1993

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**Bos indicus** Breeding Effects on Muscle Characteristics and Their Relationship With Meat Tenderness

Georgianna Whipple, Mohammad Koochmarale, Michael E. Dikeman, John D. Crouse, and Melvin C. Hunt

Introduction

Variation in meat tenderness that exists among animals may be due to genetics, diet, age, and other factors. Results from the Germplasm Evaluation (GPE) program show that rib eye steaks from many of the **Bos indicus** breeds of cattle are less tender than steaks from most of the European (**Bos taurus**). Although **Bos indicus** crossbreeding programs are advantageous due to hybrid vigor and insect and heat resistance in subtropical regions. Because consumers consider tenderness to be the principal component of cooked beef quality, it is important to determine the biological factors that regulate meat tenderness. If this knowledge can be obtained, steps could be taken to decrease the variation in meat tenderness; thus, red meat producers could consistently provide consumers with a tender product.

Factors affecting meat tenderness have been studied by MARC scientists for many years. Some factors that may affect tenderness include muscle pH (acidity), rate of temperature decline after slaughter, muscle cell length (an indicator of the state of muscle contraction), the amount of total and soluble collagen (a form of connective tissue), muscle type (red vs white muscle types), and muscle enzyme activity. Of the many enzymes found in muscle, the calpain proteolytic (enzyme) system is thought to have a major role in the meat tenderization process. These calpain enzymes occur naturally in muscle tissue as well as a specific protein, known as calpastatin, that inhibits the activity of these enzymes. Two forms of calpain exist, μ-calpain which requires low calcium concentrations for activity, and m-calpain which requires high calcium concentrations for activity. Therefore, μ-calpain is the form that is active in muscle tissue after slaughter. During the aging or storage of meat, this enzyme system is active in degrading certain muscle proteins which must occur for meat to be tender. If the activity of this system is hindered in any way or potential for activity is lost, then tenderness is ultimately affected. Therefore, much emphasis has been placed on understanding this enzyme system.

This report summarizes results from experiments which were designed to determine which mechanisms associated with tenderness can best explain the differences or variation observed in tenderness between **Bos indicus** and **Bos taurus** breeds.

Procedures

Sahiwal crossbreds were used as the representative of the **Bos indicus** breed and Hereford X Angus crossbreds served as the contemporary **Bos taurus** breed. Seven heifers and four steers of 5/8 Sahiwal X Hereford, Angus or Hereford X Angus, three heifers and three steers of 3/8 Sahiwal, and five heifers and five steers of Hereford X Angus crosses were used in this study. Calves were weaned at 6 to 8 mo of age and fed an alfalfa haylage and corn silage finishing diet until 15 to 17 mo of age. Animals were then slaughtered over a 7 wk period. Carcasses were not electrically stimulated but were chilled at 30°F for 24 hr. Longissimus muscle (rib eye, loin) temperature and pH were determined at <1, 3, 6, 9, 12, and 24 hr after slaughter. Carcass data were collected at 24 hr. Samples were taken immediately after slaughter and 24 hr post-slaughter to determine activities of the calpain enzyme system. Besides the calpain system, the activities of another enzyme system (cathepsins) were determined. In addition, samples were taken to determine muscle type, muscle calcium and zinc concentrations, muscle cell length, and total and soluble collagen. The ease of muscle fiber fragmentation under controlled homogenization, which is an indicator of the amount of muscle proteins that have been degraded, was also determined. Also, we examined which muscle proteins were degraded. One inch thick rib eye steaks were obtained 14 days after slaughter to determine tenderness by measuring the amount of force needed to sever a 1/2 inch cooked meat core (Warner-Bratzler shear force). Cooked steak samples also were served to a trained sensory panel to determine tenderness, juiciness and flavor intensity.

Results

The **Bos indicus** and **Bos taurus** cattle used had similar USDA quality and yield grades, with averages of high Select and 3.2, respectively. No differences were found for lean color, lean firmness, lean texture, maturity scores, dressing percentage, and percentage of kidney, pelvic, and heart fat. Least-squares means for growth, carcass, and loin muscle chemical fat percentage are given in Table 1. Differences were observed for average daily gain, with the 5/8 Sahiwal crosses gaining most slowly. The Hereford X Angus crosses were significantly heavier at slaughter than the 5/8 Sahiwals. Small differences occurred for adjusted fat cover and rib eye area among the breed crosses. There were no statistically significant breed cross marbling differences, which contradicts some other reports, yet shows that quality grades are not totally indicative of tenderness. The chemical fat percentages support the marbling scores in that no breed cross differences were found.

Sensory panel evaluation scores and Warner-Bratzler shear force values (the greater the shear force the less tender the meat) for rib eye steaks aged 14 days are given in Table 2. The Hereford X Angus crosses were more tender as revealed by both lower Warner-Bratzler shear values and higher sensory panel tenderness scores. The sensory panellists found no differences in steak juiciness or flavor among the breed crosses. The muscle fiber fragmentation values (ease with which the sample fragments) were lower in the 5/8 Sahiwal meat samples (Table 2). The lower muscle fiber fragmentation values indicate that less muscle protein was degraded during the aging process in the 5/8 Sahiwal cross carcasses, which can explain why steaks obtained from their carcasses were less tender.

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3The authors would like to express their gratitude for the technical support of Sue Hauver, Kathy Mihm, Pat Tammen, Kay Theer, and Jeff Waechter.
No breed cross differences occurred for temperature and pH decline after slaughter. Neither were there breed cross differences in muscle calcium and zinc concentrations, total and soluble collagen, muscle cell length, muscle type nor cathepsin enzyme activity (data not shown). Also, no differences in calpain activity were found among the breed crosses at either 0 hr or 24 hr postmortem (Table 3). Initial (0 hr) calpastatin activity (inhibitor of calpain activity) was similar among the breed crosses; however, at 24 hr, calpastatin activity was greater in muscle samples from 3/8 and 5/8 Sahiwal crosses (Table 3). The greater calpastatin activity may have inhibited calpain from degrading the muscle proteins that are necessary to make meat tender. This again is supported by the muscle fiber fragmentation values previously mentioned. Other research has shown that those muscle proteins degraded with postmortem aging of meat are also the muscle proteins degraded by calpain. In this study, when we evaluated which proteins were degraded, those proteins that are degraded in tender meat were also degraded in samples from Hereford x Angus crosses, but not in samples from the 5/8 Sahiwal crosses. Therefore, it appears that a lack of protein degradation is the reason why the Sahiwal steaks were less tender; and the greater 24 hr activity of calpastatin in the Sahiwal samples may be why this occurred.

Further statistical analyses were performed on 42 different variables obtained from this study to determine which variable(s) could accurately predict tenderness in this population of cattle. Of all variables, 24 hr calpastatin activity was able to explain 44% of the variation in tenderness. The variation in tenderness that was not explained by 24 hr calpastatin activity was explained partially by other variables. Muscle type accounted for 9 to 16%, muscle pH at 6 hr post-slaughter explained 9%, and cathepsin enzyme activity accounted for 10% of the tenderness variation. However, calpastatin 24 hr activity was the only variable that was significantly related to tenderness among and within breed subclasses.

These results suggest that differences in the amount of muscle protein degraded during the aging of meat is the major reason why steaks from Sahiwal crosses are less tender than steaks from Hereford x Angus crosses, and why the calpain-calpastatin system appears to be responsible for these differences. Therefore, knowledge of the calpain system mechanism and how it is regulated in postmortem muscle tissue is a must. Then, methodology could be developed to optimize the amount of muscle protein degraded through the action of the calpain system, thus ensuring tender meat. These methods may be through animal selection, dietary management, and/or post-slaughter handling practices.

### Table 1—Least-squares means for growth, carcass and chemical traits by breed cross

<table>
<thead>
<tr>
<th>Trait</th>
<th>H x A</th>
<th>3/8 Sah</th>
<th>5/8 Sah</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG, lb</td>
<td>2.11</td>
<td>1.94</td>
<td>1.78</td>
</tr>
<tr>
<td>Slaughter wt, lb</td>
<td>1104.6</td>
<td>1030.9</td>
<td>986.2</td>
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<tr>
<td>Hot carcass wt, lb</td>
<td>678.9</td>
<td>644.8</td>
<td>622.8</td>
</tr>
<tr>
<td>Adj. fat thickness, in</td>
<td>.60</td>
<td>.55</td>
<td>.47</td>
</tr>
<tr>
<td>Rib eye area, in²</td>
<td>11.63</td>
<td>11.01</td>
<td>10.94</td>
</tr>
<tr>
<td>Marbling</td>
<td>Small51</td>
<td>Slight76</td>
<td>Small58</td>
</tr>
<tr>
<td>Rib eye chemical fat, %</td>
<td>5.4</td>
<td>4.0</td>
<td>4.7</td>
</tr>
</tbody>
</table>

### Table 2—Least-squares means for Warner-Bratzler shear force, sensory-panel scores and muscle fiber fragmentation values

<table>
<thead>
<tr>
<th>Trait</th>
<th>H x A</th>
<th>3/8 Sah</th>
<th>5/8 Sah</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warner-Bratzler shear, lb*</td>
<td>10.3</td>
<td>14.1</td>
<td>16.9</td>
</tr>
<tr>
<td>Sensory-panel score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tendernessb</td>
<td>5.9</td>
<td>5.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Juicinessc</td>
<td>5.4</td>
<td>5.3</td>
<td>4.8</td>
</tr>
<tr>
<td>Flavor4</td>
<td>4.9</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Muscle fiber fragmentation values*</td>
<td>82</td>
<td>75</td>
<td>60</td>
</tr>
</tbody>
</table>

#### Notes:
- *A higher shear force value indicates less tender meat.
- b A score of 6=moderately tender, ... 4=slightly tough.
- c A score of 6=moderately juicy, ... 4=slightly dry.
- d A score of 6=moderately intense, ... 4=slightly bland.
- e A higher muscle fiber fragmentation value indicates that more protein has been degraded; thus, muscle fibers are easier to fragment.