LUIZ HENRIQUE PEREIRA SILVA

EFFECT OF CASTRATION AND MATURITY ON BODY GLUCOSE SENSITIVITY, CARCASS COMPOSITION, MEAT QUALITY TRAITS AND MUSCLE PROTEOME AND PHOSPHOPROTEOME OF NELLORE MALE CATTLE

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Doctor Scientiae.*

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BIOGRAFIA

Luiz Henrique Pereira Silva, filho de Germina Pereira Silva e Ricardo Pereira Matos, nasceu em 4 de abril de 1989, na cidade de Janaúba, Minas Gerais.

Ingressou-se no curso de graduação em Zootecnia na Universidade Federal de Viçosa no ano de 2007, graduando-se em Zootecnia em janeiro de 2012. Durante a graduação foi bolsista de iniciação científica do CNPq (PIBIC/CNPq) sob orientação do professor Pedro Veiga Rodrigues Paulino.

Em março de 2012 ingressou-se no mestrado em Zootecnia, no programa de pós-graduação em Zootecnia da Universidade Federal de Viçosa também sob orientação do professor Pedro Veiga Rodrigues Paulino. Obteve o título de mestre em Zootecnia no dia 21 de fevereiro de 2014.

Iniciou o doutorado em fisiologia da produção e qualidade da carne em março de 2014 no programa de pós-graduação em Zootecnia da Universidade Federal de Viçosa sob orientação do professor Mario Luiz Chizzotti. Durante o doutorado foi pesquisador visitante na University of New Hampshire sob orientação do professor André Fonseca de Brito. Submeteu tese de doutorado para defesa em 9 de março de 2018.

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ABSTRACT

SILVA, Luiz Henrique Pereira, D.Sc., Universidade Federal de Viçosa, March, 2018. **Effect of castration and maturity on body glucose sensitivity, carcass composition, meat quality traits and muscle proteome and phosphoproteome of Nellore male cattle.** Adviser: Mario Luiz Chizzotti. Co-adviser: Marcio de Souza Duarte

The current work consists of four manuscripts developed using the same animals. The objective of the first study aimed to evaluate the castration effect on carcass and meat traits of Nellore cattle harvested at different body weights (BW). Thirty-six Nellore (*Bos taurus indicus*) calves averaging 256.1 ± 3.05 kg of BW and 8.2 ± 0.07 months old were used, within then half was randomly selected for surgical castration one-week prior weaning. Weaned calves were fed with the same diet, and then six calves from each sex condition were randomly assigned to be harvested when the average BW of both sex conditions reaches 280, 380, and 480 kg. Therefore, this study was carried out as a complete randomized design following a 2 (sex condition) by 3 (weight at harvest) factorial arrangement of treatments. Beef traits were evaluated at 1, 7, and 14 d postmortem. Interaction effect was found ($P < 0.01$) for intramuscular fat and for kidney, pelvic and heart fat. Bull carcass was heavier ($P < 0.05$) than steer. Steer carcass had greater ($P < 0.05$) backfat thickness. Castration reduced shear force and increased myofibrillar fragmentation index at 14 d postmortem. Carcasses from cattle harvested at 480 kg had slower chilling, longer sarcomere, and lower shear force at 1 d postmortem. Shear force change from 1 d to 14 d postmortem reduced ($P < 0.05$) as harvest body weight increased. In conclusion, despite of carcass fatness, castration and body weight at harvest affect carcass and meat traits independently. The second study aimed to evaluate the glucose sensitivity of bulls and steers throughout body development. In addition, it was evaluated gene expression of biomarkers related to glucose metabolism, muscle growth and lipid deposition. Therefore, the same animals from the first study were used, and this study was carried out as a complete randomized design following a 2 (sex condition) by 3 (weight at harvest) factorial arrangement of treatments. Two glucose tolerance tests (GTT) were performed at 380 and 480 kg. Longissimus dorsi (LD) was sampled just after stunning for gene expression. Carcass composition and animal performance were evaluated. Bulls had greater final BW ($P = 0.02$) and tended to increase G:F ratio ($P =$

0.08) compared with steers. Bulls had greater carcass yield of lean (P = 0.02) and protein (P = 0.01) than steers. An interaction effect was found for carcass fat gain (P = 0.01), and steers gained more carcass fat than bulls only from 380 to 480 kg of BW. Carcass protein fractional accretion rate (FAR) decreased as cattle BW increased (P < 0.01). Neither glucose basal level nor area under the curve (AUC) post-infusion were affected by castration or cattle BW (P > 0.05). Expression of genes related to glucose metabolism in the LD were not affected by castration or cattle BW (P > 0.05). Castration tended (P = 0.086) to upregulate LD expression of *Acetyl-CoA carboxylase alpha* (*ACACA*). Interaction effects (P < 0.05) were found for LD expression of *IGF-1 receptor* (*IGF1R*) and *F-box protein 32* (*FBXO32*), and for both genes steers had the greatest mRNA abundance at 380 kg while bulls had the greatest abundance at 480 kg of BW. The LD expression of *serpin A3-6* tended (P = 0.08) to be downregulated as cattle BW increased from 280 to 480 kg. In conclusion, despite of the carcass fatness enhancement by castration and increasing BW, Nellore cattle whole-body sensitivity to glucose does not change. For the third study, the LD sampled at the harvest were used to compare the proteome and phosphoproteome of Nellore bulls and steers during different growth stages. Extracted muscle protein was separated in a 2D-PAGE and stained sequentially with Pro-Q Diamond and Colloidal Coomassie. Afterward, a comparative analysis of protein profile was performed, and differentially abundant protein spots were excised for protein identification by MALDI-TOF/TOF. Castration affected (P < 0.05) abundance of 6 phosphoproteins and 10 protein spots, while body weight affected (P < 0.05) abundance of 34 phosphoproteins and 29 protein spots. Castration decreased (P < 0.05) the abundance of two glycolytic enzymes of the energy-yielding phase, suggesting that glycolysis pathway enhanced glycerol-3P supply for a greater fat deposition on steers. Regarding the growth stage, despite the structural proteins myosin regulatory light chain 2 (MYLPF), and actin alpha 1 (ACTA1), most of identified proteins were related to energy metabolism, including glycogen metabolism, glycolysis, oxidative phosphorylation, creatine-phosphate metabolism, and cytosolic NADH metabolism. These results suggest that decreasing muscle growth rate decreases muscle glycolysis and ATP generation. The fourth study used only the twelve Nellore cattle harvested at 480 kg of BW. The aim of this study was to evaluate the differential proteome and phosphoproteome between bulls

and steers during conversion of muscle to meat, as well as after 14 d of aging. Twelve male Nellore calves were used (247 kg, and 8 months old) and six calves were randomly selected for surgical castration one week before weaning. Post-weaning calves were fed the same diet and were harvested after 230 d on feeding. *Longissimus* muscle was sampled just after stunning (0d postmortem), at deboning (1d postmortem) and after aging (14d postmortem) for proteome analysis. The carcass traits were evaluated at deboning and meat shear force was measured at 1, 7, and 14 d postmortem. Muscle protein extract was separated by 2D-PAGE and stained sequentially for phosphoprotein (Pro-Q Diamond) and for total protein (Colloidal Coomassie). Differentially abundant protein spots between sex condition or across postmortem time were excised for protein identification by MALDI-TOF/TOF. Castration upregulated ($P < 0.05$) the abundance of glycolytic enzymes, while the oxidative phosphorylation protein ATP5B was downregulated ($P < 0.05$). In addition, abundance of troponin T fast isoform (TNNT3) was upregulated by castration ($P < 0.05$), while the slow isoform (TNNT1) tended to decrease ($P < 0.10$) abundance. The creatine kinase M-type was markedly fragmented postmortem. Abundance of phosphorylated PGM1 increased during the first 24 h postmortem and was highly correlated with carcass pH. The abundance of one spot of heat shock cognate 71 kDa protein (HSC70) markedly increased after aging. Further, abundance of the phosphorylated myofibrillar proteins ACTA1 and MYLPF were positively correlated with sarcomere shortening. In conclusion, our finds demonstrated that abundance and phosphorylation of glycolytic enzymes affect meat quality during conversion of muscle to meat. Overall, castration markedly increased carcass fatness and intramuscular fat, whereas whole body glucose sensitivity and conversion of muscle to meat seems to be similar between bulls and steers.

RESUMO

SILVA, Luiz Henrique Pereira, D.Sc., Universidade Federal de Viçosa, março de 2018. **Efeito da castração e maturidade sobre a sensitividade corporal à glicose, composição de carcaça, qualidade da carne e sobre o proteoma e fosfoproteoma muscular de bovinos machos Nelore.** Orientador: Mario Luiz Chizzotti. Coorientador: Marcio de Souza Duarte.

O presente trabalho consiste de quatro manuscritos desenvolvidos usando os mesmos animais. O objetivo do primeiro estudo foi avaliar o efeito de castração sobre as características de carcaça e qualidade da carne de Nelore abatidos em diferentes pesos corporais (PC). Trinta e seis bezerros Nelore (*Bos taurus indicus*) com média inicial de 256,1 ± 3,05 kg de PC e 8,2 ± 0,07 meses de idade foram utilizados, sendo que a metade foi aleatoriamente selecionada para a castração cirúrgica uma semana antes do desmame. Os bezerros desmamados foram confinados recebendo uma mesma dieta, e seis bezerros de cada condição sexual foram distribuídos aleatoriamente para serem abatidos quando a média do PC atingisse 280, 380 e 480 kg. Portanto, este estudo foi realizado seguindo delineamento inteiramente casualizado com tratamentos arranchados em esquema fatorial com 2 condições sexuais (castrado vs. inteiro) e 3 pesos de abate (280, 380, e 480 kg). As características de qualidade da carne foram avaliadas aos 1, 7 e 14 dias postmortem. O efeito de interação foi encontrado ($P < 0,01$) para a gordura intramuscular e para a gordura renal, pélvica e cardíaca. A carcaça de boi inteiro foi mais pesada ($P < 0,05$) do que de castrados. A carcaça do boi inteiro teve maior ($P < 0,05$) espessura de cobertura. Castração reduziu a força de cisalhamento e aumentou o índice de fragmentação miofibrilar aos 14 dias postmortem. As carcaças do abate aos 480 kg apresentaram um resfriamento mais lento, um sarcômero mais longo e uma menor força de cisalhamento quando avaliada a 1 dia postmortem. A redução na força de cisalhamento de 1 para 14 dias post-mortem foi reduzida ($P < 0,05$) à medida que se aumentou o peso corporal ao abate. Conclui-se que, excetuando a gordura da carcaça, a castração e o peso corporal na colheita afetam as características de carcaça e carne independentemente. O segundo estudo teve como objetivo avaliar a sensibilidade à glicose de bovinos Nelore castrados e inteiros ao longo do desenvolvimento corporal. Além disso, foram avaliadas a expressão gênica de biomarcadores relacionados ao metabolismo da glicose, crescimento muscular e

deposição de lipídios. Para tanto, os mesmos animais do primeiro estudo foram utilizados, e este estudo foi realizado seguindo delineamento inteiramente casualizado com tratamentos arranchados em esquema fatorial com 2 condições sexuais (castrado vs. inteiro) e 3 pesos de abate (280, 380, e 480 kg). Dois testes de tolerância à glicose (TTG) foram realizados aos 380 e 480 kg de PC. Longissimus dorsi (LD) foi amostrado logo após a sangria para a expressão gênica. A composição da carcaça e o desempenho animal foram avaliados. Animais não castrados tiveram maior PC final (P = 0,02) e tendência para aumentar a eficiência alimentar (P = 0,08) em relação aos castrados. Os bovinos inteiros tiveram maior rendimento de carne magra (P = 0,02) e proteína (P = 0,01) do que os castrados. Efeito de interação foi encontrado para o ganho de gordura de carcaça (P = 0,01), e os castrados ganharam mais gordura de carcaça do que os inteiros apenas de 380 a 480 kg de PC. A taxa fracional de acumulação de proteína da carcaça (FAR) diminuiu à medida que os bovinos aumentaram o PC (P <0,01). Nem o nível basal da glicose nem a área sob a curva (AUC) pós-infusão foram afetados pela castração ou PC dos bovinos (P > 0,05). A expressão de genes relacionados ao metabolismo da glicose no LD não foi afetada pela castração ou PC dos bovinos (P> 0,05). Foi observada uma tendência de aumento na expressão de LD de acetil-CoA carboxilase alfa (ACACA) pela castração (P = 0,086). Efeitos de interação (P <0,05) foram encontrados para a expressão do receptor de IGF-1 (IGF1R) e da proteína F-box 32 (FBXO32) no LD, e para ambos os genes, os novilhos apresentaram a maior abundância de mRNA aos 380 kg de PC, enquanto os inteiros tiveram a maior abundância aos 480 kg de PC. A expressão de serpin A3-6 no LD tendeu (P = 0,08) a reduzir com aumento do PC de 280 para 480 kg. Em conclusão, apesar do aumento na gordura da carcaça pela castração e aumento do PC, a sensibilidade corporal de bovinos Nelore à glicose não muda. Para o terceiro estudo, as amostras de LD coletadas no momento do abate foram utilizadas para comparar o proteoma e o fosfoproteoma de bovinos Nelore castrados ou não durante os diferentes estádios de crescimento. A proteína muscular extraída foi separada em 2D-PAGE e corada sequencialmente com Pro-Q Diamond e Coomassie coloidal. Posteriormente, realizou-se uma análise comparativa do perfil protéico, e os spots diferencialmente abundantes foram excisados para identificação de proteínas por MALDI-TOF/TOF. A castração afetou (P <0,05) a abundância de 6 fosfoproteínas e 10 spots de proteína total, enquanto o peso

corporal afetou (P <0,05) abundância de 34 fosfoproteínas e 29 spots de proteína total. A castração diminuiu (P <0,05) a abundância de duas enzimas glicolíticas da fase de produção de energia, sugerindo que o aumentou na via da glicólise promove síntese de glicerol-3P para dar suporte a uma maior deposição de gordura em novilhos. Em relação ao estágio de crescimento, além das proteínas estruturais da cadeia leve reguladora de miosina 2 (MYLPF) e da actina alfa 1 (ACTA1), a maioria das proteínas identificadas estão relacionada ao metabolismo energético, incluindo o metabolismo do glicogênio, glicólise, fosforilação oxidativa, metabolismo da creatina-fosfato, e metabolismo citosólico de NADH. Esses resultados sugerem que a diminuição da taxa de crescimento muscular reduz a glicólise e a geração de ATP no músculo. No quarto estudo utilizou-se apenas os doze bovinos Nelore abatidos aos 480 kg de PC. O objetivo deste estudo foi avaliar as diferenças no proteoma e fosfoproteoma entre bovinos Nelore castrados e inteiros durante a conversão do músculo em carne, bem como após 14 dias de maturação. Foram utilizados 12 bezerros machos Nelore (247 kg e 8 meses) e seis bezerros foram selecionados aleatoriamente para a castração cirúrgica uma semana antes do desmame. Os bezerros desmamados foram alimentados com a mesma dieta e foram abatidos após 230 dias de confinamento. O músculo Longissimus foi amostrado logo após a sangria (0d pós-morte), na desossa (1d pós-morte) e após a maturação (14d pós-morte) para análise de proteoma. As características de carcaça foram avaliadas na desossa e a força de cisalhamento da carne foi medida ao 1, 7 e 14 dias postmortem. O extrato de proteína muscular foi separado por 2D-PAGE e corado sequencialmente para fosfoproteína (Pro-Q Diamond) e para proteína total (Coomassie coloidal). Os spots diferencialmente abundantes entre a condição sexual ou entre os tempo pós-morte foram excisados para identificação de proteínas por MALDI-TOF/TOF. A castração aumentou (P <0,05) a abundância de enzimas glicolíticas, enquanto que a proteína da fosforilação oxidativa ATP5B foi reduzida (P <0,05). Além disso, a abundância de troposina T isoforma rápida (TNNT3) foi aumentada pela castração (P <0,05), enquanto a isoforma lenta (TNNT1) tendeu a diminuir (P <0,10). A creatina quinase tipo-M foi marcadamente fragmentada no postmortem. A abundância de PGM1 fosforilada aumentou durante as primeiras 24 horas pós-morte e foi altamente correlacionada com o pH da carcaça. A abundância de uma proteína de choque termico 71 kDa (HSC70) aumentou marcadamente após a maturação.

Além disso, a abundância das proteínas miofibrilares fosforiladas ACTA1 e MYLPF foram positivamente correlacionadas com o encurtamento do sarcômero. Em conclusão, nossos dados demonstraram que a abundância e a fosforilação das enzimas glicolíticas afetam a qualidade da carne durante a conversão do músculo em carne. No geral, a castração aumentou acentuadamente a gordura da carcaça e a gordura intramuscular, enquanto que a sensibilidade corporal à glicose e a conversão do músculo na carne parecem ser semelhantes entre bovinos castrados e não castrados.

INTRODUCTION

Brazilian beef production has been growing, and the Brazilian cattle herd is approximately 209 million head, using around 20% of a total of 174 million ha of land available for agriculture and livestock production (Lobato et al., 2014). Interestingly, the beef production and the cattle herd have been increasing in Brazil, but the area available for raising cattle have been decreased (Martha et al., 2012). These data demonstrate that there is a continuous improvement in efficiency of beef production in Brazil. In addition, although the whole herd size has been increasing, the dynamic across the country shown that some traditional beef production region such as southeast have shown a reduction in the growth rate while the north and Midwest regions are accelerating the herd growth.

Although statistical data about slaughter cattle is scarce, greater proportion of slaughtered cattle in Brazil are intact male, once the usage of exogenous anabolic hormones for cattle is banned in Brazil, and the Brazilian carcass grading system does not evaluate marbling. In a survey about management practices adopted in the Brazilian feedlots from 2014, the authors found that around 84.5% of penned cattle were bull, and the nutritionists recommended the use of this sex conditions due to the better feed efficiency of bull comparing with steers (Oliveira and Millen, 2014). Thus, even though there are some beef packing industries paying more for steers, usually is more profitable to produce intact bull than steers.

Castration is a widely used practice on many beef production countries, mainly due to the marbling enhancement followed by castration, improving quality grading and then carcass price (Bretschneider, 2005; Jeong et al., 2013). Besides the carcass quality, castration benefits also include the easier cattle handling, avoid undesired breeding, and reduce mounting behavior (Coetzee et al., 2010). However, regarding the anabolic effect of testosterone the use of intact bulls might increase the profitability in market where the use of exogenous anabolic hormones is forbidding, and carcass premium price is not established by marbling level (Molleta et al., 2014; Moran et al., 2017). The immunocastration has recently been studied in tropical conditions and seems to be a good option to improve carcass fatness and meat quality with minor effect on performance

(Miguel et al., 2014). In addition, a growing animal welfare concern by consumers can lead to increasing in immunocastration.

Despite of carcass fatness and marbling, sex condition can also affect meat tenderness. Previous studies suggest that the great muscle hypertrophy of bulls is due decreased muscle protein degradation by increasing calpastatin activity (Morgan et al., 1993a, 1993b), resulting in decreased postmortem proteolysis (Huff-Lonergan et al., 1995). In addition, less the less tender meat produced from bull carcasses might be an effect of cold-shortening due to lower backfat thickness of bull carcass (Seideman et al., 1982). Furthermore, bulls and steers achieve the fatness to slaughter point in different weights and ages. As demonstrated by some studies animal maturity can also impact on meat tenderness not just by increasing collagen crosslinks (Duarte et al., 2011) but also increasing calpastatin activity and consequently decreasing meat tenderness (Cruzen et al., 2014). Furthermore, some studies suggested that an interaction of sex condition and maturity can impact meat quality (Hedrick et al., 1969).

Nonetheless, the magnitude of the difference between bulls and steers might vary with the age at castration and the stage of development at the evaluation (Marti et al., 2013). Furthermore, reaching the weight at maturity cattle body composition changes decreasing the lean and bone percentage whereas fat increases (Marcondes et al., 2016). It has been reported that castration decrease the weight at maturity (Valadares Filho et al., 2016), explaining the earlier fat deposition of steers compared with bulls.

Changes on body composition might affect cattle metabolism. For instance, increasing body fat has been related to decreasing insulin responsiveness in sheep (Bergman et al., 1989), while well-muscled cattle have greater glucose clearance after intravenous exogenous glucose dose (McGilchrist et al., 2011). In addition, NEFA blood level is negatively related to body glucose uptake (Burdick Sanchez et al., 2016). Decreased glucose uptake by peripheral tissue might impair muscle and adipocyte growth rate. The glucose tolerance test (GTT) have been applied recently in the animal science field to evaluate the whole-body sensitivity to a high exogenous glucose dose (González-Grajales et al., 2017). Nonetheless, muscle is the main tissue that uptake this high amount of exogenous glucose, once this tissue is approximately 36% of cattle body weight (Marcondes et al., 2012), and it has been cited that muscle glucose uptake accounts for

around 80% of the exogenous dose (McGilchrist et al., 2011; Xia et al., 2013). The muscle glucose sensitiveness is mainly controlled by amount of glucose transporter sensitive to insulin (GLUT4) at the sarcolemma, also present on adipose tissue membrane (Fitzsimons et al., 2014).

Proteomics is an "omic" tool from the post-genomic era (Bendixen, 2005). In the Animal Science field, the proteomic have been used to investigate the global proteins expression changes by muscle hypertrophy (Bouley et al., 2005), feed efficiency (Vincent et al., 2015), production system (Marco-Ramell et al., 2012), different breeds (Rodrigues et al., 2017), different genotypes (Rosa et al., 2018), and meat quality of the different species (Lana and Zolla, 2016). In the meat science field, proteomic studies have allowed to discover some potential biomarkers to increase animal efficiency and meat quality (Lana and Zolla, 2016; Picard et al., 2017, 2010) as well as to understand better the conversion of muscle to meat (Longo et al., 2015), and water holder capacity (Di Luca et al., 2013).

Therefore, the objective of this study was to evaluate the effect of castration and cattle body weight on animal performance, carcass traits and meat quality. In addition, gene expression and proteomic analysis were used to explain the differences between bull and steers. Further, a differential muscle proteome and phosphoproteome analysis was performed to study the proteome change during conversion of muscle to meat and after an aging period.

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Chapter I: Carcass and meat quality traits of Nellore bulls and steers harvested at different body weights

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ABSTRACT

This study aimed to evaluate the effect of castration on carcass and meat traits of Nellore cattle harvested at different body weights (280, 380, and 480 kg). Beef traits were evaluated at 1, 7, and 14 d postmortem. Data were analyzed as 2 x 3 factorial design with two sex conditions and three harvest weights. Interaction effect was found ($P < 0.01$) for intramuscular fat and for kidney, pelvic and heart fat. The carcass of bulls was heavier ($P < 0.05$) than steer. Steer carcass had greater ($P < 0.05$) backfat thickness. Castration reduced shear force and increased myofibrillar fragmentation index at 14 d postmortem. Carcasses from cattle harvested at 480 kg had slower chilling, longer sarcomere, and lower shear force at 1 d postmortem. Shear force change from 1 d to 14 d postmortem reduced ($P < 0.05$) as harvest body weight increased. In conclusion, despite of carcass fatness, castration and body weight affect carcass and meat traits independently.

Keywords: maturity weight; castration; fat color; cold-shortening; sarcomere length; carcass pH

1. Introduction

In vivo muscle protein turnover affects animal performance and postmortem tenderization (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002; Kristensen et al., 2002). The greater muscle hypertrophy of bulls in relation to steers may be supported by increased in vivo calpastatin activity which decreases muscle protein breakdown, enhancing fractional accretion rate and then, animal performance (Morgan, Wheeler, Koohmaraie, Crouse, & Savell, 1993). Despite the enhancement in animal gain by decreased protein breakdown, there is a possible negative impact on postmortem muscle proteolysis and beef tenderness (Huff-Lonergan, Parrish, & Robson, 1995). Additionally, growth stage can also influence muscular protein turnover and postmortem tenderization, and increased calpastatin activity have been reported in mature cows in comparison with growing calves (Cruzen, Paulino, Lonergan, & Huff-Lonergan, 2014). Therefore, body weight and castration can affect independently in vivo muscle protein metabolism and may impact on postmortem tenderization of aged meat.

The weight at maturity has been defined as the cattle empty body weight when the amount of retained body protein stabilize, then protein accretion rate is close to zero (Marcondes, Tedeschi, Valadares Filho, Silva, & Silva, 2016). Maturity body weight has been used by different nutritional systems to predict composition of body gain, and it is affected by breed and sex, in which castration decreases the weight at maturity (NRC, 2016; Owens, Dubeski, & Hanson, 1993; Valadares Filho et al., 2016). Thus, the dynamics of muscle protein turnover may differ between bulls and steers.

Nonetheless, as cattle reach the maturity weight, animal performance decreases and fat deposition increase (Costa e Silva et al., 2013), enhancing carcass fat content (Valadares Filho et al., 2016). Increasing subcutaneous fat thickness reduces carcass shrinkage and chilling rate preventing cold shortening (Duarte et al., 2011; Prado & de Felício, 2010) and enhances intramuscular fat, since there is a high correlation between these two fat depots. Marbling is an important meat trait affecting meat appearance, juiciness, and meat price and can be influenced by both body weight at harvest (Sharman et al., 2013), and castration (Moran et al., 2017).

Meat and fat color are considered the most important meat traits which influence consumer acceptability at moment of the purchase (Mancini & Hunt, 2005). Meat color could be affected by animal age and carcass pH, among other factors (Ponnampalam et al., 2017). The greater reactivity of bulls in comparison to steers can be related to the higher dark-cutting incidence on bulls' carcasses, since the pre-slaughter stress reduces muscle glycogen content required to postmortem muscle acidification (Seideman, Cross, Oltjen, & Schanbacher, 1982). Regarding fat color, even though it is also evaluated by consumer at purchasing, there are only few studies in which fat color of bulls and steers are compared.

Therefore, the objective of this study was to assess carcass traits, and beef quality of young bulls and steers harvested at three different body weights. We hypothesized that carcass and beef traits of bulls and steers differ as Nellore cattle achieve the weight at maturity.

2. Materials and Methods

The Animal Care and Ethics Committee for Use of Production Animals of the Universidade Federal de Viçosa (CEUAP-UFV), reviewed and approved all animal handling and castration surgery procedure (protocol 035/2015), following the international requirements underlined by the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010) and EU Directive 2010/63/EU (European Union, 2010).

2.1. Animals, castration surgery and housing conditions

Thirty-six Nellore male calves of 8.2 ± 0.07 months old and 256.1 ± 3.05 kg of body weight (BW) were used. Half of calves were randomly selected to be surgically castrated one week before weaning. The castration surgery was performed holding the cattle in a squeeze-chute. The castration site was cleaned with neutral soap and 2% iodine solution prior surgery. Local anaesthesia (8 ml of 2% lidocaine) was injected into each testicle and allowed to diffuse into the spermatic cord, 15 min before the surgery. One new scalpel blade was used per animal, and after the surgery silver sulfadiazine and zinc oxide were placed on surgery site. Oxytetracycline hydrochloride was intramuscularly injected in a dose of 10 mg/kg body weight. During 3 weeks after the castration the steers were checked daily, and silver sulfadiazine and zinc oxide were used until complete healing.

After weaning, the calves were ear tagged, dewormed and housed as two groups, according to sex condition (bulls or steers). Six calves from each sex condition were randomly assigned to be harvest at one of three endpoints throughout finishing phase (280, 380 or 480 kg of BW). The shrunk body weight of 480 kg represents the Nellore cattle weight at maturity (Costa e Silva et al., 2013). Therefore, the body weights of 380 and 280 kg represents about 80% and 60% of the Nellore weight at maturity, respectively. The experiment was carried out as a 2 x 3 factorial arrangement of treatments with 2 sex condition (bulls and steers) and 3 weights at harvest (280, 380 and 480 kg of BW). Each feedlot pen was equipped with three electronic feed bunks system (AF 1000 Master, Intergado LTDA, Contagem, MG, Brazil) and one water drinker/body scale (WD 1000, Intergado LDTA, Contagem, MG, Brazil) allowing to record individually the daily intake of feed, water as well as record the body weight.

Throughout the trial, both groups (bulls and steers) were kept under the same experimental conditions, receiving the same diet (Table 1). Fresh feed was provided *ad libitum* twice a day, about 60% at 7:00 h and the remain at 14:00 h. The diet was formulated according to the average nutrient requirements between bulls and steers performing 1.4 kg/day, according to BR-CORTE system (Valadares Filho, Marcondes, Chizzotti, & Paulino, 2010).

2.2. Slaughter and carcass traits

Six bulls and six steers were harvested when the average body weight achieved the endpoints of 280, 380 and 480 kg, totalizing three slaughters of 12 cattle each. Once achieved the target body weight, the selected group was slaughtered at an experimental packing plant after 16 h of fasting with free access to water. The procedures during the harvest followed humane slaughter practices and the Brazilian standards of sanitary regulation for animal products (Brasil, 1997).

The kidney, pelvic and heart fat (KPH) was trimmed at dressing. KPH fat was weighed without chilling and expressed as percentage of hot carcass. After dressing, carcasses were split, weighed and suspended by the aitch bone (tenderstretch method). Throughout chilling at 2 °C, the carcass pH and temperature were recorded at 0.5, 2, 4, 6, 8, 10, 12, 14, 18, 22, and 24 h postmortem using a previous calibrated portable pH meter with temperature compensation (Inlab® Solids PRO, Mettler-Toledo AG, Schwerzenbach, Switzerland). The pH and temperature of each carcass were measured in the *longissimus thoracis* muscle at 13th rib of the left carcass side about one-inch depth.

The cold right carcass side was used to measure carcass length. Afterward, the carcass was ribbed between the 12th and 13th rib to evaluate back fat thickness (BFT) and rib eye area (REA). A digital caliper ruler was used to measure the BFT in millimeters. The *longissimus* muscle perimeter was delimited on a transparency film and REA was measured using ImageJ software (ImageJ 1.48g, Bethesda, Maryland, USA).

2.3. Meat sampling, aging and evaluation

Longissimus muscle samples were obtained as a section from 6th to 9th rib of each right carcass side after 24 h of chilling. Subcutaneous and intermuscular fat were removed from

the *longissimus* section, and then the meat sample was divided into three portions (cranial, medial and caudal). The cranial portion was used to evaluate fresh meat color (as described below), labeled as unaged beef (1-day postmortem), vacuum packed and then frozen at -20 °C. Medial and caudal portions were vacuum packed and kept at 4 °C until 7 or 14 days postmortem (PM), respectively. Once aged, the portions were kept frozen at -20 °C until analyzes. Color of the subcutaneous fat covering the *longissimus* muscle was analyzed at deboning (1-day postmortem).

2.3.1. Instrumental color measurement

Meat and fat color parameter were obtained from an average of five reading across the surface previously exposed to air for 30 min. Immediately prior to data collection, a Hunter MiniScan EZ (4500L, Hunter Associates Laboratory, Inc., Reston, Virginia, USA) was calibrated and used to scan meat and fat color. The spectrophotometer was set to read using illuminant D65, port size of 31.8 mm and 10° standard observer. Values of lightness (L^*), redness (a^*) and yellowness (b^*) were obtained according to CIE $L^*a^*b^*$ color system (Mancini & Hunt, 2005).

2.3.2. Warner-Bratzler shear force

One-inch steaks were taken from frozen *longissimus* portions (1, 7 and 14 d PM) using a butcher band saw. Steaks were weighed, packed and thawed at 4 ºC for 16 h. Thawed steak weight was recorded allowing to calculate thaw loss as percentage of frozen sample. Unfrozen steaks were vacuum packed and cooked in a preheated water bath at 70 ºC for 40 min (Bruce, Stark, & Beilken, 2004). After cooking, steaks were cooled in running water (about 20° C) for 30 min, then stored overnight at 4 ºC. Cooked steaks were weighed for cooking loss assessment as percentage of thawed sample. Total loss was calculated as the weight difference between cooked and frozen steak. Six cores (1.27 cm diameter) were removed parallel to the longitudinal orientation of the muscle fibers from each cooked steak after cooling. Cores was sheared once, perpendicular to the longitudinal orientation of the muscle fibers by a Warner-Bratzler shear machine (G-R Electrical Manufacturing Company, Manhattan, KS, USA) coupled with a 1.016 mm tick V-shaped blade at a constant speed of 2 mm/s. Sample's WBSF was recorded as average of the maximum force (N) obtained from the six cores.

2.3.3. Intramuscular fat content

Steaks of approximately 100 g were obtained from unaged *longissimus* samples. The steak edges were trimmed avoiding any subcutaneous and intermuscular fat, minced and freeze-dried. Later, dried samples were ground using a stainless ball mill. Fat content was analyzed in duplicate by extraction with petroleum ether using Ankom XT4 filter bag and the fat extractor Ankom XT15 (ANKOM Technology, Macedon, NY, USA), following the manufacturer recommendations. Intramuscular fat (IMF) is expressed as percentage of fresh meat.

2.3.4. Myofibrillar fragmentation index

Myofibrillar fragmentation index (MFI) was evaluated by measuring the turbidity of homogenized samples at standardized protein concentration (Olson, Parrish, & Stromer, 1976). Some modifications were applied for better results including the use of shaft type homogenizer (Hopkins, Martin, & Gilmour, 2004), for more details see Silva et al. (2017). Briefly, in duplicate myofibrils were extracted from the frozen *longissimus* samples of each aging time. Afterwards, protein concentration was determined by the biuret method (Gornall, Bardawill, & David, 1949). Aliquots were then diluted in ice-cold buffer to achieve 0.5 mg of protein per milliliter of solution. A final reading was performed at 540 nm and absorbance was multiplied by 150 obtaining the MFI value.

2.3.5. Sarcomere length

The sheared cores used for WBSF at 1 d postmortem were kept for sarcomere length measurement (Wheeler, Shackelford, & Koohmaraie, 2002). Six fibers bundles were taken from each core using a fine tip tweezer and then placed on a microscope slide with a drop of fresh buffer solution contain 0.2 M Sucrose in 0.1 M $NaHPO_4$ buffered at pH 7.2. Sarcomere length was measured using a helium neon laser diffraction unit (05-LHR-021, Melles Griot, Carlsbad, CA, USA) and calculated as described by Cross, West, & Dutson (1981).

2.4. Statistical analysis

To predict carcass temperature at pH 6.0 (temp@pH6) and pH at temperature 18 °C (pH@18), exponential models were individually fitted for temperature versus postmortem

pH using NLIN procedure at SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA).

All data were analyzed as a completely randomized design following a 2 (bull and steer) x 3 (280, 380 and 480 kg at harvest) factorial arrangement of treatments. Analysis of variance (ANOVA) was performed to evaluate the effect of main factors and interaction on carcass and meat traits, using the GLM procedure of SAS. Initial body weight was included as covariate in the model, and then kept where $P < 0.10$. Once detected significant ($P < 0.05$) effect for weight at harvest or interaction, treatments least squared means were compared by Tukey's test.

3. Results

Bulls and steers were harvest with similar age at each weight endpoint. The average shrunk body weights at each harvest were 276.4, 389.3, and 488.6 kg (Table 2), which were close to target weights (280, 380 and 480). Sex condition by weight at harvest interaction effect was found only for IMF and KPH (Table 2, Figure 1, and Figure 2). Hence, the data is shown and discussed separately by sex condition and weight at harvest.

3.1. Sex condition

Bulls had greater ($P < 0.05$) body weight and cold carcass weight than steers (Table 2). Carcass length and shrinkage were similar ($P > 0.05$) between bulls and steers. Likewise, rib eye area (REA) and carcass dressing percentage did not differ ($P > 0.05$) between sex conditions. Steer carcasses had greater ($P < 0.05$) back fat thickness than bull carcasses. Interaction effect for intramuscular fat and KPH fat were found ($P < 0.01$), showing that steer carcasses had greater internal and intramuscular fat than bull carcasses only when harvested at 480 kg of body weight (Figure 1, and Figure 2). Ultimate pH and temperature (24 h postmortem) were similar ($P > 0.05$) for bulls and steers carcass. Likewise, the carcass pH at temperature 18 °C did not differ ($P > 0.05$) between bulls and steers, averaging 6.30 (Table 2). Bulls and steers carcasses achieved the pH 6.0 at the same temperature ($P > 0.05$). In addition, monitoring pH and temperature throughout chilling, there was no difference between the carcasses of bulls and steers, regardless of the evaluating time postmortem (Figure 3).

Final sarcomere length was not different between bulls and steers ($P = 0.743$), averaging 1.66 µm (Table 3). Although the WBSF and MFI of unaged meat did not differ ($P > 0.05$) between the sex conditions, steers steaks aged for 14 d PM had greater MFI and lower WBSF than bulls ($P < 0.05$). Sex condition did not affect ($P > 0.05$) the exudate loss during thawing and cooking at 1 and 14 d PM meat. However, there was a trend ($P < 0.10$) to increase thawing loss and decrease cooking loss for steers steaks at 7 d PM, nonetheless total loss at 7 d PM was not affected ($P = 0.567$) by sex condition.

Beef from bulls and steers had similar ($P > 0.05$) color characteristics (Table 4). Likewise, sex condition did not affect fat color ($P > 0.05$).

3.2. Weight at harvest

Increasing body weight at harvest improved ($P < 0.01$) hot and cold carcass weight as well carcass length (Table 2). However, carcass dressing percentage was not affected ($P = 0.385$) by weight at harvest. As weight at harvest increased, carcass shrinkage during chilling decreased ($P < 0.01$). Rib eye area and BFT improved ($P < 0.01$) as body weight at harvest increased.

Although ultimate pH was not affected by weight at harvest ($P = 0.566$), the carcass pH evaluated at 0.5, 8, 12 and 14 h postmortem were higher for harvest at 280 kg of BW (Figure 4). Furthermore, the carcass pH at temperature 18 °C was higher for cattle harvested at 280 kg of BW. Carcass temperature at 24 h postmortem was higher ($P < 0.01$) for cattle harvested at 480 kg of BW. Likewise, carcass from harvest at 480 kg of BW had higher temperature for all evaluated times throughout chilling (Figure 4). Moreover, carcass temperature at pH 6.0 was lower ($P < 0.01$) for carcass from cattle harvested at 280 kg of BW (Table 2). These results demonstrate that lighter carcasses from cattle harvested at 280 kg of BW had higher chilling rate but lower acidification rate (slow pH dropping).

Muscle from cattle slaughtered at 480 kg BW had higher ($P < 0.01$) sarcomere length at 24 h postmortem (Table 3). The WBSF 1 d PM was lower for *longissimus* samples from harvest at 480 kg of BW ($P = 0.02$). However, WBSF of aged meat for 7 or 14 d postmortem were not affected by weights at harvest ($P > 0.05$). Although the myofibrillar

fragmentation index (MFI) at 1 and 7 d PM was not different among harvest weights, the MFI 14 d PM tended ($P < 0.10$) to be higher for cattle harvested at 280 kg of BW. Increasing body weight at harvest decreased ($P = 0.035$) WBSF change from 1 to 14 d PM. The extent of MFI change from 1 to 14 d PM was similar among weights at harvest (Table 3). Meat exudate losses during thawing and cooking were affected by the weight at harvest ($P < 0.05$). Steaks from cattle of 280 kg at harvest had greater exudate losses, regardless of aging time (1, 7, and 14 d PM) or process (i.e., thawing or cooking). Consequently, the total loss which compute losses from frozen to cooked steak was higher at 280 and lower at 480 kg of body weight, for all evaluated aging period.

Fresh meat color (1 d PM) was affected by weight at harvest ($P < 0.01$), and *longissimus* samples from 280 kg of BW had lower lightness (L^*), redness (a^*), and yellowness (b^*) than other weights (Table 4). Subcutaneous fat color parameter at deboning (1 d PM) did not differ ($P > 0.05$) among weights at harvest (Table 4).

4. Discussion

Slaughter weight and castration affect carcass fatness and then meat yield and quality. Intact cattle usually produce heavier and leaner carcasses than castrated, while increasing harvest weight improves carcass fatness. Cattle muscle growth rate enhances from birth to puberty and then decreases until the maturity weight is reached (Owens, Dubeski, & Hanson, 1993). The weight at maturity is reduced when castration is applied (Valadares Filho et al., 2016), and steers achieve the maximum body protein mass at a lighter weight than bulls. Regarding the distinct body protein and fat deposition of bulls and steers, it is expected that the difference on carcass and meat traits between these sex conditions vary as body weight increases. However, only few interactions between sex condition and weight at harvest were found (i.e., intramuscular and KPH fat).

4.1. Sex condition

When fed under the same conditions, bulls usually have greater average daily gain than steers, producing heavier carcasses at harvest (Seideman, Cross, Oltjen, & Schanbacher, 1982). At the current study, bulls' carcasses were 6% heavier than steers. Although the REA was similar between the sex conditions, steers carcasses had greater BFT. These results suggest that when castrated at weaning and then fed intensively (calf-fed system),

Nellore steers have increased fat deposition but minor effect on muscular growth, since the puberty was achieved close to harvesting.

Increasing harvest weight improves carcass fat deposition, since adipose tissue has late maturity (Marcondes, Tedeschi, Valadares Filho, Silva, & Silva, 2016; Owens et al., 1993). In addition, fat deposition rate is different between bulls and steers of same BW (Valadares Filho et al., 2016). It has been shown that castration improves IMF by increasing lipid uptake and lipogenesis, and by decreasing lipolysis (Bong et al., 2012). We found similar KPH and intramuscular fat deposition for bulls and steers from 280 to 380 kg of BW, however from 380 to 480 kg, steers had greater fat deposition than bulls. These interaction effects confirm that close to puberty, the gain composition of bulls and steers are markedly different.

The frequency of carcass classified as dark-cutting or DFD meat is higher for bulls than steers (Seideman et al., 1982). This undesirable meat impacts negatively the beef industry profit, and carcasses with pH_u greater than 5.8 have their price reduced (Ponnampalam et al., 2017). It has been reported that bulls are more susceptible to pre-slaughter stress, having higher pH_u than steers (Moreira et al., 2017). Nonetheless, at the current study, carcass pH was not affected by sex condition, regardless the postmortem time evaluated and the average pH_u was of 5.78 preventing dark-cutting beef. In addition, fresh meat color *L** value and exudate losses obtained at the present study were not affected by sex condition, corroborating with the absence of difference on pH_u, since color and water hold capacity are affected by pH_u (Ponnampalam et al., 2017). It is important to highlight that calves used in the current study were intensively raised from birth to slaughter (creep-fed pre-weaning, and penned post-weaning) with daily humane management respecting animal welfare. It has been shown that positive humane contact (e.g., daily concentrate delivery in a grazing system) on the farm affect the incidence of dark-cut meat (Mounier, Dubroeucq, Andanson, & Veissier, 2006). Hence, as cattle were slaughtered in an experimental packing plant close to their housing barns, minimal pre-slaughter stress was expected and therefore our data suggest that when carefully managed the carcass pHu of bulls might be similar that of steers, in agreement with other study with similar results (Moran et al., 2017).

Although it has been shown that BFT could affect chilling rate (May, Dolezal, Gill, Ray, & Buchanan, 1992), the fatness difference between bulls and steers did not affect the carcass chilling rate. In addition, the carcass pH at 18 °C (pH@18) and carcass temperature at pH 6.0 (Temp@6.0) depicts a similar rate of pH and temperature decline between bulls and steers. Both sex conditions had Temp@6.0 higher than 12 °C minimizing cold shortening (Thompson, 2002), which can be verified by the sarcomere length greater than 1.5 µm (Bruce et al., 2004).

Tenderness is considered one of the most important meat trait, but it is also highly variable (Hopkins et al., 2011; Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002). Regarding sex condition, studies have been shown that the greater muscle hypertrophy of bulls is supported by increased calpastatin activity which decreases muscle protein breakdown (Morgan, Wheeler, Koohmaraie, Crouse, & Savell, 1993). Despite the enhancing of fractional accretion rate, and then animal performance, reduced protein breakdown can decrease postmortem proteolysis affecting meat tenderness (Huff-Lonergan et al., 1995). At the current study, although sex condition did not affect WBSF nor MFI of unaged meat, as aging time increased the difference between bulls and steers increased, suggesting that steers had greater postmortem proteolysis.

Consumers tended to reject meat with dark color, assuming undesirable alterations regarding animal health and storage (Suman & Joseph, 2013). Castration did not affect fresh meat color, which corroborates the similar carcass pH pattern between bulls and steers. Recently, Moran et al. (2017), found that the subcutaneous fat of steers is more yellow than that of bulls. In another study, early castrated cattle had greater subcutaneous fat thickness and yellowness in comparison with late castrated (Gazzola, Jeffery, White, Hill, & Reid, 2001). However, at current study fat color was not affected by sex condition.

4.2. Weight at harvest

Increasing the harvest weight improved carcass weight, and length, as well as REA and BFT, decreasing carcass shrinkage. The carcass shrinkage represents an economical issue for the meat packing industry, especially when carcass acquisition is based on hot weight. The percentage of weight lost during chilling usually varies from 0.75 to 2% of the hot carcass weight (Savell, Mueller, & Baird, 2005). However, aspects related to cooling

condition as air flow and humidity can affect the weight loss throughout chilling (Prado & de Felício, 2010). Carcass traits can also affect the shrinkage during chilling, with heavier and fatter carcass having lower chilling loss. At the current study, the carcass shrinkage decreased as weight at harvest increased, probably due to greater fat covering and smaller specific area presented by heavier carcasses (Savell, Mueller, & Baird, 2005).

Although pH_u is a good measurement to identify dark-cutting meat, a pH_u below than 5.8 does not guarantee that meat quality was not impacted during conversion of muscle into meat throughout the early postmortem stage. The weight at harvest did not affect the pH_u but greater chilling rate and reduced pH decline were found for carcass of cattle harvest at 280 kg. Reduced pH declines due to increased chilling rate have been reported (Hwang, Park, Cho, & Lee, 2004; Sammel et al., 2002), and it is probably explained by the negative impact of low temperature on activity of enzymes like glycogen debranching enzyme (GDE), related to glycogenolysis (Kylä-Puhju, Ruusunen, & Puolanne, 2005) and phosphofructokinase, related to glycolysis (Pösö & Puolanne, 2005).

It has been shown that the carcass temperature at the onset of rigor mortis can affect the final muscle contraction status (Savell, Mueller, & Baird, 2005). Regarding carcass chilling, to avoid cold shortening carcass temperature might be higher than 12 °C when the carcass achieves pH 6.0 (Thompson, 2002). However, carcass from cattle harvested at 280 kg of BW had a Temp@pH6 of 8.74 °C, and consequently the sarcomere length was shorter than cattle slaughtered at 480 kg of BW, which had a Temp@pH6 of 17.1 °C. Although carcass from the cattle harvested at 380 kg of BW had a Temp@pH6 slightly greater than 12 °C (14.5 °C), the sarcomere length was also shorter than those from cattle harvested at 480 kg of BW, suggesting some degree of cold shortening and corroborating with Sørheim & Hildrum, (2002) which reported that cold shortening starts when temperature at rigor is lower than 15 °C. The carcasses of the current study were not electrically stimulated but were suspended by the aitch bone (tenderstretch method). Although tenderstretch method is well known as an efficient tool to increase final sarcomere length of *longissimus* muscle (Sørheim & Hildrum, 2002), it was not effective to avoid myofibril shrinkage due to cold shortening.

The reduced sarcomere length found in the carcasses of cattle harvested at 280 and 380 kg could be related to the higher WBSF of unaged meat of these groups in compare with cattle harvested at 480 kg of BW. Nonetheless, after ageing for 7 or 14 d PM there was no difference for WBSF or MFI among harvest weights. These results are in accordance with Hopkins et al. (2011) who reported that sarcomere length variation explained more the 1 d aged WBSF variance than the 5 d WBSF. In addition, Starkey, Geesink, Oddy, & Hopkins (2015) pointed out that other traits related to postmortem proteolysis such as particle size and desmin degradation are better predictor for WBSF during aging than sarcomere length. The beef tenderization evaluated by WBSF changes from 1 to 14 d PM was reduced as harvest weight increased. These results suggest a higher myofibrillar proteolysis during aging for younger cattle, in agreement with previous study (Cruzen, Paulino, Lonergan, & Huff-Lonergan, 2014).

Increased water hold capacity and decreased thawing loss are expected after aging (Aroeira et al., 2016), because postmortem proteolysis can enhance the water bind and entrap by the muscle cell (Huff-Lonergan & Lonergan, 2005). In fact, unaged beef from cattle harvested at 280 and 380 kg of BW had greater exudate loss during thawing and cooking than that of cattle harvested at 480 kg, likely due to a reduced lattice space for water between the myofibrils (Bertram, Purslow, & Andersen, 2002). However, at 14 d postmortem the exudate loss of steaks from cattle harvested at 380 kg had similar loss to those harvested at 480 kg, while aging was not effective to decrease the exudate loss of steaks from cattle harvested at 280 kg of BW, even after 14 d postmortem. The lower IMF in beef from cattle harvested at 280 kg of BW may have contributed for higher exudate losses on this group, since decreasing intramuscular fat can increase beef moisture content (Duarte et al., 2011; Pflanzer & de Felício, 2011) increasing water losses.

Fresh meat color (1 d PM) from cattle harvest at 280 kg of BW had lower lightness (L^*), redness (a^*), and yellowness (b^*) in comparison with the other harvest weights. Although lightness is frequently associated with pH_u, the lack of difference between harvest weights for this trait suggests that other factor rather than WHC had affected the beef lightness. Increased L^* and b^* values have been reported in response to improving beef marbling (Duarte et al., 2011; Ponnampalam et al., 2017). Hence, lower intramuscular fat content

might be related to decreased lightness (*L**) and yellowness (*b**) of cattle harvest at 280 kg of BW. Lower redness (*a**) found for the lighter and younger cattle harvest at 280 kg of BW may be a reflection of the lower myoglobin content usually found in younger animals (Suman & Joseph, 2013). In agreement, Gardner et al. (2007) found increasing myoglobin concentration as sheep became mature, and a high correlation between *a** and meat myoglobin content.

5. Conclusions

Our results suggest that for Nellore cattle intensively raised (calf-fed system), the pre-weaning castration has no effect on carcass pH and temperature decline, as well as meat color. Regardless of weight at harvest, castration enhances subcutaneous fat, however, marbling is increased only when Nellore steers are harvested heavier and close to the weight at maturity (480 kg of BW). Castration increases postmortem tenderization rate. Lighter and leaner carcasses have faster chilling rate leading to reduce sarcomere length and increase shear-force of unaged meat.

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Table 1. Ingredient and chemical diet composition.

Item	
Ingredient	*% DM*
Corn silage	40.68
Ground corn	44.37
Soybean meal	9.97
Urea	0.96
Ammonium sulfate	0.11
Sodium bicarbonate	1.50
Magnesium oxide	0.50
Mineral premix[1]	1.91
Chemical composition	
Dry matter (g/kg as fed)	470.9
Crude protein (g/kg of DM)	138.7
NDF (g/kg of DM)	282.4
DE (MJ/kg)	13.95

[1]Providing per kg: 120 g of Ca, 87 g of P, 12 g of S, 198 g of Na, 625 mg of Cu, 45 mg of I, 50 mg of Co, 1000 mg of Mn, 7.5 mg of Se.

Table 2. Carcass traits of bulls and steers harvest at three endpoints (280, 380 and 480 kg of BW).

Item[1]	Sex condition (S)			Weight at harvest (W)				*P*-value[3]		
	Bull	Steer	SEM[2]	280	380	480	SEM[2]	S	W	S*W
Shrunk Body weight (kg)	396.1	373.5	6.46	276.4^{c}	389.3^{b}	488.6^{a}	8.20	0.022	<0.01	0.251
Hot carcass weight (kg)	236.6	224.0	4.31	157.7^{c}	233.2^{b}	299.9^{a}	5.48	0.053	<0.01	0.259
Cold carcass weight (kg)	232.1	219.7	4.20	153.9^{c}	228.7^{b}	295.1^{a}	5.33	0.049	<0.01	0.234
Carcass length (cm)	123.6	122.0	0.977	112.0^{c}	123.7^{b}	132.8^{a}	1.24	0.280	<0.01	0.568
Carcass shrinkage (%)	1.95	1.98	0.065	2.39^{a}	1.92^{b}	1.59^{c}	0.082	0.748	<0.01	0.228
Carcass dressing (%)	60.0	59.1	0.509	58.8	60.0	59.7	0.647	0.231	0.385	0.334
Rib eye area (cm^2)	70.6	66.7	1.82	56.5^{c}	67.9^{b}	81.4^{a}	2.32	0.152	<0.01	0.383
Back fat thickness (mm)	3.77	4.73	0.329	2.37^{b}	4.90^{a}	5.49^{a}	0.403	0.047	<0.01	0.235
KPH (%)	2.11	2.79	0.140	1.46^{c}	2.19^{b}	3.70^{a}	0.171	<0.01	<0.01	<0.01
Intramuscular fat (%)	3.40	3.99	0.225	2.38^{c}	3.62^{b}	5.09^{a}	0.276	0.076	<0.01	<0.01
pH_u	5.78	5.78	0.018	5.79	5.79	5.76	0.022	0.743	0.566	0.797
pH@18	6.30	6.29	0.051	6.65^{a}	6.17^{b}	6.06^{b}	0.062	0.968	<0.01	0.563
$Temperature_{24h}$ (^{o}C)	8.28	8.45	0.426	6.52^{b}	7.16^{b}	11.4^{a}	0.521	0.771	<0.01	0.783
Temp@pH6.0 (^{o}C)	13.9	13.0	0.826	8.74^{b}	14.5^{a}	17.1^{a}	1.01	0.419	<0.01	0.663

[1]KPH = Kidney, pelvic, and heart fat. $Temperature_{24h}$ = carcass temperature at 24 h postmortem. pH@18 = pH at 18 °C. Temp@pH6.0 = Temperature at pH 6.0.

[2]SEM = standard error of the mean.

[3]S = Sex condition main effect. W = Weight at harvest main effect. S*W = Sex condition by weight at harvest interaction.

[abc]LS means with uncommon superscript letter differ significantly ($P < 0.05$) by Tukey's test.

Table 3. Unaged and aged (7 and 14 d PM) meat traits of bulls and steers harvest at three endpoints (280, 380 and 480 kg).

Item[1]	Sex condition (S)			Weight at harvest (W)				*P*-value[3]		
	Bull	Steer	SEM[2]	280	380	480	SEM[2]	S	W	S*W
Sarcomere 1 d (µm)	1.67	1.65	0.046	1.56^{b}	1.51^{b}	1.92^{a}	0.057	0.743	<0.01	0.983
WBSF 1 d (N)	55.9	55.8	2.44	60.7^{a}	58.7^{a}	48.3^{b}	3.11	0.970	0.020	0.309
WBSF 7 d (N)	49.1	45.5	2.20	45.9	49.8	46.2	2.80	0.275	0.524	0.581
WBSF 14 d (N)	44.6	38.2	1.66	40.3	44.2	39.8	2.10	0.013	0.249	0.476
WBSF change 1-14 (N)	11.3	17.6	2.32	20.3^{a}	14.5^{ab}	8.4^{b}	2.95	0.074	0.035	0.446
MFI 1 d	20.9	24.3	1.39	24.2	21.1	22.5	1.77	0.104	0.464	0.336
MFI 7 d	30.0	37.8	2.52	36.6	31.4	33.7	3.20	0.042	0.509	0.898
MFI 14 d	39.6	49.8	3.18	51.3	38.6	44.3	4.04	0.036	0.092	0.196
MFI change 1-14	18.8	25.5	3.03	27.2	17.5	21.8	3.85	0.134	0.212	0.110
Thaw loss 1 d (%)	8.40	8.12	0.618	9.67^{a}	9.26^{a}	5.85^{b}	0.756	0.747	<0.01	0.899
Cook loss 1 d (%)	17.4	17.0	0.567	18.6^{a}	17.9^{a}	15.2^{b}	0.694	0.605	<0.01	0.331
Total loss 1 d (%)	24.4	23.7	0.574	26.5^{a}	25.5^{a}	20.1^{b}	0.703	0.423	<0.01	0.470
Thaw loss 7 d (%)	6.88	8.13	0.473	8.54^{a}	8.09^{a}	5.89^{b}	0.579	0.070	<0.01	0.770
Cook loss 7 d (%)	18.4	16.7	0.669	20.3^{a}	16.4^{b}	16.1^{b}	0.819	0.083	<0.01	0.570
Total loss 7 d (%)	24.0	23.5	0.676	27.1^{a}	23.1^{b}	21.1^{b}	0.828	0.567	<0.01	0.410
Thaw loss 14 d (%)	6.75	6.36	0.391	8.50^{a}	5.54^{b}	5.63^{b}	0.478	0.480	<0.01	0.988
Cook loss 14 d (%)	17.7	16.6	0.631	18.7^{a}	17.2^{ab}	15.5^{b}	0.773	0.262	0.023	0.472
Total loss 14 d (%)	23.2	21.9	0.703	25.6^{a}	21.8^{b}	20.3^{b}	0.861	0.199	<0.01	0.569

[1]WBSF = Warner-Bratzler shear force evaluated at 1, 7 and 14 days postmortem, MFI = Myofibrillar Fragmentation Index evaluated at 1, 7 and 14 days postmortem.
[2]SEM = standard error of the mean.
[3]S = Sex condition main effect. W = Weight at harvest main effect. S*W = Sex condition by weight at harvest interaction.
[abc]LS means with uncommon superscript letter differ significantly ($P < 0.05$) by Tukey's test.

Table 4. Fresh meat and fat color of bulls and steers harvest at three endpoints (280, 380 and 480 kg of BW).

Item[1]	Sex condition (S)			Weight at harvest (W)				*P*-value[3]		
	Bull	Steer	SEM[2]	280	380	480	SEM[2]	S	W	S*W
Meat color										
*L**	40.2	40.6	0.476	37.5^{b}	42.3^{a}	41.4^{a}	0.583	0.561	<0.01	0.86
*a**	13.6	13.9	0.209	13.1^{b}	14.1^{a}	14.0^{ab}	0.256	0.263	0.018	0.21
*b**	11.9	12.4	0.269	11.0^{b}	13.2^{a}	12.3^{a}	0.330	0.198	<0.01	0.92
Fat color										
*L**	67.2	67.3	1.08	66.5	67.4	67.9	1.33	0.957	0.749	0.533
*a**	11.0	10.5	0.800	11.6	10.8	9.85	0.979	0.665	0.472	0.694
*b**	22.7	23.9	0.736	22.8	23.7	23.5	0.902	0.279	0.781	0.713

[1]Color measurements of the muscle and fat evaluated at 1, 7 and 14 days postmortem.
[2]SEM = standard error of the mean.
[3]S = Sex condition main effect. W = Weight at harvest main effect. S*W = Sex condition by weight at harvest interaction.
[abc]LS means with uncommon superscript letter differ significantly ($P < 0.05$) by Tukey's test.

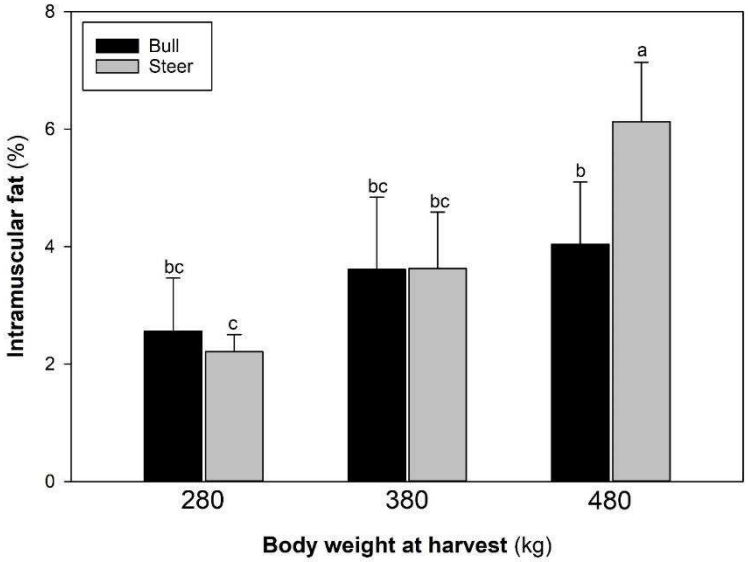

Figure 1. Intramuscular fat content of fresh meat from bulls and steers harvest at three endpoints (280, 380 and 480 kg). [abc]LS means with uncommon superscript letter differ significantly ($P < 0.05$) by Tukey's test. Bars denote standard error.

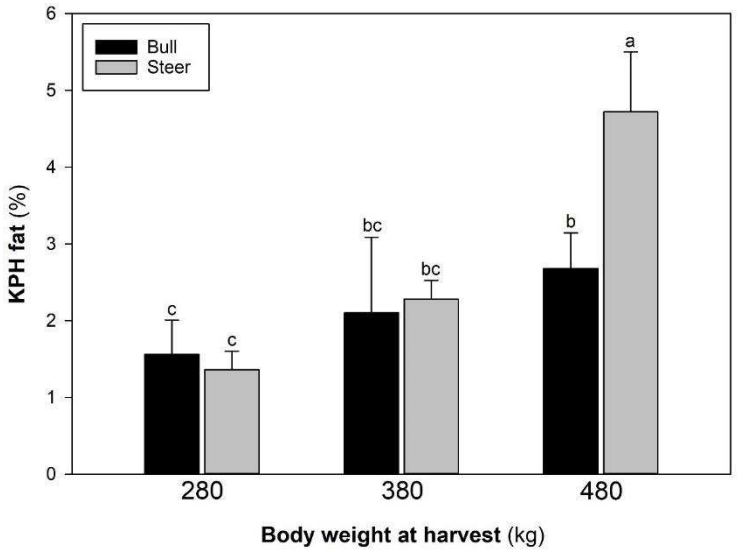

Figure 2. Kidney, pelvic, and heart fat (% of hot carcass) of bulls and steers harvest at three endpoints (280, 380 and 480 kg). [abc]LS means with uncommon superscript letter differ significantly ($P < 0.05$) by Tukey's test. Bars denote standard error.

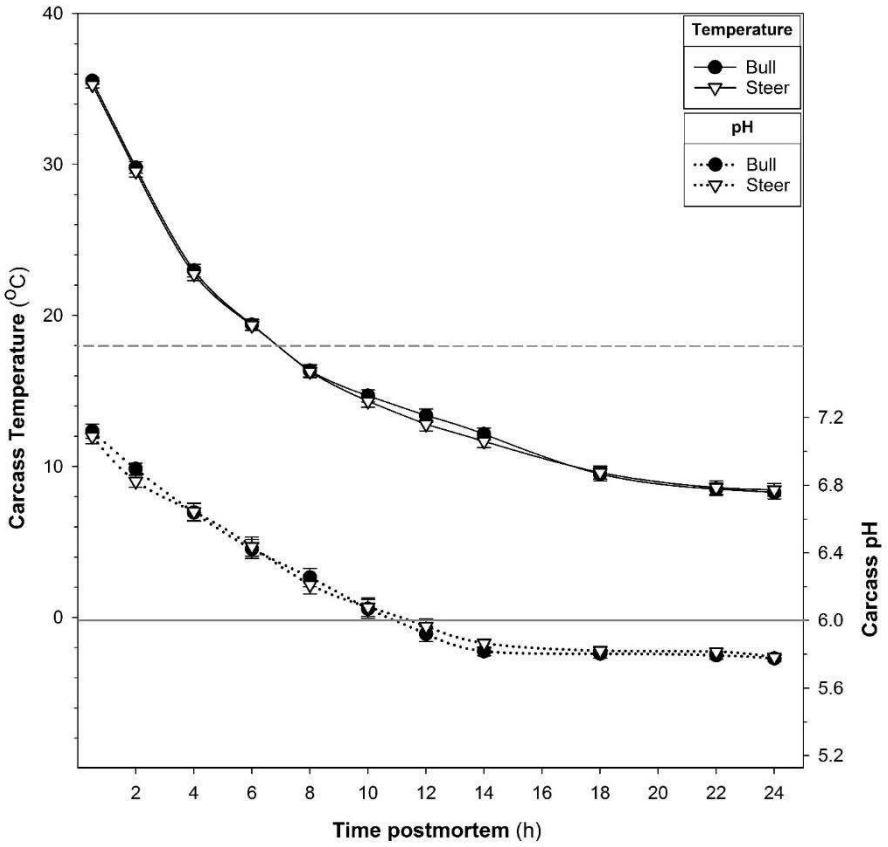

Figure 3. Carcass temperature and pH throughout chilling of bulls and steers. There was no difference between bulls and steers carcass pH and temperature within any evaluated time ($P > 0.05$). Dashed line (---) represent temperature 18 °C, while solid line (—) represent de pH 6.0. Error bars denote standard error of the mean.

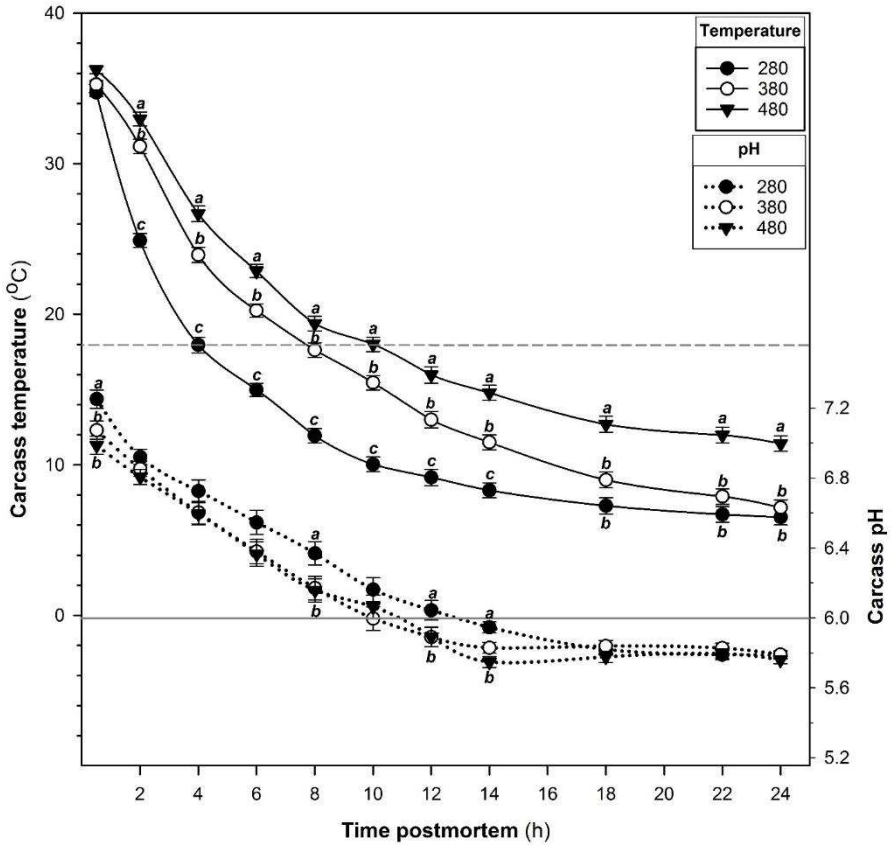

Figure 4. Carcass temperature and pH throughout chilling of cattle harvest at 280, 380 and 480 kg of BW. [abc]LS means with uncommon superscript letter differ significantly within the time postmortem ($P < 0.05$) by Tukey's test. Dashed line (---) represent temperature 18 °C, while solid line (—) represent de pH 6.0. Bars denote standard error of the mean.

Chapter II: Castration and development stage affecting Nellore muscle expression of genes related to glucose sensitivity and body composition[1]

Manuscript prepared following the Journal of Animal Science guidelines

Running head: Glucose tolerance of growing bulls and steers

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ABSTRACT

Castration and development stage modify muscle and adipose tissue growth affecting cattle body composition. Increasing body fatness might impair whole-body sensitivity to glucose, which is mainly mediated by reduced muscle glucose uptake. Therefore, the aim of the current study was to evaluate the glucose sensitivity of bulls and steers throughout body development. In addition, were evaluated the gene expression of biomarkers related to glucose metabolism, muscle growth and lipid deposition. Thirty-six Nellore calves (256.1 ± 3.05 kg of BW) were used, within then half was randomly selected for surgical castration one wk prior weaning. Weaned calves were penned and then six calves from each sex condition were randomly assigned to be harvested when the average BW of both sex conditions reaches 280, 380, and 480 kg. Therefore, this study was carried out as a complete randomized design following a 2 (sex condition) by 3 (weight at harvest) factorial arrangement of treatments. Two glucose tolerance tests (GTT) were performed at 380 and 480 kg. Longissimus dorsi (LD) samples were taken just after stunning for gene expression. Carcass composition and performance were evaluated. Bulls had greater final BW (P = 0.02) and tended to increase G:F ratio (P = 0.08) compared with steers. Bulls had greater carcass yield of lean (P = 0.02) and protein (P = 0.01) than steers. An interaction effect was found for carcass fat gain (P = 0.01), and steers gained more carcass fat than bulls only from 380 to 480 kg of BW. Carcass protein fractional accretion rate (FAR) decreased as cattle BW increased (P < 0.01). Neither glucose basal level nor area under the curve (AUC) post-infusion were affected by castration or cattle BW (P > 0.05). Expression of genes related to glucose metabolism in the LD were not affected by castration or cattle BW (P > 0.05). Castration tended (P = 0.086) to upregulated LD expression of *Acetyl-CoA carboxylase alpha* (*ACACA*). Interaction effects (P < 0.05) were

found for LD expression of *IGF-1 receptor* (*IGF1R*) and *F-box protein 32* (*FBXO32*), and for both genes steers had the greatest mRNA abundance at 380 kg while bulls had the greatest abundance at 480 kg of BW. The LD expression of *serpin A3-6* tended (P = 0.08) to be downregulated as cattle BW increased from 280 to 480 kg. In conclusion, the data suggest that Nellore muscle glucose uptake is not affected by neither castration nor cattle body weight. In addition, the greater intramuscular fat of steers may be supported by increased *de novo* fatty acid biosynthesis form glucose.

Key words: carcass composition, castration, glucose tolerance test, maturity weight, Nellore cattle

INTRODUCTION

Castration is widely applied on beef production, mainly due to the marbling enhancement, which improves quality grading and carcass value (Jeong et al., 2013). However, the anabolic effect of testosterone on bulls might increase the profitability in markets where the use of exogenous anabolic hormones is prohibited, and where the carcass grading system does not measure marbling level (Molleta et al., 2014).

Castration effects on performance and body composition are well established, and steers present greater carcass fatness but reduced growth rate (Seideman et al., 1982; Bretschneider, 2005). Nonetheless, the magnitude of the difference between bulls and steers might vary with the age at castration and the stage of development (Marti et al., 2013). Furthermore, with the advance of the maturity, body composition changes decreasing the lean and bone percentage whereas fat increases (Marcondes et al., 2016).

Increasing body fat has been related to decreased insulin responsiveness in sheep (Bergman et al., 1989), while well-muscled cattle have greater glucose clearance after intravenous exogenous glucose dose (McGilchrist et al., 2011). The glucose tolerance test (GTT) has been applied to evaluate the whole-body sensitivity to a high exogenous glucose dose (González-Grajales et al., 2017). Muscle glucose uptake accounts for around 80% of the exogenous dose (Xia et al., 2013) and glucose sensitiveness is mainly controlled by amount of glucose transporter sensitive to insulin (GLUT4) at the sarcolemma (Fitzsimons et al., 2014).

Finally, considering the economic importance of carcass muscularity and adiposity, study the effect of castration as cattle BW increases might help to better clarify the pathways related to muscle growth and fat deposition in beef cattle. The aim of this study was to evaluate the effect of castration and body weight on glucose sensitivity and muscle expression of genes related to glucose metabolism, muscle growth and fat deposition. Therefore, we hypothesized that as cattle reach the maturity weight the body responsiveness to glucose decrease in response to reduced muscle glucose uptake. In addition, reduced muscle glucose utilization should stimulate intramuscular fat synthesis from the available glucose.

MATERIALS AND METHODS

The Animal Care and Use Committee of the Department of Animal Science of the Universidade Federal de Viçosa (CEUAP-UFV) reviewed and approved all animal handling and the castration surgery procedure (protocol 035/2015).

Animals, experimental design, and feed management

Thirty-six male Nellore calves averaging 256.1 ± 3.05 kg of BW and 8.2 ± 0.07 mo old were used. Half of calves were randomly selected to be surgically castrated one wk before weaning. The castration surgery was performed by a veterinary using a squeeze-chute to hold and restrain the animal. The castration site was cleaned with neutral soap and 2% iodine solution prior surgery. One new scalpel blade was used per animal, and after the surgery silver sulfadiazine and zinc oxide were placed on surgery site. Oxytetracycline hydrochloride was intramuscularly injected (10 mg/kg of BW). Steers were checked daily during three wk post-surgery, and silver sulfadiazine and zinc oxide were used until complete healing.

Weaned calves were ear tagged, dewormed and penned as two groups, according to sex condition (bulls or steers). Calves were gradually adapted to the diet through four wk. Six calves from each sex condition were randomly assigned to be harvested when the average BW of both sex conditions reaches 280, 380, and 480 kg. Therefore, this study was carried out as a complete randomized design following a 2 (sex condition) by 3 (weight at harvest) factorial arrangement of treatments, which consisted of bulls harvested at 280 kg of BW (**B-280**), steers harvested at 280 kg of BW (**S-280**), bulls harvested at 380 kg of BW (**B-380**), steers harvested at 380 kg of BW (**S-380**), bulls harvested at 480 kg of BW (**B-480**), steers harvested at 480 kg of BW (**S-480**). Nellore cattle reach the maturity weight at 437 kg of empty body weight (**EBW**), as previous reported (Costa e Silva et al., 2013). Hence, as EBW usually represents 90% of shrunk body weight (**SBW**), Nellore reaches maturity weight at approximately 480 kg of SBW. Thus, the body weights of 380 and 280 kg represent around 80% and 60% of the Nellore weight at maturity, respectively.

Throughout the experiment, both groups (bulls and steers) were kept under the same experimental conditions, receiving the same diet (Table 1). Fresh feed was provided ad libitum twice a day, about 60% at 0700 h and the remain at 1400 h. The diet was formulated regarding the average nutrient requirements between bull and steer performing 1.4 kg/d, according to BR-CORTE system (Valadares Filho et al., 2010). Each feedlot pen was equipped with three electronic feed bunk system (AF 1000 Master, Intergado LTDA, Contagem, MG, Brazil) and one water drinker/body scale (WD 1000, Intergado LDTA, Contagem, MG, Brazil) allowing to record individually the daily intake of feed and water as well as record the body weight.

Cattle performance

Cattle harvested at 280 kg of BW reached the target weight just five d after the adaptation period. Consequently, the performance was not estimated for these animals. The remaining cattle performance was evaluated separately from 280 to 380 kg of BW and from 380 to 480 kg of BW. Once cattle reached the targets BW, calves were individually weighed after 16 h of fasting for SBW estimation, and then ADG.

Concentrate feedstuffs were sampled every batch, while corn silage was daily sampled and pooled by week. The pooled corn silage samples were weighed, oven dried at 55°C for 72 h, and then weighed again to quantify the moisture loss. The ground corn, soybean meal, and dried corn silage samples were ground through a 1-mm screen using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA, USA). All ground feedstuffs were analyzed for DM content (method 934.01; AOAC International, 2005). Therefore, DMI was obtained as the individual daily intake (as fed) obtained from the electronic feed bunk system multiplied by diet dry matter content. The relative DMI (%BW/d) was individually

estimated using the average BW during the evaluated period. Furthermore, gain to feed ration (**G:F**) could be calculated dividing ADG by DMI.

Glucose tolerance test

The glucose tolerance tests were performed one wk prior the harvest at 380 kg of BW, and one wk prior the harvest at 480 kg of BW. Due to facility limitations and to minimize variations within animals, these two glucose tolerance tests (**GTT**) were performed using only the six bulls and the six steers selected to be harvested at 480 kg of BW. As our facility has two squeeze-chutes, to minimize the difference on fasting prior the GTT cattle were divided into two groups of six (3 bulls and 3 steers) to be submitted to GTT in two consecutive days. The animal sequence to GTT was balanced for sex condition, allowing to submit simultaneously one bull and one steer to GTT, having the same number of bulls and steers at each squeeze-chute.

Cattle were weighed after 14 h of fasting to calculate the individual glucose dose of 200 mg/kg of BW (McGilchrist et al., 2011). It was used a sterile physiological saline solution with 50% of glucose (Isofarma Industrial Farmacêutica Ltda, Eusébio, CE, Brazil). The animal was carefully held and restrained in the squeeze-chute, then a catheter was fitted into the right jugular vein. Blood samples for glucose basal level concentration were obtained from the catheter at -5 and 0 min relative to infusion. Then the glucose dose was injected into the left jugular vein, within two min. Blood samples were obtained from the catheter at 5, 10, 15, 20, 30, 45, 60, and 120 min post-infusion. The whole blood glucose concentration was determined in real time using a glucometer (Accu-chek performa nano, Roche Diagnostics GmbH, Mannheim, Germany). A natural logarithmic function was

individually adjusted for blood glucose concentration post-infusion, then the area under the curve (**AUC**) was obtained integrating the function from 5 to 120 min post-infusion.

Slaughter, longissimus muscle sampling, and carcass composition

Six bulls and six steers were harvest when the average BW of both sex conditions reached the endpoints of 280, 380 and 480 kg, totalizing three slaughters of 12 cattle each. Once the target BW was achieved the selected group was slaughtered after 16 h of fasting with free access to water. Due to the facility limitations, a balanced group of six cattle (3 bulls and 3 steers) were harvested during two consecutive days. Procedures during the harvest followed humane slaughter practices, as described at Brazilian standards of sanitary regulation for animal products (Brasil, 1997).

Just after stunning and blooding, the longissimus dorsi (**LD**) muscle samples were obtained from the right carcass side making an incision through the hide at the 13th rib. The LD samples were trimmed for epimysium and subcutaneous fat, minced, and then snap frozen in liquid nitrogen. Minced LD samples were ground using a mortar with liquid nitrogen, placed into identified cryovial, and then stored at -80°C until the RNA extraction.

After dressing, the EBW was estimated by individually weighting all body components (e.g., blood, head, skin, legs, tail, organs and hot carcass) including the emptied gastrointestinal tract. The mesenteric fat was physically separated from the gastrointestinal tract and weighed with the kidney, pelvic and heart fat (**KPH**) composing the visceral fat (**VF**). Hot carcasses were weighed (**HCW**), suspended by the aitch bone (tenderstretch method), and chilled for 24 h at 2°C. Afterward, the 9th-11th rib section

was removed from the left side of the cold carcass for carcass physical and chemical composition estimation (Hankins and Howe, 1946).

The 9th-11th rib samples were dissected into lean, fat, and bone to estimate carcass physical composition according to Marcondes et al. (2012). Afterwards, these samples were freeze-dried and ground for analyzes of CP (method 984.13; AOAC, 2005), DM (method 934.01; AOAC International, 2005), and EE in a XT15 extractor (Ankom, Macedon, NY, USA). Carcass chemical composition was estimated by equations suggested by Marcondes et al. (2012). To estimate carcass gain (**CG**), cattle harvested at 280 kg of BW were used as a reference initial group allowing to access initial carcass composition from 280 to 380 kg, while cattle harvested at 380 kg of BW were a reference group to the period from 380 to 480 kg of BW. The gains in carcass physical and chemical components were then calculated from 280 to 380 kg of BW and from 380 to 480 kg of BW. In addition, the carcass protein fractional accretion rate (**FAR**) was calculated dividing the carcass protein gain by the average protein pool of the period.

RNA extraction, cDNA synthesis, and RT-qPCR analysis

Fifty milligrams of powdered whole LD muscle were used for total RNA extraction with Trizol reagent (Invitrogen, Carlsbad, CA). To remove DNA contamination, total RNA samples were then treated with DNase I, Amplification Grade (Invitrogen, Carlsbad, CA, USA). The spectrophotometer NanoVue Plus (GE Healthcare, Freiburg, Germany) was used to estimate RNA concentration at 260 nm, and RNA quality was verified by 260/280 nm ration. Finally, RNA integrity was checked through presence of 18s and 28s bands in 1% agarose gel electrophoresis. The cDNA synthesis was performed using the GoScript

Reverse Transcriptase kit (Promega, Madison, WI, USA). Samples were stored at -20°C until analysis.

The primers were designed by the web tool Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast) using the *Bos taurus* reference sequences from GenBank database. The primer sequences of the 14 target genes and the 18S rRNA endogenous control are shown at Table 2. The real-time quantitative PCR was performed using a 7300 Real-Times PCR unit (Applied Biosystems, Carlsbad, CA, USA) and SYBR Green RT-PCR GoTaq Master Mix (Promega, Madison, WI, USA) following the cycle parameters: 95°C for 3 min and 40 cycles at 95°C for 10 s and 60°C for 30 s. The cycle threshold (**Ct**) values were recorded for target and endogenous genes.

Statistical analyzes

The gene expression statistical analysis was performed as proposed by Steibel et al. (2009), which consists of a linear mixed model that uses target and endogenous Ct to analyze the relative quantification real-time PCR. The sex condition (bull, and steer) and weights at harvest (280, 380, and 480 kg of BW) were included in the model as a fixed effect, while animal was included as a random effect. Gene expression is reported as fold-change calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

All data, were analyzed as a completely randomized design following a two (bull and steer) by Three (280, 380 and 480 kg at harvest) factorial arrangement of treatments, using the MIXED procedure of SAS. Initial body weight was included as covariate in the model, and then kept where $P < 0.10$. Once ANOVA pointed significant ($P < 0.05$) effect for weight at harvest or interaction between sex condition by weight at harvest, treatments

least squared means were compared by Tukey's test. The GTT data were analyzed as a repeated measurement, once both trials were performed using the same group of cattle.

RESULTS

Cattle performance

Post-weaned calves were fed during 33, 133, and 230 d to reach the target BW of 280, 380, and 480 kg respectively. The average SBW for both sex conditions at the harvests were 276.4 ± 11.4, 389.3 ± 11.1, and 488.6 ± 11.4 kg, which were close to target BW. At harvests of 280, 380, and 480 kg of BW cattle were 9.3 ± 0.09, 12.6 ± 0.05, and 15.7 ± 0.07 mo old. Regardless of weight at harvest, bulls had greater SBW ($P = 0.02$), EBW ($P = 0.03$), and have greater HCW ($P = 0.053$) than steers (Table 3). An interaction effect was found for VF ($P < 0.01$), and steers had greater VF than bulls only at 480 kg harvest. Sex condition did not affect DMI ($P = 0.42$) as kg/d, but steers had greater DMI expressed as percentage of BW ($P = 0.01$). Although increasing BW from 380 to 480 kg lightly increased DMI expressed as kg/d ($P = 0.047$), the DMI as a percentage of BW markedly decreased ($P < 0.01$). Neither ADG nor carcass gain (CG) were affected by sex condition ($P > 0.05$). Increasing cattle BW from 380 to 480 kg decreased ADG ($P = 0.03$), though CG was not affected ($P = 0.17$). Bulls tended to have great G:F ratio than steers ($P = 0.08$). Feed efficiency was negatively affected by increasing cattle BW ($P < 0.01$). Likewise, the efficiency to convert feed into carcass tended to be higher for bulls ($P = 0.07$) and decreased as cattle became heavier ($P < 0.01$). Neither glucose baseline blood concentration nor glucose AUC response were affected by sex condition or weight at harvest ($P > 0.05$).

Carcass composition

Interaction effects were found ($P < 0.05$) for carcass bone, carcass EE, fat gain, and EE gain (Table 4). The increase in cattle BW increased ($P < 0.01$) carcass lean, carcass fat, lean:bone ratio, and the yield of carcass protein and water. Increasing cattle BW from 380 to 480 did not affected ($P > 0.05$) carcass gain of any physical and chemical component, except water which decreased ($P < 0.01$).

Regardless of the weight at harvest, bulls had greater lean quantity than steers ($P = 0.02$), although lean daily gain from 380 to 480 kg did not differ between the sex conditions ($P = 0.15$). Castration did not affect the carcass fat amount ($P = 0.29$), even though B-480 had decreased carcass fat gain. Bulls had greater carcass bone gain than steers ($P = 0.02$). Castration negatively affected ($P < 0.05$) the carcass protein, and water, and tended to decrease carcass gain of protein ($P = 0.07$), and water ($P = 0.07$). Although castration did not affect the carcass protein fractional accretion rate ($P > 0.05$), it decreased ($P < 0.01$) as cattle became heavier (Figure 1).

Longissimus muscle gene expression

Interaction effect were found ($P < 0.05$) for mRNA abundance of *IGF1R* and *FBXO32* (Table 5). Sex condition did not affect ($P > 0.05$) LD gene expression, except for *ACACA*, which tended to increase in the LD of steers compared with bulls ($P = 0.08$). Increasing cattle BW did not affect ($P > 0.05$) the gene expression in the LD, except for *SERPINA3-6*, which tended to be downregulated as cattle became heavier ($P = 0.08$).

DISCUSSION

As cattle reach the weight at maturity the body composition changes as a result of the decreasing muscle growth rate with simultaneous acceleration of fat tissue development (Owens et al., 1993). Castration may speed up this body composition change, as steers

have lower maturity body weight than bulls (Valadares Filho et al., 2016). Therefore, the degree of maturity and castration are two independent factors that can help to better understand the pathways related to economically important traits, such as carcass lean yield and fatness. Hence, at the current study we evaluated the difference between bulls and steers on carcass composition, glucose tolerance, and muscle gene expression as cattle reached the weight at maturity.

Cattle performance

The SBW and EBW differences between bulls and steers increased from 6 kg when cattle were harvested with 280 kg, to 18 kg at 380 kg harvest and to around 40 kg when harvested with 480 kg of BW. Likewise, carcass weight difference between sex conditions increased from 3 up to 26 kg, when animals were harvested at 280 and 480 kg of BW respectively. These results demonstrate a negative impact of castration on BW and carcass weight, which increases as cattle became heavier.

The weight loss post-surgery and the performance reduction due to absence of androgens from testicles are the two main reasons for the reduced BW of steers (Bretschneider, 2005). Castration-related weight loss is minimal close to birth but is maximum after puberty (Bretschneider, 2005). Likewise, the performance difference between bulls and steers increases after puberty due to the enhancement of testicular androgens production (Biagini and Lazzaroni, 2011). It has been reported that Nellore reaches puberty around 15 mo old (Freneau et al., 2006), though well-fed cattle may have earlier puberty (Renaville et al., 2000). Thus, at this study bulls may have reached the puberty between 380 to 480 kg of BW, when they were around 13 and 16 mo old respectively.

The ADG did not differ between sex condition, even though bull's average was 10% greater than steers. The carcass gain followed the same pattern. Although bulls usually have greater growth rate than steers, the superiority of bulls can vary from 7.5 up to 18.7% (Paulino et al., 2008; Marti et al., 2013). Castration did not affect DMI, corroborating with Azevêdo et al. (2016). Nonetheless, bulls tended to have greater feed efficiency (i.e., G:F ratio) than steers agreeing with previous reports (Seideman et al., 1982; Sales, 2014). Regardless of sex condition, as cattle BW increased the DMI increased and ADG decreased leading to reduction on G:F ratio. However, the DMI as percentage of BW markedly decreased as cattle BW increased, indicating a reduction on feed intake capacity. Interestingly, although ADG decreased around 17% as cattle BW increase, the carcass gain (CG) reduced only 10% and did not differ between cattle at 380 and 480 kg of BW. These results indicate the later maturity of carcass compared with non-carcass components (Owens et al., 1993; Kern et al., 2014).

Muscle growth

Skeletal muscle represents around 37% of Nellore cattle EBW (Marcondes et al., 2012), and this tissue accounts for great proportion of the animal daily requirements of energy and protein (Goll et al., 2008; Wang et al., 2010). Previous studies have shown that castration negatively impacts muscle growth, since testosterone is an anabolic hormone stimulating muscle hypertrophy directly and indirectly (Gentile et al., 2010; Dayton and White, 2014). Furthermore, increasing cattle age and BW reduce muscle growth rate (Marcondes et al., 2016). At the current study, we evaluated the effect of castration on muscle growth and gene expression as cattle became heavier.

Although bull had only around 3 kg greater lean carcass tissue than steers when harvested at 280 and 380 kg of BW, a greater difference (i.e., 23 kg) was found at 480 kg of BW. Regarding the carcass lean gain, both sex condition had similar muscle growth from 280 to 380 kg averaging 448 g/d, whereas from 380 to 480 kg of BW bulls maintained the lean growth rate (463 g/d) and steers tended to decrease (328 g/d). Besides, the carcass protein gain, which is mainly from muscle, tended to decrease following castration. These results indicate that castration decreased skeletal muscle growth as cattle BW increased. Similar results have been previously found, in which muscle mass and metabolic characteristics did not differ between bulls and steers at 5 mo post-surgery but differed at 13 mo post-surgery (Picard et al., 1995). However, the carcass protein FAR decreased as cattle became heavier, regardless of sex condition, indicating that difference between protein synthesis and breakdown decreases as cattle reach the weight at maturity.

Myostatin has been reported as a biomarker for muscle mass, once increasing muscle expression of *MSTN* is associated to muscle growth inhibition (Bouley et al., 2005). It has been proposed that muscle upregulation of *MSTN* and *PPARγ* are related to reduced muscle growth rate and accelerated fat deposition as the animal reaches the mature BW (Kern et al., 2014). However, at the current study even though castration slightly decreased muscle growth and increased carcass adiposity at 480 kg of BW, muscle expression of *MSTN* and *PPARγ* did not differ among treatments. Furthermore, muscle expression of *GSK3B* was negatively correlated to muscle growth (Busato et al., 2016), whereas neither castration nor harvest BW affected muscle mRNA abundance of *GSK3B*.

The GH/IGF1 axis have a key role in the regulation of skeletal muscle mass by stimulating protein synthesis (Velloso, 2008). The interaction effect found for muscle expression of

IGF1R pointed out that while this gene was upregulated as Nellore bulls increased the BW from 280 to 480 kg, in steers *IGF1R* expression increased from 280 to 380 kg and then decreased from 380 to 480 kg of BW. These findings indicate that an early inhibition of muscle growth in steers in comparison with bulls could be regulated by reduction on muscle sensitivity to IGF1 hormone. Furthermore, besides the direct effect of testosterone on muscle cells, it has been proposed an indirect effect of testosterone on muscle anabolism mediated by IGF1 (Gentile et al., 2010; Dayton and White, 2014).

Muscle protein turnover affects muscle hypertrophy, growth efficiency and may impair meat quality (Koohmaraie et al., 2002). Ubiquitin-proteasome is the main proteolytic system related to skeletal muscle protein turnover, and protein breakdown by this pathway may account for 80 up to 90% of in vivo muscle proteolysis (Goll et al., 2008). The *F-box protein 32* (*FBXO32*), also known as *Atrogin-1*, is an important subunit of the E3 ubiquitin-protein ligase (Foletta et al., 2011). Upregulation of muscle *Atrogin-1* has been related to muscle atrophy (Anthony, 2016). Nellore muscle *FBXO32* expression enhanced as cattle BW increased, and steers reached a maximum expression at 380 kg while in bulls muscle the expression increased by 480 kg of BW. These data may indicate that the ubiquitin-proteasome system is associated to muscle growth and remodeling and that the reduced muscle growth rate as cattle reach the maturity weight could be related to increased muscle protein breakdown.

Besides proteasome, caspases are another group of endogenous muscle proteases involved on muscle protein turnover and remodeling (Anthony, 2016). As Nellore cattle reached the weight at maturity, muscle mRNA abundance of *CASP3* did not change while a trend was found for decreased *SERPINA3-6* abundance, suggesting an increase on the caspase

proteolytic system. These data are corroborating with a previous observation that usually the protease inhibitor level is a better predictor of the system activity than their target protease (Boudida et al., 2014).

Finally, the greater bone growth of bulls compared with steers may be due to the androgens stimulus, as previous confirmed in rats (Gentile et al., 2010; Xia et al., 2013). In agreement, a previous study also reported greater bone proportion on bull carcass regardless of castration method (Marti et al., 2013; Moreira et al., 2017).

Fat tissue growth

Castration markedly enhanced body fat growth, regardless of the adipose tissue depot. However, greater difference was found comparing bulls and steers at 480 kg of BW. An interaction effect was found for visceral fat (VF) as percentage of EBW, indicating that bulls and steers did not differ when harvested at 280 and 380 kg of BW, whereas steers had 45% more VF than bulls when harvested at 480 kg (7.82 vs. 5.38% of EBW). The same pattern was found for carcass EE yield, and steers had 15% greater carcass EE than bulls harvested at 480 kg of BW (67.8 vs. 58.7 kg of EE). These results confirm that body composition is affected by castration only at heavier weights, probably close and after puberty.

The intramuscular fat (IMF) differed between sex condition only at 480 kg of BW harvest, and steers had 52% greater IMF than bulls (6.13 vs. 4.04% of fresh meat). However, regarding the expression of genes related to adipogenesis and lipid metabolism, only the *ACACA* tended to differ between sex condition, and steers had greater muscle mRNA abundance than bulls. The enzyme ACACA catalyzes the carboxylation of acetyl-CoA to

malonyl-CoA, a key step during fatty acid biosynthesis in ruminants (Shin et al., 2011). High and positive correlation has been found between *ACACA* muscle expression and IMF, suggesting that ACACA plays an important role in the intramuscular fat deposition in ruminants (da Costa et al., 2013). Therefore, this result confirms the enhancement of fat synthesis following cattle castration. In agreement, it has been reported that castration upregulated LD muscle *ACACA* expression in Korean cattle, supporting the greater IMF found for steers (Bong et al., 2012).

The *PPARγ* is a transcription factor promoting expression of target genes related to adipocyte differentiation and lipid storage, then it is a biomarker for adipogenesis (Bionaz et al., 2013). It was expected an enhancement on muscle expression of *PPARγ* following castration (Serra et al., 2013) and in response to increasing cattle BW (Kern et al., 2014). Nevertheless, the muscle abundance of *PPARγ* mRNA did not differ among treatments. In agreement, Cesar et al. (2015) did not find any difference on LD muscle *PPARγ* expression between Nellore steers grouped as low and high IMF content. Likewise, it has been reported weak correlation between muscle expression of *PPARγ* target genes and IMF in Brahman, a zebu breed (De Jager et al., 2013).

It has been shown that the increased intramuscular fat of steers is supported by enhancement of lipid uptake and decreased lipolysis (Jeong et al., 2013; Baik et al., 2017). However, at the current study neither castration nor weight at harvest affected the expression of genes related to lipid uptake (*LPL*, and *SLC27A1*) or lipolysis (*LIPE*).

Glucose/glycogen metabolism

Glucose is an important energetic source, and some body tissues such as brain, and red blood cells can only use glucose as energy fuel (Huntington and Richards, 2005). However, even though muscle can use other energy sources, it has been estimated in ruminants that muscle accounts for approximately 20-40% of glucose uptake (Dijkstra et al., 2005). Others reported in cattle that muscle metabolizes 50-55% of the blood glucose (Ortigues-Marty et al., 2003). Furthermore, in rats up to 90% of an exogenous glucose dose was taken up by skeletal muscle tissue (Xia et al., 2013). These results indicate that muscle plays an important role on blood glucose homeostasis. Besides that, muscle glucose uptake rate can be affected by castration (Xia et al., 2013) and by body composition changes (Huntington and Richards, 2005).

The GTT is applied to study the whole-body sensitivity to an exogenous glucose dose (González-Grajales et al., 2017). In this study, the GTT data pointed out that neither sex condition nor cattle BW affected blood glucose baseline concentration or clearance of exogenous glucose. The GTT results are in accordance with the lack of difference among treatments for muscle expression of *GLUT4*. In agreement, cattle with different residual feed intake (RFI) presented similar glucose AUC as well as muscle expression of *GLUT4* (Fitzsimons et al., 2014). Further, a similar glucose clearance has been found for Angus with different muscling genotypes (McGilchrist et al., 2011). It has been reported that blood NEFA level is negatively corelated to glucose sensitiveness (Burdick Sanchez et al., 2016). At the current study NEFA was not evaluated but the mRNA abundance of the *hormone-sensitive lipase* (*LIPE*) in the LD did not differ among treatments, suggesting a similar TAG hydrolysis between bulls and steers regardless of cattle BW.

Just after uptake into muscle cell, glucose molecule is phosphorylated to glucose-6P, which then can follow the glycolysis pathway or be stored as glycogen. Glucose-6P must be converted to glucose-1P to be stored as glycogen, and this reaction is catalyzed by phosphoglucomutase (PGM1). The glycogen synthase kinase (GSK3B) modulates the glycogen synthase activity and is a key enzyme in glycogen metabolism. Finally, the glycogen phosphorylase (PYGM) catalyzes the first step of glycogen breakdown. At the current study, the mRNA abundance of *PGM1*, *GSK3B*, and *PYGM* were not affected, suggesting that neither castration nor increases in cattle BW affect muscle glycogen metabolism at gene expression level.

Decreased muscle glucose sensitivity can may impair muscle glycogen storage, and then postmortem carcass pH decline (Aoki et al., 2007). However, in the current study a lack of difference among cattle groups for the GTT was found, suggesting that muscle glucose metabolism was not affected by castration and cattle BW. Moreover, the postmortem ultimate pH also did not differ among treatments (data not shown).

In conclusion, the lack of difference for GTT and muscle glucose gene expression suggest that Nellore muscle glucose uptake was not affected by neither castration nor cattle body weight. In addition, the upregulation of muscle *ACACA* by castration suggests that the greater intramuscular fat of steers may be supported by increased *de novo* fatty acid biosynthesis from glucose. Finally, the downregulation of muscle *serpin A3-6* and upregulation of *Atrogin-1* as cattle reached the weight at maturity indicates that reduced protein fractional accretion rate (FAR) as cattle became heavier might is related to increased protein degradation by Ubiquitin-proteasome and caspase systems.

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Table 1. Ingredient and chemical diet composition.

Item	
Ingredient	*% DM*
Corn silage	40.68
Ground corn	44.37
Soybean meal	9.97
Urea	0.96
Ammonium sulfate	0.11
Sodium bicarbonate	1.50
Magnesium oxide	0.50
Mineral premix[1]	1.91
Chemical composition	
DM, g/kg as fed	470.9
CP, g/kg of DM	138.7
NDF, g/kg of DM	282.4
DE, MJ/kg	13.95

[1]Providing per kg: 120 g of Ca, 87 g of P, 12 g of S, 198 g of Na, 625 mg of Cu, 45 mg of I, 50 mg of Co, 1000 mg of Mn, 7.5 mg of Se.

Table 2. Primers sequences of genes analyzed by qPCR.

Gene	Abbreviation	GenBank access code	Forward (**F**) and reverse (**R**) sequences
Glycogen synthase kinase 3 beta	*GSK3B*	NM_001101310.1	F: TACCAAATGGGCGAGACAC R: CCGAGCATGAGGAGGAATAAG
Glycogen phosphorylase	*PYGM*	NM_175786.2	F: CCCAAACAGCCTGACCTATT R: TCCACTCTCTCGGGTTCTT
Phosphoglucomutase 1	*PGM1*	NM_001076903.1	F: CACTGGAAGATGGAGGTTGAG R: AAGGCTCACCAAGGAGAAAG
Insulin-regulated glucose transporter	*GLUT4*	AY458600.1	F: CCTTGGTCCTTGCCGTATT R: CCAGCCAGGTCTCATTGTAG
Peroxisome proliferator activated-receptor gamma	*PPARγ*	NM_001098905.1	F: TGGAGACCGCCCAGGTTTGC R: AGCTGGGAGGACTCGGGGTG
Fatty acid transporter, member 1	*SLC27A1*	NM_001033625.2	F: CTCGTGTCTGTGTGTCTGTC R: GGAGGAGAGATGGAGGAAGA
Lipoprotein lipase	*LPL*	NM_001075120.1	F: CCTGAAGACTCGTTCTCAGATG R: AAGGCCTGGTTGGTGTATG
Acetyl-CoA carboxylase alpha	*ACACA*	NM_174224.2	F: GAAGTGATGGGCTGCTTCT R: GGGACCTTGTCTTCGTCATAC
Lipase, hormone- sensitive	*LIPE*	NM_001080220.1	F: GAGGGTGATGAGAGGGTAATTG R: AGGTGTGAACTGGAAACCC
Myostatin	*MSTN*	AF019620.1	F: CCACGGAGTCTGATCTTCTAAC R: TCCACAGTTGGGCCTTTAC
Insulin like growth factor 1 receptor	*IGF1R*	NM_001244612.1	F: CTCAACCCAGGGAACTACAC R: GTCTTGGCCTGAACGTAGAA
Caspase 3	*CASP3*	NM_001077840.1	F: CGTCCCTTTCTGCCATCC R: CAGACCATTAGGCCACACTC
Serpin A3-6	*SERPINA3-6*	NM_001146302.1	F: CTGGGCTGGTTCTGGTAAA R: TGACTGCTGTGCCATCTT
F-box protein 32 (Atrogin-1)	*FBXO32*	NM_001046155.1	F: CCCAGAGAGCTGTTCCATTT R: CTCTGGATTCCCAACCATCC
18 S ribosomal	*18S*	DQ222453.1	F: CCTGCGGCTTAATTTGACTC R: AACTAAGAACGGCCATGCAC

Table 3. Performance of Nellore bulls and steers harvested at 280, 380, and 480 kg of BW.

Item[1]	Treatments[5]							*P*-value[6]		
	B-280	S-280	B-380	S-380	B-480	S-480	SEM	S	W	S*W
SBW, kg	279	273	398	380	510	467	11.5	0.022	<0.01	0.251
EBW, kg	258	252	371	353	476	438	11.6	0.033	<0.01	0.346
HCW, kg	159	156	237	230	313	287	7.81	0.053	<0.01	0.259
VF, %EBW	3.31[c]	3.06[c]	5.50[b]	5.08[b]	5.38[b]	7.82[a]	0.37	0.056	<0.01	<0.01
DMI, kg/d	-	-	6.70	7.31	7.69	7.52	0.27	0.420	0.047	0.160
DMI, %BW/d	-	-	2.04	2.27	1.68	1.75	0.06	0.015	<0.01	0.177
ADG, g/d	-	-	1,170	1,077	983	891	80.6	0.266	0.031	0.997
CG,[2] g/d	-	-	736	701	696	592	51.3	0.183	0.171	0.494
G:F	-	-	0.17	0.14	0.13	0.12	0.01	0.080	<0.01	0.344
Carcass FE	-	-	0.11	0.10	0.09	0.08	0.01	0.074	<0.01	0.810
Glucose,[3] mg/dL	-	-	92.6	94.4	87.1	91.5	3.92	0.449	0.310	0.753
Glucose AUC[4]	-	-	16,997	17,287	16,266	17,000	955	0.599	0.607	0.817

[1]SBW = shrunk body weight; EBW = empty body weight; VF = visceral fat;

[2]CG = carcass gain. Cattle harvested at 280 and 380 were used to estimate initial carcass weight to harvest at 380 and 480, respectively.

[3]Baseline whole blood glucose concentration.

[4]Area under the curve post intravenous glucose infusion (200mg/kg of BW).

[5]B-280 = bull harvested at 280 kg of BW; S-280 = steer harvested at 280 kg of BW; B-380 = bull harvested at 380 kg of BW; S-380 = steer harvested at 380 kg of BW; B-480 = bull harvested at 480 kg of BW; S-480 = steer harvested at 480 kg of BW.

[6]S = main effects of sex condition; W = main effect of weight at harvest; S*W = sex condition by weight at harvest interaction effect.

[abc]Least squares means without common superscript differ by Tukey test ($P < 0.05$).

Table 4. Carcass physical and chemical composition of bulls and steers harvested at 280, 380, and 480 kg of BW.

Item	Treatments[1]							*P*-value[2]		
	B-280	S-280	B-380	S-380	B-480	S-480	SEM	S	W	S*W
Carcass physical composition										
Lean, kg	106	103	151	147	201	178	5.04	0.021	<0.01	0.090
Bone, kg	34.2[a]	32.8[a]	42.7[b]	40.2[b]	54.6[a]	44.2[b]	1.55	0.001	<0.01	<0.01
Fat, kg	15.7	16.9	39.2	38.3	53.2	59.2	2.45	0.293	<0.01	0.311
Lean:bone, kg/kg	3.1	3.1	3.5	3.6	3.7	4.1	0.10	0.053	<0.01	0.219
Lean gain,[3] g/d	-	-	442	455	463	328	40.9	0.153	0.205	0.085
Bone gain,[3] g/d	-	-	88.2	77.4	111.5	50.8	14.6	0.024	0.908	0.103
Fat gain,[3] g/d	-	-	209[a]	190[a]	120[b]	217[a]	21.1	0.073	0.162	0.011
Carcass chemical composition										
Protein, kg	28.9	28.8	42.6	40.1	55.2	48.2	1.76	0.013	<0.01	0.072
EE, kg	20.6[d]	18.7[d]	43.6[c]	41.6[c]	58.7[b]	67.8[a]	2.99	0.389	<0.01	0.039
Water, kg	94.2	94.0	133.4	130.4	171.2	154.2	4.85	0.049	<0.01	0.088
Protein gain,[3] g/d	-	-	121	109	114	83.3	11.2	0.066	0.179	0.412
EE,[3] g/d	-	-	205[b]	223[ab]	124[c]	267[a]	20.3	0.001	0.376	<0.01
Water gain,[3] g/d	-	-	382	372	342	242	28.8	0.071	<0.01	0.132

[1]B-280 = bull harvested at 280 kg of BW; S-280 = steer harvested at 280 kg of BW; B-380 = bull harvested at 380 kg of BW; S-380 = steer harvested at 380 kg of BW; B-480 = bull harvested at 480 kg of BW; S-480 = steer harvested at 480 kg of BW.

[2]S = main effects of sex condition; W = main effect of weight at harvest; S*W = sex condition by weight at harvest interaction effect.

[3]Cattle harvested at 280 and 380 were used to estimate initial carcass composition to harvest at 380 and 480, respectively.

[abc]Least squares means without common superscript differ by Tukey test ($P < 0.05$).

Table 5. Normalized gene expression in the LD of Nellore bulls and steers harvested at 280, 380, and 480 kg of BW.

Gene[1]	Treatments[2]							*P*-value[3]		
	B-280	S-280	B-380	S-380	B-480	S-480	SEM	S	W	S*W
Glucose/Glycogen metabolism										
GSK3B	1.00	1.83	0.97	1.94	1.80	1.20	0.50	0.148	0.941	0.158
PYGM	1.00	1.12	0.71	1.24	1.01	0.66	0.48	0.686	0.549	0.354
PGM1	1.00	1.41	0.64	1.18	1.36	0.88	0.65	0.507	0.610	0.510
GLUT4	1.00	1.06	0.72	0.86	0.95	0.51	0.49	0.512	0.262	0.291
Adipogenesis and lipid metabolism										
PPARy	1.00	1.26	0.61	1.24	0.97	0.64	0.55	0.430	0.394	0.243
SLC27a1	1.00	0.87	0.82	1.26	1.24	0.67	0.51	0.596	0.903	0.450
LPL	1.00	1.60	1.01	1.71	1.67	0.92	0.55	0.542	0.978	0.338
LIPE	1.00	1.56	1.13	1.77	2.29	1.41	0.53	0.518	0.374	0.263
ACACA	1.00	1.71	0.81	1.27	0.89	1.03	0.53	0.086	0.458	0.394
Muscle protein turnover										
MSTN	1.00	1.29	1.08	2.78	2.12	1.72	0.77	0.289	0.353	0.356
IGF1R	1.00^{c}	1.71^{abc}	1.31^{bc}	2.73^{a}	2.41^{ab}	1.32^{bc}	0.48	0.252	0.258	0.035
CASP3	1.00	1.06	1.24	1.28	1.53	1.31	0.57	0.923	0.518	0.908
SERPINA3-6	1.00	1.03	0.75	0.62	0.30	0.18	1.34	0.692	0.083	0.364
FBXO32	1.00^{c}	1.87^{bc}	1.73^{bc}	4.01^{a}	3.50^{ab}	2.25^{ab}	0.55	0.126	0.018	0.008

[1]*GSK3B* = glycogen synthase kinase 3 beta; *PYGM* = glycogen phosphorylase; *PGM1* = phosphoglucomutase 1; *GLUT4* = insulin-regulated glucose transporter; *PPARγ* = peroxisome proliferator activated-receptor gamma; *SLC27A1* = fatty acid transporter, member 1; *LPL* = lipoprotein lipase; *LIPE* = lipase, hormone-sensitive; *ACACA* = acetyl-CoA carboxylase alpha; *MSTN* = myostatin; *IGF1R* = insulin like growth factor 1 receptor; *CASP3* = caspase 3; *SERPINA3-6* = serpin A3-6; *FBXO32* = F-box protein 32 (Atrogin-1).

[2]B-280 = bull harvested at 280 kg of BW; S-280 = steer harvested at 280 kg of BW; B-380 = bull harvested at 380 kg of BW; S-380 = steer harvested at 380 kg of BW; B-480 = bull harvested at 480 kg of BW; S-480 = steer harvested at 480 kg of BW.

[3]S = main effects of sex condition; W = main effect of weight at harvest; S*W = sex condition by weight at harvest interaction effect.

[abc]Least squares means without common superscript differ by Tukey test ($P < 0.05$).

Figure captions:

Figure 1. Carcass protein fractional accretion rate (FAR) of Nellore bulls and steers from 280 to 380 kg of BW, and from 380 to 480 kg of BW. Error bar represents SEM. NS = Bulls had similar FAR than steers ($P > 0.05$).

Figure 2. Whole blood glucose concentration prior (-5, and 0 min) and post intravenous glucose infusion (200 mg/kg of BW) of Nellore bull and steer. Error bar represents SEM.

Figure 3. Whole blood glucose concentration prior (-5, and 0 min) and post intravenous glucose infusion (200 mg/kg of BW) of Nellore cattle weighting 380 and 480 kg of BW. Error bar represents SEM.

Figure 1.

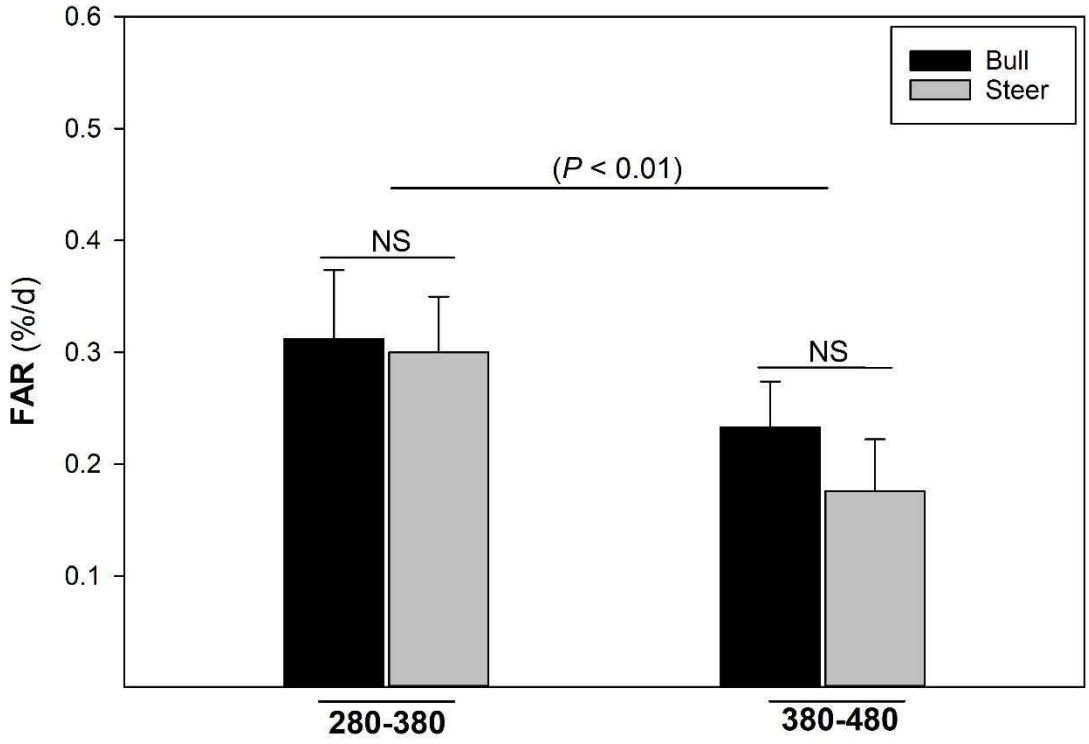


Figure 2.

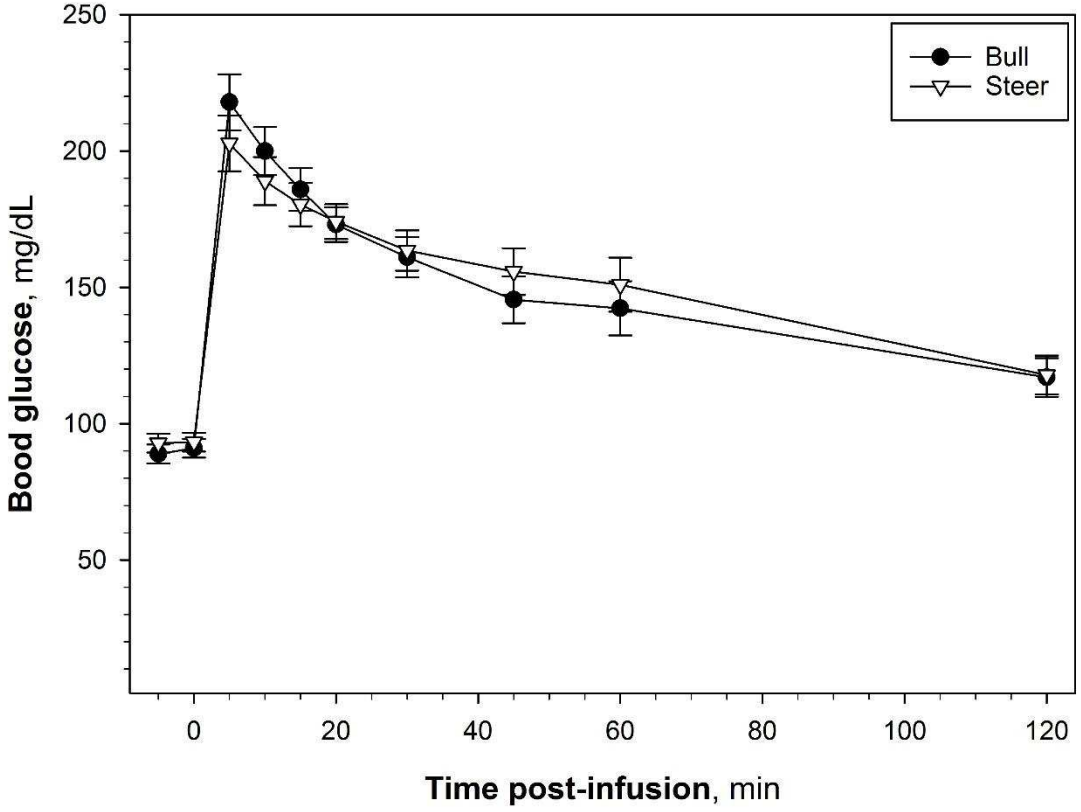
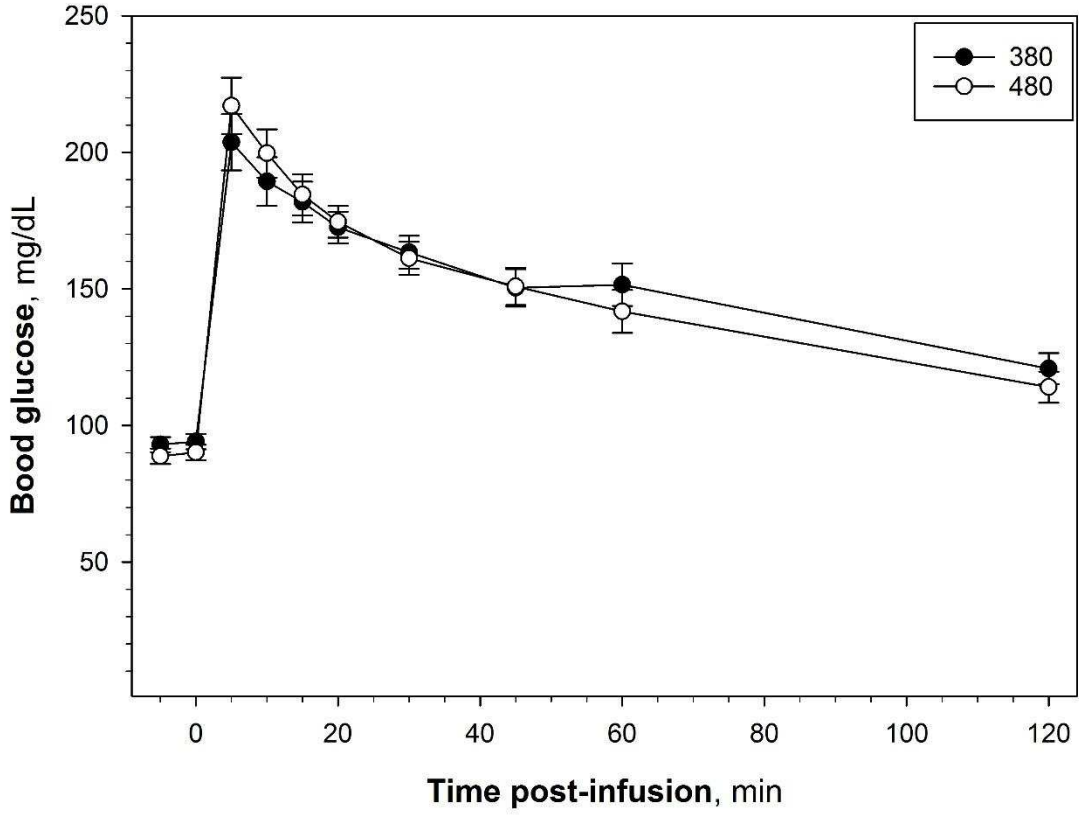

Bull
Steer
Bood glucose, mg/dL
250
200
150
100
50
0
20
40
60
80
100
120
Time post-infusion, min

Figure 3.


250
200
150
100
50
Bood glucose, mg/dL
0
20
40
60
80
100
120
Time post-infusion, min
380
480

Chapter III: Castration and body weight affecting the proteomic and phosphoproteomic profiles of *longissimus* muscle of Nellore cattle (*Bos taurus indicus*)

Manuscript prepared following the PLoS ONE journal guidelines

Short title: Muscle proteome and phosphoproteome as affected by castration and body weight

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Abstract

Skeletal muscle accounts for about 40% of cattle's body weight, presenting a high metabolic and economic significance. Castration and slaughter weight impact carcass composition. Muscle growth rate decreases as animal approach their physiological maturity and castration negatively impact muscle growth but enhance fat deposition, nonetheless the molecular mechanisms are not fully elucidated. Therefore, the aim of this study was to compare the proteome and phosphoproteome of Nellore bulls and steers during different growth stages. Thirty-six Nellore calves averaging 256.1 ±3.05 kg of body weight were used, and half were surgically castrated one-week prior to weaning. Calves were fed the same diet after weaning and then six bulls and six steers were randomly selected and harvested when their average body weight reached 280, 380, or 480 kg. *Longissimus* muscle was sampled just after stunning for proteome analysis. Extracted muscle protein was separated in a 2D-PAGE and stained sequentially with Pro-Q Diamond and Colloidal Coomassie. Afterward, a comparative analysis of protein profile was performed, and differentially abundant protein spots were excised for protein identification by MALDI-TOF/TOF. Castration affected ($P < 0.05$) abundance of 6 phosphoproteins and 10 protein spots, while body weight affected ($P < 0.05$) abundance of 34 phosphoproteins and 29 protein spots. Castration decreased ($P < 0.05$) the abundance of two glycolytic enzymes of the energy-yielding phase, suggesting that glycolysis pathway enhanced glycerol-3P supply for a greater fat deposition on steers. Regarding the growth stage, despite the structural proteins myosin regulatory light chain 2, and actin alpha 1, most of identified proteins were related to energy metabolism, including glycogen metabolism, glycolysis, oxidative phosphorylation, creatine-phosphate metabolism, and

cytosolic NADH metabolism. These results suggest that decreasing muscle growth rate decreases muscle glycolysis and ATP generation.

Introduction

The skeletal muscle tissue usually represents around 37% of cattle body weight [1], 44% of the lean pig body weight [2], and around 34% of human body weight [3]. Despite other organs with higher specific metabolic rate than muscle (e.g., brain, liver, heart, and kidney), the greater muscle mass turns this tissue the body component with the highest energy demand and the greater proportion of whole body protein [4].

Regarding livestock production, muscle is the most economically relevant tissue, since constitutes the major source of meat. Muscle growth rate varies among animal's development, increasing from birth to puberty and then decreasing until the maturity [5], while fat deposition rate increases as muscle growth rate decreases [6]. Endogenous steroids hormones like testosterone can also stimulate muscle growth [4]. However, despite the greater growth rate and carcass weight typically reported for bulls, there is an increased frequency of undesirable carcass and meat traits such as insufficient subcutaneous fat [7], and dark-cuts [8] in bulls' carcasses compared with steers, particularly at heavier slaughter weights [9]. Although the sex condition effect on animal growth and carcass quality traits are well described, there are only few studies evaluating cattle's muscle proteome changes by castration [10,11] and none evaluated the dynamic of these changes throughout the growth. At the current study we evaluated the difference on proteome and phosphoproteome of Nellore cattle harvested at 280, 380, and 480 kg of BW, which represent around 60, 80, and 100% of the reported weight at maturity for this breed [6].

The two-dimensional gel electrophoresis (2D-PAGE) is a proteomic approach that allows to obtain a snapshot of the functional molecules in a tissue under some experimental situation [12]. Proteomic have been used to investigate the global protein expression changes by muscle hypertrophy [13], feed efficiency [14], production system [15], and meat quality of the different species [16], and to identify some potential biomarkers to increase animal efficiency and meat quality [16–18]. Protein phosphorylation is one of the most important post-translational modifications, enriching the information about protein modulation. Therefore, the aim of the present study was to evaluate the differential muscle proteome and phosphoproteome profiles of Nellore bulls and steers at different growth stages. This exploratory study may support a better understanding of pathways related to muscle growth and intramuscular fat deposition.

Materials and methods

All the procedures related to animal handling were reviewed and approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa (CEUAP-UFV, protocol # 035/2015).

Animal housing and management

Thirty-six male Nellore (*Bos taurus indicus*) calves were used. The calves were intensively raised on tropical pasture and supplemented at 0.7% of body weight with concentrate. Half of calves were randomly selected to be surgically castrated one week before weaning. At weaning, the Nellore calves averaged 256.1 ±3.05 kg of body weight (BW) and 8.2 ±0.07 months old.

Post-weaning calves (young bulls and steers) were ear tagged, dewormed and fed in feedlot with the same diet containing 138.7 2g/kg of crude protein and 13.95 MJ/kg of digestible energy. The diet was formulated considering the average nutrient requirements of bulls and steers performing 1.4 kg/day, according to BR-CORTE system [19]. The diet was provided twice a day, as a total mixed ration *ad libitum.*

The animal's weight was monitored daily by an automatic scale system (WD1000, Intergado Ltda, Contagem, Brazil). Six calves from each sex condition were randomly assigned to be harvested when the average shrunk body weight (SBW) reached 280, 380 or 480 kg. It is expected that Nellore cattle reach their physiological maturity at 480 kg of SBW [6]. Therefore, the body weights of 380 and 280 kg represent 80% and 60% of the weight at maturity, respectively.

Harvest and muscle sampling

Once the average body weight reached the endpoints of 280, 380 and 480 kg, six bulls and six steers were harvested after 16 h of feed fasting with free access to water. The procedures during the harvest followed humane slaughter practices and the Brazilian standards of sanitary regulation for animal products [20]. The *longissimus* muscle samples were obtained from the right carcass side at the 13th rib, within 30 min after stunning and blooding. The *longissimus* samples were trimmed for epimysium and subcutaneous fat, minced, and then snap frozen by immersion into liquid nitrogen. Minced *longissimus* samples were ground using a mortar pestle with liquid nitrogen, transferred into cryovials, and then stored at -80˚C until protein extraction.

Protein extraction

Fifty milligrams of pulverized muscle sample were placed into a test tube containing 1mL of cold extraction buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% 3-10 IPG-buffer, 40 mM DTT, 0.5mM Phenylmethanesulfonyl fluoride, and 0.5mM Benzamidine), and then homogenized for 15 s using a Homogenizer (LabGEN 125, Cole-Parmer, Bunker Hill, IL, USA) at 9,500 rpm. The homogenate was kept in ice bath for 15 s and then centrifuged at 20,200 g for 30 min at 4˚C. After centrifugation, the supernatant was collected, and stored at -80˚C. The protein concentration of each sample was determined by the Bradford protein method [21] using premixed reagents (BioRad, Hercules, CA, USA).

Two-dimensional gel electrophoresis

The first dimension was carried out on immobilized pH gradient (IPG) strips of pH 3-10, and 24 cm long (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Strips were rehydrated for 16 h with 450 µL of solution containing 1,500 µg of extracted protein, DeStreak Rehydration Solution (GE Healthcare Bio-Sciences), and 3% pH 3-10 IPG-buffer. The isoelectric focusing (IEF) of rehydrated strips was performed using a Ettan IPGphor 3 System (GE Healthcare Bio-Sciences) at 20˚C following the 5-steps program: 200 V for 2 h, 500 V for 1 h, voltage linear increasing to 1,000 V to reach 800 V/h, voltage linear increasing to 10,000 V to reach 16,500 V/h, hold 10,000 V to reach 27,500 V/h. The current limit was 50 µA per strip. After the IEF, strips were equilibrated for 20 min in 5 mL of equilibration buffer solution (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, and 0.002% bromophenol blue) containing 1% DTT. Afterwards, strips were equilibrated in the dark room for 20 min in 5 mL of equilibration buffer solution containing 2.5% iodoacetamide. The second-dimension electrophoresis was performed in a 12.5% sodium dodecyl sulfate polyacrylamide gel (25.5 × 19.6 cm, and 1 mm thick)

using an Ethan DALTsix unit (GE Healthcare Bio-Sciences). Strips and the weight calibration kit (GE Healthcare Bio-Sciences) were placed on top of the SDS-PAGE and sealed with melted 0.5% agarose solution containing 0.002% of bromophenol blue. Electrophoresis was performed at 8˚C using two steps, first for 45 min with 20 mA/gel and 80 V, and then with 40 mA/gel and 500 V until the dye reached the bottom of the gel (about 6 h).

Gel staining and scanning

After second dimension, gels were washed in double-distilled water and fixed overnight using fresh solution containing 10% of acetic acid, and 50% methanol. Later, gels were washed two times with deionized-distilled water and stained with Pro-Q Diamond dye (Invitrogen Molecular Probes, Eugene, OR, USA) for phosphoproteins detection, according the modified protocol suggested by Agrawal and Thelen [22]. Gels images at 300 dpi were obtained using a Fuji Film scanner (Fuji Film 5100 FLA, Fuji Medical Systems, Hanover Park, IL, USA) in fluorescent mode (excitation 532 nm, emission 580 nm).

After scanning for phosphoprotein, gels were stained for total protein using Colloidal Coomassie Blue solution (8% ammonium sulfate (w/v), 0.8% phosphoric acid (v/v), 0.08% Coomassie blue G-250 (v/v), and 20% methanol (v/v)) for 72 h. Afterward, gels were washed three times with distilled water for 30 min and once overnight with 1% acetic acid (v/v). Gel imagens for total protein were obtained using a pre-calibrated Image Scanner III (GE Healthcare Bio-Sciences) with resolution of 300 dpi. Gels were then stored into 2% acetic acid (v/v) at 20˚C until spots excision.

Image analyses and differential abundance detecting

A total of 36 gels were made (one per animal) and stained with two dyes totalizing 72 gel imagens. To avoid technical bias, all analytical steps was performed using a set of samples balanced for treatments. Digitalized gel images were analyzed using Image Master 2D Platinum 7.0 software (GE Healthcare Bio-Sciences). Corresponding spots were detected, matched and individually checked within gels of each dye. The normalized spot volume was obtained for each gel and exported. Data was then analyzed as a completely randomized design following a 2 (bull and steer) x 3 (280, 380, and 480 kg of body weight) factorial arrangement of treatments, using the GLM procedure of SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA). Once detected significant effect ($P < 0.05$) by ANOVA for sex condition, weight at harvest or interaction, treatments least squared means were compared by Tukey's test.

Protein identification

Images from a reference gel stained with both dyes (Pro-Q and Coomassie) were overlapped allowing to identify at the Coomassie image the corresponding selected spots at the Pro-Q Diamond image. Differentially abundant protein spots were manually excised following the Coomassie blue image. Excised spots were then in-gel digested using trypsin (Trypsin V511, Promega, Madison, WI, USA) as described by Shevchenko [23]. Tryptic peptides obtained were then recovered, dried in a SpeedVac centrifuge (AG-22331, Eppendorf, Germany) and then resuspended with α-cyano-4-hydroxycinnamic acid matrix solution (Bruker Daltonics, Germany). Afterward, 1 µL of the peptides solution was placed on a MALDI target plate. Peptide mass spectra was obtained using a

matrix assisted laser desorption/ionization time-of-flight mass spectrometer (Ultraflex III MALDI- TOF/TOF system, Bruker Daltonics, Bremen, Germany). The MS/MS peaks list was generated by Flexcontrol software (version 3.3, Bruker Daltonics, Germany).

Protein was then identified by MASCOT software (version 2.4.0, Matrix Science, London, UK) following the restrictions: enzymatic tryptic digestion; maximum one missed cleavage; maximum mass error of 0.2 Da for MS and 0.5 Da for MS/MS mode, cysteine carbamidomethylation as a fixed modification, and methionine oxidation as a variable modification. The Bovidae family information in the UniProt database was used. Protein identification by MASCOT was then validated using SCAFFOLD software (version 3.6.4, Proteome Software, Portland, OR, USA). The Protein Prophet algorithm was used for protein validation [24]. The criteria used for the validation was: at least one unique peptide, with identification probability greater than 90%.

Protein–protein interaction analysis

All identified differentially abundant protein spots from both dyes (i.e., Colloidal Coomassie and Pro-Q Diamond) were uploaded together in the web tool STRING 10.5 (http://string- db.org) for protein-protein interaction analysis. The network was then generated using the following restrictions: *Bos taurus* as reference organism; textmining, experiments, database, and co-expression as active interaction source; 0.90 as the minimum required interaction score; and no more than ten interactions for both shells.

Results and discussion

Animal growth

Throughout the experiment, no clinic symptoms were reported for digestive disorder or any illness. After weaning, calves were fed during 33, 133, and 230 days to achieve the target body weights (280, 380, and 480 kg).

Bulls usually have greater average daily gain than steers, producing heavier and leaner carcass at similar age [8]. However, the difference between bull and steer performance is greater as harvest age increases [25]. In the current study, growth rate did not differ between the sex conditions ($P = 0.26$), even though bulls average daily gain (ADG) was 9% greater than steers (1.08 vs. 0.98 kg/d). However, this small difference was enough to increase ($P < 0.05$) bull's body weight at 480 kg harvest (Fig 1). The rib eye area (REA) was similar ($P = 0.15$) for both sex condition, and steer carcass had 27% greater ($P = 0.047$) back fat thickness than bull carcass. The intramuscular fat at 480 kg of BW was higher in steer steaks than bull (4.04 vs. 6.13% of fresh beef).

Fig 1. Body weight of Nellore bulls and steers throughout the experiment. Black arrows represent the harvests at 280, 380, and 480 kg of averaging body weight.

It has been shown that the performance differences between bull and steer are more evident after puberty [26,27].

Longissimus proteome and phosphoproteome

The 24-cm long strip with IPG of pH 3-10 allowed us to detect 391 protein spots at gel images stained with Colloidal Coomassie, and 43 spots were differentially abundant across treatments (S1 Fig). Among them, 7 spots were affected by interaction effect of sex condition by body weight, while 29 were affected by body weight, and 10 were affected

by sex condition. However, due to small protein content of some spots, only 19 spots were excised within nine protein spots were identified by MALDI-TOF/TOF (Fig 2, Table 1, and S1 Table).

Fig 2. A representative 12.5% SDS-PAGE (IPG 3-10, 24 cm) of Nellore cattle *longissimus* muscle stained with Colloidal Coomassie for total protein. Numbers indicate the identified protein spots differently abundant among treatments.

Table 1. Differentially abundant proteins in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight.

					Theoretical		Experimental	
Spot	**Protein name**	**Abbr.[a]**	**UniProt[b]**	**Prob.[c]**	Mass	pI	Mass	pI
1	Myosin regulatory light chain 2, skeletal muscle isoform	MLCPF	Q0P571	95	19.1	4.91	19.4	4.30
3	Actin, alpha 1, skeletal muscle	ACTA1	A4IFM8	100	42.3	5.23	40.0	5.45
2	Malate dehydrogenase, cytoplasmic	MDH1	Q3T145	95	36.7	6.16	36.8	6.25
4	Fructose-bisphosphate aldolase	ALDOA	A6QLL8	100	39.9	8.45	42.2	9.40
6	Pyruvate kinase	PKM2	L8I1M9	100	63.6	7.26	60.7	7.90
9	Pyruvate kinase	PKM2	L8I1M9	100	63.6	7.26	60.5	8.36
8	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	P10096	98	36.1	8.50	39.1	8.04
5	Cytochrome b-c1 complex subunit 1, mitochondrial	UQCRC1	G1K1X0	99	53.5	5.94	50.4	5.60
7	ATP synthase subunit beta, mitochondrial	ATP5B	P00829	100	56.2	5.15	54.1	4.83

[a] Protein name abbreviation.
[b] Accession number at UniProt database.
[c] Protein identification probability (%) by Scaffold software.

Regarding the gel images stained with Pro-Q Diamond a total of 456 spots were detected, and 52 spots were differentially abundant across treatments (S2 Fig). A total of 12

phosphoproteins spots were affected by interaction of sex condition by body weight, while 34 spots were affected by body weight, and only 6 phosphoproteins spots were affected by sex condition. A total of 18 spots were excised then submitted to protein identification by MALDI-TOF/TOF, and eight differentially abundant protein spots were identified (Fig 3, Table 2, and S2 Table).

Fig 3. A representative 12.5% SDS-PAGE (IPG 3-10, 24 cm) of Nellore cattle *longissimus* muscle stained with Pro-Q Diamond for phosphoprotein. Numbers indicate the identified phosphoprotein spots differently abundant among treatments.

Table 2. Differentially abundant phosphoproteins in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight.

					Theoretical		Experimental	
Spot	**Protein name**	**Abbr.[a]**	**UniProt[b]**	**Prob.[c]**	Mass	pI	Mass	pI
23	Actin, alpha 1, skeletal muscle	ACTA1	A4IFM8	100	42.3	5.23	45.8	4.85
21	Triosephosphate isomerase	TPI1	Q5E956	95	26.9	6.45	27.9	6.32
24	Triosephosphate isomerase	TPI1	Q5E956	100	26.9	6.45	27.4	6.95
22	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	P10096	100	36.1	8.50	39.1	9.47
25	Phosphoglucomutase-1	PGM1	Q08DP0	100	61.8	6.36	65.0	6.30
26	Creatine kinase M-type	CKM	Q9XSC6	100	43.2	6.63	42.6	7.13
27	Alpha-1,4 glucan phosphorylase	PYGM	B0JYK6	100	97.7	6.67	103.3	7.09
28	Fructose-bisphosphate aldolase	ALDOA	A6QLL8	100	39.9	8.45	42.8	7.81

[a] Protein name abbreviation.
[b] Accession number at UniProt database.
[c] Protein identification probability (%) by Scaffold software.

Within all detected spots, around 66% were found between the pH 4-7 (S3 Fig). Regarding the molecular mass, around 64% of the spots were found from 20 to 60 kDa. Nevertheless, even though a great proportion of spots are at these pH and molecular mass ranges some

important spots detected as differentially abundant at the present study were out or at the limit of this window (e.g., spots 4, 6, 9, 22, 25, 27, and 28).

Interaction effects were found ($P < 0.05$) for total protein abundance of fructose-bisphosphate aldolase (ALDOA), and malate dehydrogenase cytoplasmic (MDH1). Regarding phosphoprotein abundance, only one interaction effect was found ($P < 0.05$) for Alpha-1,4 glucan phosphorylase (PYGM) abundance. Therefore, data is shown and discussed separately by sex condition and weight at harvest.

Effect of sex condition on proteome and phosphoproteome profile

Castration affected the abundance of ten spots of total protein and six phosphoproteins, while animal's body weight affected twenty-nine and thirty-four, respectively. These lower proteome changes by sex condition in relation to body weight are in accordance with the greater effect of body weight rather than sex condition on animal performance. Although bulls are frequently reported to have greater performance than steers [8], the difference between these two sex conditions increases as cattle reach their weight at maturity [9]. At a companion paper using the same animals of the current study [Silva, 2018], castration markedly enhanced fat deposition, regardless of the body location (e.g., subcutaneous fat, internal fat, and intramuscular fat). Differential proteome analysis indicated that castration decreased ($P < 0.05$) the *longissimus* muscle abundance of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and pyruvate kinase (PKM2), two important enzymes of the energy-yielding step of glycose metabolism (Table 3).

Table 3. Normalized spots value of proteins abundantly different in *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight.

		Sex condition (S)			Body Weight (W)				*P*-value[3]		
Spot	Protein[1]	Steer	Bull	SEM[2]	280	380	480	SEM[2]	S	W	S*W
1	MYLPF	0.137	0.150	0.011	0.173[a]	0.121[b]	0.135[ab]	0.014	0.412	0.034	0.962
3	ACTA1	0.055	0.062	0.003	0.059[ab]	0.051[b]	0.067[a]	0.004	0.150	0.034	0.522
2	MDH1	0.107	0.115	0.004	0.114	0.107	0.112	0.005	0.194	0.573	0.022
4	ALDOA	1.77	1.73	0.101	1.76	1.74	1.75	0.123	0.776	0.993	0.046
6	PKM2	0.698	0.643	0.039	0.792[a]	0.638[b]	0.581[b]	0.048	0.327	0.012	0.820
9	PKM2	0.231[b]	0.276[a]	0.014	0.250	0.233	0.278	0.017	0.028	0.185	0.386
8	GAPDH	0.320[b]	0.414[a]	0.026	0.328	0.363	0.410	0.032	0.015	0.199	0.518
5	UQCRC1	0.054	0.058	0.005	0.071[a]	0.050[b]	0.047[b]	0.006	0.563	0.026	0.483
7	ATP5B	0.143	0.147	0.007	0.163[a]	0.135[b]	0.135[b]	0.008	0.690	0.032	0.651

[1]Myosin regulatory light chain 2, skeletal muscle isoform (MYLP); Malate dehydrogenase, cytoplasmic (MDH1); Actin, alpha 1, skeletal muscle (ACTA1); Fructose-bisphosphate aldolase (ALDOA); Cytochrome b-c1 complex subunit 1, mitochondrial (UQCRC1); Pyruvate kinase (PKM2); ATP synthase subunit beta, mitochondrial (ATP5B); Glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
[2]SEM = Standard error of the mean.
[3]S = main effect of sex condition; W = main effect of body weight at harvest; S*W = interaction effect of sex condition by body weight at harvest.
[abc] Lsmeans of normalized spot value followed by different superscript letter differ ($P < 0.05$) by Tukey's test.

The GAPDH catalyzes the interconversion of glyceraldehyde-3P to 1,3 Bis P-glycerate at glycolysis/gluconeogenesis pathway, generating NADH [28]. Steers had the *longissimus* muscle abundance of GAPDH reduced by 23% compared with bulls. The GAPDH is frequently identified in proteomic studies of domestic animals, and its increased abundance have been related to fast pH decline in post mortem muscle [29], higher proportion of fast-twitch glycolytic muscle fibers [18], and more tender meat [30]. However, at the current study the carcass pH declines throughout 24 h postmortem was similar between bulls and steers, and tenderness was greater for steers after 14 days of aging [Silva, 2018].

The muscle pyruvate kinase (PKM2) abundance was reduced 16% by castration. Pyruvate kinase is a rate-limiting glycolysis enzyme, catalyzing the transfer of a phosphate from phosphoenolpyruvate to ADP producing pyruvate and ATP [28] and an important kinase mediating the effects of growth signals by directing metabolites derived from glycolysis to either the biosynthesis or energy production pathways [31]. It has been reported that cattle with high genetic merit for carcass weight have greater muscle abundance of PK [32], suggesting that pyruvate kinase is associated to muscle growth control. Bulls usually have greater muscle growth, producing heavier carcasses with higher muscle proportion than steers [7]. At the current study, bulls had heavier carcasses and greater muscle mass than steers [Silva, 2018], supporting the link between muscle growth and PK abundance, probably in order to sustain mitochondrial synthesis of ATP demanded by protein synthesis. Interestingly, sex condition affected spot 9 but not spot 6 of PKM2, which presented similar mass but a lower pI (7.9 versus 8.36), indicating a more phosphorylated isoform. Phosphorylation of PKM2 in response to different growth stimulus has been reported and regulates its protein kinase and pyruvate kinase activities, controlling cell growth and proliferation [31].

At the preparatory phase of glycolysis pathway two ATP molecules are consumed and the glucose is converted into glyceraldehyde-3P and dihydroxyacetone-P [28]. Nevertheless, only the glyceraldehyde-3P can be metabolized to pyruvate at the second phase of glycolysis pathway (energy-yielding phase). Castration decreased the abundance of GAPDH and PK, which catalyzes important steps of the energy-yielding phase. These results suggest that steer's muscle downregulates the production of pyruvate from glyceraldehyde-3-phosphate, shifting to a greater synthesis of dihydroxyacetone-P and

then glycerol-3P likely to support a higher triglyceride synthesis, since the glycerol-3P synthesis is a limiting step [33]. A greater abundance of glycerol-3-phosphate dehydrogenase (G3PD) in pig breed with increased intramuscular fat was reported [34] supporting this hypothesis.

The interaction effect on fructose-bisphosphate aldolase (ALDOA, Fig 4) also suggest greater synthesis of dihydroxyacetone-P, since ALDOA was more abundant in steers than bulls only at 480 kg of body weight, in accordance with the greater intramuscular fat found for steers at 480 kg of BW. In agreement, greater muscle abundance of ALDOA have been found for cattle breed with higher intramuscular fat content [34].

Fig 4. Fructose-bisphosphate aldolase (ALDOA, spot 4) abundance in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight. Error bar represents the standard error of the mean. ^{abc} Lsmeans of normalized spot value followed by different superscript letter differ ($P < 0.05$) by Tukey's test.

The cytosolic form of the enzyme malate dehydrogenase (MDH1) catalyzes the NAD/NADH-dependent interconversion of malate to oxaloacetate [30] and is a key regulator of the malate/aspartate shuttle, transporting NADH from cytosol to the mitochondrion [35]. The interaction effect found for cytoplasmic MDH1 abundance in the *longissimus* muscle (Fig 5) demonstrate that steers has less abundance of MDH1 than bulls only at 480 kg of BW, where the fat synthesis was the highest. This result along with decreased abundance of GAPDH and PKM2 suggests that castration reduces mitochondrial energy generation from glucose, since castration decreased abundance of enzymes related to pyruvate synthesis and NADH transport to mitochondrion.

Fig 5. Malate dehydrogenase cytoplasmic (MDH1, spot 2) abundance in the *longissimus* muscle of Nellore bulls and steers at 280, 380, and 480 kg of body weight. Error bar represents the standard error of the mean. [abc] Lsmeans of normalized spot value followed by different superscript letter differ ($P < 0.05$) by Tukey's test.

The alpha-1,4 glucan phosphorylase (PYGM) is also known as glycogen phosphorylase (GP), and catalyzes the first step for utilization of glucose from muscle glycogen, and its activity is controlled by phosphorylation. The non-phosphorylated form of glycogen phosphorylase (GPb) is inactive, while the phosphorylated form (GPa) is active [36]. An interaction effect of sex condition by body weight was found ($P < 0.05$) for muscle abundance of PYGM, and steers harvested at 380 kg had greater abundance than all other treatments (Table 4, and Fig 6). Catecholamines released in response to animal stress, quickly stimulate the phosphorylation of PYGM inducing a fast glycogen breakdown [36]. Even though bulls and steers were managed under the same pre-slaughter conditions the steers at 380 kg may had greater pre-slaughter stress inducing PYGM phosphorylation and then interaction effect observed. Nonetheless, steers had higher ($P = 0.052$) abundance of phosphorylated PGMY than bulls, suggesting higher glycolytic activity in steers. Supporting our findings, the glycolytic enzymes enolase 1 and 3 and the fast-twitch myosin heavy chain type IIX were reported to be more abundant in steers than in young bulls [37].

Table 4. Normalized spots value of phosphoproteins abundantly different in *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight.

		Sex condition (S)			Weight at harvest (W)				*P*-value[3]		
Spot	Protein[1]	Steer	Bull	SEM[2]	280	380	480	SEM[2]	S	W	S*W
23	ACTA1	0.396	0.372	0.021	0.315^{b}	0.387^{ab}	0.449^{a}	0.026	0.438	0.004	0.708
21	TPI1	0.155	0.145	0.009	0.123^{b}	0.150^{ab}	0.177^{a}	0.011	0.448	0.005	0.670
24	TPI1	0.044	0.041	0.003	0.036^{b}	0.043^{ab}	0.049^{a}	0.003	0.589	0.033	0.228
22	GAPDH	0.115	0.118	0.012	0.085^{b}	0.147^{a}	0.117^{ab}	0.015	0.869	0.024	0.412
25	PGM1	0.273	0.239	0.015	0.225^{b}	0.248^{ab}	0.296^{a}	0.018	0.115	0.027	0.259
26	CKM	0.128	0.122	0.011	0.099^{b}	0.121^{ab}	0.156^{a}	0.013	0.695	0.017	0.763
27	PYGM	0.094	0.072	0.008	0.069	0.102	0.078	0.009	0.052	0.053	0.020
28	ALDOA	1.13	1.10	0.081	0.993^{b}	1.00^{b}	1.35^{a}	0.099	0.806	0.027	0.597

[1]Triosephosphate isomerase (TPI1); Glyceraldehyde-3-phosphate dehydrogenase (GAPDH); Actin, alpha 1, skeletal muscle (ACTA1); Phosphoglucomutase-1 (PGM1); Creatine kinase M-type (CKM); Alpha-1,4 glucan phosphorylase (PYGM); Fructose-bisphosphate aldolase (ALDOA).
[2]SEM = Standard error of the mean.
[3]S = main effect of sex condition; W = main effect of weight at harvest; S*W = interaction effect of sex condition by weight at harvest.
[abc] Lsmeans of normalized spot value followed by different superscript letter differ ($P < 0.05$) by Tukey's test.

Fig 6. Phosphorylated Alpha-1,4 glucan phosphorylase (PYGM, spot 27) abundance in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight. Error bar represents the standard error of the mean. [abc] Lsmeans of normalized spot value followed by different superscript letter differ ($P < 0.05$) by Tukey's test.

Effect of body weight on proteome and phosphoproteome

Five proteins differentially abundant ($P < 0.05$) among weights at harvest were identified. Among them, two were related to muscle contraction (Table 2). The abundance of skeletal muscle isoform of the myosin regulatory light chain 2 (MYLPF) was higher ($P < 0.05$) in

cattle at 280 kg of BW than at 380 kg. The MYLPF is a component of the thick myofilament and is involved on regulation of muscle contraction. A decreased abundance of MYLPF spot, at a similar gel position observed in this study, was related with tougher beef of Nellore cattle genotyped for undesirable polymorphism on calpastatin gene (UOGCAST) [38], indicating that MYLPF might also be associated with a myofibrillar protein turnover.

The *longissimus* muscle abundance of alpha actin 1 (ACTA1) was greater at cattle with 480 kg than at 380 kg of BW. Greater ACTA1 abundance on heavier cattle has also been reported in Charolais bulls [39] and its abundance could be suggested as marker of maturity. Interestingly, old skeletal muscle also exhibited greater levels of ACTA1 in women [40].

In addition, phosphorylated alpha actin 1 abundance increased as cattle became heavier (Table 3). The spot 3 at the Coomassie stained images was identified as ACTA1, however the small abundance and greater pI and smaller mass than the theoretical suggest that this spot represent the ACTA1 with some modification. Regarding the spot identified as phosphorylated ACTA1 (spot 23), the experimental pI was smaller than the theoretical (4.85 vs. 5.23) as expected for phosphorylated proteins [41]. Greater abundance of phosphorylated ACTA1 has been reported in tougher beef [29,42] and its phosphorylation was linked to the onset of apoptosis [16]. Nellore bulls presented a higher abundance of phosphorylated ACTA1 than Angus bulls [44] and a lower rate of growth and tougher beef, suggesting that phosphorylation of ACTA1 could also be related to muscle protein turnover.

Despite of these two structural proteins (MLCPF, and ACTA1), the stage of growth affected abundance of proteins related to muscle energetic metabolism, including glycogenolysis (PYGM, and PGM1), glycolysis (ALDOA, TPI1, GAPDH, and PKM2), oxidative phosphorylation (ATP5B, and UQCRC1), creatine metabolism (CKM), and malate/oxaloacetate shuttle (MDH1), as depicted by the protein-protein interaction network (Fig 7).

Fig 7. Protein-protein interaction network of the differentially abundant proteins and phosphoproteins in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight. Elaborated using the web tool String 10.5.

Increasing cattle BW from 280 to 380 kg decreased ($P < 0.05$) the abundance of pyruvate kinase (PKM2), cytochrome b-c1 complex subunit 1 mitochondrial (UQCRC1), and ATP synthase subunit beta mitochondrial (ATP5B). Nevertheless, the abundance of these proteins did not change ($P > 0.05$) from 380 to 480 kg of BW.

Reduced PKM2 abundance from 280 to 380 and 480 kg of BW can suggest a decrease on growth stimulation factors, as overexpression of PKM2 activates mammalian target of rapamycin complex 1(mTORC1) signaling by phosphorylating its inhibitor AKT1 substrate 1 (AKT1S1), promoting protein synthesis [43].

The UQCRC1, which is part of the mitochondrial respiratory chain, was more abundant on pig breeds with lesser diameter of muscle fascicles [44] and its gene expression was highly correlated with muscle mass in rats [45]. The ATP5B, a subunit of the ATP

synthase complex, catalyzes the rate-limiting step of ATP generation at the mitochondria of eukaryote cells [46], and its reduced mRNA expression was related to a decrease of mitochondrial biogenesis in pig fetuses [47]. Taken together, the reduction of mitochondrial proteins UQCEC1 and ATP5B indicate a shift toward the anaerobic metabolism in skeletal muscle with advancing of maturity, corroborating with a gradual decrease on muscle oxidative metabolism during postnatal development [48].

Cattle body weight markedly affected ($P < 0.05$) the phosphorylation of enzymes related to glycolysis in the *longissimus* muscle. In general, increasing BW enhances the phosphorylation of glycolytic enzymes (Table 3). Picard et al. found that the carbohydrate metabolism, and more specifically the glycolysis pathway has high importance to muscle growth and regeneration [17]. In addition, the *longissimus* muscle abundance of phosphorylated creatine kinase M-type, an enzyme of the creatine metabolism, increased from 280 to 480 kg of BW.

Increase on BW also enhanced ($P < 0.05$) the abundance of the phosphorylated form of phosphoglucomutase-1 (PGM1), which catalyzes the interconversion of glucose-1P to glucose-6P, an important step during glycogen metabolism [49]. In the *longissimus* muscle of cattle with different tenderness, Anderson et al. found six spots identified as PGM1, and among them five were detected as phosphorylated, suggesting that this enzyme can be multi-phosphorylated at different sites [41]. It has been reported that phosphorylation of PGM1 at threonine 466 by kinase p21-activated kinase 1 (Pak1) enhances its enzymatic activity [49]. In addition, when dephosphorylated, the PGM1 activity decrease [50]. The abundance of this enzyme has been related to increased muscle mass and higher proportion of fast-twitch muscle fiber on cattle [13]. Therefore, the

increase on the abundance of phosphorylated PGM1 as cattle reach maturity corroborate an increase on glycolytic metabolism of skeletal muscle.

The abundance of the phosphorylated form of ALDOA was also increased ($P < 0.05$) when cattle BW increased from 380 to 480 kg. High correlation between ALDOA abundance and muscle ultimate pH have been reported, evidencing the importance of this enzyme activity to muscle phenotype and glycolysis postmortem [51]. Phosphorylated ALDOA [52] was correlated with a higher pH decline post-mortem in pigs [53].

Two of the differentially abundant phosphoproteins spots (i.e., spot 21, and 24) were identified as Triosephosphate isomerase (TPI1). These two spots presented the same pattern, increasing ($P < 0.05$) abundance from 280 to 480 kg of BW (Table 3). The TPI1 is a key enzyme of glycolysis pathway catalyzing the reversible interconversion of dihydroxyacetone phosphate (DHAP) and glucose aldehyde-3-phosphate (G3P) [28]. Only G3P can further proceed the glycolytic pathway, while DHAP can be converted into glycerol-3P supporting triglyceride synthesis as previously highlighted [54]. Phosphorylation decreased the catalytic activity of TPI1 in Hela cells [55]. Therefore, the reduced activity of TPI1 by phosphorylation may result in greater availability of DHAP to be converted to 3-P-glycerol supporting an increased lipid synthesis found in heavier cattle. In agreement, two spots of TPI1 were also differently abundant on heavier Charolais cattle [39].

The abundance of phosphorylated glyceraldehyde-3-phosphate dehydrogenase (GAPDH) increased ($P < 0.05$) from 280 to 380 kg of BW, and then decreased to an intermediary value at 480 kg of BW. It has been proposed that the phosphorylation of GAPDH at the amino acid residues Y94, S98, and T99 increases its enzymatic activity [56]. In meat

animals, the increased abundance of GAPDH phosphorylated at early postmortem may enhance glycolysis and then promote a fast muscle pH decline [52]. Despite of the carcass temperature effect on pH decline, cattle at 480 kg of BW had greater abundance of GAPDH phosphorylated, and an increased carcass acidification compared with cattle at 280 kg.

Creatine kinase M-Type (CKM) is a muscle cytosolic enzyme that catalyzes the reversibly transference of phosphate between ATP and creatine. This reaction is important for ATP regeneration in muscle tissue [57]. It has been shown that obese women have muscle increased CKM activity and that post-translation modification modulates its activity [58]. In addition, CKM phosphorylation inhibited the creatine/phosphocreatine shuttle in cardiac muscle of rat [59]. Therefore, the gradual increasing on CKM phosphorylation as cattle reach the mature body weight indicates a reduction in muscle capacity to regenerate ATP from ADP by the creatine/phosphocreatine shuttle. The pathways of the metabolic enzymes differentially abundant across the treatments are shown at Fig 8.

Fig 8. Pathways of the identified differentially abundant proteins in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight. In red the identified proteins as following: Alpha-1,4 glucan phosphorylase (PYGM), Phosphoglucomutase-1 (PGM1), Fructose-bisphosphate aldolase (ALDOA), Triosephosphate isomerase (TPI1), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Pyruvate kinase (PK), Malate dehydrogenase, cytoplasmic (MDH1), ATP synthase subunit beta, mitochondrial (ATP5B), Cytochrome b-c1 complex subunit 1, mitochondrial (UQCRC1), Creatine kinase M-type (CKM). Pathways adapted from

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways. Underlined proteins abbreviation represents effect of castration by body weight interaction. Open arrows represent total protein downregulation by castration. Solid black arrows represent total protein downregulation in heavier cattle. Solid red arrows represent phosphoprotein upregulation in heavier cattle.

The consistent increase of glycolytic enzymes with advancing of maturity suggest that muscle hypertrophy decreases oxygen availability favoring anaerobic/glycolytic dependent metabolism of skeletal muscle.

Conclusions

In conclusion, castration and body weight affected proteomic and phosphoproteomic profile, and proteins related to muscle energy metabolism were markedly modified. These results help to better clarify which pathways are related to reducing muscle growth and increasing intramuscular fat deposition by castration and during cattle development.

Steers presented decreased muscle abundance of GAPDH and PK, and when heavier than 480 kg have decreased MDH1 and increased ALDOA, suggesting an enhancement on glycerol-3P production from glycolysis to support the greater intramuscular fat synthesis.

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Supporting information

S1 Fig. A representative Coomassie stained gel image of Nellore cattle *longissimus* muscle, showing all 391 detected spots (A), and all 43 spots differentially abundant among the treatments (B).

S2 Fig. A representative Pro-Q Diamond stained gel image of Nellore cattle *longissimus* muscle, showing all 456 detected spots (A), and all 52 spots differentially abundant in the Nellore cattle *longissimus* muscle among the treatments (B).

S3 Fig. Frequency of spot pI (A) and mass (B) from the 2D-PAGE of Nellore *longissimus* muscle stained with Colloidal Coomassie and Pro-Q Diamond.

S1 Table. Descriptive information of the differentially abundant total protein spots in the longissimus muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight. [a]Protein name abbreviation. [b]Protein identification probability by Scaffold. [c]Peptide identification probability by Scaffold.

S2 Table. Descriptive information of the differentially abundant phosphoprotein spots in the longissimus muscle of Nellore bulls and steers harvested at 280, 380, and

480 kg of body weight. [a]Protein name abbreviation. [b]Protein identification probability by Scaffold. [c]Peptide identification probability by Scaffold.

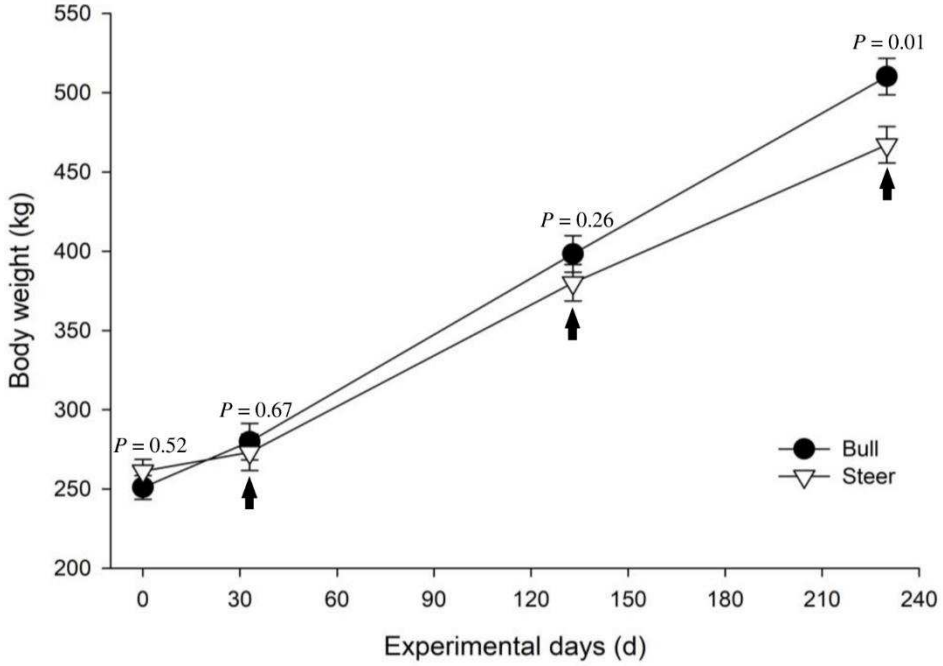


Fig 1. Body weight of Nellore bulls and steers throughout the experiment. Black arrows represent the harvests at 280, 380, and 480 kg of averaging body weight.

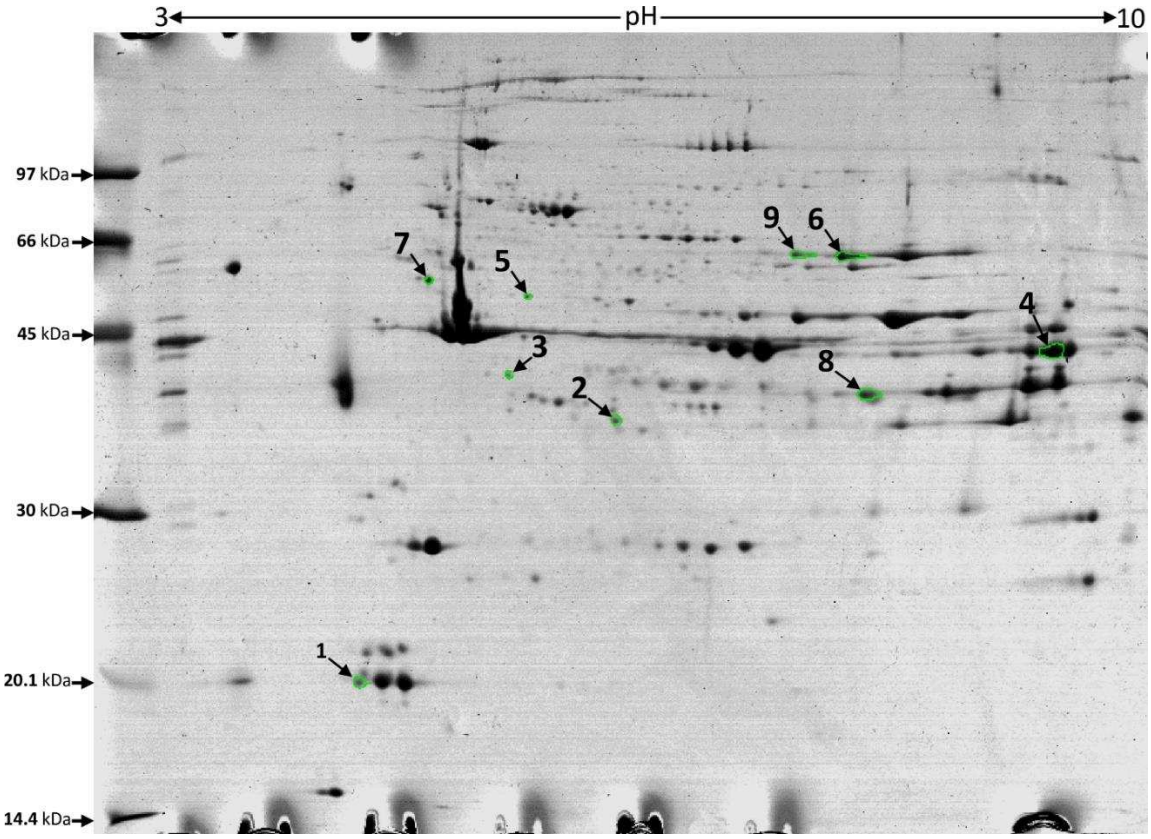


Fig 2. A representative 12.5% SDS-PAGE (IPG 3-10, 24 cm) of Nellore cattle *longissimus* muscle stained with Colloidal Coomassie for total protein. Numbers indicate the identified protein spots differently abundant among treatments.

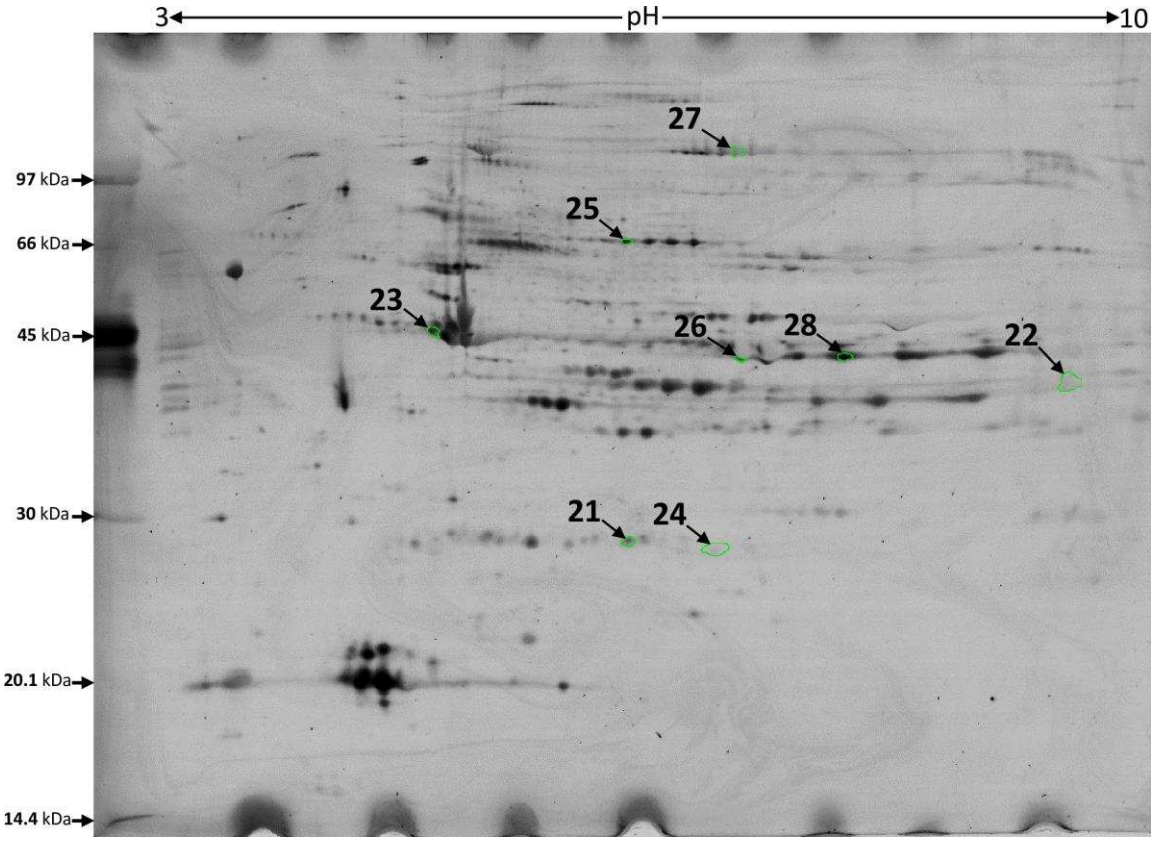


Fig 3. A representative 12.5% SDS-PAGE (IPG 3-10, 24 cm) of Nellore cattle *longissimus* muscle stained with Pro-Q Diamond for phosphoprotein. Numbers indicate the identified phosphoprotein spots differently abundant among treatments.

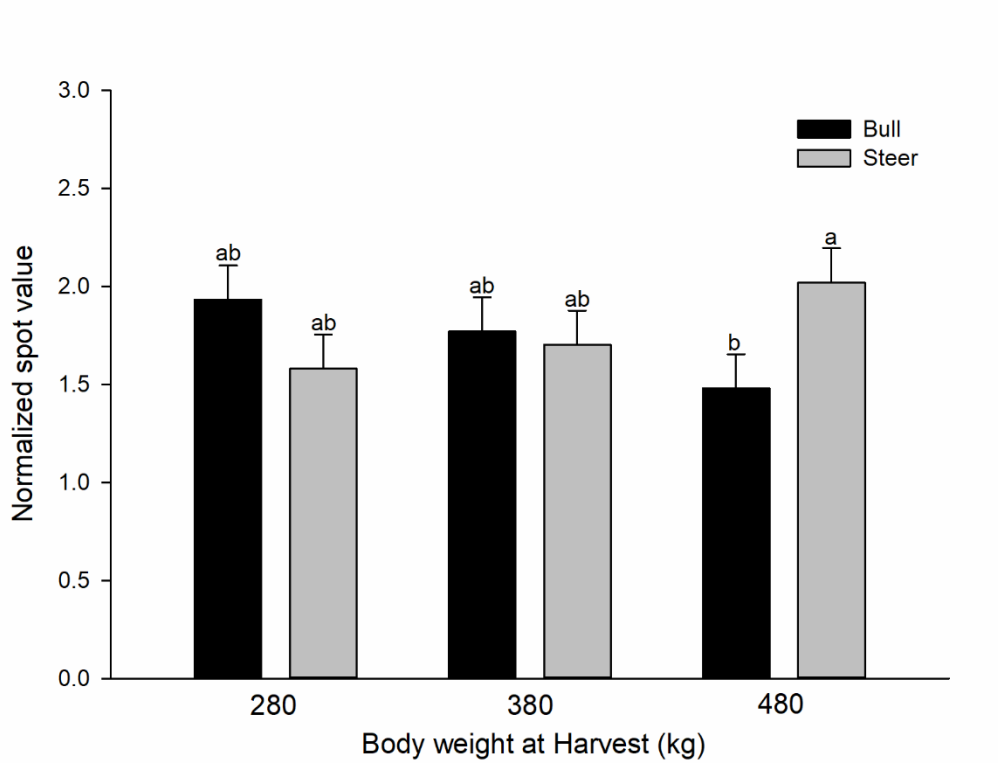


Fig 4. Fructose-bisphosphate aldolase (ALDOA, spot 4) abundance in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight.

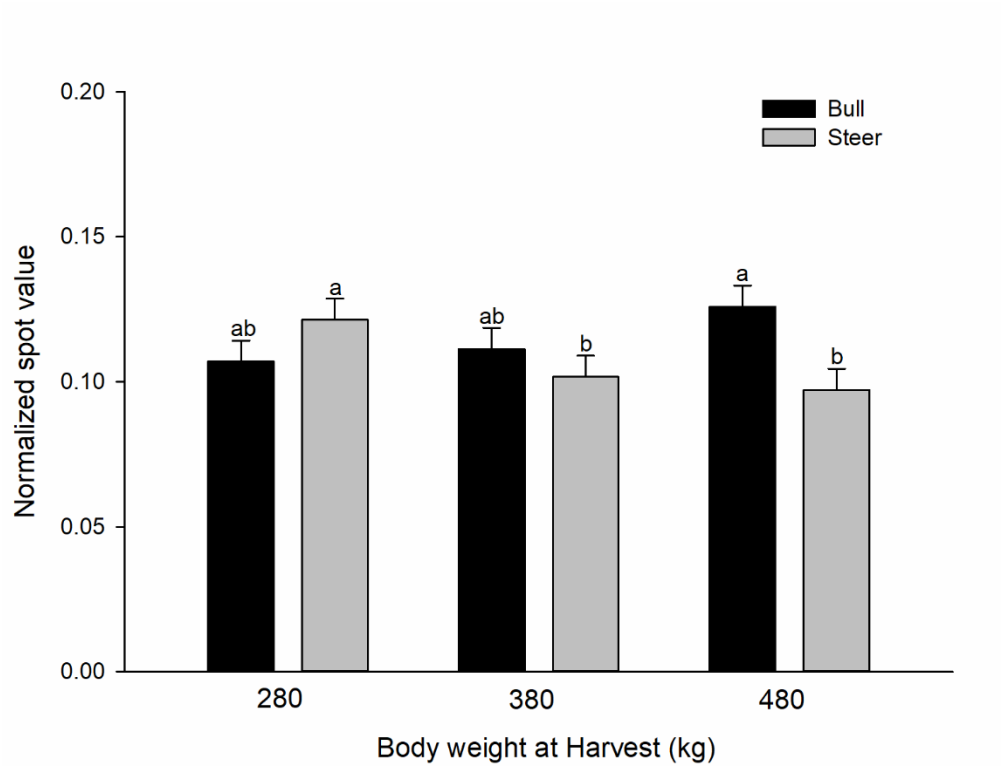


Fig 5. Malate dehydrogenase cytoplasmic (MDH1, spot 2) abundance in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight

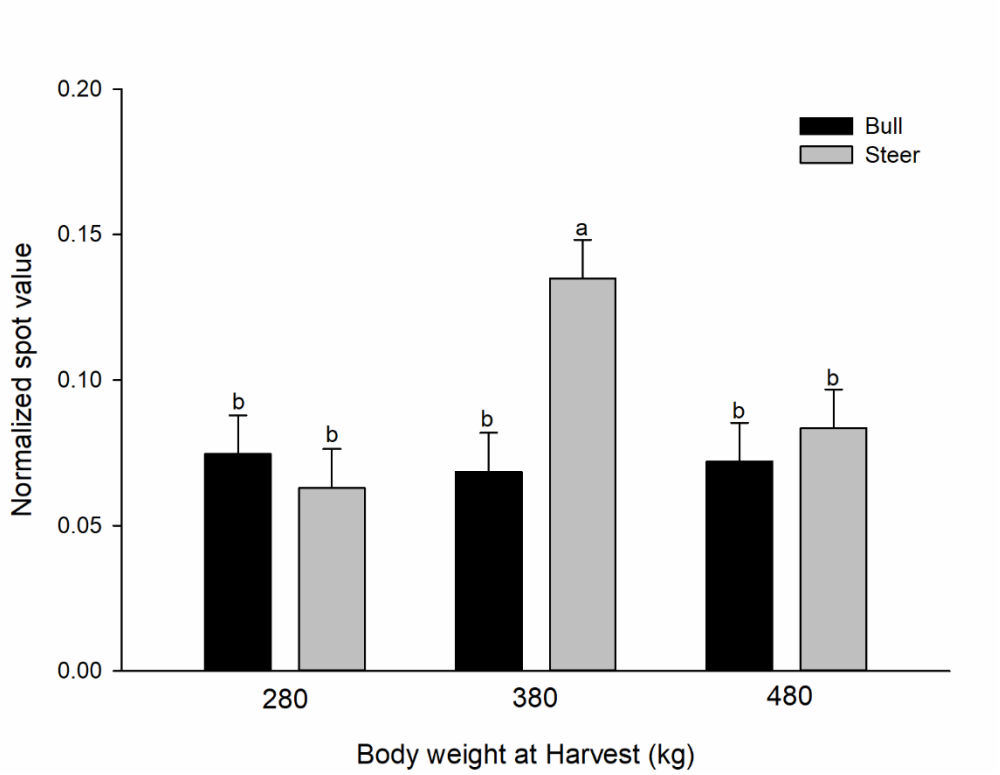


Fig 6. Phosphorylated Alpha-1,4 glucan phosphorylase (PYGM, spot 27) abundance in the longissimus muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight

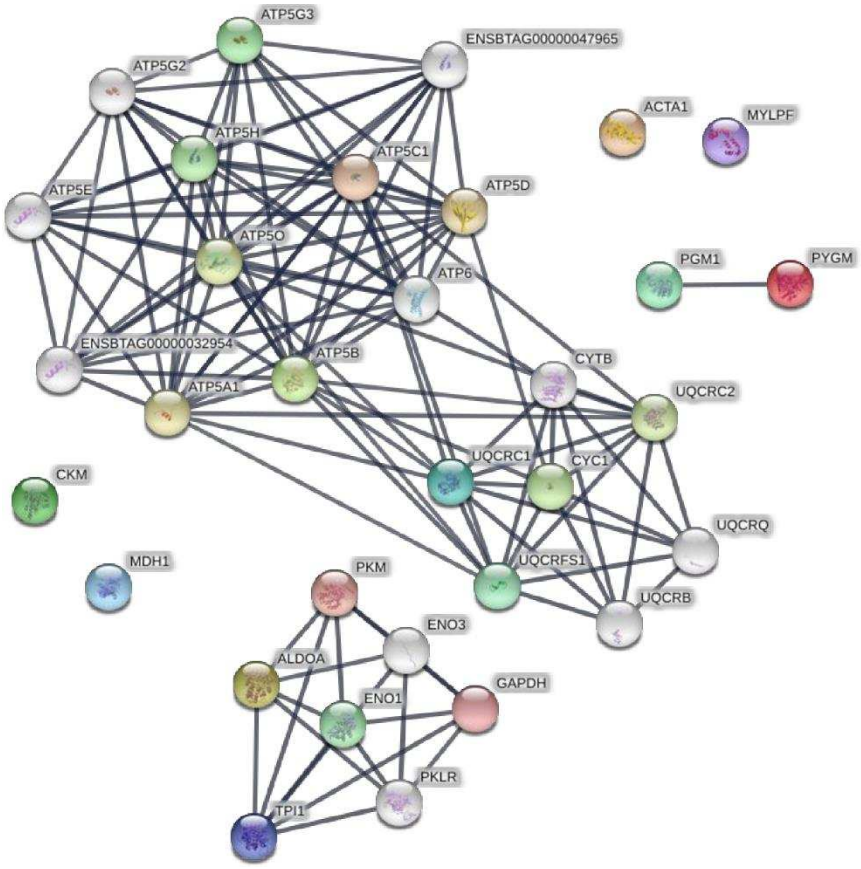


Fig 7. Protein-protein interaction network of the differentially abundant proteins and phosphoproteins in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight. Graphic generated by String 10.5.

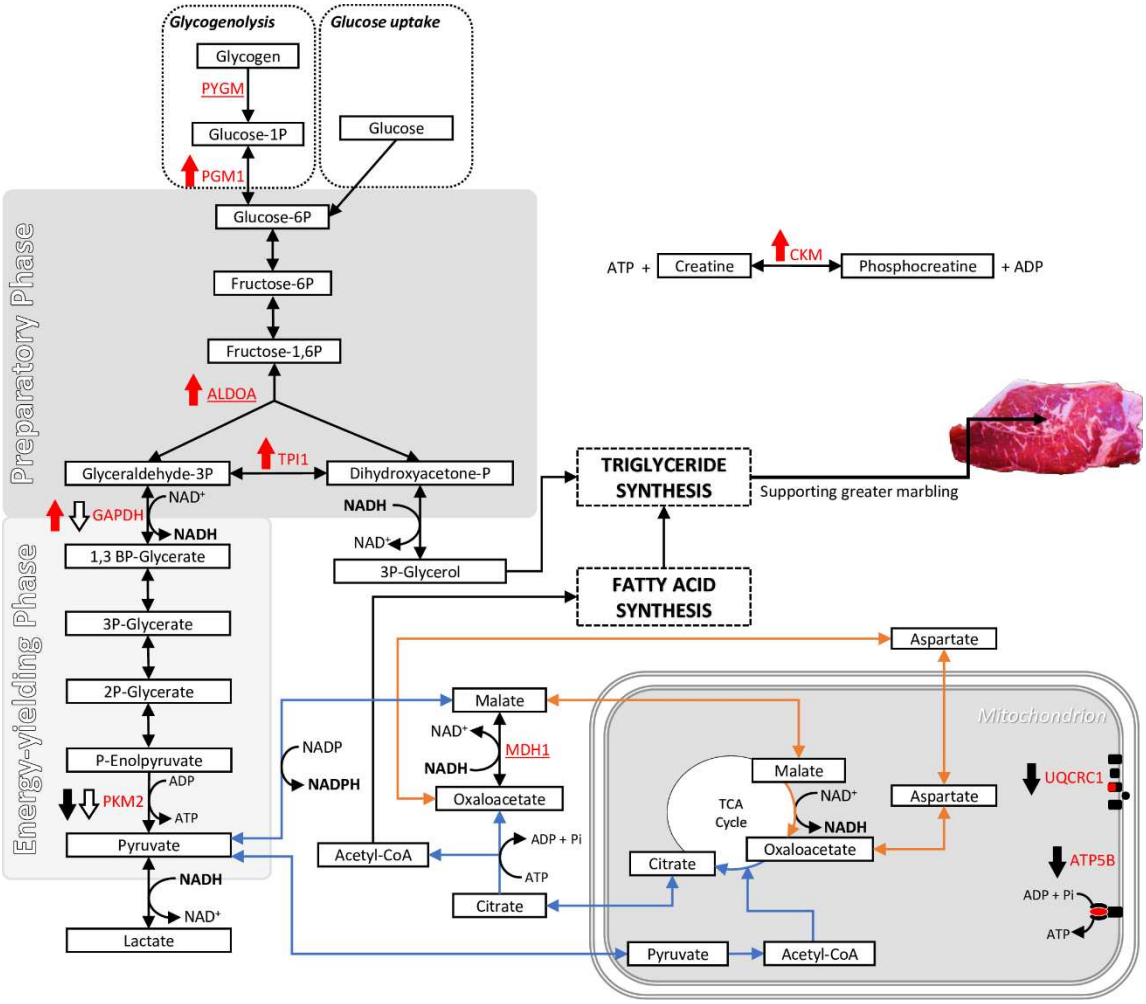
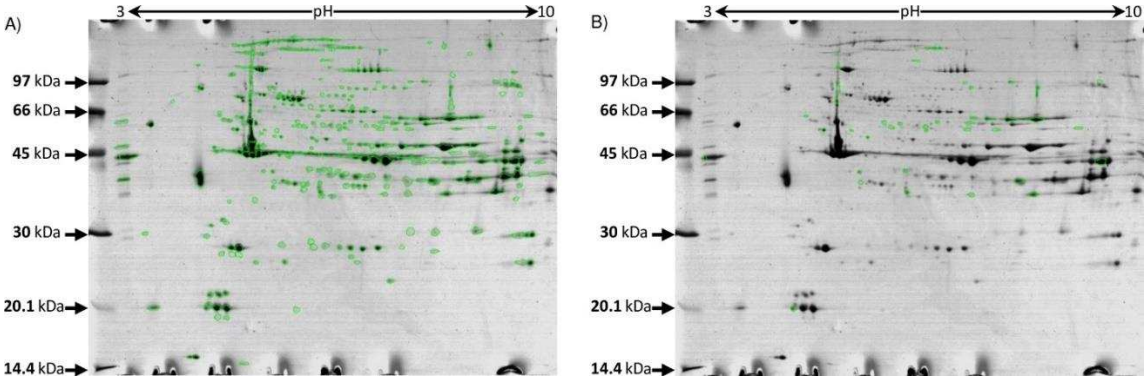


Fig 8. Pathways of the identified differentially abundant proteins in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weights. Underlined proteins abbreviation represents effect of castration by body weight interaction. Open arrows represent total protein downregulation by castration. Solid black arrows represent total protein downregulation in heavier cattle. Solid red arrows represent phosphoprotein upregulation in heavier cattle. The pathway in orange is the malate-aspartate shuttle, while in blue is the citrate-pyruvate shuttle.

S1 Fig.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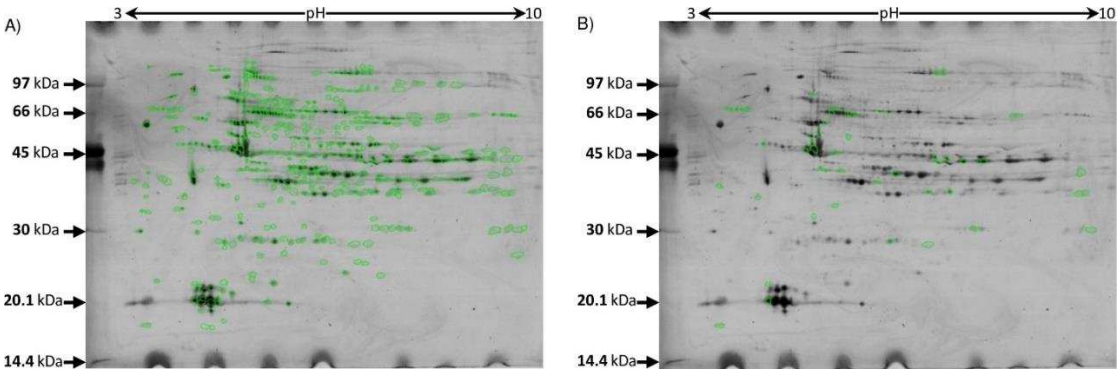
S2 Fig.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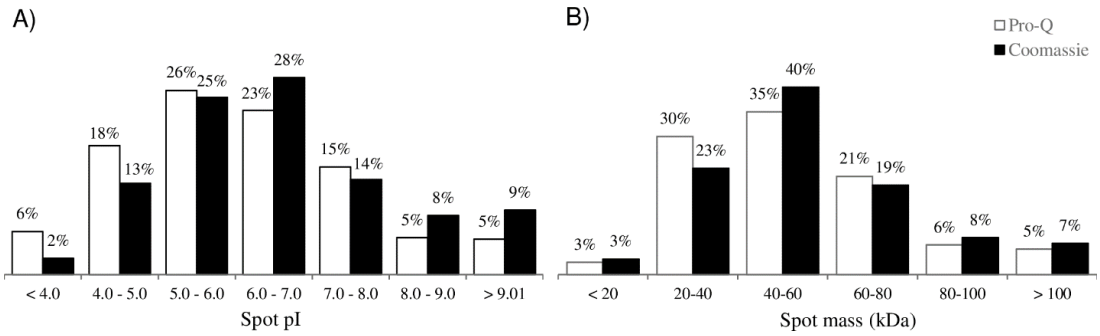
S3 Fig.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20-40
40-60
60-80
80-100
> 100
Spot mass (kDa)

S1 Table. Descriptive information of the differentially abundant total protein spots in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight.

Spot	Protein name	Abbr.[a]	UNIPROT Accession number	Score	Protein Prob. (%)[b]	Sequence coverage (%)	Identified peptides	Peptides sequence	Peptide Prob. (%)[c]
1	Myosin regulatory light chain 2, skeletal muscle isoform	MLC2F	Q0P571	112	95	7	1	FLEELLTTQCDR	95
2	Malate dehydrogenase	MDH1	Q3T145	106	95	7	2	DLDVAILVGSMPR VVEGLPINDFSR	92 95
3	Actin, alpha 1, skeletal muscle	ACTA1	A4IFM8	125	100	7	2	IWHHTFYNELR SYELPDGQVITIGNER	94 95
4	Fructose-bisphosphate aldolase	ALDOA	A6QLL8	306	100	16	4	PHQYPALTPEQK ADDGRPFPQVIK IGEHTPSSLAIMENANVLAR YSHEEIAMATVTALR	95 95 95 95
5	Cytochrome b-c1 complex subunit 1, mitochondrial	UQCRC1	G1K1X0	67	99	4	1	NALVSHLDGTTPVcEDIGR	95
6	Pyruvate kinase	PKM2	L8I1M9	327	100	7	3	NTGIICTIGPASR FGVEQNVDMVFASFIR DPVQEAWAEDVDLR	95 95 95
7	ATP synthase subunit beta, mitochondrial	ATP5B	P00829	366	100	11	4	LVLEVAQHLGESTVR AHGGYSVFAGVGER VALTGLTVAEYFR DQEGQDVLLFIDNIFR	95 95 95 94
8	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	P10096	116	98	4	1	LISWYDNEFGYSNR	95
9	Pyruvate kinase	PKM2	L8I1M9	295	100	10	4	NTGIIcTIGPASR FGVEQNVDMVFASFIR CLAAALIVLTESGR DPVQEAWAEDVDLR	91 95 90 95

[a] Protein name abbreviation. [b] Protein identification probability by Scaffold. [c] Peptide identification probability by Scaffold.

S2 Table. Descriptive information of the differentially abundant phosphoprotein spots in the *longissimus* muscle of Nellore bulls and steers harvested at 280, 380, and 480 kg of body weight.

Spot	Protein name	Abbr.[a]	UNIPROT Accession number	Score	Protein Prob. (%)[b]	Sequence coverage (%)	Identified peptides	Peptides sequence	Peptide Prob. (%)[c]
21	Triosephosphate isomerase	TPI1	Q5E956	66	95	5	1	DLGATWVVLGHSER	95
22	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	P10096	215	100	8	2	VPTPNVSVVDLTcR	95
								LISWYDNEFGYSNR	95
23	Actin, alpha 1, skeletal muscle	ACTA1	A4IFM8	505	100	21	6	AVFPSIVGRPR	94
								IWHHTFYNELR	95
								VAPEEHPTLLTEAPLNPK	95
								GYSFVTTAER	93
								SYELPDGQVITIGNER	95
								QEYDEAGPSIVHR	95
24	Triosephosphate isomerase	TPI1	Q5E956	66	95	5	1	DLGATWVVLGHSER	95
25	Phosphoglucomutase-1	PGM1	Q08DP0	255	100	9	3	TGEHDFGAAFDGDGDR	95
								LSLCGEESFGTGSDHIR	95
								IDNFEYSDPVDGSISR	95
26	Creatine kinase M-type	CKM	Q9XSC6	508	100	20	5	DLFDPIIQDR	95
								LSVEALNSLTGEFK	95
								SFLVWVNEEDHLR	95
								RGTGGVDTAAVGSVFDVSNADR	95
								LGSSEVEQVQLVVDGVK	95
27	Alpha-1,4 glucan phosphorylase	PYGM	B0JYK6	700	100	17	11	DYYFALAYTVR	95
								ARPEFTLPVHFYGR	95
								DFNVGGYIQAVLDR	95
								LKQEYFVVAATLQDIIR	95
								HLQIIYEINQR	95
								IGEEYIADLDQLRK	95
								LLSYVDDESFIR	95
								VHINPNSLFDIQVK	95

Continuation…

Spot	Protein name	Abbr.[a]	UNIPROT Accession number	Score	Protein Prob. (%)[b]	Sequence coverage (%)	Identified peptides	Peptides sequence	Peptide Prob. (%)[c]
27 (continuation)								QLLNCLHVITLYNR	95
								LITAIGDVVNHDPVVGDR	95
								VIFLENYR	95
28	Fructose-bisphosphate aldolase	ALDOA	A6QLL8	157	100	7	2	ADDGRPFPQVIK	95
								YSHEEIAMATVTALR	95

[a] Protein name abbreviation. [b] Protein identification probability by Scaffold. [c] Peptide identification probability by Scaffold.

Chapter IV: Explaining meat quality of bulls and steers by differential muscle proteome and phosphoproteome

Manuscript prepared following the Journal of Proteomics guidelines

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Abstract

The intramuscular fat (IMF) and meat tenderness are considered two of the most important beef traits. Previous reports have shown that castration improves both intramuscular fat content and tenderness. In addition, tenderness can be enhanced throughout aging by postmortem proteolysis. Therefore, the aim of this study was to evaluate the differential proteome and phosphoproteome between bulls and steers during conversion of muscle to meat, as well as after 14 d of aging. Twelve male Nellore (*Bos taurus indicus*) calves were used (247 kg, and 8 months old) and six calves were randomly selected for surgical castration one week before weaning. Post-weaning calves were fed the same diet and were harvested after 230 d on feeding. *Longissimus* muscle was sampled just after stunning (0d postmortem), at deboning (1d postmortem) and after aging (14d postmortem) for proteome analysis. The carcass traits were evaluated at deboning and meat shear force was measured at 1, 7, and 14 d postmortem. Muscle protein extract was separated by 2D-PAGE and stained sequentially for phosphoprotein (Pro-Q Diamond) and for total protein (Colloidal Coomassie). Differentially abundant protein spots between sex condition or across postmortem time were excised for protein identification by MALDI-TOF/TOF. Castration upregulated ($P < 0.05$) the abundance of glycolytic enzymes, while the oxidative phosphorylation protein ATP5B was downregulated ($P < 0.05$). In addition, abundance of troponin T fast isoform (TNNT3) was upregulated by castration ($P < 0.05$), while the slow isoform (TNNT1) tended to decrease ($P < 0.10$) abundance. The creatine kinase M-type was markedly fragmented postmortem. Abundance of phosphorylated PGM1 increased during the first 24 h postmortem and was highly correlated with carcass pH. The abundance of one spot of heat shock cognate 71 kDa protein (HSC70) markedly increased after aging. Further, abundance of the phosphorylated myofibrillar proteins ACTA1 and MYLPF were positively correlated with sarcomere shortening. Overall, our finds demonstrated that abundance and phosphorylation of glycolytic enzymes affect meat quality during conversion of muscle to meat.

Significance: The design of the preset study allowed to clarify the key proteins related to changes during conversion of muscle to meat such as pH decline and sarcomere shortening. Further, the proteins and pathways related to enhancement of intramuscular fat by castration were identified.

Keywords: aging, castration, intramuscular fat, phosphoprotein, zebu cattle

1. Introduction

Meat tenderness and intramuscular fat content are two of the most important beef quality traits. Several carcass grading systems evaluate animal maturity as an indicator of tenderness and marbling score for estimating intramuscular fat. Therefore, these two meat traits are highly desirable for consumer, and have economic importance for beef industry. The intramuscular fat content can be accurately estimated by visual scores or video image analysis [1]. However, predict meat tenderness is a challenging task demanding more laborious and expensive analysis, which sometimes are not able to explain more than 50% of tenderness variation [2,3]. The meat tenderness is affected by pre-slaughter factors (e.g., breed, age, sex, handling) and by post-mortem factors such as carcass handling, conditioning and storage length [4].

It has been reported that castration affect meat yield and quality, and overall steers have greater carcass fatness, while retail cuts yield is lower comparing with bulls [5]. The less tender meat produced from bull carcasses have been related mainly to scarce backfat thickness leading to cold-shortening [6], and lower endogenous proteases activity reducing tenderization rate [7,8]. The sarcomere length seems to be a good tenderness indicator in unaged meat, while after an aging period the myofibrillar fragmentation become a better tenderness predictor [2]. Hence, castrated cattle constitute a good biological model to better clarify the pathways related to marbling and tenderness, two of the most important meat traits.

After slaughter, muscles cells do not die immediately and instead of this the cell change its metabolism trying to maintain the homeostasis [9]. Therefore, important chemical and physical changes take place at the initial hours postmortem when muscle is being converted into meat. Among these changes are the temperature and pH decline, which affect the rigor development [4]. Thus, carcass handling from slaughter to deboning might affect not only meat tenderness, but also color and water holder capacity. Likewise, meat aging after deboning allows to improve tenderness by postmortem proteolysis, and the myofibrillar fragmentation index is a good indicator [10]. It has been reported that the greater beef tenderization occurs until 14 d postmortem [11]. Nevertheless, although the

effect of meat storing on tenderness is well established, the knowledge about global protein change during aging has growing only in the last years by using "omics" tools [12].

Proteomic studies have allowed to better understand the meat differences among breeds [13], difference genotypes [14], conversion of muscle to meat [15] as well as water holder capacity [16]. Indeed, the different proteomic approaches have permitted to discover and confirm biomarker for meat quality [17]. Furthermore, studying proteome throughout conversion of muscle to meat have allowed to identify the postmortem proteolysis target proteins [18]. Interestingly, proteomic has allowed to detect the posttranslational modifications that happen during postmortem, such as protein phosphorylation. Recently, using 1-DE gel to study the ovine *longissimus* muscle phosphoproteome from 0.5 to 24 h postmortem, Chen et al. [19] found that nine of the twenty-one detected bands increased the phosphorylation throughout the conversion of muscle to meat.

The two-dimensional gel electrophoresis (2D-PAGE) allows to obtain a snapshot of the muscle functional molecules (i.e., proteins) as well as to study posttranslational modifications like phosphorylation. Therefore, the aim of this study was to evaluate the differential proteome and phosphoproteome between bulls and steers during conversion of muscle to meat and after 14 d of aging.

2. Material and methods

All the procedures related to animal management and handling followed the protocol (protocol # 035/2015) previously reviewed and approved by the Animal Care and Use Committee of the *Universidade Federal de Viçosa* (CEUAP-UFV).

2.1. Animal housing and management

Twelve male Nellore (*Bos taurus indicus*) calves were used. The calves were intensively raised on tropical pasture using a creep-feed system (0.7% of body weight in concentrate). Six calves were randomly selected for surgical castration one week before weaning. At weaning, the Nellore calves averaged 247.83 ± 3.90 kg of body weight (BW) and 8.1 ± 0.10 months old.

Weaned calves (young bulls and steers) were ear tagged, dewormed and fed in feedlot with the same diet containing 138.72 g/kg of crude protein and 13.95 MJ/kg of digestible energy. The diet was formulated considering the average nutrient requirements of bulls and steers performing 1.4 kg/day, according to BR-CORTE system [20]. Fresh feed was provided twice a day, as a total mixed ration ad libitum.

2.2. Harvest, carcass traits, and muscle sampling

Bull and steers were harvested after 230 d on feeding. Cattle were slaughtered after 16 h of feed fasting with free access to water, following humane slaughter practices. The *longissimus* muscle samples (0 d postmortem) were obtained from the right carcass side at the 13th rib, just after stunning and blooding. *Longissimus* samples were trimmed for epimysium and subcutaneous fat, minced, and then snap frozen in liquid nitrogen. Minced *longissimus* samples were ground using a mortar pestle with liquid nitrogen, transferred into cryovials, and then stored at -80˚C until protein extraction.

After dressing, carcasses were weighed, suspended by the aitch bone (tenderstretch method), and chilled at 2 °C. Throughout chilling, a portable pH meter (Inlab® Solids PRO, Mettler-Toledo AG, Schwerzenbach, Switzerland) was used to measure the carcass pH and temperature in the *longissimus* muscle at 0.5, 2, 4, 6, 8, 10, 12, 14, 18, 22, and 24 h postmortem. After 24 h of chilling, cold carcass was ribbed between the 12th and 13th rib and back fat thickness (BFT) was measured in millimeters using a digital caliper ruler. The *longissimus* muscle perimeter was delimited on a transparency film and rib eye area (REA) was measured by ImageJ software (ImageJ 1.48g, Bethesda, Maryland, USA).

Longissimus muscle was sampled from the right side of the cold carcass. The meat sample was trimmed of all subcutaneous and intermuscular fat, and then divided into three portions (cranial, medial and caudal). The cranial portion was labeled as unaged meat (1-day postmortem), vacuum packed and then frozen at -20 °C. Medial and caudal portions were vacuum packed and kept at 4 °C until 7 or 14 days postmortem (PM), respectively. Once aged, the portions were kept frozen at -20 °C until analyzes. Samples of unaged meat (1d postmortem) and aged meat (14d postmortem) for proteomic analysis were obtained from cranial and caudal portions, respectively. The samples for proteomic analysis were minced and powdered as previous described.

2.3. Meat quality assessment

Unaged meat (1d postmortem) samples were used to measure the sarcomere length using the laser diffraction method (Wheeler, Shackelford, & Koohmaraie, 2002) and to quantify the intramuscular fat (IMF) by petroleum ether extraction using a fat extractor Ankom XT15 unit (ANKOM Technology, Macedon, NY, USA). In addition, myofibrillar fragmentation index (MFI) and Warner-Bratzler shear force (WBSF) were evaluated in the unaged and aged meat, as previously described [(SILVA, Meat Science manuscript)].

2.4. Proteome and phosphoproteome analyzes

2.4.1. Protein extraction

Fifty milligrams of powdered *longissimus* sample were placed into a test tube containing 1mL of cold extraction buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% 3-10 IPG-buffer, 40 mM DTT, 0.5mM Phenylmethanesulfonyl fluoride, and 0.5mM Benzamidine), and then homogenized for 15 s using a shaft type homogenizer (LabGEN 125, Cole-Parmer, Bunker Hill, IL, USA) at 9,500 rpm. The homogenate was kept in ice bath for 15 s and then centrifuged at 20,200 g for 30 min at 4˚C. After centrifugation, the supernatant was collected, and stored at -80˚C. Extracted protein concentration was determined by the Bradford protein method [21] using premixed reagents (BioRad, Hercules, CA, USA).

2.4.2. Two-dimensional gel electrophoresis

The isoelectric focusing (IEF) was carried out using IPG strips of pH 3-10, and 24 cm long (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Strips were rehydrated for 16 h with 450 µL of solution containing 1,500 µg of extracted protein, DeStreak Rehydration Solution (GE Healthcare Bio-Sciences), and 3% of pH 3-10 IPG-buffer. Rehydrated strips were placed on an Ettan IPGphor 3 System (GE Healthcare Bio-Sciences) for IEF following the 5-steps program: 200 V for 2 h, 500 V for 1 h, voltage linear increasing to 1,000 V to reach 800 V/h, voltage linear increasing to 10,000 V to reach 16,500 V/h, hold 10,000 V to reach 27,500 V/h. The current limit was 50 µA per strip. After first dimension, strips were equilibrated for 20 min in 5 mL of equilibration buffer solution (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, and 0.002% bromophenol blue) containing 1% DTT. Afterwards, a second equilibration was

performed for 20 min in 5 mL of equilibration buffer solution containing 2.5% iodoacetamide. The second-dimension electrophoresis was performed in a 12.5% SDS-PAGE using an Ethan DALTsix unit (GE Healthcare Bio-Sciences). Previously equilibrated strips and the molecular weight calibration kit (GE Healthcare Bio-Sciences) were placed on top of the SDS-PAGE and sealed with melted 0.5% agarose solution containing 0.002% of bromophenol blue. Electrophoresis was performed at 8˚C, initially with 20 mA/gel and 80 V for 45 min, and then with 40 mA/gel and 500 V until the dye reached the bottom of the gel.

2.4.3. Gel staining and scanning

After second dimension, gels were washed three times in double-distilled water and fixed overnight using fresh solution containing 10% of acetic acid (v/v), and 50% methanol (v/v). Later, gels were washed twice with deionized-distilled water and stained with Pro-Q Diamond dye (Invitrogen Molecular Probes, Eugene, OR, USA) for phosphoproteins detection [22]. Gels were scanned using a Fuji Film scanner (Fuji Film 5100 FLA, Fuji Medical Systems, Hanover Park, IL, USA) in fluorescent mode (excitation 532 nm, emission 580 nm). Subsequently, the gels were stained for total protein using Colloidal Coomassie Blue solution [8% ammonium sulfate (w/v), 0.8% phosphoric acid (v/v), 0.08% Coomassie blue G-250 (v/v), and 20% methanol (v/v)] for 72 h. Afterward, gels were washed three times with distilled water for 30 min and then overnight with 1% acetic acid (v/v). Gel imagens for total protein were obtained using a pre-calibrated Image Scanner III (GE Healthcare Bio-Sciences) with resolution of 300 dpi. Gels were stored in 2% acetic acid (v/v) at 20˚C until spots excision.

2.4.4. Image analyses and differential abundance detecting

A total of 12 gels were made (one per animal) and stained with two dyes totalizing 24 gel imagens. To avoid technical bias, all analytical steps was performed using a set of samples balanced for treatments. Corresponding spots were detected, matched and individually checked within gels of each dye using Image Master 2D Platinum 7.0 software (GE Healthcare Bio-Sciences). When the corresponding spot was not present in a gel the spot was propagated ensuring that all gels have the same number of spots. The normalized spot volume was obtained for each spot of all gels. To detect abundance difference, ANOVA

was performed for normalized spot volumes using the MIXED procedure of SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA). Data were analyzed as a completely randomized design with two treatments (bull and steer) and six biological replicates. Postmortem time (0, 1, and 14 d postmortem) were included in the model as a repeated measurement with the animal as subject. Due to the great number of spots differentially abundant across postmortem time, only the spots with fold-change greater than 1 were selected for identification. Once detected significant effect ($P < 0.05$) by ANOVA, treatments least squared means were compared by student's t test.

2.4.5. Protein identification

Reference gel imagens from the same sample stained with both dyes (Pro-Q and Coomassie) were overlapped allowing to identify the corresponding spots between Coomassie and Pro-Q Diamond image. Differentially abundant protein spots were manually excised, and then digested in-gel using trypsin (Trypsin V511, Promega, Madison, WI, USA) as previous described protocol [23]. Tryptic peptides were then recovered, dried using a SpeedVac centrifuge (AG-22331, Eppendorf, Germany) and then resuspended with CHCA matrix solution (Bruker Daltonics, Germany). Later, 1 µL of the peptides solution was placed on a MALDI target plate. Peptide mass spectra was obtained using a MALDI- TOF/TOF system (Ultraflex III, Bruker Daltonics, Bremen, Germany). The MS/MS peaks list was generated by Flexcontrol software (version 3.3, Bruker Daltonics, Germany).

Protein was then identified by MASCOT software (version 2.4.0, Matrix Science, London, UK) with the following restrictions: enzymatic tryptic digestion; maximum one missed cleavage; maximum mass error of 0.2 Da for MS and 0.5 Da for MS/MS mode, cysteine carbamidomethylation as a fixed modification, and methionine oxidation as a variable modification. The information from the Bovidae family in the UniProt database was used. The SCAFFOLD software (version 3.6.4, Proteome Software, Portland, OR, USA) was used to validate the protein identification by MASCOT. Validation was performed using the Protein Prophet algorithm [24], and the criteria used for the validation was: at least one unique peptide, with identification probability greater than 90%.

2.5. Statistical analyzes of carcass and meat traits

To estimate carcass temperature at pH 6.0 (temp@pH6) and carcass pH at temperature 18 °C (pH@18), exponential models were individually fitted for temperature versus postmortem pH using NLIN procedure of SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA).

To detect difference between bull and steer for carcass and meat quality traits the ANOVA was performed using GLM procedure of SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA). Initial body weight was included as covariate in the model and kept where $P < 0.10$. The value of 0.05 was used as a critical threshold for type I error. In addition, correlation coefficients between meat quality traits and normalized spots volume were calculated using the CORR procedure of SAS 9.4 and significant correlation was assumed where $P < 0.05$, and trends where $0.05 < P < 0.10$.

3. Results

3.1. Carcass and meat quality traits

The Nellore cattle was fed in feedlot during 230 d to reach the average target BW of 480 kg. There were trends ($P < 0.10$) toward lower BW and carcass weight on castrated cattle (Table 1). Similarly, castration tended to increase ($P < 0.089$) the backfat thickness. The rib eye area was not affected ($P = 0.22$) by castration. The postmortem decline rate of carcass pH and temperature were similar between bulls and steers ($P > 0.05$), and the final sarcomere length did not differ between sex conditions ($P = 0.97$). Steer had greater intramuscular fat than bulls ($P < 0.01$). Although the WBSF and MFI did not differ between bulls and steers at 1 d postmortem ($P > 0.05$), throughout aging steers had greater tenderization reducing the WBSF and enhancing the MFI in comparison with bulls.

3.2. Proteome change by castration and postmortem

The image analysis of Colloidal Coomassie stained gels, allowed to revolve 582 protein spots. Among them, 517 spots were common across postmortem time, and there was none detected spot exclusively for sex condition. Among the postmortem time, five spots were detected only in muscle (0d postmortem) and unaged meat (1d postmortem), while another five were detected only in unaged and 14d aged meat. In addition, two protein spots were detected only in muscle samples, while two were exclusive to unaged meat, and 51 spots

were detected only in 14 d aged meat. These results demonstrate the dynamic proteome change during postmortem time. Nonetheless, almost all these spots uncommon across postmortem time had low abundance making it difficult to identify later by MALDI-MS/MS (Figure S1). Therefore, only two spots exclusively of 14 d postmortem were detected (spots 270 and 396).

Interaction effect of sex condition by postmortem time was found for 23 spots ($P < 0.05$), while 38 protein spots were differentially abundant ($P < 0.05$) between bulls and steers. Initially, a total of 217 spots were differentially abundant ($P < 0.05$) across postmortem time, whereas considering fold-change ≥ 1 as a threshold, 56 spots were selected for identification (Figure S2). Among them, 36 proteins spots were identified by MALDI-MS/MS (Figure 1 and Table 2), corresponding to 15 different proteins. Interaction effect of sex condition by postmortem time was found ($P < 0.05$) for spot 35 identified as carbonic anhydrase (CA3), and steers had greater abundance than bulls at 1d postmortem (Figure 2). The spot 246 identified as actin alpha 1 (ACTA1) was also affected by interaction ($P < 0.05$), and bulls had greater abundance than steers only in muscle samples (Figure 3). The postmortem time differentially affected spots from the same protein (Figure 4), mainly for phosphoglucomutase-1 (PGM1), heat shock cognate 71 kDa protein (HSC70), fructose-bisphosphate aldolase (ALDOA), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Abundance of the seven spots identified as creatine kinase M-type (CKM) did not differ from muscle to meat ($P > 0.05$), however during aging from 1 to 14 d postmortem, the four heavier spots have their abundance decreased ($P < 0.05$), while abundance of the three lighter spots increased (Figure 5). The identified proteins are mainly related to muscle contraction (ACTA1, ACTN3, MYL1, MYLPF, TNNI2, and TNNT1), glycolysis pathway (ALDOA, GAPDH, PGK1, and TPI1), glycogen metabolism (PGM1), oxidative phosphorylation (ATP5B), cell defense (HSC70), creatine metabolism (CKM), and cytoprotection (CA3).

The correlation analyzes shown that in muscle samples, only 24 trends ($P < 0.10$) and 19 significant ($P < 0.05$) correlations were found between the identified proteins and meat traits (Figure 6). The correlation between meat quality traits and protein abundance were inconsistent across postmortem time, except for spots 406, 127, 4, and 168. The

intramuscular fat content positively correlated with muscle abundance of glycolytic enzymes GAPDH (spot 322, $P < 0.05$), PGK1 (spot 105, $P < 0.05$), and ALDOA (spot 88, $P < 0.10$). Regarding the correlations between meat quality traits and protein abundance in unaged meat (Figure 7), the spots 407 (CKM), 4 (MYLPF), and 168 (PGM1) negatively correlated with WBSF ($P < 0.05$) and positively with MFI ($P < 0.05$). Looking at correlation coefficients between meat traits and protein abundance in 14d aged meat (Figure 8), the WBSF evaluated at 14d postmortem negatively corelated with spot 270 identified as CKM, and positively correlated ($P < 0.05$) with three spots identified as GAPDH (68, 69, and 322).

3.3. Phosphoproteome changes by castration on postmortem

The 2-DE stained with Pro-Q Diamond allow to revolve 573 phosphoprotein spots, within 516 spots were common among all gels (Figure S3). Similar to Coomassie images, none phosphoprotein spot was detected only in a sexual condition. Four spots were exclusively found at muscle (0d postmortem) and unaged meat (1d postmortem), while 35 were detected only in unaged and aged meat. There was no phosphoprotein spot exclusively detected in muscle, while one spot was found in unaged meat and seventeen spots were exclusively detected in 14d postmortem samples. However, due to low abundance of these phosphoproteins spot detected exclusively on some treatments were not identified.

Analyzing the abundance of the phosphoprotein spots, 23 spots were affected by interaction effect of sex condition by postmortem time, while 27 phosphoprotein spots were differentially abundant between bulls and steers. Initially, 310 spots changed the abundance throughout postmortem time, within 78 spots had fold-change ≥ 1 and were selected for identification (Figure S4). A total of 44 spots were excisable and used for MS/MS analysis allowing to identify 18 phosphoprotein spots, corresponding to 12 proteins (Figure 9, and Table 3). Overlapping Pro-Q and Coomassie images shown that six identified protein in both dyes are corresponding spots, confirming the protein identification (Table S1). The identified differentially abundant phosphoproteins are mainly related with glycolysis pathway (ALDOA, GAPDH, PKM2, and TPI1), glycogen metabolism (PGM1), muscle contraction (ACTA1, MYL1, MYLPF, TMOD1, TNNT3, and TNNT1), and creatine metabolism (CKM).

Correlation analyzes revelated that only few phosphoproteins abundance in muscle samples were correlated with meat traits (Figure 10), and muscle abundance of PGM1 (spot 149) and TPI (spot 45) were highly and positively correlated with MFI. Interestingly, the spot 149 detected at phosphoproteome is equivalent to spot 168 at Coomassie images (Table S1), and both were positively ($P < 0.05$) correlated to MFI. The intramuscular fat did not correlate with any phosphoprotein in muscle samples ($P > 0.05$), though positively correlated ($P < 0.05$) with ALDOA (spots 43 and 210) and GAPDH (spots 208 and 211) at 1d postmortem. The phosphorylated ACTA1 (spot 109) negatively correlated with pH@18 and WBSF at 1d and 7d postmortem ($P < 0.05$). The abundance of phosphorylated GAPDH (spots 208 and 211) and TNNT1 (spots 81 and 82) in 14d aged meat correlated ($P < 0.05$) positively with WBSF14 and negatively with MFI14.

4. Discussion

4.1. Castration enhanced subcutaneous fat with minor effect on muscle tissue and postmortem pH

Bulls usually are heavier and leaner than steers owing to the anabolic effect of testosterone on muscle tissue, while steers had greater carcass fat content [25]. At the current study, castration markedly increased the carcass fat content, and steer carcass had 45% more backfat thickness (BFT) with similar rib eye area. In addition, steers carcasses were only 11% lighter than bulls. These results suggest that when castrated at weaning and intensively raised, Nellore steers have greater fat deposition with minor effect on muscle tissue.

The carcass weight and subcutaneous fat might affect the muscle temperature and pH decline postmortem. Previous report shown that greater carcass fat reduces temperature decline rate which increases acidification rate during conversion of muscle to meat [26]. Therefore, it was expected greater pH decline rate for steer carcasses since it had more BFT than bulls. In addition, lesser pH declines rates in bull's carcass has been reported due to the greater stress responsiveness, leading to higher pH at 24 h postmortem [6]. Nonetheless, the pH and temperature 24 h postmortem was similar between bulls and steers carcasses. Further, the pH at temperature 18 °C (pH@18C) and the temperature at pH 6 (Temp@6) are two indicators used for evaluating pH and temperature falling rate

respectively, and both were similar between bulls and steers carcasses. Hence, these results suggest a similar conversion of muscle to meat for bulls and steers explaining the lack of difference for sarcomere length, WBSF and MFI measured at 1 d postmortem.

4.2. Castration enhanced intramuscular fat

The intramuscular fat (IMF), also known as marbling, is one of the most important meat quality traits affecting quality grading and carcass price. Hence, several studies have been developed to better understand the pathways involved in IMF deposition, providing information to manipulate this desirable meat trait [27]. The IMF content is affected by cattle breed [28], diet composition [29], animal age [30], and castration [31]. At the current study steers steak had 45% greater intramuscular fat than bulls, and even though Nellore cattle is considered a lean cattle breed steers reached a Modest marbling score [1].

Previous transcriptome study reported that castration increases the intramuscular adipogenesis, lipogenesis and lipolysis [31]. In another transcriptome study was found increased lipid uptake, lipogenesis and reduced lipolysis following castration [32], supporting the great lipid accumulation of steers. At the current study none of the differentially abundant identified protein are directly related to fat metabolism to confirm these previous reports. Nevertheless, castration upregulated muscle abundance of some glycolytic enzymes (i.e., ALDOA, GAPDH, and TPI) indicating a greater muscle glucose metabolism in steers.

It has been proposed that glucose is the main carbon source for *de novo* fatty acid synthesis of intramuscular fat [33]. In addition, glycolysis also is a pathway used to produce glycerol-3P required to triglyceride synthesis [34]. Further, castration downregulated the mitochondrial ATP synthase subunit beta (ATP5B), indicating a reduction in oxidative phosphorylation pathway in steers. Therefore, the greater glycolysis along with reduced oxidative phosphorylation in steers muscle suggest a greater synthesis of glycerol-3P and fatty acid from glucose, supporting the higher IMF level in steers steaks (Figure 11).

In agreement, muscle total protein abundance of the glycolytic enzymes ALDOA, GAPDH, and PGK1 were positively correlated with muscle intramuscular fat, confirming the association between glycolysis and intramuscular triglyceride synthesis. In line,

previous muscle proteome study has been reported upregulation of GAPDH in *logissimus* muscle of pig breed with higher IMF [35]. In another muscle proteomic study, not only glycolytic enzymes were upregulated in pig breed with greater carcass fattening, but also glycerol-3-phosphate dehydrogenase (G3PD), the enzyme that catalyzes the conversion of dihydroxyacetone phosphate (DHAP) from glycolysis to glycerol-3P [36]. In addition, the metabolomic analyzes in this study confirmed the greater muscle abundance of glycerol-3P and glycerol, supporting the importance of glycolysis for glycerol biosynthesis [36].

4.3. Steers had greater postmortem tenderization rate

Several studies pointed out that tenderness is the most important beef quality trait affecting consumers satisfaction, and some consumers are willing to pay a premium for this desirable meat trait [37]. However, beef tenderness is a highly variable characteristic leading its prediction a challenging task. Regarding castration, it has been reported that steers beef is more tender than bulls owing to the greater activity of proteolytic system following castration [8,38].

The lack of difference between bulls and steers for WBSF and MFI at 1d postmortem indicate that background tenderness and conversion of muscle to meat were similar for both sex condition. However, steers shown a higher tenderization rate throughout aging, increasing the MFI and decreasing the WBSF after storing at 4 °C. Therefore, we analyzed the proteome and phosphoproteome changes of bulls and steers during conversion of muscle to meat and after aging of 14d postmortem, to clarifying which proteins are the targets for proteolytic systems during postmortem.

Castration increased the abundance of glycolytic enzymes, and three total protein spots identified as TPI1 was more abundant in steer muscle. Previous reports have listed TPI1 as a biomarker for meat tenderness, negatively related to WBSF [39,40]. Indeed, steers had also greater abundance of phosphorylated TPI1 (spot 45) which had highly and positive correlated with MFI regardless of postmortem time. The GAPDH is another glycolytic enzyme upregulated by castration that was previously confirmed as a biomarker for meat tenderness [40]. The interaction effect found for carbonic anhydrase 3 (CA3) shown that steers had greater abundance than bull after 24 h postmortem, and previous

report have shown that meat abundance of CA3 is positively related to meat tenderness [41].

Regarding the myofibrillar proteins, castration downregulated total abundance of the most alkaline ACTA1 spot (406) which was highly and negatively correlated with sarcomere and MFI, and positively correlated to WBSF, even in muscle samples. In addition, the interaction effect found for ACTA1 (spot 246) shown that bull had greater muscle abundance of this protein which was also negatively correlated with MFI. Steer muscles also has approximately 20% greater MYL1 than bulls. Previous muscle proteomic studies with cattle have shown that higher MYL1 abundance was found in less tender meat [13,42].

4.4. Castration shift muscle fiber toward fast-twitch and glycolytic metabolism

Muscle is a heterogeneous tissue having different types of muscle fibers. The myofiber type IIB also known as white fiber, has a fast twitch and preferentially glycolytic metabolism, while type I myofiber (red fiber) uses preferentially the oxidative phosphorylation to regenerate ATP and has a slow twitch [43]. Cattle usually have more oxidative muscle fiber than non-ruminant animals, leading to higher cold-shortening risk in beef carcass once type I fiber has lower acidification capacity. However, muscle have phenotypic plasticity allowing muscle to convert the fiber type in response to environmental conditions such as feeding intensity, exercise, age and castration [44]. Previous reports shown that castration increased muscle fiber type IIB, reducing types IIA [45].

The comparative proteome and phosphoproteome shown that three of the four spots identified as ALDOA were upregulated by castration, while three of the six spots identified as GAPDH were upregulated by castration. Further, all three spots identified as TPI1 and one PGM1 spot were also upregulated by castration. These results suggest a greater glycolysis flux, and then greater pyruvate synthesis. As product of glycolysis, pyruvate can enter the mitochondria for complete phosphorylation or be converted to lactate. However, the downregulation of ATP5B, a member of oxidative phosphorylation, by castration indicates reduction on oxidative phosphorylation capacity in steers muscle, and the greater pyruvate production in steers muscle may be used for other pathways rather

than oxidative phosphorylation. Our data support the previous reported change from oxidative to glycolytic metabolic after castration [45].

Regarding the contractive proteins, the phosproteomic approach used at this study allowed to find greater abundance of fast troponin T isoform (TNNT3) in steers muscle, while slow troponin T isoform (TNNT1) tended to decrease, comparing with bulls. The myosin light chain 1 isoform (MYL1) is more expressed in slow- twitch muscle fiber [46], and at this study greater abundance was found in bulls' muscle comparing with steers. In addition, castration upregulated total protein abundance of CKM, an enzyme related to ATP regeneration by creatine phosphate, which is a metabolism more used by IIB fiber type [43].

The muscle fiber type was not directly evaluated at this study, whereas the proteome and phosphoproteome changes pointed out that castrated Nellore cattle shifted from slow to fast twitch and from oxidative to glycolytic metabolism, as steers muscle had greater abundance of proteins expressed in fast-twitch and glycolytic fiber type [46].

4.5. Abundance of protein and phosphoproteins correlated with carcass pH and sarcomere length

Bovine muscle is typically known as a low glycolytic potential muscle, leading to slow postmortem carcass pH decline [43], and consequently higher risk of cold-shortening. It has been suggested that negative impact of sarcomere length on meat tenderness is greater when the ultimate sarcomere length is shorter than 1.5 µm [47]. The Temp@pH6 have been proposed as an indicator for cold-shortening, and to minimize sarcomere shortening carcass temperature should be greater than 12 °C when carcass pH reach 6 [3]. At the current study Temp@pH6 was not affected by castration, averaging 17 °C, consequently sarcomere length was similar between bulls and steers and was greater than the threshold of 1.5µm. Although castration did not affect carcass pH falling throughout conversion of muscle into meat (first 24 h), the correlation analysis revealed some protein spots related with muscle pH postmortem.

The abundance of creatine kinase M-type 37.1 kDa fragment (CKM, spot 407) in unaged meat was highly and negatively correlated with pH@18C, suggesting that carcass pH

decline is faster with greater CKM proteolysis. In agreement, the abundance of this spots was highly and positively correlated with MFI1, indicating that meat with greater postmortem proteolysis also had higher CKM breakdown. The CKM catalyzes the transference of a phosphate between ATP and creatine, and this is an important ATP regeneration in muscle. After slaughter, the absence of oxygen lead muscle to regenerate ATP using other pathways rather than oxidative phosphorylation. The ATP regeneration by CKM maintain constant the muscle ATP concentration during a short time, delaying lactate production from glycolysis and then pH falling [12]. Therefore, the negative correlation between muscle abundance of CKM fragment and pH@18C, suggests that early CKM breakdown allow faster pH decline postmortem due to reduced ATP regeneration from creatine phosphate. In addition, when the phosphate is transferred from phosphocreatine to ADP one proton is consumed, proving the importance of this pathway to buffer pH postmortem [9].

The phosphoglucomutase 1 (PGM1, spot 168) abundance in unaged meat sample was negatively correlated with pH@18C, WBSF1, and WBSF7, and positively correlated with sarcomere length. Interestingly, the phosphoprotein spot 149 is the correspondent to 168, and had the same pattern throughout postmortem time and similar correlations with meat traits, confirming the results from Coomassie stained gel. The PGM1 is an important enzyme related to muscle glycogen metabolism, and during glycogenolysis PGM1 converts glucose-1P to glucose-6P which follow glycolysis pathway [48]. Phosphorylation increased PGM1 enzymatic activity in cell culture [49]. Therefore, these data indicate that greater PGM1 activity results in faster pH decline preventing sarcomere shortening during conversion of muscle to meat.

However, as previous reported PGM1 has multiples phosphorylation sites generating a set of spots with similar mass and different pI, as the more phosphorylated the protein the lower is its pI [48]. At the current study, it was identified three PGM1 spots for total protein abundance. During conversion of muscle to meat the abundance of the more alkaline spot (169) decreased while the more acidic increased (168, and 165), suggesting early postmortem activation of PGM1 by phosphorylation. However, the unaged meat abundance of the more acid PGM1 spot (165) and presumed more phosphorylated did not

correlated with muscle pH or WBSF, while the more alkaline PGM1 spot (169) correlated positively with WBSF and negatively with MFI.

Interestingly, even in muscle samples (at slaughter) the abundance of this less phosphorylated form (spot 168 in Coomassie and 149 in ProQ) positively correlated with MFI1 and negatively with WBSF1 and WBSF7, suggesting that this could be a biomarker to predict meat tenderness before slaughter. In fact, studying the effect of PGM1 phosphorylation on meat tenderness, Anderson et al [48] found that unphosphorylated PGM1 did not differ between tender and touch meat, and among the five phosphorylated forms only the least phosphorylated differed between tender and tough meat. These results indicate that phosphorylation of PGM1 is important to promote pH decline, whereas high phosphorylated PGM1 did not correlate with pH postmortem.

Three total protein spots were identified as actin, alpha 1 (ACTA1, spots 246, 396, and 406) while one phosphorylated ACTA1 was differentially abundant across postmortem time (spot 109). The more alkaline ACTA1 spot (406) had a consistent and negative correlation with sarcomere and MFI7, even in muscle samples. Contrarily, the phosphorylated ACTA1 spot abundance in unaged and aged meat was positively correlated with sarcomere length and MFI and was highly and negatively correlated with sarcomere length ($r = -0.85$) and WBSF ($r = -0.81$). These results suggest that ACTA1 phosphorylation might increase post rigor sarcomere length and meat tenderness. Interestingly, a phosphorylated ACTA1fragment of 30 kDa was upregulated in tough meat [18], whereas at this study all ACTA1 spots have around 43 kDa.

The abundance of myosin regulatory light chain 2 (MYLPF, spot 4) in unaged meat positively correlated to sarcomere length ($r = -0.72$) and negatively correlated with WBSF. Interestingly this MYLPF spot has not correspondent on ProQ Diamond image, suggesting that this is a unphosphorylated MYLPF spot. The MYLPF is a regulatory component of the thick myofilament, and it has been reported that its phosphorylation increases skeletal muscle contraction force [50]. Therefore, the positive correlation between unphosphorylated MYLPF and sarcomere length suggests that greater MYLPF phosphorylation may increases sarcomere shrinkage during conversion of muscle to meat, decreasing tenderness. In agreement, the phosphorylated MYLPF spot (12) had a negative

correlation with sarcomere length. Furthermore, in previous proteomic studies, greater abundance of phosphorylated MYLPF has been found in tough meat in sheep [19] and beef cattle [13].

The muscle abundance of heat shock cognate 71 kDa protein (HSC70, spot 182) was positively correlated with pH@18C. Previous proteomic study reported increased abundance of heat shock proteins in pork samples with low postmortem muscle exudate [16]. Furthermore, the abundance of HSC70 was highly correlated (r = + 0.948) with beef pH [51]. Although studies have suggested HSC70 as a biomarker for meat tenderness [52], at the current study only some poor correlation were found between HSC70 abundance and meat traits. In agreement, in a study using dot blot to validate biomarkers for meat traits, Guillemin et al. [53] found that muscle abundance of HSC70 differed between tough and tender meat only in *semitendinosus* muscle, but not in *longissimus*.

The ATP synthase subunit beta, mitochondrial (ATP5B, spot 127), was highly and negatively correlated to pH@18C, and fast pH decline. However, it was expected a positive rather than the negative found correlation, once ATP5B is a mitochondrial protein and more abundant in oxidative muscle, which usually have reduced pH decline [54]. However, although oxidative muscle fiber has lower glycolytic capacity, this type of fiber also has reduced buffering capacity and lower phosphocreatine concentration [43], which at least in part contribute to accelerated muscle acidification. In agreement, previous report found a negative correlation (r = - 0.95) between *longissimus* muscle ATP5B abundance and meat pH, in Chinese Luxi yellow cattle [51].

4.6. Proteome and phosphoproteome changes during conversion of muscle to meat and aging

During the conversion of muscle to meat several physical and chemical changes happen impacting meat quality [55]. Throughout aging, meat is stored at 2-6 °C allowing to reduce spoilage and maintain activity of endogenous proteases that breakdown muscle proteins including myofibrillary enhancing meat tenderness [56]. It has been reported that beef has a greater tenderization during the first 14 days [11]. At the current study, the WBSF reduction throughout the 14-d aging period was around 11% for bull and 31% for steers steaks, indicating that steers had greater tenderization during meat storing.

During the first 24 h postmortem, only few of the identified protein spots abundance changed, including some metabolic enzymes. Among the six total protein spots identified as GAPDH only the spot 323 has its abundance reduced from 0 to 24 h postmortem. The PGM1 spots markedly changes during the conversion of muscle to meat, and the abundance of the more alkaline spot (169) decreased 60% while the abundance of the more acids spots (165, and 168) increased around 60%. In addition, abundance of phosphorylated PGM1 spot (149) increased around 55% during this period, indicating a postmortem phosphorylation of PGM1, as previous reported [57], indicating enhancement of PGM1 catalytic activity early postmortem. The PKM2 was another metabolic protein that change during first 24 h postmortem, and the phosphorylated form of PKM2 (spot 329) markedly increased during this time. Phosphorylation of pyruvate kinase has been reported as posttranslational modification that maintain the enzymatic activity in lower pH [57]. The change of these enzymes abundance and phosphorylation during early postmortem may be related to activating glycolytic pathway to produce ATP.

Interestingly, among the eight myofibrillar proteins identified, only the phosphorylated fast (TNNT3) and slow (TNNT1) isoforms of troponin T, had the abundance reduced during conversion of muscle to meat. These data confirm the troponin T as a target protein for early postmortem proteolysis [55]. Nonetheless, the proteolysis of these troponin T isoforms seems to maintain throughout aging up to 14d postmortem, once the abundance decreased from 1 to 14d postmortem, as well as the troponin I2 (TNNI2). In fact, the intensity of the phosphorylated troponin spots at 14d aged meat was positively correlated to meat toughness, indicating that breaking down of troponin is important to develop meat tenderness during aging. In agreement, studying myofibrillar proteins throughout aging by western-blotting Marino et al. [58] found a progressive decreasing of intact bands (38 and 36 kDa) along with increasing of fragment bands (33 and 30 kDa). The authors also pointed out that the dynamic troponin T degradation throughout beef aging was a good predictor of proteolytic potential among muscles.

The creatine kinase M-type was also a target protein for proteolysis early postmortem, and the phosphorylated CKM fragment with 36.4 kDa (spot 386) had a two-fold increased abundance during first 24 h postmortem. Furthermore, analyzing the total protein

abundance of the seven CKM spots at 14d postmortem meat, the intensity of the four heavier spots decreased while the three lighter increased, indicating progressive proteolysis of CKM. Interestingly, the abundance of this CKM fragment spot (407) at 14 d postmortem was positively correlated with WBSF, while the smaller CKM fragment (26.3 kDa) was negatively correlated with WBSF14. Previous reports confirm CKM fragmentation in beef during postmortem [59,60]. These data suggest that CKM is a target for postmortem proteolysis, and continuously CKM breakdown is a good indicator of meat tenderization. Besides, these results demonstrate that correlation between the abundance of a proteolysis target protein fragment and meat tenderness might change within the time, as result of the dynamic and progressive postmortem proteolysis.

Although the abundance of ALDOA did not change during the first 24 h postmortem, the total protein spots with greater pI (85, and 88) reduced while the spot with smaller pI (91) increased from 1 to 14 d postmortem. In addition, phosphorylated ALDOA spots (210, and 43) had their intensity were increased by meat aging. These results indicate enhancement on ALDOA phosphorylation throughout meat aging. Previous phosphoproteome studies have reported increasing ALDOA phosphorylation in pork [61] and lamb [19].

5. Conclusions

The design of this study allowed to study the muscle proteome and phosphoproteome changes by sex condition, conversion of muscle to meat, as well as after aging period. Although castration did not affect pH decline during conversion of muscle to meat, the data suggest that early postmortem phosphorylation of phosphoglucomutase 1 (PGM1) can increase the pH decline rate increasing the final sarcomere length. In addition, the greater beef tenderization followed by castration may is related to increased muscle abundance of glycolytic enzymes. Finally, the correlation between some biomarker and meat quality traits were confirmed here.

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Table 1. Carcass and meat quality traits of Nellore bulls and steers.

Item[1]	Bull	Steer	SEM[2]	*P*-value
Final body weight, kg	504.2	455.2	15.9	0.070
Hot carcass weight, kg	310.1	278.2	11.1	0.089
Back fat thickness, mm	4.47	6.51	0.76	0.089
Rib eye area, cm^2	82.4	77.5	2.63	0.224
pH@18C	6.02	6.10	0.11	0.634
Temp@pH6, ℃	18.2	16.0	2.09	0.479
pH24	5.75	5.78	0.04	0.565
Temp24, ℃	10.3	12.5	1.11	0.219
Sarcomere, µm	1.92	1.92	0.09	0.971
Intramuscular fat, % of wet beef	4.04	6.13	0.42	<0.01
WBSF 1 d postmortem, kgf	5.27	4.55	0.39	0.246
WBSF 7 d postmortem, kgf	4.94	3.96	0.41	0.144
WBSF 14 d postmortem, kgf	4.68	3.12	0.28	<0.01
WBSF change1_14, kgf	0.66	1.37	0.26	0.079
MFI 1 d postmortem	23.4	22.2	2.67	0.770
MFI 7 d postmortem	27.7	42.7	3.29	0.014
MFI 14 d postmortem	32.5	59.2	4.10	<0.01
MFI change1_14	9.98	36.10	4.34	<0.01

[1]pH@18C = carcass pH at temperature 18 ℃; Temp@pH6 = carcass temperature at pH 6; pH24 = carcass pH at 24 h postmortem; Temp24 = carcass temperature at 24 h postmortem; WBSF = Warner-Bratzler shear force; MFI = myofibrillar fragmentation index.

[2]SEM = standard error of the mean.

Table 2. Differentially abundant proteins by castration and postmortem time.

Protein name	Abbre.[1]	UniProt	Spot	Prob.[2]	Mr, kDa Teor/Exp	pI Teor/Exp	Fold change Steer / Bull[3]	Meat / Muscle[4]	Aged / Unaged[5]
Actin, alpha 1, skeletal muscle	ACTA1	A4IFM8	246[x]	100	42.3/43.6	5.23/5.17	0.74	0.75	1.22
			396	99	42.3/43.1	5.23/4.93	0.87	0.96	4.25***
			406	100	42.3/44.2	5.23/5.29	0.47*	1.01	1.07
Alpha-actinin-3	ACTN3	Q0III9	426	98	104/107	5.31/5.29	1.07	0.90	3.38***
ATP synthase subunit beta, mitochondrial	ATP5B	P00829	127	100	56.2/53.5	5.15/4.82	0.84**	1.03	1.08
Carbonic anhydrase 3	CA3	Q3SZX4	35[x]	95	29.6/29.8	7.71/8.74	1.13	0.91	1.05
Creatine kinase M-type	CKM	Q9XSC6	96	100	43.2/43.3	6.63/6.95	1.12$^{\#}$	0.97	0.83*
			98	100	43.2/42.9	6.63/7.10	1.10$^{\#}$	0.95	0.81**
			104	100	43.2/42.7	6.63/7.28	1.06	1.03	0.80**
			238	95	43.2/43.7	6.63/6.82	1.16*	1.06	0.90
			270	94	43.2/26.3	6.63/7.29	1.22	0.80	5.59***
			407	100	43.2/37.1	6.63/7.29	0.89	1.40	5.67***
			58	95	43.2/37.1	6.63/7.07	0.95	1.07	3.03***
Fructose-bisphosphate aldolase	ALDOA	A6QLL8	85	100	39.9/42.0	8.45/9.39	1.41**	1.09	0.66**
			87	100	39.9/42.4	8.45/9.22	1.35*	0.91	0.80
			88	100	39.9/42.2	8.45/9.51	1.37**	0.93	0.72*
			91	98	39.9/42.6	8.45/8.28	1.28	1.06	2.15***
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	P10096	68	99	36.1/38.7	8.50/9.21	1.03	0.96	0.59**
			69	98	36.1/38.8	8.50/8.73	1.21	1.17	0.59**
			72	100	36.1/38.7	8.50/8.61	1.15	0.92	0.62***
			73	99	36.1/38.8	8.50/8.50	1.21*	0.92	0.76**
			322	100	36.1/39.1	8.50/9.44	1.26*	0.98	0.64***
			323	98	36.1/40.3	8.50/9.22	1.42*	0.70*	0.66$^{\#}$
Heat shock cognate 71 kDa protein	HSC70	P19120	182	99	71.4/77.0	5.37/5.35	1.12	1.00	6.04***
			184	100	71.4/76.1	5.37/5.27	1.00	1.03	0.76***
Myosin light chain 1/3	MYL1	A0JNJ5	222	100	21.0/26.5	4.96/4.83	0.83*	1.05	1.20
Myosin regulatory light chain 2	MYLPF	Q0P571	4	100	19.1/18.9	4.91/4.61	0.92	1.02	1.73***
Phosphoglucomutase-1	PGM1	Q08DP0	165	100	61.8/65.1	6.36/6.41	1.22**	1.69***	0.99
			168	100	61.8/64.7	6.36/6.74	1.07	1.66***	0.96
			169	100	61.8/65.4	6.36/6.87	1.00	0.42***	1.20
Phosphoglycerate kinase 1	PGK1	Q3T0P6	105	100	44.9/44.9	8.48/9.45	1.15	0.97	0.71**
Triosephosphate isomerase	TPI1	Q5E956	27	100	26.9/27.2	6.45/6.92	1.2***	1.04	1.02
			28	100	26.9/27.4	6.45/6.72	1.14**	1.06	1.01
			29	100	26.9/27.5	6.45/7.15	1.13*	1.01	1.02
Troponin I2, fast skeletal type	TNNI2	A5PJM2	325	95	23.7/24.6	9.13/9.71	1.36	1.03	0.28**
Troponin T, slow skeletal muscle	TNNT1	Q8MKH6	558	91	31.3/38.1	5.71/5.68	1.01	0.96	0.73***

[1]Protein abbreviation; [2]Protein identification probability (%) by Scaffold software; [3]value greater than one represents upregulation in steers; [4]value greater than one represents upregulation in meat (1 d postmortem); [5]value greater than one represents upregulation in 14 d aged meat.

[x]Spots affected by interaction effect of sex condition by time postmortem.

$^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; $^{\#}P < 0.10$

Table 3. Differentially abundant phosphoproteins by castration and postmortem time.

Protein name	Abbre.[1]	UniProt	Spot	Prob.[2]	Mr, kDa Teor/Exp	pI Teor/Exp	Fold change Steer / Bull[3]	Fold change Meat / Muscle[4]	Fold change Aged / Unaged[5]
Actin, alpha 1, skeletal muscle	ACTA1	A4IFM8	109	100	42.3/44.9	5.23/4.81	0.87	0.87	2.13***
Creatine kinase M-type	CKM	Q9XSC6	386	95	43.2/36.4	6.63/6.97	0.95	2.20***	0.34***
			101	98	43.2/41.7	6.63/7.05	1.15	0.92	2.52***
			201	95	43.2/42.3	6.63/7.38	1.14	0.81	4.79***
Fructose-bisphosphate aldolase	ALDOA	A6QLL8	210	100	39.9/42.4	8.45/8.21	1.10	0.93	2.89***
			43	100	39.9/42.4	8.45/8.78	1.15	0.92	1.82***
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	P10096	208	98	36.1/38.4	8.50/8.01	0.92	0.86	0.49***
			211	98	36.1/38.8	8.50/8.69	0.95	0.88	0.40***
Myosin light chain 1/3, skeletal muscle isoform	MYL1	A0JNJ5	296	100	21.0/25.9	4.96/4.74	0.98	1.18	2.28**
Myosin regulatory light chain 2	MYLPF	Q0P571	12	99	19.1/19.3	4.91/4.36	1.02	0.92	0.86$^{\#}$
			185	90	19.1/20.1	4.91/4.18	1.30**	0.90	0.86
Phosphoglucomutase-1	PGM1	Q08DP0	149	100	61.8/62.3	6.63/6.71	1.01	1.55**	1.11
Pyruvate kinase	PKM2	L8I1M9	329	100	63.6/60.9	7.26/7.60	1.28	2.56***	0.97
Triosephosphate isomerase	TPI	Q5E956	45	95	26.9/27.5	6.45/6.21	1.14*	0.90	1.15
Tropomodulin-1	TMOD1	A0JNC0	251	95	40.5/48.4	5.07/4.26	1.33$^{\#}$	0.93	0.13***
Troponin T, fast skeletal muscle	TNNT3	Q8MKI3	90	95	32.1/39.6	5.99/6.73	1.48*	0.76*	0.28***
Troponin T, slow skeletal muscle	TNNT1	Q8MKH6	82	99	31.3/37.9	5.71/5.62	0.92	0.75**	0.53***
			81	98	31.3/37.8	5.71/5.73	0.84$^{\#}$	0.76**	0.43***

[1]Protein abbreviation; [2]Protein identification probability (%) by Scaffold software; [3]value greater than one represents upregulation in steers; [4]value greater than one represents upregulation in meat (1 d postmortem); [5]value greater than one represents upregulation in 14 d aged meat.

[X]Spots affected by interaction effect of sex condition by time postmortem.

$^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; $^{\#}P < 0.10$

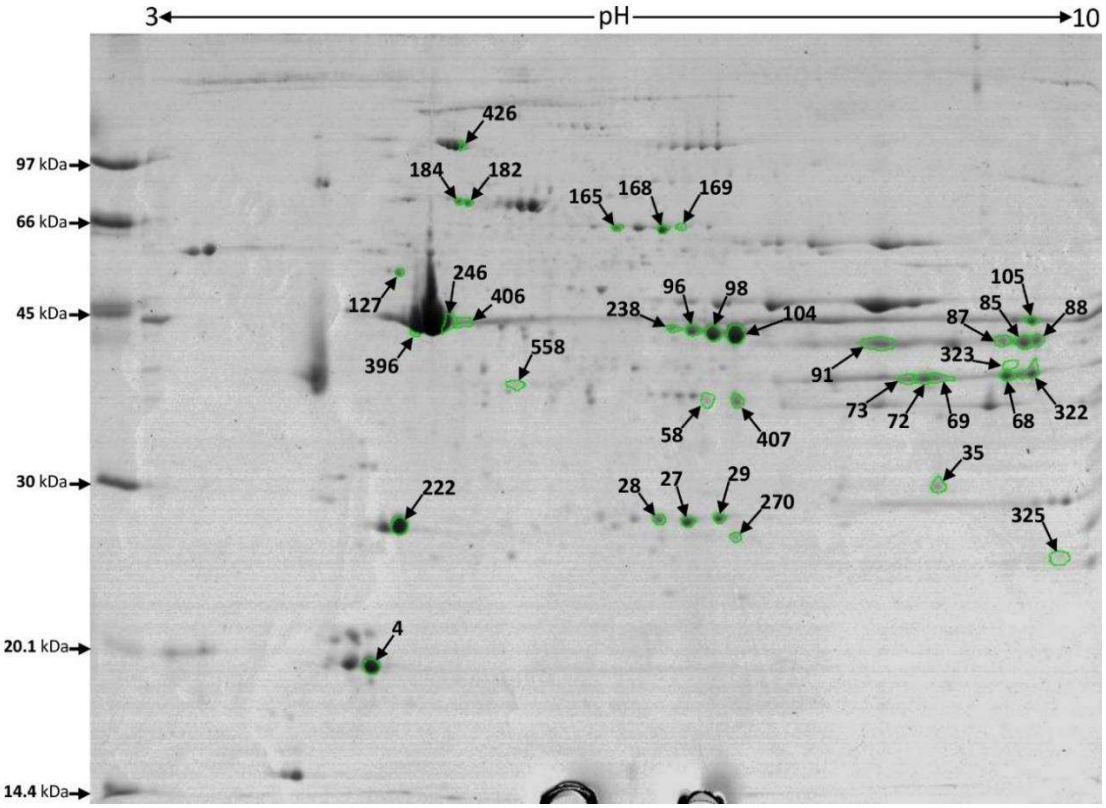


Figure 1. A representative Coomassie stained 2-DE image of Longissimus muscle of Nellore bull and steer.

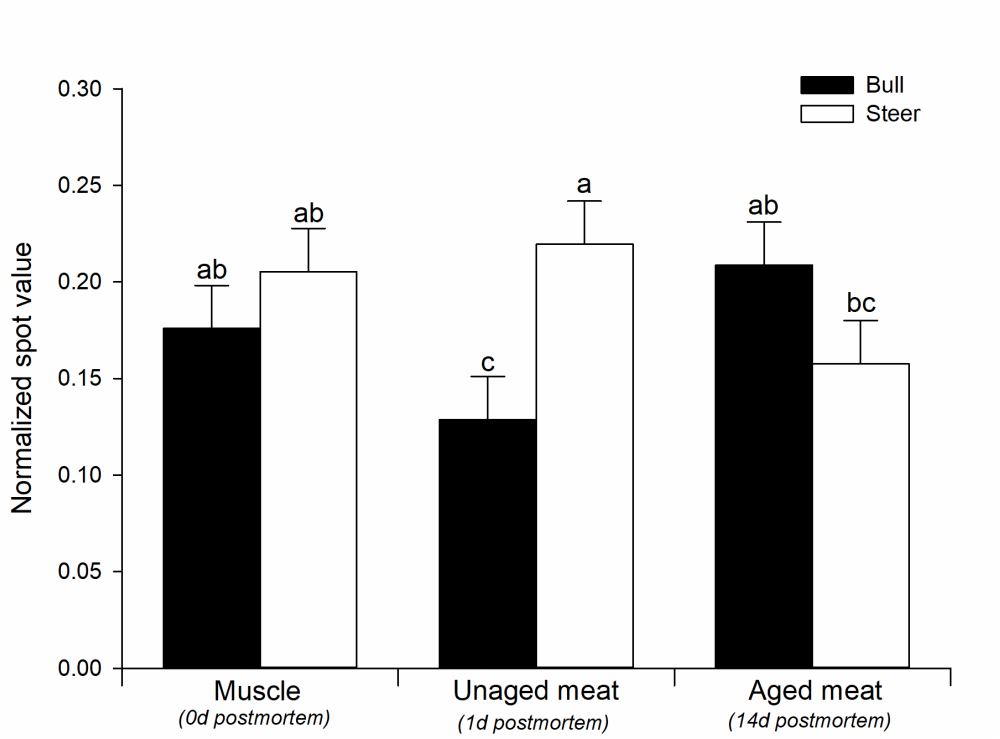


Figure 2. Abundance of carbonic anhydrase 3 (CA3, spot 35) in Longissimus muscle of Nellore bull and steer during postmortem time. Error bar represents standard error of the mean. [abc]Least squared means with different letter are different by student's t test ($P < 0.05$).

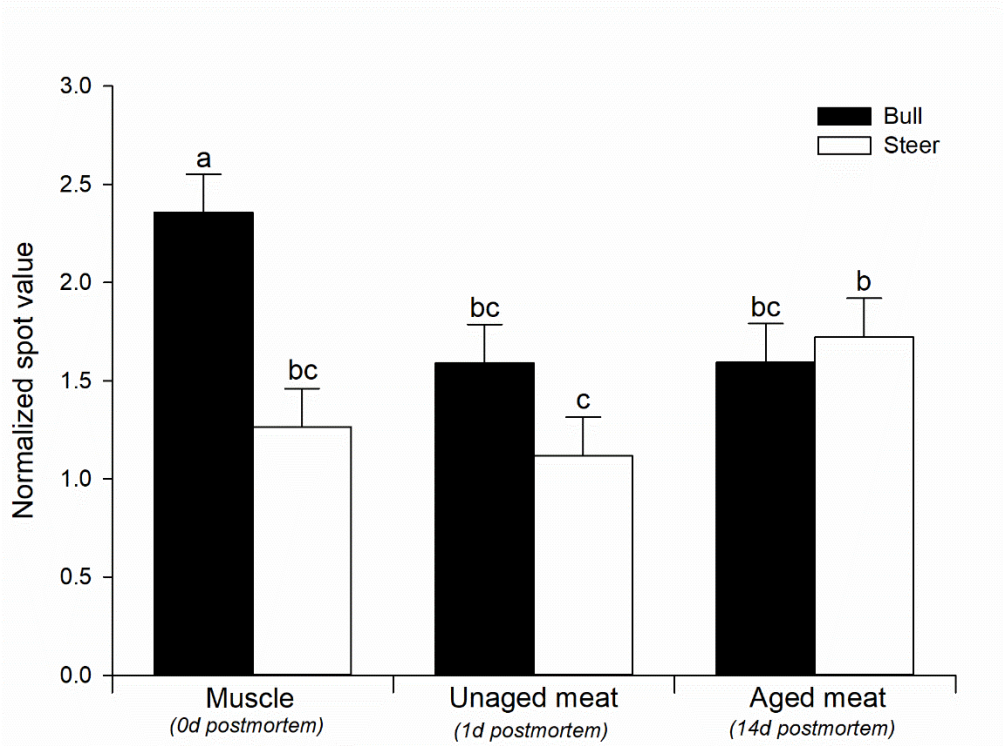


Figure 3. Abundance of actin, alpha 1, skeletal muscle (ACTA1, spot 246) in Longissimus muscle of Nellore bull and steer during postmortem time. Error bar represents standard error of the mean. [abc]Least squared means with different letter are different by student's t test ($P < 0.05$).

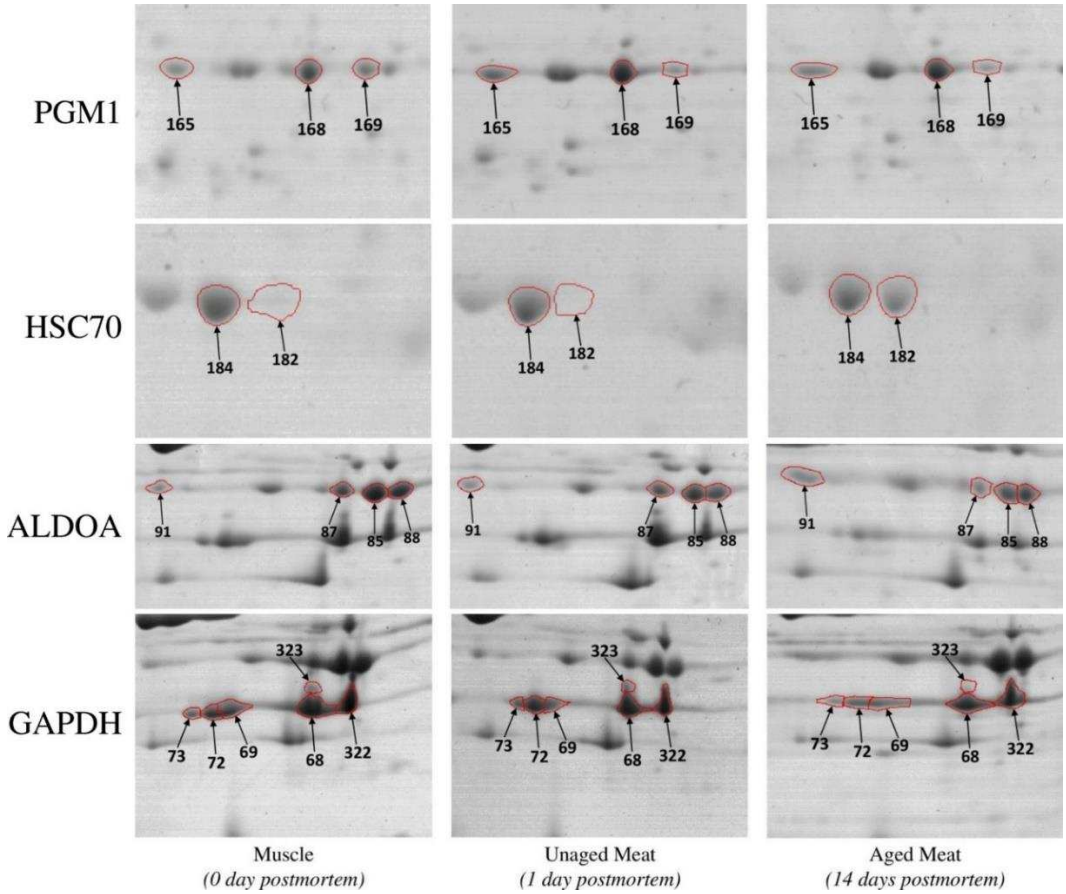
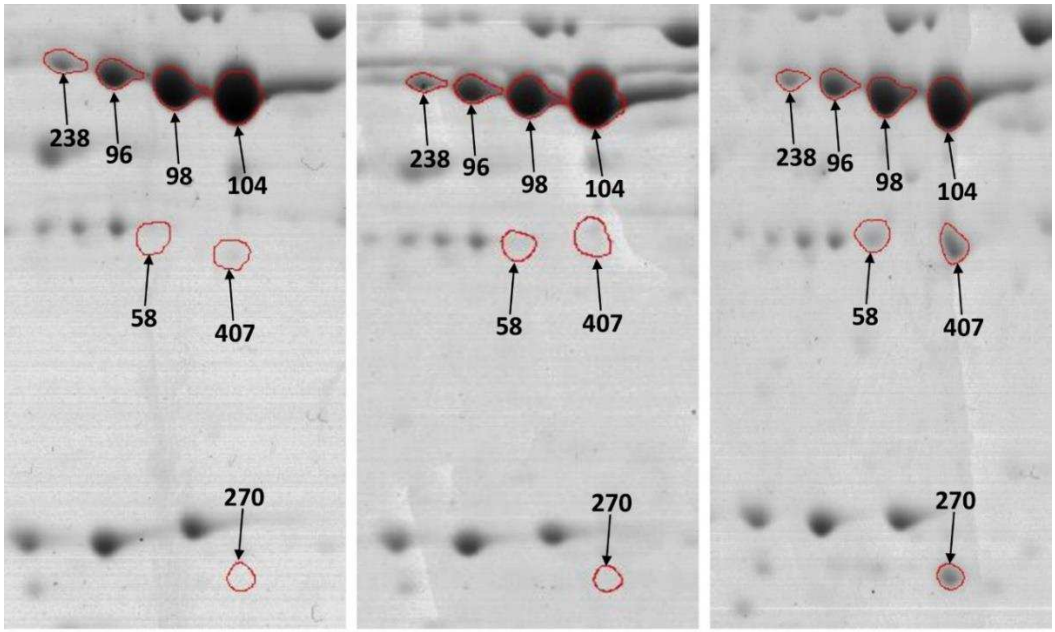


Figure 4. Protein abundance change in Nellore cattle Longissimus muscle during postmortem. Phosphoglucomutase-1 (PGM1), Heat shock cognate 71 kDa protein (HSC70), Fructose-bisphosphate aldolase (ALDOA), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH).



Muscle
(0 day postmortem)

Unaged Meat
(1 day postmortem)

Aged Meat
(14 days postmortem)

Figure 5. Nellore Longissimus muscle abundance of creatine kinase M-type (CKM) during postmortem.

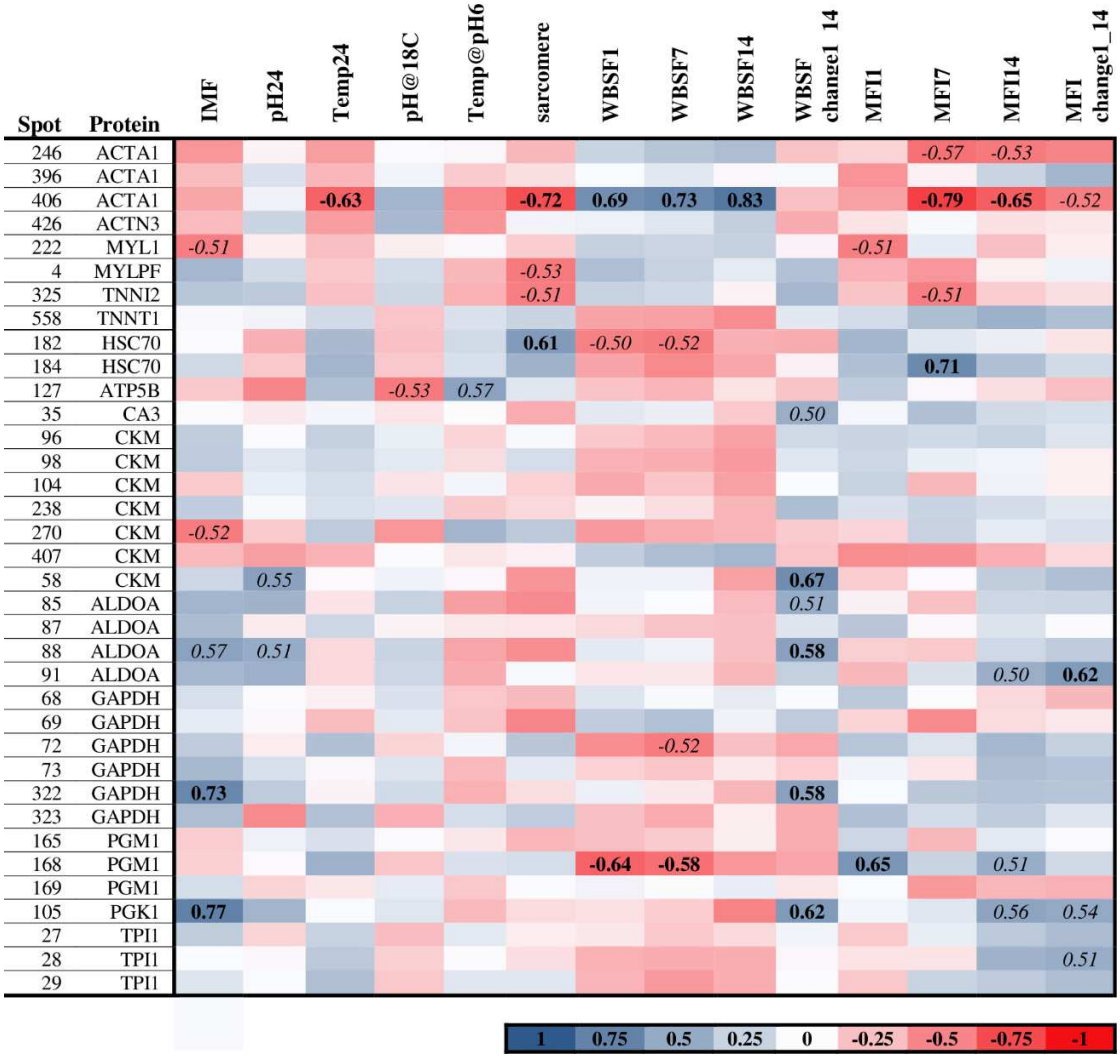


Figure 6. Correlation between total protein abundance in the muscle at the harvest (o d postmortem) and meat quality traits. Bold correlation coefficients are significantly ($P <$ 0.05) different than zero, while italic tended ($P < 0.10$) to differ than zero.

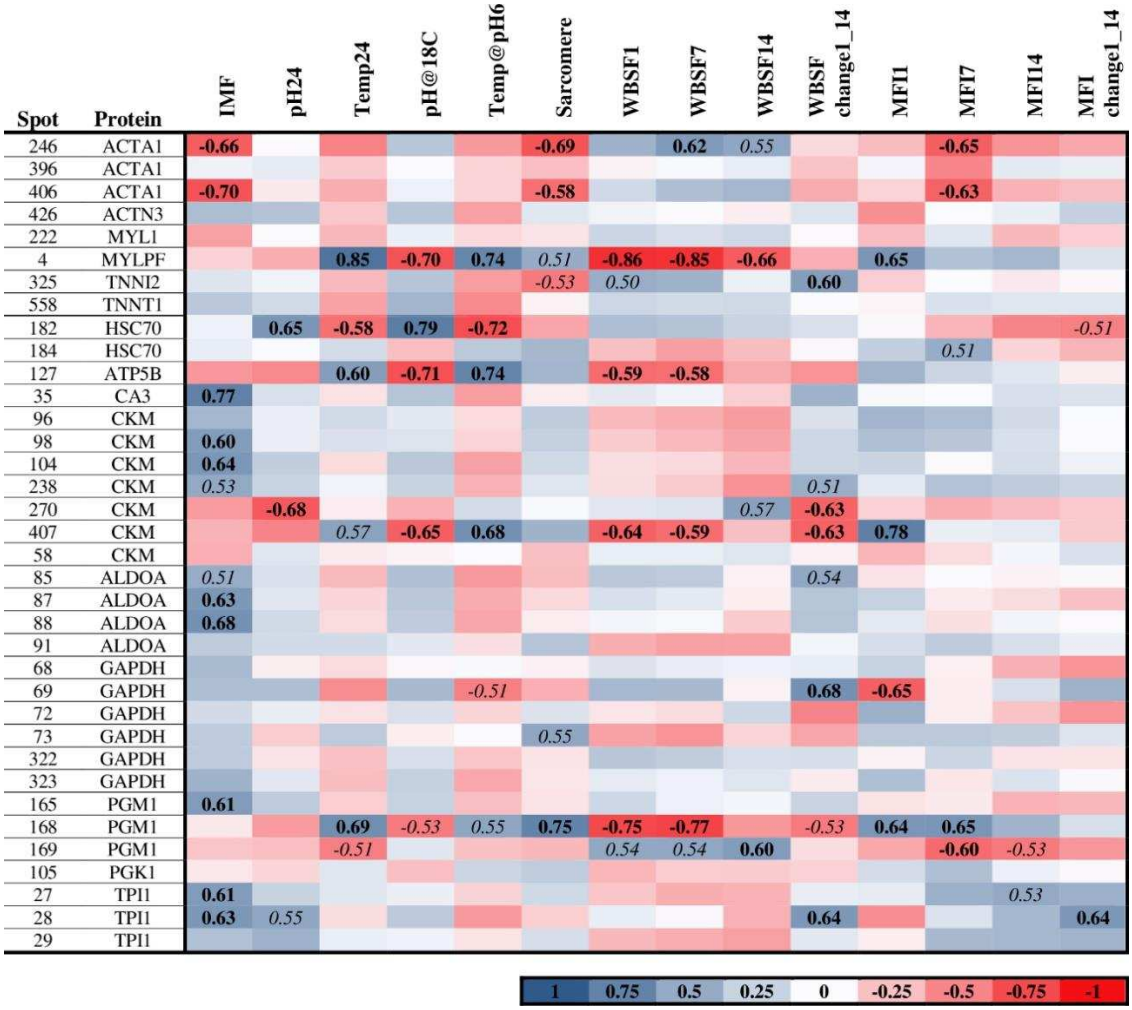


Figure 7. Correlation between total protein abundance in the meat (1 d postmortem) and meat quality traits. Bold correlation coefficients are significantly ($P < 0.05$) different than zero, while italic tended ($P < 0.10$) to differ than zero.

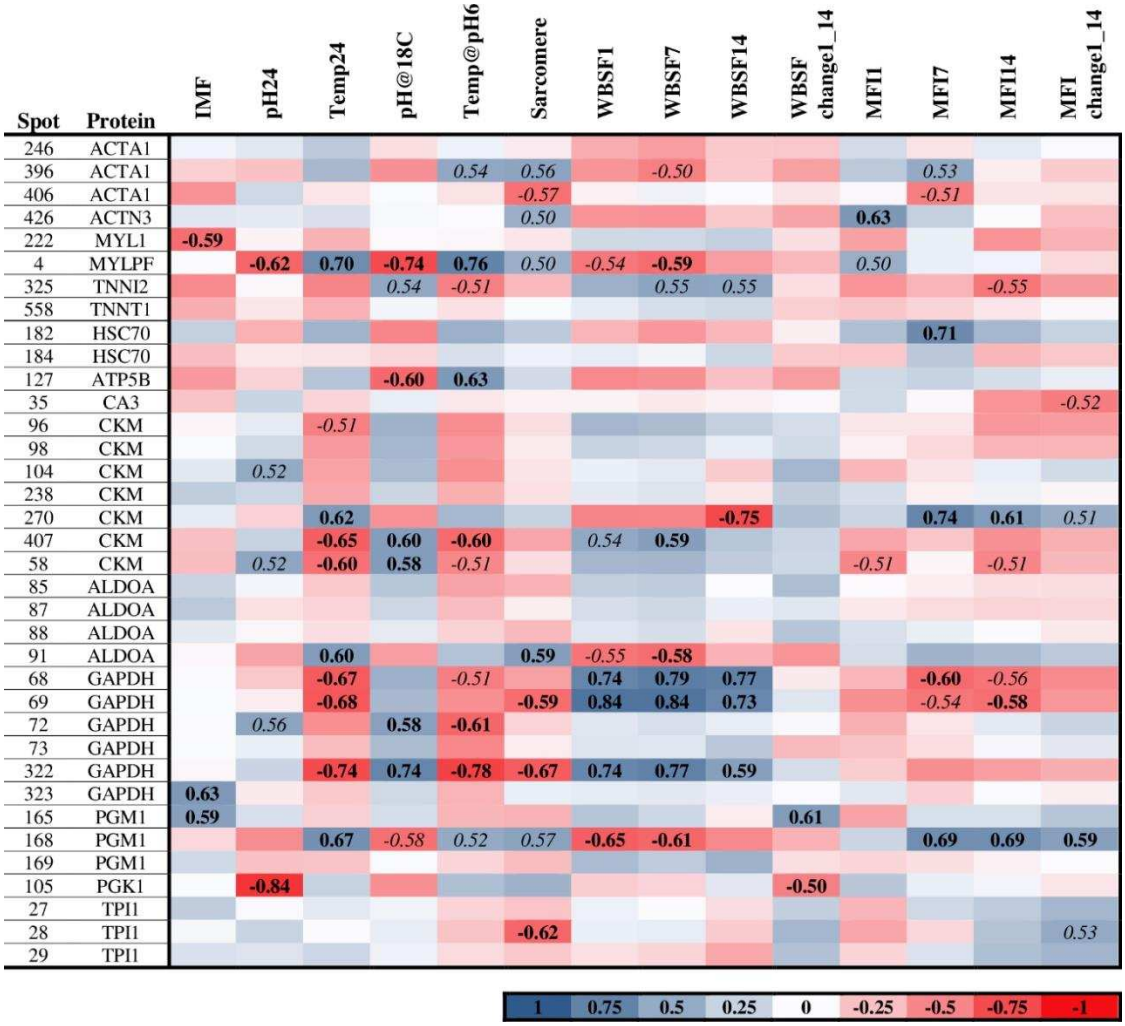


Figure 8. Correlation between total protein abundance in the aged meat (14 d postmortem) and meat quality traits. Bold correlation coefficients are significantly ($P < 0.05$) different than zero, while italic tended ($P < 0.10$) to differ than zero.

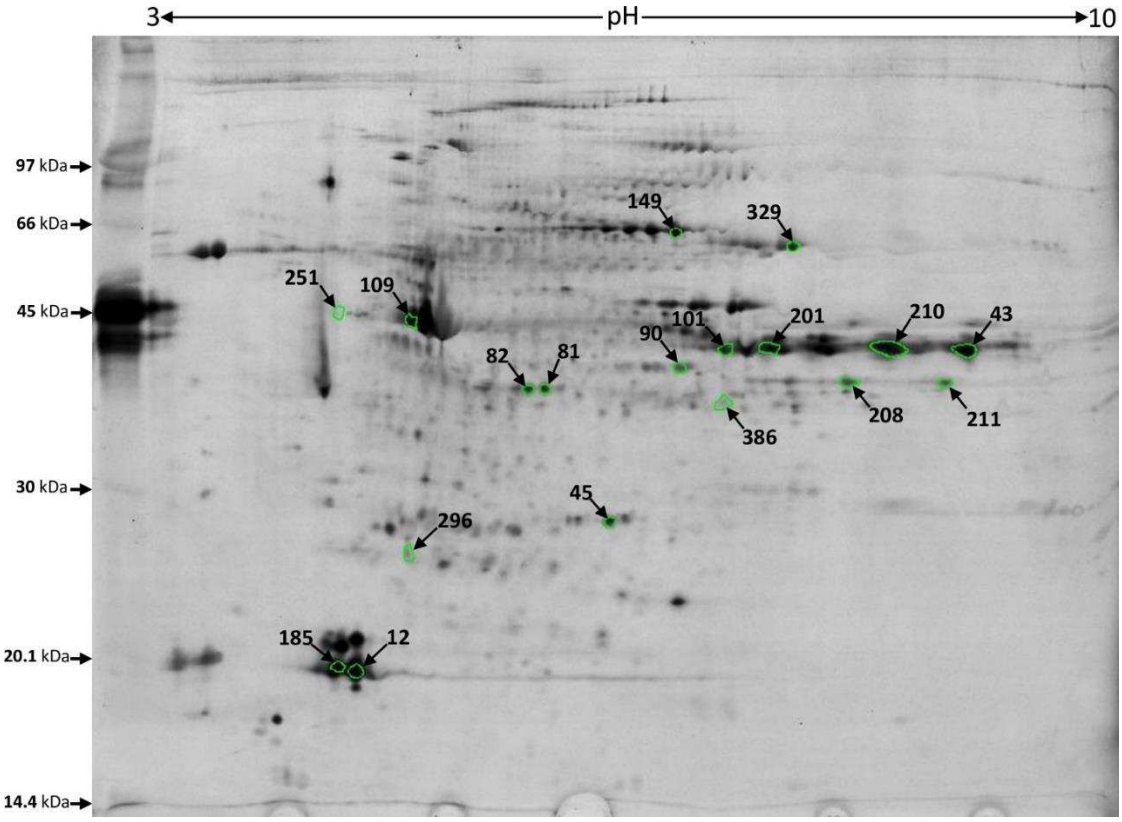


Figure 9. A representative Pro-Q Diamond stained 2-DE image of Longissimus muscle of Nellore bull and steer.

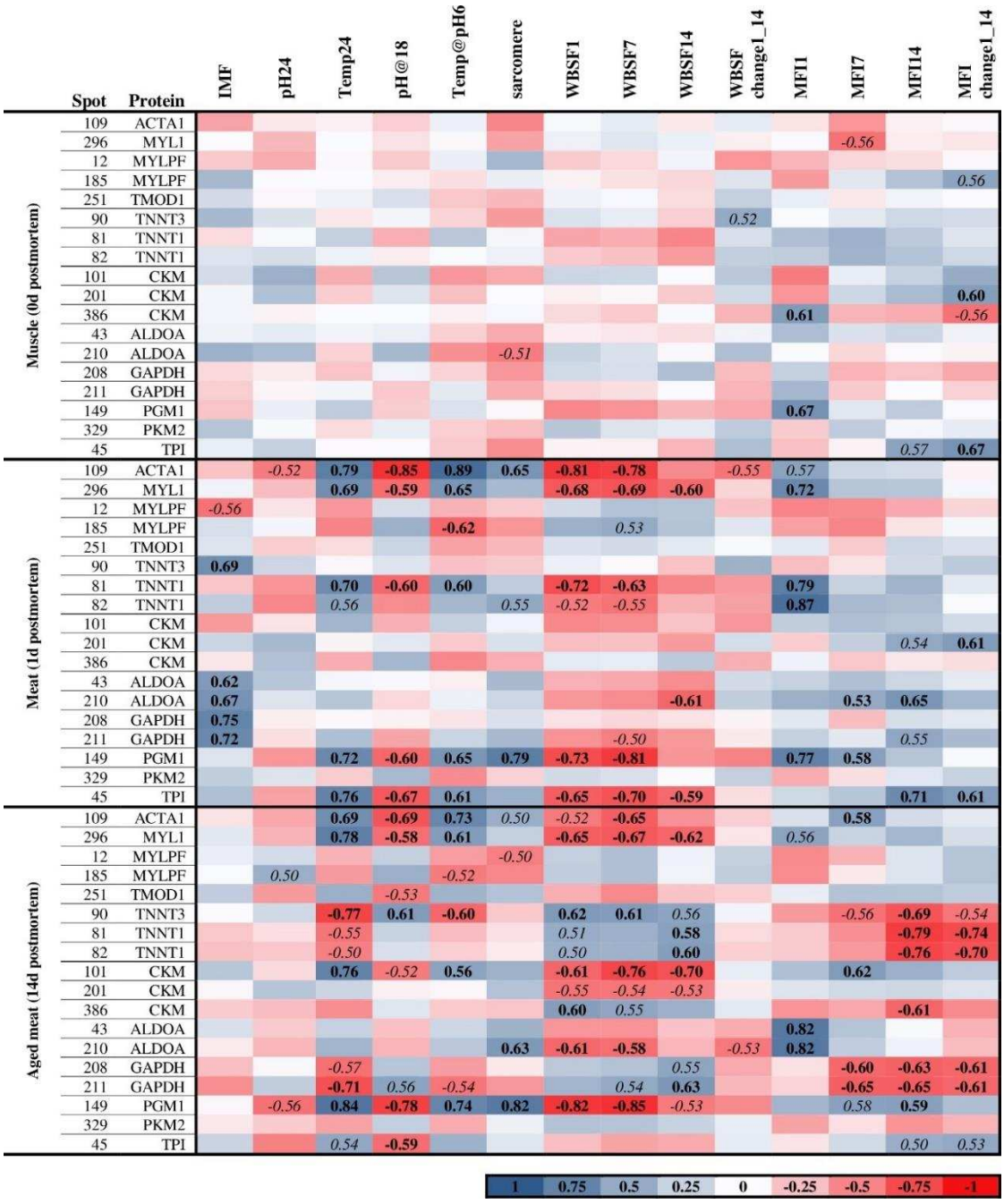


Figure 10. Correlation between Longissimus muscle phosphoprotein abundance during three timepoints postmortem (0, 1, and 14 d postmortem) and meat quality traits. Bold correlation coefficients are significantly ($P < 0.05$) different than zero, while italic tended ($P < 0.10$) to differ than zero.

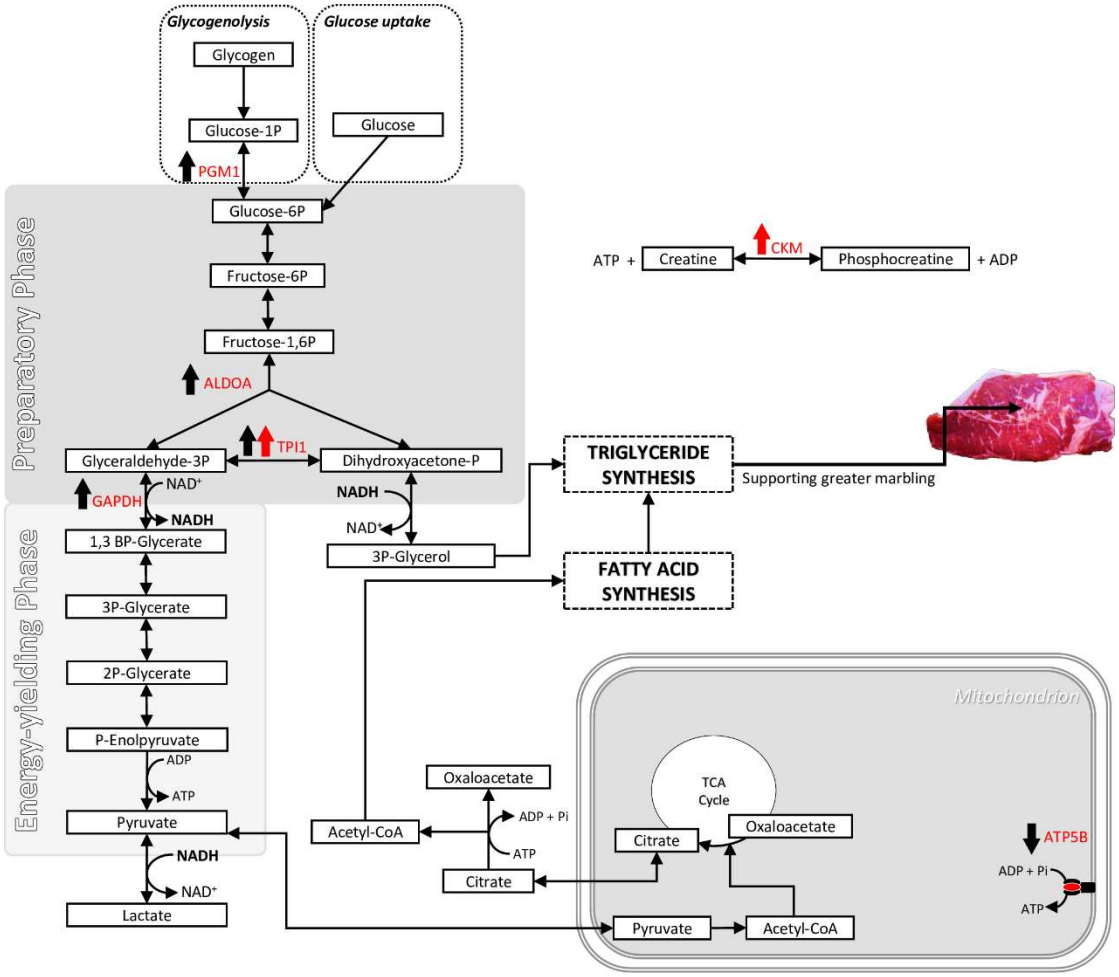


Figure 11. The hypothesis of marbling enhancement by modulating glycolytic enzymes and oxidative phosphorylation. Black arrows indicate up or downregulation of total protein abundance by castration. Red arrow indicates upregulation of phosphoprotein abundance by castration.

GRAPHICAL ABSTRACT:

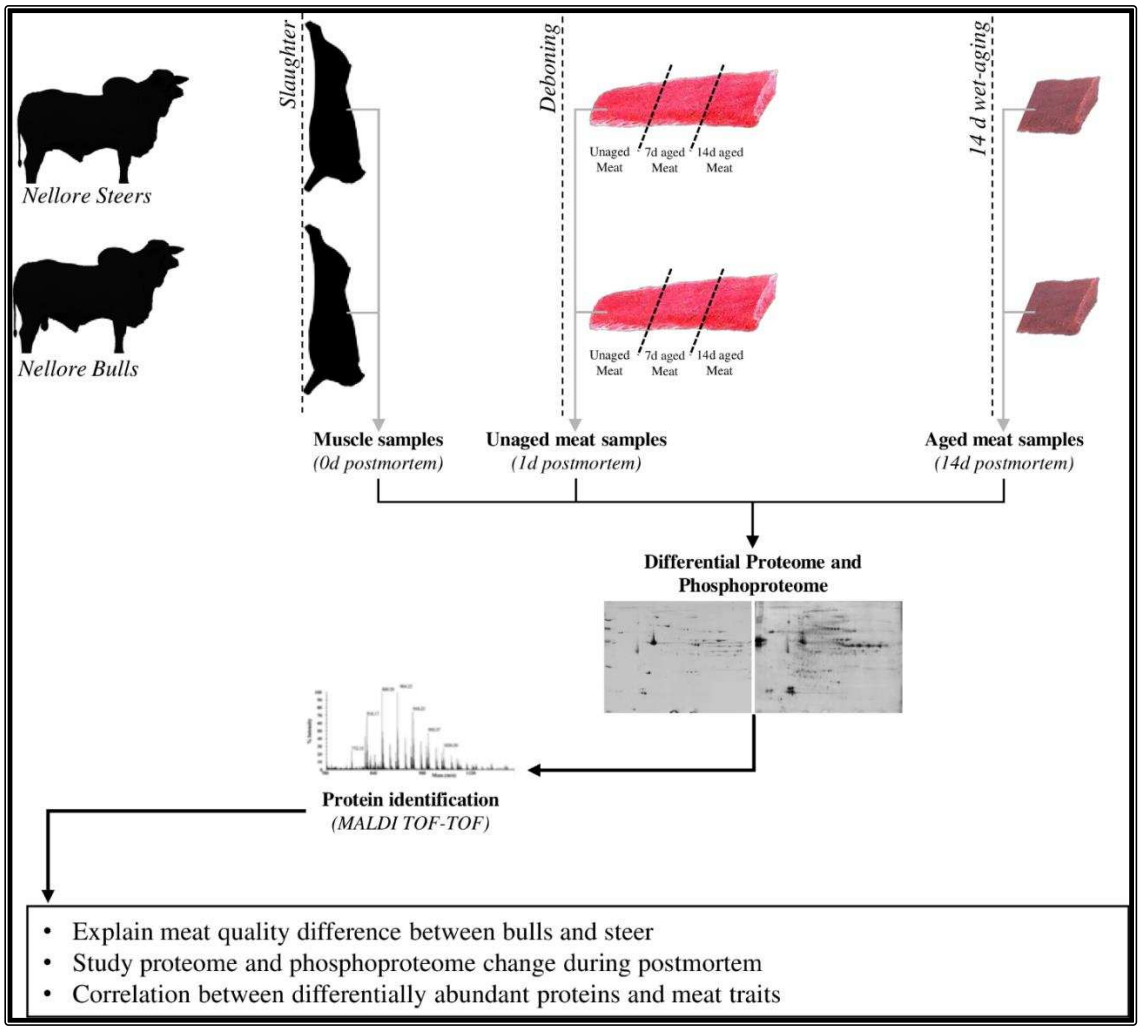


Table S1. Descriptive data of correspondent spots pairs in the both dyes

Pair	Protein[a]	Dye	Spot	UNIPROT Accession number	Score	Protein Prob. (%)[b]	SC (%)	Mr, kDa Teor/Exp		pI Teor/Exp		Peptides sequence	Peptide Prob. (%)[c]
1	TNNT1	Coomassie	558	Q8MKH6	42	91	3	31.3	5.71	38.1	5.68	YEINVLYNR	91
		Pro-Q	82	Q8MKH6	77	99	6	31.3	5.71	37.9	5.62	DLLELQTLIDVHFEQR	94
2	CKM	Coomassie	58	Q9XSC6	68	95	3	43.2	6.63	37.1	7.07	SFLVWVNEEDHLR	95
		Pro-Q	386	Q9XSC6	69	95	3	43.2	6.63	36.4	6.97	SFLVWVNEEDHLR	95
3	PGM1	Coomassie	168	Q08DP0	300	100	6	61.8	6.36	64.7	6.74	LSLcGEESFGTGSDHIR	95
												IDNFEYSDPVDGSISR	95
		Pro-Q	149	Q08DP0	289	100	6	61.8	6.63	62.3	6.71	LSLCGEESFGTGSDHIR	95
												IDNFEYSDPVDGSISR	95
4	CKM	Coomassie	96	Q9XSC6	144	100	6	43.2	6.63	43.3	6.95	DLFDPIIQDR	95
												LSVEALNSLTGEFK	94
		Pro-Q	101	Q9XSC6	75	98	3	43.2	6.63	41.7	7.05	SFLVWVNEEDHLR	95
5	GAPDH	Coomassie	72	P10096	140	100	4	36.1	8.50	38.7	8.61	LISWYDNEFGYSNR	95
		Pro-Q	211	P10096	132	98	4	36.1	8.50	38.8	8.69	LISWYDNEFGYSNR	95
6	ALDOA	Coomassie	91	A6QLL8	94	98	4	39.9	8.45	42.6	8.28	YSHEEIAMATVTALR	95
		Pro-Q	210	A6QLL8	125	100	10	39.9	8.45	42.4	8.21	IGEHTPSSLAIMENANVLAR	92
												YSHEEIAMATVTALR	95

[a]TNNT1 = Troponin T, slow skeletal muscle; CKM = Creatine kinase M-type; PGM1 = Phosphoglucomutase-1; GAPDH = Glyceraldehyde-3-phosphate dehydrogenase; ALDOA = Fructose-bisphosphate aldolase.

[b]Protein identification probability (%) by Scaffold software.

[c]Peptide identification probability (%) by Scaffold software

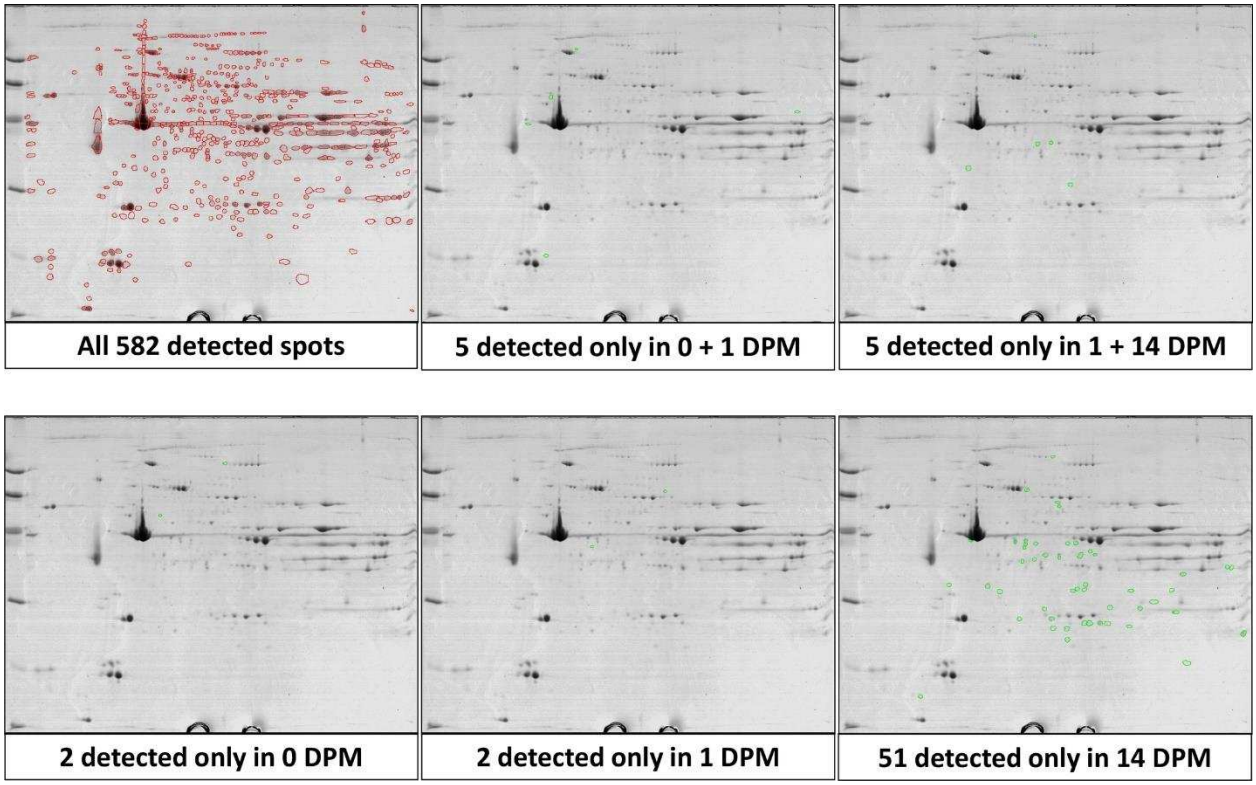


Figure S1. Representative Coomassie stained 2-DE gel showing the spots exclusively detected in specific days postmortem (DPM).

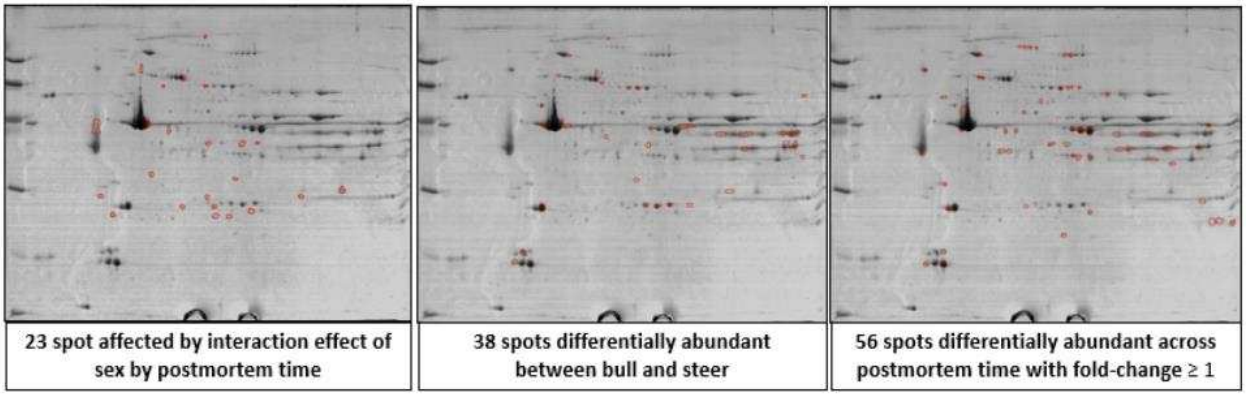


Figure S2. Representative Coomassie stained 2-DE gel showing the spots differentially abundant among treatments.

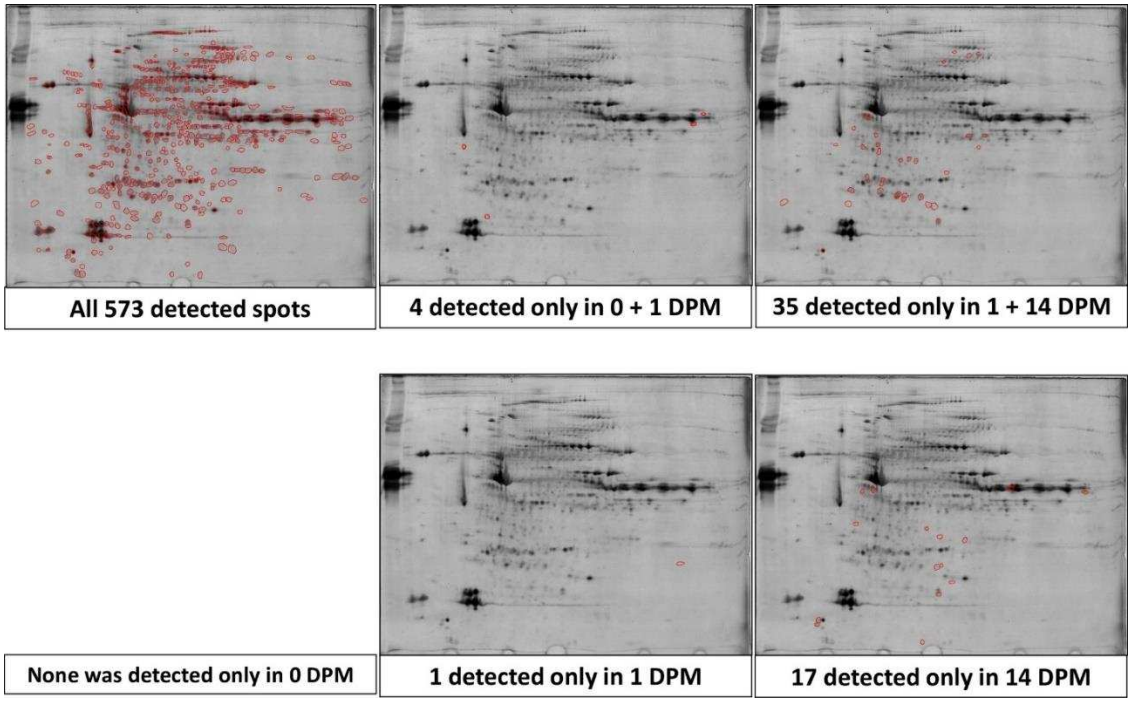


Figure S3. Representative Pro-Q Diamond stained 2-DE gel showing the spots exclusively detected in specific days postmortem (DPM).

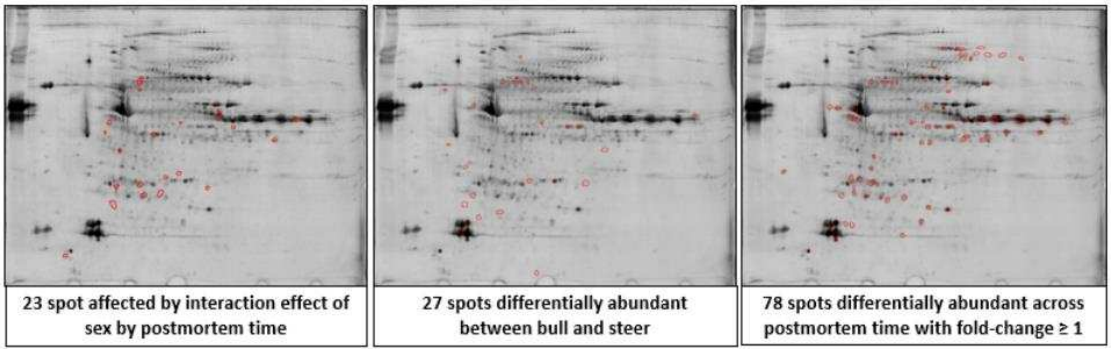


Figure S4. Representative Pro-Q Diamond stained 2-DE gel showing the spots differentially abundant among treatments.

CONSIDERAÇÕES FINAIS – Castrar ou não os bovinos de corte?

Introdução

Apesar da castração ser alvo de estudos científicos publicados desde a década de 50, demonstrando os efeitos sobre o desempenho e características de carcaça, frequentemente nos deparamos com o questionamento sobre a utilização ou não da castração para bovinos destinados à produção de carne. Esta pergunta é muitas vezes levantada tanto na academia quanto por técnicos e produtores nas condições práticas de produção. A resposta para esta pergunta, não muito diferente de outras na área da produção animal, geralmente é um não muito conclusivo "DEPENDE". Isto porque, em determinadas condições a utilização da castração pode ser fundamental, enquanto em outras não há justificativa nem vantagem econômica para sua utilização. Assim, este capítulo final tem como objetivo descrever de forma sucinta o impacto da castração sobre o sistema produtivo e sobre a qualidade da carcaça e da carne.

Porque castrar?

O primeiro questionamento que devemos nos fazer é: Qual a finalidade da castração dos bovinos destinados à produção de carne? Aqui, esta pergunta será tratada em tópicos, abordando os impactos no sistema de produção (porteira para dentro) e na qualidade da carcaça e da carne (do frigorífico ao consumidor).

Impacto no sistema de produção *(porteira a dentro)*

Melhoria no manejo e comportamento animal

A castração normalmente facilita o manejo dos bovinos devido ao aumento na docilidade. Notavelmente, a castração reduz as brigas e montas excessivas (sodomia), que são problemas comuns quando machos não castrados são agrupados. Este efeito da castração em "acalmar" o bovino é visível, aceito como senso comum e comprovado cientificamente. Price et al. (2003) demonstraram uma redução de pelo menos 50% nas montas e brigas após a castração, independentemente do tipo de castração utilizada (cirúrgica ou imunológica). Os pesquisadores destacaram ainda que a energia gasta pelos machos inteiros durante as disputas e montas poderia ser convertida em ganho de peso, apesar de não terem medido esta perda energética.

Este comportamento menos dócil e sexualmente ativo dos machos inteiros não impactam apenas o desempenho produtivo, mas também pode trazer prejuízos econômicos para a propriedade como um todo. Alguns produtores que possuem ciclo completo (cria-recria-engorda) ou propriedades com atividades leiteiras e criação de animais para abate optam pelo uso da castração para evitar cópulas indesejadas e prejuízos com reforma de cercas e instalações.

Aumento na precocidade de acabamento

Sem dúvidas, o principal motivo para a castração de bovinos é o aumento na deposição de gordura. O consumo de matéria seca e consequentemente energia pelo animal não é influenciado pela castração, porém, a forma como a energia é depositada do corpo é marcadamente alterada pela castração. A energia consumida acima do requerimento de mantença pode ser retida no corpo na forma de gordura ou proteína. Bovinos não castrados apresentam um ganho mais "magro" com menor percentagem de gordura, enquanto os castrados depositam percentualmente mais gordura e menos proteína.

Esta alteração na composição do ganho gera as mudanças na composição final da carcaça. Porém, a composição do ganho também pode ser alterada pela taxa de ganho e pelo peso corporal. Normalmente, animais com maior ganho médio diário (GMD) apresentam maior percentagem de gordura no ganho. Esta mudança na composição do ganho foi observada em um trabalho prévio do nosso grupo de pesquisa (Silva, 2014). Na Figura 1 pode se observar que ao aumentar o GMD há um aumento na deposição de proteína e gordura na carcaça, porém o incremento na deposição de gordura é maior do que o de proteína.

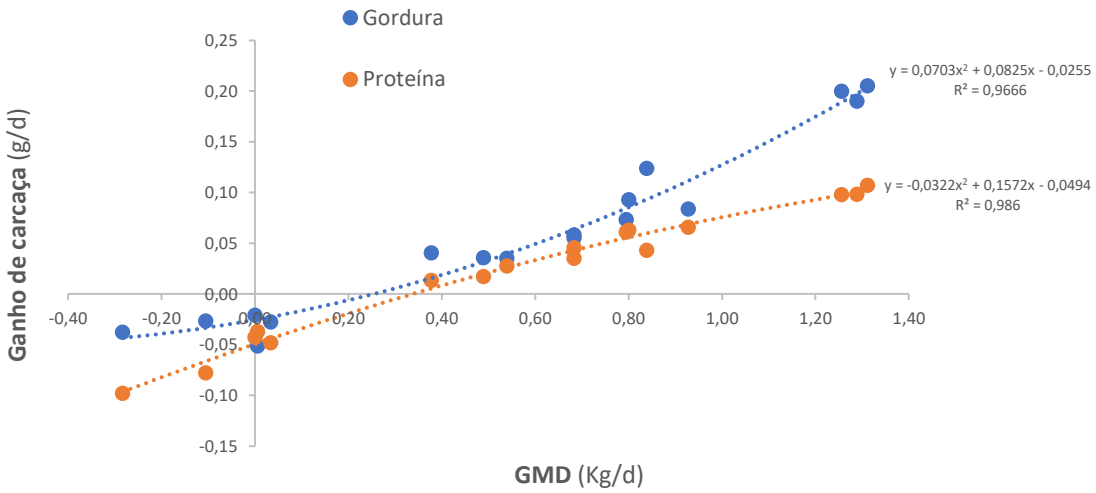


Figura 1. Ganho de proteína e gordura na carcaça de tourinhos Nelore em função do ganho de peso. Dados não publicados, adaptado de Silva, 2014.

Na Figura 2 está apresentada uma simulação da composição do ganho de bovinos castrados e não castrados baseada nas equações do sistema BR-CORTE 2016 (Valadares Filho et al., 2016). A composição do ganho foi estimada considerando a equações para bovinos Nelore em confinamento pesando 150 ou 400 kg de peso corporal. Foi utilizado uma faixa de desempenho de 0,1 a 1,4 kg/d. Esta simulação mostra que ao aumentar o ganho médio diário (GMD) há um leve aumento na percentagem de gordura no ganho, demonstrando que animais com maior taxa de ganho apresentam um ganho mais "gordo". Note que este padrão é similar entre castrado e inteiro. Outro ponto interessante demonstrado nesta simulação é que quanto mais pesado o animal maior é a proporção de gordura no ganho. Levando em consideração os valores obtidos nesta simulação, machos não castrados apresentam em média uma redução de 5 pontos percentuais na deposição de gordura.

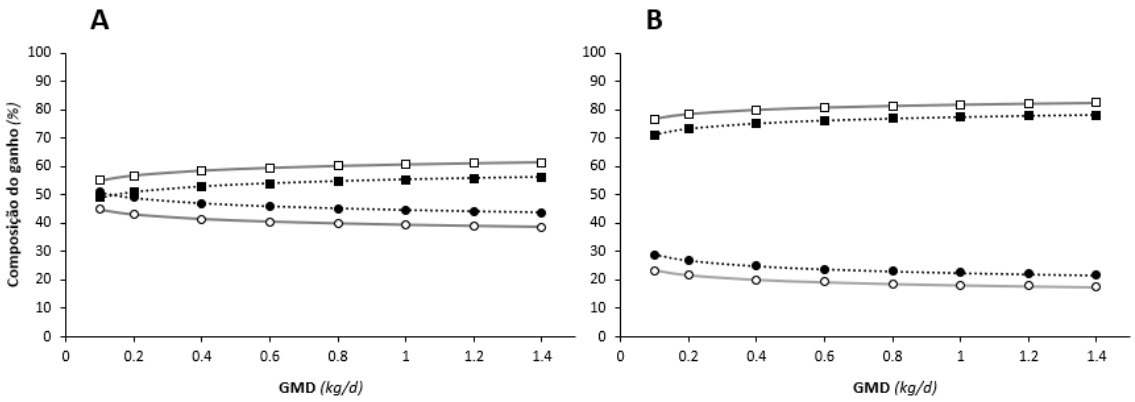


Figure 2. Percentagem da energia retida como gordura (quadrado) ou proteína (círculo) no ganho de bovinos Nelore castrados (quadrado e círculo aberto) ou não castrados (quadrado e círculo fechado) ao 150 kg (A) ou 400 kg (B) de peso corporal. Simulação com base no sistema BR-CORTE 2016.

Estes dados demonstram que a castração não é a única forma de aumentar a deposição de gordura na carcaça. O incremento na taxa de ganho não só aumenta a proporção de gordura no ganho, mas leva ao rápido aumento no peso corporal o que também auxilia na deposição de gordura. Apesar de ser inegável a superioridade dos castrados em depositar mais gordura, um animal castrado em uma dieta com baixa densidade energética pode depositar menos gordura do que o não castrado recebendo uma dieta alta em energia. De forma mais direta, a castração promove a maior deposição de gordura, mas é possível melhorar a deposição de gordura dos animais não castrados aumentando a taxa de ganho.

Como conceito de composição "ideal" da carcaça, temos que a carcaça deve ter o máximo de tecido muscular, mínimo de tecido ósseo e quantidade "adequada" de gordura. Em outras palavras, a quantidade de gordura dever ser o suficiente para atingir as exigências do mercado consumidor. Em países como Estados Unidos e Japão que possuem uma alta bonificação por carne marmorizada, a utilização de bovinos castrados se faz necessária para se obter melhor preço pela carcaça comercializada. No Brasil, aparentemente está havendo um aumento na procura por carnes marmorizadas, haja visto as marcas de carne que foram criadas recentemente. Porém, apesar da bonificação de carcaças por marmoreio ainda estar um pouco distante da realidade em nosso país, há uma bonificação por parte de alguns frigoríficos para a gordura de acabamento. O ágio normalmente é pago para as

carcaças com cobertura de gordura maior de 3 milímetros na região do contrafilé, e carcaças muito gordas (mais de 10 mm) e muito magras (menos de 3 mm) recebem preço de balcão ou até mesmo um deságio.

Desta forma, tendo esta faixa de 3-10 mm de gordura como o nível adequado de gordura para o mercado da carne brasileira, este nível de gordura na carcaça pode ser alcançado utilizando animais Nelore não castrados em confinamento. Esta afirmação é embasada em trabalhos realizados em diversas instituições de pesquisa no Brasil utilizando animais da raça Nelore. Freitas et al. (2008) comparando desempenho produtivo de machos Nelore castrados cirurgicamente ou não observaram aumento na espessura de gordura subcutânea pela castração, como esperado, porem os machos não castrados atingiram em média 3 mm de gordura subcutânea ao 395 kg de peso corporal. Em outro estudo comparando classes sexuais, Paulino et al. (2009) observaram similar taxa de deposição de gordura entre machos castrados e inteiros, sendo os machos inteiros abatidos com 435 kg de peso corporal (PC) apresentando 5,82 mm de gordura subcutânea. Este mínimo grau de acabamento foi obtido mesmo após um curto período de confinamento de 63 dias utilizando a dieta do grão inteiro (Dias et al., 2016). Esta meta de acabamento pode ainda ser atingida mais facilmente utilizando cruzamento com raças de taurinos britânicos (como Angus).

No entanto, os estudos com bovinos Nelore terminados em pastejo mostram que o acabamento pode não ser facilmente atingido sem a castração, quando os bovinos são mantidos sem ou com baixa suplementação. Acabamento abaixo do mínimo (<3mm) foi observado por Moreira et al. (2017) utilizando bovinos Nelore x Angus a pasto suplementados com 0,3% do peso corporal, e mesmo sendo abatidos mais pesados (523 kg de peso corporal) os machos inteiros produziram carcaças com em média 1,8 mm de gordura. Dados similares fora m obtidos por Ribeiro et al. (2004) em um estudo com Nelore a pasto sem suplementação, onde os machos inteiros apresentaram um peso de abate de 517 kg e 2,9 mm de gordura subcutânea.

Todavia, cabe aqui ressaltar que no caso de machos inteiros não basta somente atingir o acabamento mínimo para obter a bonificação, normalmente os frigoríficos estreitam a faixa de maturidade desta categoria para 0 dente incisivo permanente (DIP). Ou seja,

normalmente para obter a bonificação o produtor tem que entregar os machos não castrado com menos de 24 meses de idade e com acabamento mediano (3-6mm) ou uniforme (6-10mm). Caso o pecuarista castre seus animais, ele "ganha" mais um ano para atingir o nível de 3 mm de gordura na carcaça, e pode abate seus animais com 2 DIP, ou seja, entorno de 36 meses de idade. Em conclusão, apesar de ser possível atingir as características de carcaça demandadas atualmente pelo mercado brasileiro utilizando machos não castrados, a castração pode tornar mais fácil a obtenção na bonificação.

Embora os consumidores brasileiros não serem exigentes quanto ao marmoreio, aparentemente há uma crescente parcela da população brasileira que está buscando carnes diferenciadas, estando disposta a pagar mais por carne marmorizada. Tendo como objetivo produzir carne marmorizada, a castração se apresenta como uma boa ferramenta. Os resultados científicos se mostram bem consistentes quanto ao aumento do marmoreio em animais castrados. Isto porque, a castração aumenta a deposição de gordura como um todo na carcaça, como previamente demonstrado aqui, antecipando também a deposição de gordura de marmoreio, que normalmente é o depósito de gordura mais tardio.

Interessantemente, em nosso estudo apresentado nesta tese, observamos animais com bom grau de marmorização (6% de gordura na carne fresca) mesmo sendo da raça Nelore, considerada de baixo potencial para deposição deste tipo de gordura. Em nossa pesquisa observamos que quando leves e com pouca gordura corporal, não há diferença entre macho castrado e não castrado, porém ao atingirem o ponto de abate de 480 kg aos 16 meses de idade os bovinos castrados apresentaram carnes com 50% mais marmoreio (Figura 3). Demonstrando a maior precocidade dos animais castrados para depositarem esta gordura de marmoreio (ou gordura intramuscular). Neste estudo utilizamos ferramentas de ponta para avaliar a mudança na expressão de genes e de proteínas que pudessem nos esclarecer melhor o porquê da maior deposição intramuscular de gordura nos animais castrados. Os resultados indicam que mudanças no metabolismo da glicose e no tipo de fibra muscular favorecem a maior deposição de gordura intramuscular nos animais castrados (Silva, 2018).

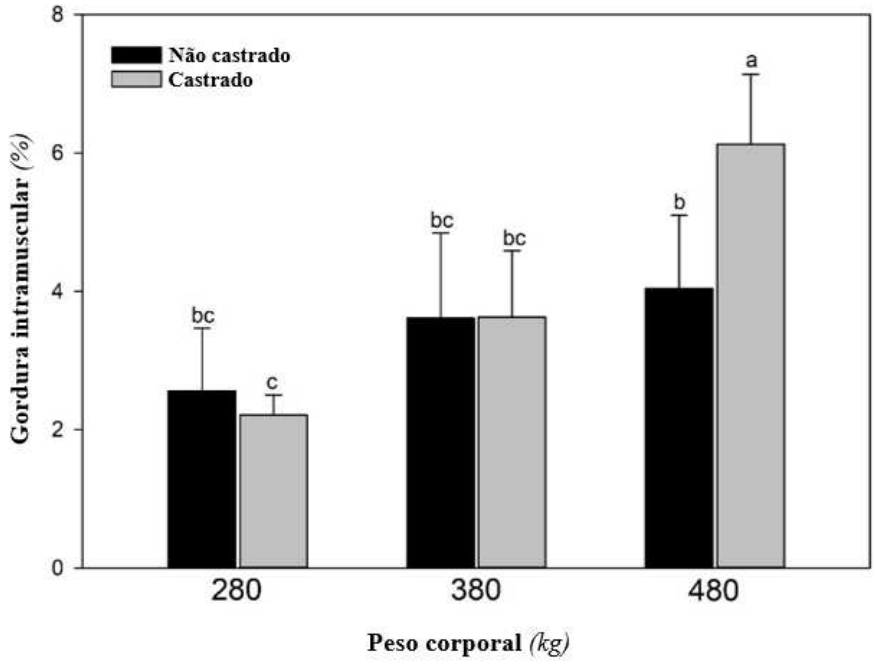


Figura 3. Conteúdo de gordura intramuscular da carne fresca de bovinos Nelore castrados ou nãos e abatidos com 280, 380 e 480 kg de peso corporal. [abc]Médias com diferentes letras diferem pelo teste Tukey ($P < 0.05$). (Silva, 2018).

Deste modo, a castração de bovinos de corte se faz necessária quando se deseja atingir maior grau de marmorização da carne. Esta produção de carne diferenciada atualmente se limita aos nichos de mercado existentes no Brasil, onde a carne é um ingrediente gourmet ao invés de commodity.

Castração reduz o ganho de peso e eficiência alimentar

Contudo, a maior precocidade de acabamento de animais castrados tem um preço, como diz o dito popular: "Não existe almoço grátis". Essa maior deposição de gordura resulta em reduções na taxa de ganho e eficiência alimentar. Bovinos castrados apresentam normalmente uma redução de até 17% no ganho de peso e de 13% na eficiência alimentar (Seideman et al., 1982). A mudança na composição do ganho é a principal causa para a redução no ganho e na eficiência alimentar de bovinos castrados. Isto porque, como os animais inteiros depositam mais proteína há também uma maior deposição de água no corpo, ou seja, é mais eficiente depositar tecido magro do que tecido gordo. Considerando

a conversão de matéria seca (MS) em carcaça, Pedro Veiga encontrou em seu estudo que machos Nelore não castrados consumiram 13,2 kg de MS para cada quilo de carcaça depositada enquanto o castrado precisou consumir 15,9 Kg de MS para depositar a mesma quantidade de carcaça (Paulino et al., 2009). Em nosso trabalho, observamos uma eficiência alimentar para deposição de carcaça de 0,10 para machos inteiros e 0,09 para machos castrados, insto significa que o macho inteiro converteu 10% da MS consumida em carcaça, enquanto o castrado converteu 9% da MS ingerida em peso de carcaça (Silva, 2018).

Este menor desempenho produtivo de bovinos castrados pode ser corrigido com o uso de anabolizantes exógenos, que mantem o animal com comportamento e acabamento de castrado, porém com desempenho similar de inteiro (Hunt et al., 1991). Contudo, em países como Brasil e os países do bloco da União Europeia a utilização dos anabolizantes exógenos é proibida. Sendo assim, a perda em desempenho dos animais castrados não pode ser corrigida utilizando a tecnologia dos anabolizantes exógenos.

A testosterona é um hormônio anabolizante endógeno, que estimula o crescimento animal e a diferenciação sexual (dimorfismo sexual). No tecido muscular, as evidências científicas indicam que este hormônio pode atuar diretamente no tecido muscular estimulando seu crescimento ou ainda de forma indireta aumentando a síntese e liberação do hormônio do crescimento (Dayton & White, 2014). Desta forma, a castração reduz os níveis circulantes deste hormônio, diminuindo o estímulo para o crescimento animal.

A produção de testosterona pelas células de Leydig nos testículos aumenta gradativamente durante a puberdade em respostas aos pulsos do hormônio LH. Assim, pouca diferença é observada entre bovinos machos castrados e não castrados do nascimento até a puberdade, pois a produção de testosterona ainda é muito baixa nos bezerros não castrados (Bretschneider, 2005). Da mesma forma, a aplicação de testosterona em bezerros prepuberes não melhora o desempenho e ainda atrasa a puberdade (Godfrey et al., 1992). Por outro lado, após a puberdade é mais evidente a diferença no desempenho entre machos castrados e inteiros.

Quando castrar?

Há distintas opiniões quanto a idade "certa" para realizar a castração, e encontramos recomendações que variam desde próximo ao nascimento até faltando poucas arrobas para o peso de abate. Do ponto de vista produtivo o mais interessante seria manter o animal na condição de inteiro até próximo do abate, tendo assim um animal mais eficiente na maior parte da vida produtiva, e então castrar já próximo ao peso de abate apenas para melhorar as características de carcaça e da carne. Porém, em idade avançada o estresse sofrido pelo animal é bem maior, gerando queda no consumo e elevada perda de peso pós cirurgia. Desta forma, o melhor desempenho do macho inteiro antes da castração pode ser parcialmente ou totalmente perdido logo após a cirurgia (Bretschneider, 2005). Neste sentido, a imunocastração tem se tornado uma interessante ferramenta para explorar por um maior período a eficiência de produção do macho inteiro, devido a testosterona endógena, e então castrar o animal no período de engorda para melhor deposição de gordura na carcaça. As pesquisas realizadas no Brasil confirmam as vantagens deste tipo de castração nas nossas condições de criação (Miguel et al., 2014; Moreira et al., 2017).

Tendo como exemplo a indústria de suínos, a castração de leitões próximo ao nascimento é altamente utilizada devido a alteração no sabor da carne. Nesta fase o animal sofre menos e o prejuízo econômico será menor caso algum animal venha a óbito. Este mesmo raciocínio pode ser utilizado para bovinos, e os estudos comprovam menor estresse, ou seja, menor liberação de cortisol, em bovinos castrados próximos ao nascimento (Bretschneider, 2005). Desta forma, a castração até os três meses parece ser uma boa recomendação reduzindo o estresse do animal e a perda pós-cirúrgica. Além disto, pode ser utilizada a castração com elastrador, que é um método simples e prático de castração. No Brasil, é mais comum a castração em torno dos 7 meses coincidindo com o manejo do desmame.

Impacto na qualidade da carcaça e da carne *(do frigorifico ao consumidor)*

Resfriamento da carcaça

A gordura de cobertura é um importante aspecto qualitativo da carcaça, isto porque após a desossa os principais cortes da carcaça serão comercializados com esta porção de gordura, melhorando a aparência e sabor da carne. Além disto, a camada externa de gordura também auxilia no adequado resfriamento da carcaça. Quando a carcaça se resfria

muito rápido há um impacto negativo para a maciez da carne e este fenômeno é conhecido na área de ciência da carne como encurtamento pelo frio. Então, carcaças muito magras, isto é, com espessura de gordura subcutânea menor do que 3 mm, estão mais susceptíveis a este tipo de problema.

A falta de acabamento das carcaças bovinas aparentemente é um problema presente em nosso sistema produtivo. O estudo de Pflanzer e Felício (2009) demonstra que na situação produtiva brasileira a maciez da carne produzida pode ser mais afetada pelo acabamento do que pela maturidade do animal, isto é, animais mais velhos com bom acabamento podem produzir carnes mais macias do que os animais jovens abatidos com gordura de acabamento escassa. Neste contexto, a castração pode melhorar a maciez da carne aumentando a gordura de acabamento e reduzindo a incidência do encurtamento pelo frio. Em nosso estudo, avaliando o resfriamento e a queda do pH após o abate, não observamos diferença na queda de temperatura e pH das carcaças de machos castrados e não castrados (Figura 4). Este resultado pode ser explicado pelo bom acabamento dos machos não castrados, que apesar de apresentarem gordura subcutânea 24% menor que os castrados (3.8 vs 4.7 mm), a camada de gordura subcutânea dos machos inteiros foi em média superior a 3 mm. Da mesma forma, a maciez da carne não maturada medida por um instrumento demonstrou que os bovinos inteiros produziram uma carne com maciez similar a dos castrados. Estes resultados comprovam que bovinos bem terminados minimizam o problema de encurtamento pelo frio que afeta a maciez da carne, independentemente se a castração foi aplicada ou não.

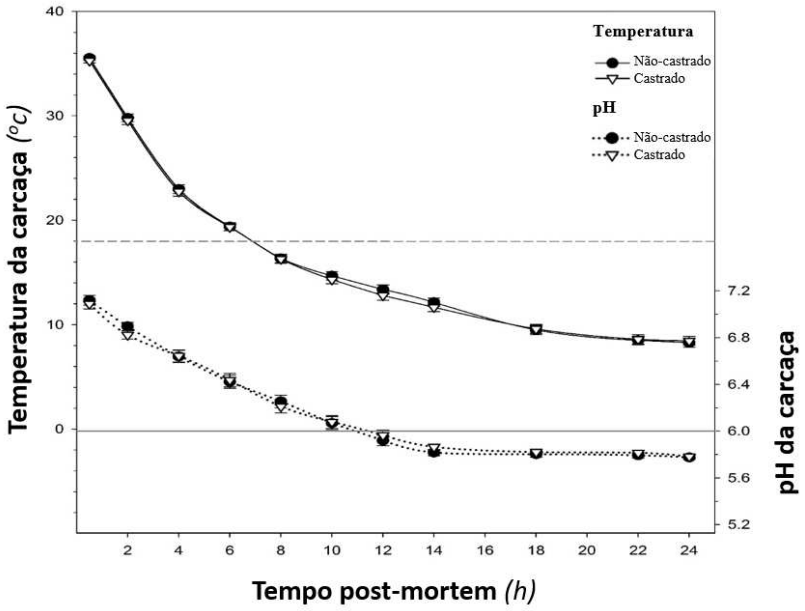


Figura 4. Temperatura e pH da carcaça de machos Nelore castrados e não-castrados. (Silva, 2018).

Sabor da carne

Em algumas espécies como suínos e caprinos a castração do macho é essencial para produção de carne com sabor e odor agradável. Interessantemente, devido à crescente preocupação por parte dos consumidores sobre o bem-estar animal, até mesmo a indústria da suinocultura, tem buscado formas de contornar esta limitação do uso de animais não castrados (Strathe et al., 2013). Considerando a carne de bovino, consumidores muitas vezes são incapazes de distinguir se a carne provem de um bovino castrado ou inteiro, assim a castração dos bovinos não de baseia em uma limitação dos animais não castrados quanto ao sabor e odor da carne. Porém, em diversos estudos as carnes de bovinos castrados receberam maiores notas para suculência e sabor, sendo este resultado principalmente atribuído ao maior grau de marmoreio da carne de bovinos castrados em relação aos inteiros (Seideman et al., 1982). Desta forma, os bovinos não castrados podem produzir carnes com sabor e odor desejável, no entanto, o maior marmoreio na carne de bovinos castrados proporciona maior intensidade do sabor e suculência a carne.

Maciez da carne

Alguns resultados de pesquisa têm demonstrado que bovinos castrados produzem uma carne mais macia do que bovinos inteiros, mesmo quando o problema do encurtamento pelo frio é minimizado (Huff-Lonergan et al., 1995; Morgan et al., 1993). Este aumento na maciez da carne dos machos castrados está ligado a maior atividade de enzimas que atuam no amaciamento da carne durante o processo de maturação.

Em nosso trabalho, realizamos a avaliação instrumental da dureza do contrafilé, por meio de um equipamento que registra a força necessária para cortar a carne, ou Força de Cisalhamento como é conhecida na área da ciência da carne. A dureza da carne foi avaliada sem maturação e após a maturação por 7 e 14 dias a 4 graus célsius. Como resultado, foi constatado uma similar maciez na carne não maturada de bovinos castrados e não castrados. Porém, após a maturação, a carne dos animais castrados se tornou mais macia do que a proveniente de bovinos não castrados (Figura 5). Nossos dados comprovam os resultados de pesquisa anteriores, onde a carne do bovino castrado apresenta maior taxa de amaciamento após o abate, e com o processo de maturação esta diferença fica mais evidente. Contudo, é importante destacar aqui que diversos trabalhos de pesquisa têm demonstrado que é possível produzir carne macia utilizando bovinos não castrados jovens. Em conclusão, é possível produzir carne com maciez adequada (força de cisalhamento < 50 N) utilizando machos inteiros, porém há um incremento na maciez quando a castração é aplicada, principalmente se a carne for maturada.

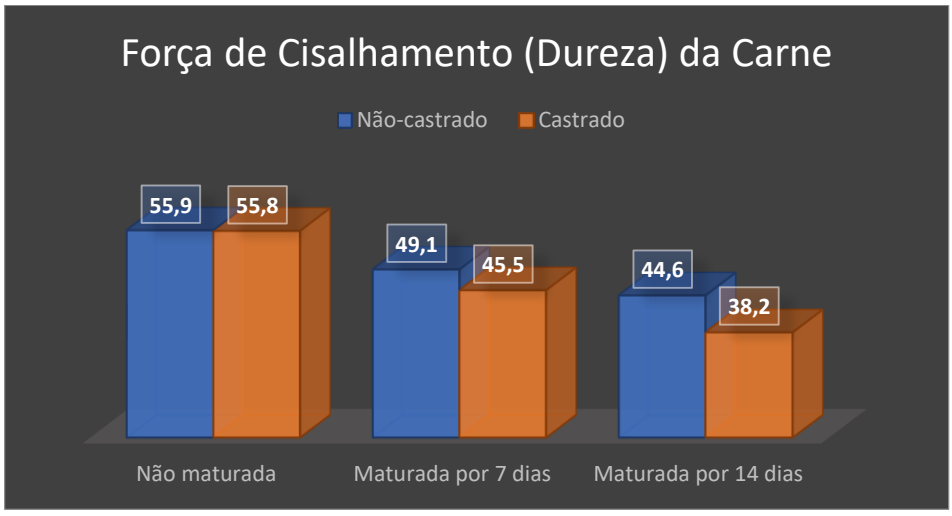


Figura 5. Força de Cisalhamento da carne de bovinos Nelore de acordo com o uso ou não da castração e da maturação. Valores em Newton. Adaptado de Silva, 2018.

Coloração da carne

A cor da carne é considerada como uma das características mais decisivas no momento da compra. Consumidores normalmente preferem carnes com coloração característica e mais claras. Os consumidores usualmente consideram que as carnes escuras não são frescas ou são provenientes de animais mais velho, apesar do escurecimento ocorrer, e é aceitável, nas carnes embaladas a vácuo e maturadas.

Mioglobina é o nome que se dá ao principal pigmento responsável pela coloração das carnes. É esperado que em mesmas condições de criação os machos inteiros apresentem maior concentração deste pigmento, uma vez que os músculos destes animais normalmente apresentam maior proporção de fibra muscular vermelha. Desta forma, a carne de bovinos não castrados tende a ser mais vermelho escura. Outro ponto relacionado a coloração da carne que pode ser alterada pela castração é a incidência de carnes escuras, também conhecidas como DFD sigla do inglês (Dark, Firm and Dry) que pode ser traduzida como carne escura, firme e seca. Este tipo indesejável de carne é produzido em decorrência da queda atípica do pH da carcaça onde 24 horas após o abate o pH é maior do que 6. Esta queda atípica do pH por sua vez está ligada ao estresse pré-abate, e como os machos inteiros são mais reativos estão mais sujeitos ao estresse relacionado ao manejo pré-abate, e então, há uma maior incidência de carnes tipo DFD. É importante destacar que a castração não elimina o problema de produção de carnes escuras, e além de fatores genéticos, o bom manejo na fazenda pode auxiliar na redução deste problema. Como exemplo, em nosso estudo não foi detectado nenhuma carne DFD (pH > 6, Figura 4), porém, os bovinos Nelore utilizados foram bem manejados desde o nascimento, recebendo diariamente suplemento no regime de pastejo na fase de cria e dois tratos diários na fase de confinamento logo após a desmama.

Conclusões

Os resultados obtidos em nossa pesquisa confirmam que é possível produzir carcaças e carne de qualidade utilizando machos Nelore não castrados criados intensivamente. A castração aumenta a taxa de amaciamento da carne durante a maturação, bem como o grau de marmoreio, porém leva a uma redução na eficiência produtiva. Desta forma, a castração

pode ser uma ferramenta para melhorar a qualidade e padronização da carne destinada a linha de cortes especiais.

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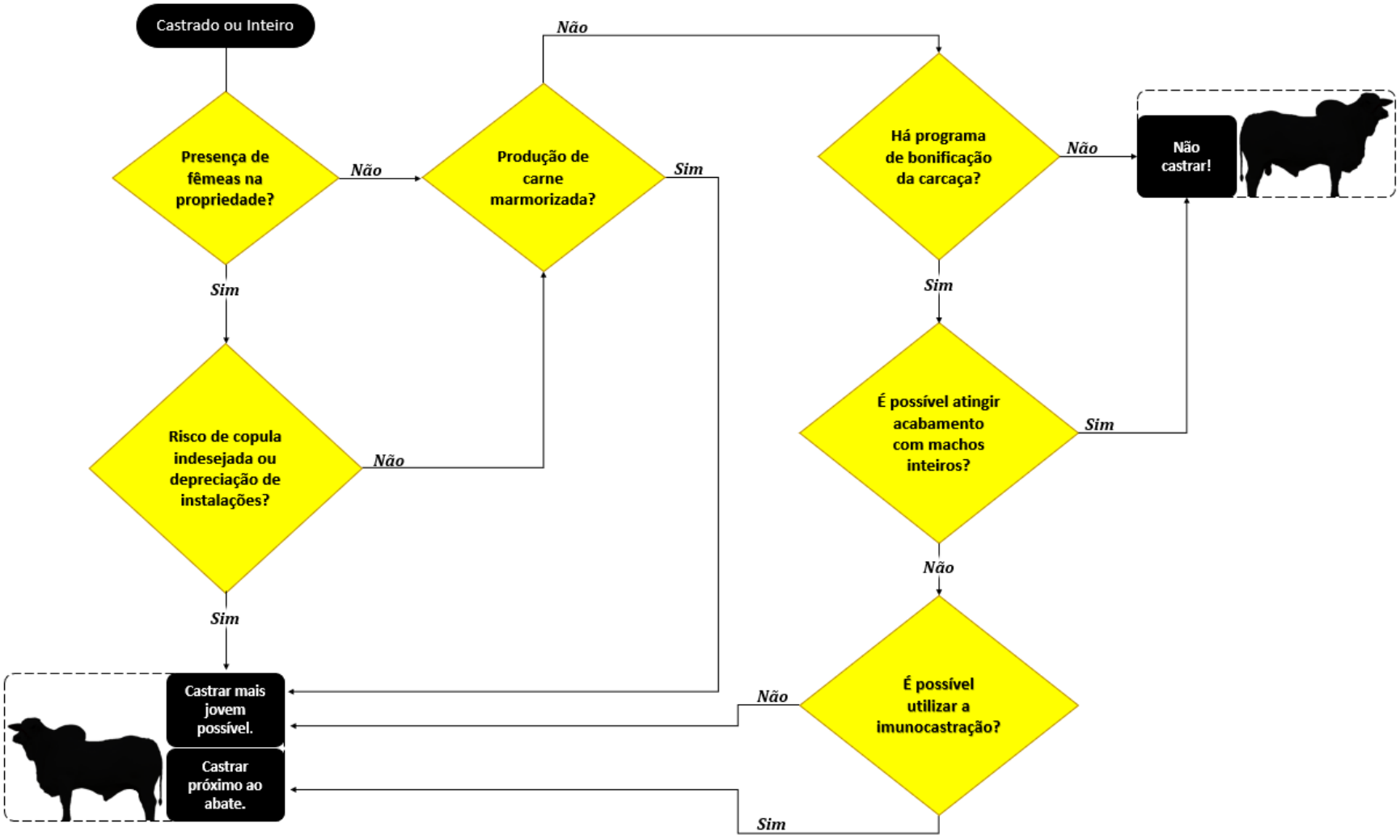

Fluxograma para orientar na decisão do uso ou não da castração de bovino destinados a produção de carne.