ABDU IBRAHIM

EFFECT OF ELECTRICAL STIMULATION AND GENOTYPE ON MEAT QUALITY TRAITS OF NELLORE AND CROSSBREED CULL COWS

Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of Magister Scientiae.

Adviser: Mario Luiz Chizzotti

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2023
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Assent:

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Abdu Ibrahim
Author

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Mario Luiz Chizzotti
Adviser
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ABSTRACT


The Brazilian beef cattle supply chain has undergone technological modernization in its production systems, resulting in better productivity, meat quality and competitiveness. Electrical stimulation (ES) has been used as an innovation in the meat industry to improve meat tenderness and colour of beef carcass. The study aimed to evaluate ES and genotype’s effects on carcass pH and temperature decline and meat quality attributes of Nellore and crossbred beef cattle. Ten cows (5 Nellore and 5 ¾ Nellore × ¼ Holstein) were used. Following slaughter, exsanguinations, dressing, and evisceration processes, the carcasses were halved by splitting along the vertebral column, and the right sides of the carcasses were electrically stimulated with 5 impulses (7 s duration each, with a 2 s interval between pulses), constant voltage (300 V) at variable amps, and 45 Hz. Meat quality analyses were performed on samples obtained from longissimus dorsi muscle. There were no effects (P>0.05) of ES, genotype and their interaction on carcass pH and temperature, carcass weight loss, thawing and cooking losses, chemical composition, colour: L* (lightness), a* (redness), and b* (yellowness) values and shear force. Sarcomere length was influenced (P=0.029) by ES × genotypes interaction. The highest sarcomere length (2.13 µm) was observed in the ES crossbred genotype group, while NES Nellore genotype group, and ES Nellore group did not differ. The practice of ES should be adopted in crossbred genotype carcasses to avoid the cold shortening of the sarcomere.

Keywords: Carcass pH. Meat colour. Meat tenderness
RESUMO


A cadeia produtiva da bovinocultura de corte brasileira tem passado por modernização tecnológica em seus sistemas produtivos, resultando em melhor produtividade, qualidade da carne e competitividade. A estimulação elétrica (ES) tem sido utilizada como uma inovação na indústria da carne para melhorar a maciez da carne e a cor da carcaça bovina. O estudo teve como objetivo avaliar os efeitos do ES e do genótipo no declínio do pH e da temperatura da carcaça e nos atributos de qualidade da carne de bovinos Nelore e mestiços de corte. Foram utilizadas 10 vacas (5 Nelore e 5 ¾ Nelore × ¼ Holandês). Após os processos de abate, sangria, preparação e evisceração, as carcaças foram divididas ao meio ao longo da coluna vertebral e os lados direitos das carcaças foram eletricamente estimulados com 5 impulsos (7 s de duração cada, com 2 s de intervalo entre os pulsos), tensão constante (300 V) em amperes variáveis e 45 Hz. As análises de qualidade da carne foram realizadas em amostras obtidas do músculo longissimus dorsi. Não houve efeito (P>0,05) de ES, genótipo e sua interação sobre o pH e temperatura da carcaça, perda de peso da carcaça, perdas por descongelamento e cozimento, composição química, cor: L* (luminosidade), a* (vermelho) e b* (amarelo) e força de cisalhamento. O comprimento do sarcômero foi influenciado (p<0,05) pela interação ES × genótipos, onde o maior comprimento de sarcômero (2,13 µm) foi observado no tratamento mestiço ES, entretanto os tratamentos NES Nelore ES Nelore não diferiram entre si. A prática de ES pode ser adotada em carcaças de animais mestiços para evitar o encurtamento do sarcômero pelo frio.

Palavras-chave: Cor da carne. Maciez da carne. pH da carcaça.
<table>
<thead>
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<th>Acronym</th>
<th>Definition</th>
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<td>DM</td>
<td>Dry Matter</td>
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<tr>
<td>ES</td>
<td>Electrical stimulation</td>
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<tr>
<td>NES</td>
<td>No Electrical stimulation</td>
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<tr>
<td>WBSF</td>
<td>Warner-Bratzler shear force</td>
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1 Introduction

Brazil ranks as the largest beef exporter in the world (USDA, 2022) and most of the beef produce are from the Nellore breed (Bos indicus) which is generally, leaner and tougher than Bos taurus beef (RODRIGUES et al., 2017). Carcass traits such as colour and tenderness are important criteria determining meat quality. Beef consumers regard tenderness as a very important quality characteristic (MILLER et al., 2001 & VERBEKE et al., 2010), while colour is the main factor that is observed when purchasing the product (MANCINI & HUNT, 2005). The cause of variation in meat quality attributes is complex and depends on factors such as species, breed type, age, bodyweight, gender, nutrition, pre- and post-slaughter handling and technological characteristic (GUERRERO, 2013; EROGLU, et al, 2011 and NAZLI et al., 2010)

The Brazilian beef cattle supply chain has undergone technological modernization in its production systems, resulting in better productivity, meat quality and competitiveness (GUILHERME et al, 2021). Electrical stimulation (ES) of carcasses is one of the major practices that is used to improve meat quality (ADEYEMI & SAZILI, 2014). The ES has been used as an innovation in the meat industry to improve meat tenderness and colour of beef, lamb, and goat carcasses (POLIDORI et al, 1999 & BISWAS et al, 2007). The ES causes muscles to contract, resulting in high anaerobic glycolytic rate and hastening pH decline. This leads to early onset of rigor mortis before the carcass temperature drops to values that cause cold shortening and toughening (SIMMONS et al., 2008; DEVINE et al., 2014). The ES of carcasses has been used to improve tenderness and colour in beef (MCKENNA et al., 2003; NAZLI et al., 2010; MOMBENI et al., 2013; AGBENIGA & WEBB, 2014).

The focus of the Brazilian meat industry is to produce a product of good quality and consistent supply. Presently, there is lack of studies that evaluate the use of ES technology to improve quality of Nellore cows and crossbred’s carcasses. Therefore, we hypothesized that electrically stimulated carcasses of Nellore and crossbred cull cows would have better meat quality traits and rapid pH declines than non-electrically stimulated carcasses. Thus, this study aimed to evaluate ES and genotype’s effects on carcass pH and temperature decline and meat quality attributes of Nellore and crossbred (Nellore × Holstein) cull cows.

2 Materials and Methods

2.1 Experimental animals.

A total of 10 cull cows (5 Nellore and 5 ¾ Nellore x ¼ Holstein) with an average weight of 500.6 ± 111 kg were obtained from the farm and transported to the slaughterhouse
one day before the slaughter. The animals were kept in lairage for 14 h. During this period, they were provided with ad libitum water and kept without feed. After the rest period, they were sent for slaughter.

2.2 Slaughter and electrical stimulation

The animals were stunned with pneumatic gun and then slaughtered by the jugular and carotid venesection. Following exsanguinations, dressing, and evisceration processes, the carcasses were halved by splitting along the vertebral column, and the right sides of the carcasses was electrically stimulated (ES) with 5 impulses (7 s duration each, with a 2 s interval between pulses), constant voltage (300 V) at variable amps, and 45 Hz (UFX 7, Fluxo Eletronica Industrial). While the corresponding left carcasses were used as controls (no electrical stimulation, NES).

2.3 Carcass pH and temperature measurement

Carcass pH and temperature were measured at 0, 1, 2, 3, 4, 12, and 24 h on all halved carcasses between 12th and 13th rib, using a pH meter (HI99163, Hanna Instruments) for pH and temperature, during this time, the carcasses were refrigerated at 4 °C.

2.4 Sample collection

Longissimus dorsi muscle samples were cut between the 9th and 12th ribs of each side of the of the chilled carcasses at 24 hours post slaughter and individually vacuum packaged, frozen and stored at -20 °C for meat quality analysis.

2.5 Carcass weight loss

Carcass weight loss was determined as

\[
\frac{\text{HotCarcassweight} - \text{Coldcarcassweight}}{\text{HotCarcassweight}} \times 100
\]

2.6 Chemical composition

The meat samples were analyzed according to the standard analytical procedures of the AOAC (1990) for Brazilian National Institute of Science and Technology in Animal Science (INCTCA; DETMANN et al., 2012) for dry matter (dried overnight at 105 °C; method INCT-CA number G003/1) and Fat (method INCT-CA number G-004/1)

2.7 Meat quality evaluation

2.7.1 Meat color measurement
Meat color measurement was performed on three steaks 24 h after thawing at 4 °C. Steaks were exposed to air 30 min prior measurements. Values of L* (lightness), a* (redness), and b* (yellowness) were obtained from five readings performed at different points on the surface of each steak, using a Hunter MiniScan EZ colorimeter (4500L, Hunter Associates Laboratory, Inc., Reston, Virginia, USA).

2.7.2 Thawing, cooking loss, and shear force

Thawing loss were estimated by the weight difference between frozen and thawed steaks. The same steaks previously thawed for meat color measurements were weighted, vacuum packed and cooked in a water bath at 70 °C for 40 minutes. Then, steaks were placed in an ice bath for 5 minutes to stop cooking and kept in refrigerator for 16 h. Thereafter, they were removed from the package and weighed again to obtain water cooking loss. The results of water loss by thawing and cooking were used to estimate the total loss of water of each steak, using the following equation: Total water loss (%) = [(frozen steak weight – cooked steak weight) / frozen steak weight] x 100.

Warner-Bratzler shear force (WBSF) was determined using the cooked steaks after cooling for 16 h at 4 °C (AMSA, 1995). Five cylindrical samples with 1.27 cm in diameter were removed from each steak parallel to the long axis of the muscle fibers, using a stainless-steel device for the extraction of samples (AMSA, 1995). Shear force was determined by perpendicular incision of the muscle fibers of each cylinder of meat by Warner-Bratzler shear device (G-R Electrical Manufacturing Company, Manhattan, KS, USA).

2.7.3 Sarcomere length

Sarcomere length was estimated according to the laser diffraction technique (Cross et al., 1981). Six individual muscle fiber were teased from the muscle bundle and placed on a microscope slide with a drop of 0.2 M sucrose solution (0.2 M glucose and 0.1 M NaHPO4 with pH 7). Sarcomere length was measured by laser diffraction using a 05-LHR-021 laser 7 (Melles Griot, Carlsbad, CA) and calculated by using the following equation: Sarcomere length (µm) = [0.6328 x D x √(T/D)2 + 1]/T; in which: D = distance (mm) from the specimen-holding device to the screen (throughout this experiment, D had a constant value of 131 mm) and T = the separation (mm) between the zero and the first maximum band.

2.8 Statistical Analysis

Data generated were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS, and means were compared using LS-MEANS option.
Statistical differences were considered at P<0.05.

The model utilized was as follow:

\[ Y = U + E_i + G + EG + e \]

Where \( Y \) = Observation on independent variables, \( U \) = Common Mean, \( E \) = effect of electrical stimulation, \( G \) = effect of genotype, \( EG \) = interaction effects of Electrical stimulation and genotype, \( e \) = random error term.

The experiment was conducted in a 2×2 factorial design and carcass were divided into 4 groups, with 5 halves in each group. The first factor was electrical stimulation (ES or NES), and the second one was genotype (Nellore or Crossbred).

3. Results and Discussion

3.1 Carcass pH and temperature

Electrical stimulation and genotype had no significant effect (P > 0.05) on carcass pH decline (Table 1). The final values obtained at 12 and 24-hours post-mortem were within the ideal range (5.5 to 5.8) for beef carcass (FERGUSON and GERRARD, 2014). The result agrees with the findings of Poul (1990) who detected that carcass pH was not affected by ES in bovine. In other species, Kadim et al. (2010) reported that carcass pH decline was not influence (P >0.05) by ES up 24 hours in Dofari goat breed. However, Agbeniga and Webb (2014) and Biraima et al. (2019) noticed influence (P < 0.05) of ES on post-mortem pH decline in beef cattle up to 12 and 24 h, respectively and Ferguson et al. (2008) among ovine muscle.

The absence of genotype effect on pH decline in this study agrees with the reports of Biraima et al. (2019) and Agbeniga and Webb (2014) in beef cattle and that of Cetin et al. (2012) in lamb and goat. In general, the degree of acidification of post-mortem muscle depends on the muscle glycogen concentrations (Pösö & Puolanne, 2005). Postmortem glycolysis accelerated the production of lactic acid by using ES which led to reduce meat pH lower than 6 before the temperature of muscle to arrive 10 °C (Lang et al., 2016). In the current study, there was no effect of ES and genotype on carcass pH which were in the ideal range in all the groups analyzed before temperature reaches cold shortening development (10 °C), indicating that all the groups had high anaerobic glycolytic rate and fast pH decline, which may be related to the absence of ante mortem stress.

The present study indicated that ES and genotype had no significant effect (P>0.05) on the carcass temperature of Nellore and Crossbred cull cows (Table 1). Similarly, Li et al. (2006) realized that the rate of carcass temperature decline was not influenced by ES in...
Chinese Yellow crossbred bulls. Agbeniga and Webb (2014) and Biraima et al., 2019 also reported that carcass temperature decline was not affected by ES in Baggara beef types.

3.2 Carcass, Thawing and cooking losses

Electrical stimulation, genotype and their interaction did not influence (P > 0.05) carcass, thawing, cooking and total losses (Table 2). However, genotype tended to affect (P=0.09) thawing loss. Meat samples from Nellore group lose more water as thawing loss than samples from the crossbred genotype (7.07 vs 5.63 %).

The fact that the carcass weight loss was not influenced by ES is in line with the findings of Bond et al. (2004) and McGeehin et al. (2002) in sheep and that of Biswas et al. (2007) in Bengal goats. However, findings from of Biraima et al. (2019) and Li et al. (2006) reported that ES of beef carcasses resulted an increased carcass weight loss. Carcass pH is one of the factors that affect water holding capacity and carcass loss. Therefore, the absence of influence of ES and genotype on carcass weight loss observed in this study may be attributed to the similar final pH detected.

Thawing, cooking and total losses were not affected by ES and genotype. Bakker, et al. (2021) indicated that weight lost during cooking did not differ between Control and ES treated side of beef carcass. Furthermore, Biraima et al. (2019) observed that ES and genotypes had no effect on cooking loss in Sudanese Baggara beef types. However, earlier observations made on beef cattle by Agbeniga & Webb (2014), Li et al. (2006) and Savell et al. (1978) contradict the findings of this study. Reports from these authors indicated significant differences between ES and NES. Water holding capacity varies mainly with carcass pH (Huff-Lonergan & Lonergan, 2005), majority of moisture in muscle is held within the structure of the muscle and muscle cells (Offer & Cousins, 1992). The pH reduction on post mortem induced the myosin denaturation (Offer, 1991) and an increase in extracellular fluid (Guignot et al., 1993), which increase water loss. The similar losses observed in this study may be related to the similar pH decline among treatments.
### Table 1: Effect of Electrical Stimulation and Genotype on Carcass pH and Temperature of Nellore and Crossbred cull cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment/ Genotypes</th>
<th>P- value</th>
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<tr>
<td></td>
<td>ES</td>
<td>NES</td>
</tr>
<tr>
<td>Initial pH</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>Initial T (ºC)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>1 h pH</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>1 h T (ºC)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>2 h pH</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>2 h T (ºC)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>3 h pH</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>3 h T (ºC)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>4 h pH</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>4 h T (ºC)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>12 h pH</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>12 h T (ºC)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>24 h pH</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>24 h T (ºC)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
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</table>

ES: Electrical stimulation, NES: No electrical stimulation, Gen: Genotype
Gen x ES: Genotype x Electrical stimulation interaction, h: Hours, T: Temperature, SEM: standard error mean

### Table 2: Effect of Electrical Stimulation and Genotype on Carcass, Thawing, Cooking and Total Losses of Nellore and Crossbred cull cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment/ Genotypes</th>
<th>P- value</th>
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<tbody>
<tr>
<td></td>
<td>ES</td>
<td>NES</td>
</tr>
<tr>
<td>Carcass loss (%)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>Thawing loss (%)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
<tr>
<td>Total loss (%)</td>
<td>Nellore</td>
<td>Crossbred</td>
</tr>
</tbody>
</table>

ES: Electrical stimulation, NES: No electrical stimulation, Gen: Genotype
Gen x ES: Genotype x Electrical stimulation interaction, SEM: standard error mean

### 3.3 Chemical Composition and Meat quality traits

Dry matter and fat percentages were not affected (P>0.05) by ES and genotype (Table 3). Vanessa *et al.* (2022) reported similar findings comparing the compositions of Nellore and ½ Nellore x ½ Angus beef cattle. Kerth *et al.* (1999) observed that ES did not affect fat percentage in different breed of sheep. nevertheless, the findings of Silvera *et al.* (2009) contradicts our results. The authors reported higher fat percentage in Nellore than in Charolais
breed. The amount of deposited fat is determined by the balance between dietary energy and metabolic requirements (NRC 1996). In our study, the absence of genetic effect on fat content may be related to the possible similarities of their rates of intake and nutrient requirements due to the high percentage of Nellore composition among crossbred genotypes.

The colour parameters lightness (L*) redness (a*) yellowness (b*) were not influenced (P>0.05) by ES and genotype (Table 3). However, all values recorded were considered satisfactory based on Muchenje et al. (2009) classifications. He describes average lightness (L*) between 33.2 and 41.0, redness (a*) between 11.1 and 23.6, and yellowness (b*) between 6.1 and 11.3. Biraima et al. (2019) found similar results on the influence of ES on b* but noticed contrary in terms of a* and L*. Additionally, report from McKenna et al. (2003) and Bakker et al. (2021) showed no significant influence of ES on lightness (L*) redness (a*) and yellowness (b*) in beef cattle. However, findings of Ehsan et al. (2013) from beef carcasses and Cetin et al. (2012) and King et al. (2004) from sheep and goats contradict the observations of the current study. The authors detected significant influence ES on lightness (L*) redness (a*) and yellowness (b*).

The non-significant effect of genotype realized on instrumental colour in our study concurred with the findings of Biraima et al. (2019) among Sudanese Baggara beef types and that of Teixeira et al. (2022) when compared Nellore, Angus and their crossbred. Results from these investigators showed no colour variability among the breeds investigated. Meat color is related to glycogen content and muscle pH (Jorquera-Chavez et al., 2019; Santos et al., 2021). The non-significant effect observed in this study may be linked to the possible similar glycogen content among the groups and the homogeneous carcass pH decline detected among treatments and genotypes.

Electrical stimulation and genotypes did not affect (P>0.05) Warner-Bratzler Shear Force (Table 3). Kerth et al. (1999) noticed that WBSF was not influenced by ES when compared Hampshire × Rambouillet crossbred lambs with the callipyge phenotype. Similar result was obtained by Cetin et al. (2012). However, report from Agbeniga and Webb (2014) indicated lower WBSF in ES treated carcasses than NES. Several authors (Simmons et al., 2008; Muhammad et al., 2021; Devine et al., 2006; Geesink et al., 2001) observed that ES treated carcasses exhibited lower WBSF when compared to NES.

The non-significant effect of genotypes on WBSF detected in this study for cull cows
agrees with the findings of Vanessa et al. (2022) in bulls, which observed that WBSF of Nellore and ½ Nellore x ½ Angus were not affected by their genetic composition. However, observation made by Teixeira et al. (2022) contradicts our findings. The authors realized beef from Angus bulls had a lower shear force value than Nellore. Additionally, Barcellos et al. (2017) observed lower shear force value among ½ Nellore x ½ Angus bull carcasses than Nellore. The total fat content of muscle has a role in the tenderness of cooked meat although the strength of the correlation varies considerably between studies (Wood et al., 2008). Fat is a significant factor that explain the variance in shear force (Starkey et al., 2016). Intramuscular fat may increase beef tenderness because fat dilutes the effects of tougher myofibrillar elements or reduces rigidity of the muscle structure (Warriss, 2010). The non-significant influence of ES and genotype observed in this study may be attributed to higher composition (¾) of Nellore genotype and similar fat content observed among the cull cows.

Sarcomere length was affected (P < 0.05) by ES x genotype interaction. The highest sarcomere length (2.13 µm) was observed in ES crossbred genotype group, nonetheless NES Nellore genotypes groups did not differ from ES Nellore. (Table 3). Teixeira et al. (2022) reported higher sarcomere length in Angus than Nellore. However, Maciel et al. (2021) observed that the sarcomere was not influenced by genotype of Red Angus and crossbred steers. Sarcomere length also contributes to the variation in beef tenderness. A reduction in the sarcomere length leads to a decrease in tenderness (Starkey et al., 2016).

Table 3: Effect of Electrical Stimulation and Genotype on Chemical Composition and Meat Quality Traits of Nellore and Crossbred Carcasses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment/ Genotypes</th>
<th>ES</th>
<th>NES</th>
<th>SEM</th>
<th>P- value</th>
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<tbody>
<tr>
<td></td>
<td>Nellore</td>
<td>Crossbred</td>
<td>Nellore</td>
<td>Crossbred</td>
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<tr>
<td>Dry matter (%)</td>
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<td>Fat (% of DM)</td>
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<td>Colour L*</td>
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<td>Colour a*</td>
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<td>Colour b*</td>
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<td>WBSF, N</td>
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<td>Sacromere Length (µm)</td>
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Means having different superscripts abcd in the same row are significantly different p<0.005.
ES: Electrical stimulation, NES: No electrical stimulation, Gen: Genotype
Gen x ES: Genotype x Electrical stimulation interaction, SEM: standard error mean, DM: Dry matter,
WBSF: Warner-Bratzler shear force.

Electrical stimulation x genotype interaction showed that muscles from ES crossbred
carcass had higher sarcomere length while ES Nellore had the least. However, all values recorded for sarcomere length were within the normal range (1.3-2.1 μm) reported by (Starkey et al. 2016), which may explain the absence of difference in tenderness, observed in this study.

4 Conclusion

Electrical stimulation, genotype and their interactions in beef cull cows did not influence most of the meat quality traits measured except sarcomere length. It was shown that the crossbred genotype’s carcass responded better to ES in terms of sarcomere length compared with carcass from the Nellore. The ES practice should be adopted to improve the tenderness of crossbred beef carcass.

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Appendix

FIGURES
Fig 1. Graphics of Comparisons for Genotype * Treatment (Electrical stimulation) for initial pH, 1, 2, 3, 4, 12 and final pH
Fig 2. Comparisons for Genotype * Treatment (Electrical stimulation) for initial Temperature, 1, 2, 3, 4, 12 and final Temperature.
Fig 3. Comparisons for Genotype * Treatment (Electrical stimulation) for Carcass, Thawing, Cooking and Total losses.
Fig 4. Comparisons for Genotype * Treatment (Electrical stimulation) for Dry matter, Fat, colour: L* (lightness), a* (redness), and b* (yellowness) and shear force
Fig 5. Comparisons for Genotype * Treatment (Electrical stimulation) and their Interaction for Sarcomere