>9.77</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Day 21</td>
<td>7.12</td>
<td>8.02</td>
<td>8.53</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Day 35</td>
<td>6.60</td>
<td>6.79</td>
<td>7.74</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Soluble collagen4, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>12.3</td>
<td>12.1</td>
<td>11.8</td>
<td>0.54</td>
<td>0.90</td>
</tr>
<tr>
<td>Day 7</td>
<td>12.0</td>
<td>11.3</td>
<td>11.5</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>11.8</td>
<td>11.9</td>
<td>11.6</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Day 21</td>
<td>12.1</td>
<td>12.3</td>
<td>12.6</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Day 35</td>
<td>12.1</td>
<td>13.4</td>
<td>12.3</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Insoluble collagen5, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>87.7</td>
<td>87.9</td>
<td>88.2</td>
<td>0.54</td>
<td>0.90</td>
</tr>
<tr>
<td>Day 7</td>
<td>88.0</td>
<td>88.7</td>
<td>88.5</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>88.2</td>
<td>88.1</td>
<td>88.4</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Day 21</td>
<td>87.9</td>
<td>87.7</td>
<td>87.4</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Day 35</td>
<td>87.9</td>
<td>86.6</td>
<td>87.7</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>

1 Crossbred heifers (group 1, n = 33; group 2, n = 32) were raised during two different time periods and were subjected to one of three treatments: (1) no implant and no Optaflexx (Elanco Animal Health, Greenfield, IN) supplementation, control; (2) implanted with Component TE-200 (Elanco Animal Health) on d 0 of feeding, no Optaflexx supplementation, implant; and (3) implanted with Component TE-200 on d 0 of feeding, and supplemented with 400 mg/day per heifer Optaflexx for 28 days (group 1) or 29 days (group 2), Optaflexx. Final body weight was 946 and 1,025 lb for group 1 and 2 heifers, respectively.

2 Cooking procedures for collagen solubility determination consisted of heating tissue in a hot water bath at 158°F for 80 minutes. Warner-Bratzler shear force steaks were cooked to an internal temperature of 152°F with a Cuisnart Griddler Deluxe (Cuisnart, East Windsor, NJ) set at 450°F.

3 SEM = standard error of the mean.

4 Calculated using the equation (soluble collagen/total collagen) × 100.

5 Calculated using the equation (insoluble collagen/total collagen) × 100.
Table 2. Muscle fiber characteristics of strip loin steaks from crossbred heifers\(^1\) subjected to three exogenous growth promoting programs

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Implant</th>
<th>Optaflexx/implant</th>
<th>SEM(^2)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional area, µm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>2,082.9</td>
<td>2,278.5</td>
<td>2,292.6</td>
<td>75.9</td>
<td>0.10</td>
</tr>
<tr>
<td>Type IIA</td>
<td>3,003.7(^a)</td>
<td>3,540.9(^b)</td>
<td>3,485.8(^b)</td>
<td>127.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Type IIX</td>
<td>3,750.2</td>
<td>4,192.9</td>
<td>4,164.1</td>
<td>161.2</td>
<td>0.10</td>
</tr>
</tbody>
</table>

\(^a,b\) Means within a row with a different superscript are different (\(P < 0.05\)).

\(^1\) Crossbred heifers (group 1, \(n = 33\); group 2, \(n = 32\)) were raised during two different time periods and were subjected to one of three treatments: (1) control, no implant and no Optaflexx (Elanco Animal Health, Greenfield, IN) supplementation; (2) implant, implanted with Component TE-200 (Elanco Animal Health) on day 0 of feeding, no Optaflexx supplementation; and (3) Optaflexx, implanted with Component TE-200 on day of feeding, and supplemented with 400 mg/day per heifer Optaflexx for 28 days (group 1) or 29 days (group 2). Final body weight was 946 and 1,025 lb for group 1 and 2 heifers, respectively.

\(^2\) SEM = standard error of the mean.