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# Post-mortem muscle proteome of crossbred bulls and steers: Relationships with carcass and meat quality


## Abstract

This study investigated the skeletal muscle proteome of crossbred bulls and steers with the aim of explaining the differences in carcass and meat quality traits. Therefore, 640 post-weaning Angus-Nellore calves were fed a high-energy diet for a period of 180 days. In the feedlot trial, comparisons of steers ($n$=320) and bulls ($n$=320) showed lower ($P$<0.01) average daily gain (1.38 vs. 1.60 ± 0.05 kg/d), final body weight (547.4 vs. 585.1 ± 9.3 kg), which resulted in lower hot carcass weight (298.4 vs. 333.7 ± 7.7 kg) and ribeye area (68.6 vs. 81.0 ± 2.56 cm²). Steers had higher ($P$<0.01) carcass fatness, meat color parameters (L*, a*, b*, chroma, hue) and lower meat pH. Moreover, lower ($P$<0.01) Warner-Bratzler shear force (WBSF) were observed in steers compared to bulls (WBSF = 3.68 vs. 4.97 ± 0.08 kg; and 3.19 vs. 4.08 ± 0.08 kg). The proteomic approach using two-dimensional electrophoresis, mass spectrometry and bioinformatics procedures revealed several differentially expressed proteins between steers and bulls ($P$<0.05). Interconnected pathways and substantial changes were revealed in biological processes, molecular functions, and cellular components between the post-mortem muscle proteomes of animals. Steers had increased ($P$<0.05) abundance of proteins related to energy metabolism (CKM, ALDOA, and GAPDH), and bulls had greater abundance of proteins associated with catabolic (glycolysis) processes (PGM1); oxidative stress (HSP60, HSPA8 and GSTP1); and muscle structure and contraction (TNNI2 and TNNT3). The better carcass (fatness and marbling degree) and meat quality traits (tenderness and color parameters) of steers were associated with higher abundance of key proteins of energy metabolism and lower abundance of enzymes related to catabolic processes, oxidative stress, and proteins of muscle contraction.

**Keywords:** Meat quality; Beef tenderness; Feedlot, Meat color, Proteomics, Biological Mechanisms; Proteome; Mass spectrometry.

## Introduction

Beef cattle producers routinely use castration to simplify the rearing of stock, which helps reducing unwanted breeding (or animals), and to modify the potential quality of the carcasses [1]. Moreover, the achievability to control the gender status of cattle is a topic of great interest due to the role it counts in the variation of meat quality traits such as tenderness, marbling and meat color [2]. The search for practices and management methods that improve meat quality to meet an increasingly demanding consumer market with high quality raises the issue of castration as the subject of several studies [3–6]. Moreover, there are indications that non-castrated animals (bulls) in the finishing phase are more susceptible to stress and injuries to the carcasses, hence reducing meat quality compared to castrated cattle [1,7].

Castration can further change some other traits such as growth rate, carcass yield, feed efficiency and carbon efficiency [8], due to the reduce anabolic potential of the animals [9]. Studies evaluated the effects of castration (conventional or immunological) in zebu (*Bos indicus*) and crossbred cattle finished in feedlot and showed that castration is able to improve carcass fatness and certain meat quality traits such as color and tenderness [7,10,11]. However, the biochemical and molecular mechanisms that control and regulate these traits are not fully understood, especially in intensive production systems. Few studies used young animals (<24 months) and feedlot finishing of 180 days, which support the need for more studies using such a biological model.

Besides that, beef cattle producers and the meat industry lack an objective and fast method to estimate the meat quality both in the live animals or using the carcass/meat cuts. Thus, an in-depth description of the gene and/or protein networks involved in the development of desired meat quality phenotypes is crucial toward the development of simple, reliable and fast tools [2]. Addressing this knowledge and technology gap would greatly increase the efficiency of the beef industry and promote the ability to generate better quality

products [12]. In this sense, the use of an optimized protocol for quality monitoring using rapid methods to record the abundance of specific proteins as biomarkers would offer an advantage to predict early post-mortem meat quality before the meat cuts reach the consumers [13]. Moreover, a better prediction of meat quality traits and molecular signatures of beef will allow the development of management tools to produce beef with superior quality. Accordingly, proteomics among the proposed approaches has been applied in meat science to explore the basis of variation in quality traits over the last ten years [14–17], as proteins are the main constituents of muscle tissue and major contributors to the regulation of the metabolic pathways involved in the conversion of muscle into meat [18,19]. Therefore, the present study aimed to investigate post-mortem muscle proteome of crossbred bulls and steers finished under feedlot and its relationships to both carcass and meat quality traits.

## Material and Methods

### *Ethics committee statement*

The animal study protocol No. 07594/2019 was approved by Ethics Committee (CEUA) of the São Paulo State University.

### *Animals and Experimental design*

The experiment was carried out in the feedlot facilities of Fazenda Turbilhão, municipality of Estrela D'Oeste, São Paulo, Brazil. A total of 640 male animals (320 steers and 320 bulls) F1 Angus-Nellore from the same herd were used. The animals were of an average initial body weight of 298.15 ± 28.58 kg. Calves were castrated using a standard surgical procedure, holding the cattle in a squeeze-chute, as previously described [3]. During the two first weeks after the castration, the pens with steers were verified daily, and proper medication (based on silver sulfadiazine and zinc oxide) were used until complete curative.

The animals were divided into two experimental groups (non-castrated/bulls and castrated/steers) and distributed in a completely randomized design, being allocated in eight collective pens (n = 4 pens/ treatment; 80 animals/ pen), equipped with a concrete bunk and an automatic water though. Each pen had a capacity for 100 animals in which they remained housed for 180 days, with 14 days of adaptation to the diet and experimental installation. An experimental high-energy diet was common to both treatments (Table S1), being provided *ad libitum* twice a day, at 9:00 AM and 3:00 PM.

*Slaughter, sample collection and carcass traits*

After 180 days on feed, all animals were slaughtered in a commercial slaughterhouse (Estrela D'Oeste, São Paulo, Brazil), located 10 km from the experimental feedlot, following the regular procedures of federal inspection. Fifteen animals were selected per pen, 60 animals from each experimental group, totaling 120 animals for the study of carcass and meat quality traits. Due to the large number of animals, two slaughter groups were formed, and the slaughter took place on two different days. Regular federal inspection procedures were followed, preceded by water and feed fasting for 16 hours. The animals were stunned (brain concussion using a captive dart gun), followed by bleeding, hide removal and evisceration.

Immediately after slaughter (hot carcass), sample biopsies of approximately 20 g of the *Longissimus thoracis* (LT) muscle were collected between the 12$^{th}$ and 13$^{th}$ ribs of the right half carcass and preserved in liquid nitrogen. Subsequently, these samples were stored in an ultra-freezer (-80 ºC) until proteomics analyses.

All carcasses were identified, washed, divided into two symmetrical parts through a longitudinal cut of the vertebral column, individually weighed and cooled at 4 °C for 48 h. Hot carcass weight (HCW) and cold carcass weight (CCW) were recorded before and after cooling, respectively. At deboning room, to measure the ribeye area (REA), the LT samples

were individually demarcated on transparencies, which were digitized and the area in $cm^2$ measured with the aid of ImageJ® software version 1.5 (National Institute of Health, Bethesda, Maryland, United States).

Backfat thickness (BFT) was measured with a digital caliper, simulating an angle of 45º between the spinous and transverse processes of the 12th thoracic vertebra and expressed in millimeters (mm), according to protocols of the United States Department of Agriculture (USDA) – Beef Quality and Yield Grade [20]. Visual marbling scores were determined by two trained panelists at deboning, using official USDA marbling photographs as a reference on a scale from 1 to 9 [20]. The marbling categories were 1 = devoid, 2 = practically devoid, 3 = traces, 4 = slight, 5 = small, 6 = modest, 7 = moderate, 8 = slightly abundant, and 9 = moderately abundant.

In the left half carcass, a sample of approximately 10 cm of the LT muscle was collected, between the 11th and 13th ribs (cranial direction). These samples were transported to the laboratory and subsequently sectioned into two 2.54 cm steaks. These were individually vacuum packed, and aged for 3 and 10 days in a refrigerator at 2 °C. After this period, the samples were frozen until meat quality evaluation.

*Meat pH, color, cooking losses and Warner-Bratzler shear force*

The beef steaks were thawed at 4 °C for 24 hours, removed from the packaging and exposed to oxygen for 30 min at 4 °C (blooming time). Then, the pH of the meat was measured using a digital pH gauge (Model HI 99163, Hanna Instruments, Woonsocket, Rhode Island, United States) equipped with a penetration probe, calibrated with pH 4.0 and 7.0 buffers at room temperature of 25 °C.

Meat color (L* = lightness, a* = redness, b* = yellowness) was evaluated using the a CR-400 colorimeter (light source A, absorbance angle 10, Y, 0, 01 at 160.00% reflectance;

Konica Minolta Sensing, Inc., Tokyo, Japan), following the procedures previously described [21]. The unit was calibrated using a black and a white standard plate and color readings were taken at three locations of the LT muscle sample after a 30 min bloom time. An average of the three measurements was generated for L*, a* and b*. The chroma colorimetric index was calculated using the formula $\sqrt{(a*)^2 + (b*)^2}$, and hue angle was calculated using $tan(\frac{b*}{a*})^{-1}$ [21,22].

For the analysis of cooking losses, the samples were placed on a grid on a glass refractory and weighed, keeping the respective identifications. A thermocouple was inserted in the geometric center of each steak, which were coupled to a digital thermometer model DT-612 (ATP Instrumentation, Ashby-de-la-Zouch, United Kingdom) to monitor the internal temperature of the samples. The steaks were roasted in an oven (Feri90; Venâcio Aires, Rio Grande do Sul, Brazil) preheated to 170 °C. equipped with a thermostat to avoid temperature variations. When the internal temperature of the steak reached 40 °C, the sample was turned over and remained in the oven until the internal temperature reached 71 °C, according to the methodology described [23]. Then, the samples were removed and kept at room temperature for 15 min, weighed and cooled at 4 °C for 24 h. Thus, cooking loss values were obtained in the same steaks by the difference in the weights before and after cooking. Total cooking losses (TL) were measured from drip (DL) and evaporation losses (EL). The DL was obtained by weighing only the glass refractory before and after cooking the sample. The EL was obtained by weighing the sample before and after cooking.

After 24 hours of cooling, eight cylinders with a diameter of 1.27 cm were removed parallel to the muscle fiber with a hollow punch coupled to an industrial drill. The cylinders were sectioned in a mechanical Warner-Bratzler Shear Force (WBSF) device (G-R Manufacturing, Manhattan, Kansas, United States), equipped with a 1.18 mm thick, 126.77 mm high stainless steel blade containing a cutting edge, with a capacity of 25 kg and speed of

20 cm/min. Thus, an average of the eight cylinders was calculated and the WBSF results were reported in kilograms (kg).

*Proteome characterization*

Samples from 24 animals were used (12 steers and 12 bulls; randomly selected) from the subsampling of the 120 animals mentioned above. The muscle tissue proteome of the animals was investigated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and electrospray ionization-mass spectrometry (ESI-MS/MS) following procedures described previously [24,25]. Only hot carcass samples were used to investigate the post-mortem muscle proteome.

*Protein extraction and precipitation*

Approximately 0.2 g of each muscle sample was ground twice in 1.0 mL a lysis buffer (8 M urea, 2 M thiourea, 2% 3-[(3-cholaminopropyl)-dimethylammonium]-1-propanesulfonate, 50 mM dithiothreitol) using an Ultra-Turrax high shear mixer (Marconi – MA102/E, Piracicaba, São Paulo, Brazil) at 20,000 rpm for 30 seconds. The protein extracts were separated from the solid part by centrifugation at 10,000 rpm for 15 min at 4 ºC. The protein extracts were stored in 80% (v/v) acetone solution in a refrigerator at 5 ºC for 2-3 h. Subsequently, the precipitated proteins were centrifuged at 10,000 rpm for 25 min at 4 ºC. A portion of these precipitated proteins was resolubilised in 0.50 M NaOH for total protein quantification. Another part was resolubilised in a specific buffer at 7 M with 2 M of thiourea, 2% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid, 0.5% (w/v) ampholytes pH 3 and 0.02% bromophenol blue. Subsequently, 2.8 mg of dithiothreitol were added to this buffer, and this mixture was used in electrophoretic separations.

*Protein separation by two-dimensional polyacrylamide gel electrophoresis*

The total protein concentration of the bovine muscle tissue samples was quantified by the Biuret method [26]. The protein concentrations obtained were used to calculate the volume of sample and solution needed for electrophoresis, considering a protein mass of 375 µg and a volume of 250 µL to be applied to the strip, resulting in a total protein concentration of 1.5 µg/µL. An aliquot of each sample of 250 µL was added to a 13-cm isoelectric focusing strip containing an immobilized ampholyte and a pH gradient from 3 to 10 for 12 h.

After this period, the strips were submitted to the first step/dimension (1D) of two-dimensional electrophoresis, using Ettan IPGphor 3 device (GE Healthcare, Chicago, Illinois, United States). The strips were placed in equilibrium solutions for reduction and alkylation and subjected to the second dimension (2D) of electrophoresis 12.5% polyacrylamide gel (dimensions: 15 × 15 cm). At the end of the 2D run, approximately 500 mL of colloidal Coomassie stain was used to stain the protein spots of the gels.

The destained gels were acquired and then analyzed with ImageMaster Platinum software to obtain information on number of spots per gel, % matching (correspondence between protein spots in the gels), isoelectric point (pI), molecular weight (MW), and spot volume. Gel correspondence (matching) within each sample (three technical replicates) was higher than 95%, i.e., 95% of the spots were present in the technical replicates, indicating good reproducibility. For image comparison, one reference gel per treatment was selected [27], which contained the largest number and best definition of spots. The reference gel of the treatment was compared with each gel of the other treatment.

*Tryptic digestion of protein spots and identification of proteins by ESI-MS/MS*

Protein spots from the experimental groups (steers versus bulss) were selected based on their volume, MW and pI obtained by image analysis, cut out (fragments of approximately

1 $mm^3$), and prepared according to the method described [28].

The samples were transferred to microtubes and submitted to the following four steps: 1) Removal of the dye with 25 mM ammonium bicarbonate (Ambic)/acetonitrile (ACN, 50:50, v/v). 2) Reduction and alkylation in which the gel fragments were rehydrated in a reducing solution and incubated for 40 min at 56 ºC. After removal of the reducing solution, alkylating solution was added and the fragments were incubated in the dark for 30 min at room temperature. 3) Trypsin digestion consisting of overnight incubation at 37 ºC with 10 ng/µL trypsin in 25 mM Ambic (Trypsin Gold Mass Spectrometry, Promega, Madison, United States). 4) Elution of peptides extracted from the gel in three steps: A) 50% ACN with 1% formic acid incubated for 15 min at 40 ºC under sonication; the supernatant was collected and transferred to a new tube. B) 60% methanol with 1% formic acid incubated for 15 min at 40 ºC under sonication; the supernatant was collected and transferred to a new tube. C) 100% ACN; the extracts were dried in a vacuum centrifuge and peptides were dissolved in 10 µL of 3% ACN with 0.1% formic acid.

The mass spectra of the peptides were obtained by analyzing aliquots of the solutions in a nanoACQUITY UPLC-Xevo TQ-MS System (Waters, Manchester, United Kingdom). The proteins were identified in the UniProt database (UniProtKB/Swiss-Prot, www.uniprot.org) for the *Bos taurus* genome.

*Bioinformatics procedures*

Bioinformatics analyses were conducted, first for the classification of differentially expressed proteins in muscle tissues from steers versus bulls in terms of biological processes (BP), molecular function (MF), and cellular components (CC). For this purpose, the proteins identified by ESI/MS/MS were analyzed using the OMICSBOX v.2.0 (https://www.biobam.com/omicsbox) and Blast2GO tools [29]. Subsequently, Metascape®

(https://metascape.org/) open-source tool was used to identify the main enriched Gene Ontology (GO) terms among the protein lists, following the procedures described by Gagaoua et al. [12]. The *Bos taurus* taxonomy is not available in Metascape®, therefore orthologs to *Homo sapiens* were retrieved using the HCOP (www.genenames.org/tools/hcop/). Hierarchical heatmap clustering comparing the significantly enriched GO terms was generated.

Further bioinformatics analysis between the proteins identified in the LT of bulls and steers were analyzed using the STRING database (Search Tool for Retrieval of Interacting Genes, v. 11.0 - https://string-db.org). Protein-Protein Interactions (PPI) network relating the differential proteins according to the pathways to which they belong was then generated. Moreover, the list of the identified proteins that differ among the groups were compared to the repertoire of Gagaoua et al. [12] to identify the extent of overlap with the previously identified beef tenderness biomarkers. The extent of isoforms identification (multiple proteins with the same gene name) was investigated on the full list of DEP proteins using the online tool Bionic Visualizations-Proteomaps (*Homo sapiens* database) (http://bionic-vis.biologie.uni-greifswald.de/) [30]. We performed the online Proteomaps to observe the proteins (and their isoforms) linked with the Kyoto Encyclopedia Genes and Genomes (KEGG) pathway on the whole protein lists.

*Statistical Analysis*

Data were analyzed for residual normality using the Shapiro Wilk test in PROC UNIVARIATE of the statistical software SAS 9.4 (SAS Institute Inc., Cary, North Carolina, United States). The day of slaughter was used as a covariate and analysis of variance (ANOVA) was used to test the effects of sex class for the variables of performance and carcass traits using the PROC GLM (SAS 9.4) procedure according to the model:

$$Y_{ij} = \mu + d_i + sc_i + \varepsilon_{ij}$$

Where: $\mu$ = overall mean, $d_i$ = slaughter day, $sc_i$ = sex class effect and $\varepsilon_{ij}$ = experimental error.

For meat quality variables, the effect of aging time was also tested, according to the model:

$$Y_{ij} = \mu + sc_i + at_j + \varepsilon_{ij}$$

Where: $\mu$ = overall mean, $sc_i$ = sex class effect, $at_j$ = aging time effect and $\varepsilon_{ij}$ = experimental error.

Protein representation data were analyzed using ImageMaster Platinum v. 7.0 (GE Healthcare, Chicago, Illinois, United States). The spot volume data were tested for homogeneity of variances and normality using the Levene and Shapiro–Wilk tests, respectively. Subsequently, the differences in the means between treatments were analyzed using Student's t-test. Additionally, the Mann–Whitney test (Wilcoxon rank-sum test) was used when the normality criteria were breached in any of the treatments. For both tests (Student or Mann–Whitney), significance was detected at the 0.05 level. For all data, trends were considered at $0.05 < P \leq 0.10$.

## Results

*Performance, carcass traits and meat quality*

Castration influenced the performance and carcass traits of the animals ($P<0.05$) (Table 1). As expected, steers had lower average daily gain (ADG), final body weight (FBW), HCW and CCW carcass weights, carcass yield (CY) and REA. On the other hand, they had a greater carcass fatness, measured as BFT and marbling degree.

There was an effect of castration on the variables of cooking losses and WBSF ($P<0.05$) (Table 2), with lower EL, TL and WBSF in the beef of steers, regardless aging time.

However, steers had higher DL when compared to bulls. As expected, there was an effect of days of aging on the meat ($P$<0.05), in which meats aged for 10 days had lower values for the variables EL, TL and WBSF, regardless experimental group.

For meat color (Table 2), it was noted that steers had higher values ($P$<0.05) for the variables L*, a*, b*, chroma and hue. As expected, the meat of the bulls showed a higher pH in relation to the steers ($P$<0.05). Meats aged for 10 days had higher values for L*, a*, chroma and pH, regardless of the experimental group.

*Muscle tissue proteome*

Images of the 2D-PAGE gels of bulls and steers were analyzed and the correspondence of the gels within each experimental group was greater than 95% compatibility. Master gels obtained from LT muscle samples (sampled early post-mortem) of the bulls and steers groups are illustrated in Figures 1A and 1B, respectively.

Overall, 56 protein spots were found differentially expressed between the two experimental groups ($P$<0.05), whereby 20 proteins were identified by ESI-MS/MS (Table 3). Steers had positive regulation ($P$<0.05) of energy metabolism proteins in LT muscle such as CKM, ALDOA, and GAPDH, as well as ADSL, PYGM and ENO1 (Table 3). The proteins (ADSL) and ENO1 were observed only in LT samples of steers. On the other hand, in bulls, we observed positive regulation of the proteins related to oxidative stress and cell response to stress such as HSP60, HSPA8 and GSTP1, other proteins belonging to energy metabolism (ATP5F1B, MDH1, PGM1, PDHB, and TPI1) and contractile and structure such as TNNI2 and TNNT3, were identified.

The main enriched terms and pathways identified in this study using the differentially abundant proteins for bulls and steers are summarized in Figure 2 and Figure 3. Based on GO terms, the generation of precursor metabolites was highly and significantly up-regulated in

steers compared to bulls. Such pathways of generation of metabolites and energy metabolism in steers help to explain the higher BFT and marbling degree found in the meat of these animals.

Moreover, there is two major sub-networks (Figure 2B): proteins related to energy metabolism (GAPDH, CKM, ALDOA, PGM1, MDH1, ADSL, TPI1, ATP5B, ENO1, PDHB, and PYGM) and those related to response to stress (HSPA8, HSPD1, GSTP1 and PRDX2). Furthermore, a small interaction network involving transport and binding (MB, HBB and HBA) and muscle structure (TNNT3 and TNNI2) was identified (Figure 2B). Glycolysis and amino acid metabolism were the main pathways highly enriched by the Proteomap analysis, with significant role of CKM (Figure 2C,D). Overall, a unique GO network was identified (Figure 2E), highlighting the degree of interconnectedness among the pathways and changing proteins.

When comparing the enriched GO terms within bulls and steers, as well the main biological process, pathways related with primary and catabolic processes, oxidative stress, and muscle contraction (peroxidase activity, molecular carrier activity, tricarboxylic acid cycle, ficolin-1-rich granule lumen and troponin complex) were highly and significantly up-regulated in bulls, which can be associated with muscle growth of these animals. The cluster pathways related to the ATP metabolic process and protein refolding were more enriched (Figure 3), which helps us to explain the greater HCW and REA (and less fatness) observed in bulls compared to steers.

The protein overlap in terms of beef tenderness biomarkers based on the database in Gagaoua et al. [12] revealed 17 from the 20 changing proteins as being already identified as meat quality biomarkers. Thus, we also found proteins related to energy metabolism (CKM, ALDOA, ENO1, PYGM, GAPDH, MHD1, PHDB, ATP5F1B, TPI1 and PGM1), responses to stress (HSPA8, GSTP1 and PRDX2), muscle structure (TNNT3 and TNNI2), as well as

transport and binding (MB and HBB). The 17 proteins were organized based on the molecular functions to which they belong as in Figure 2B.

The amount of BP, MF and CC of the identified proteins differed between bulls (Figure 4) and steers (Figure 5). Considering the distribution of the top 20 levels of Gene Ontology, BPs were identified in greater quantities in bulls, among which cellular metabolism (20), nitrogen compounds (18), ATP formation and response to stimulus (16) stand out. Divergent processes were observed in smaller amounts in steers, the main ones being those related to ATP (6), primary and catabolic processes (6).

Regarding MF, a predominance of membrane transport (12) and ionic binding (8) proteins was observed in bulls. Proteins with hydrolysis and catalytic function were also found in greater amounts in bulls compared to steers. Regarding the CC, several proteins of organization of the cellular structure stand out in the bulls, while in the steers, more cytosol and membrane proteins were identified.

**Discussion**

Improving quality attributes such as color and tenderness is a key challenge for producers and beef industry [21,32]. In this context, several studies evaluated the effects of castration (conventional or immunological) in zebu (*Bos indicus*) and crossbred cattle feedlot finished, which evidenced substantial effects on the carcass and meat quality traits [7,10,11]. These experiments report results with Nellore [7,11] or F1 crossbred cattle [10] with an initial average age of 24 months and feedlot finished for 90 to 100 days, which is the traditionally feeding period in Brazilian feedlots.

However, there are no studies comparing bulls and steers feedlot finished for a longer feeding period in tropical conditions. The carcass weights and fatness in Brazil are lower than those produced in United States. Increasing days on feed can be a strategy to improve both

carcass weight and fatness in beef cattle. According to a national survey, a similar feedlot period is used for bulls and steers (107.6 and 110.5 days, respectively) in Brazilian production systems located in tropical regions [33]. In these locations, researchers described results of beef quality of crossbred cattle feedlot finished using a proteomic approach combining 2D-PAGE, mass spectrometry and bioinformatics [18,34]. However, this is the first study that used a proteomics approach to decipher the biochemical and molecular mechanisms that regulate carcass and meat quality traits in crossbred steers compared to bulls submitted to post-weaning feedlot finishing for a period of 180 days. Such molecular biology approach can help identify biomarkers to improve meat quality traits of this biological model [2,25,35].

*Performance and carcass traits*

The better performances of bulls compared to steers were mainly a consequence of the anabolic effects of testosterone [5,36]. As described in the literature, castration reduces the production of androgenic hormones [37,38] and, consequently, reduces the anabolic power of bovines [9]. Castrated animals show less muscle development, however, when fed intensively, they increase fat deposition, as reported [3]. The results found in the present study are in agreement with those described in the literature, in which lower ADG, FBW, HCW, CCW and REA and higher BFT were also observed in castrated animals in relation to non-castrated ones [36].

*Meat quality traits*

The lower pH value of the steers meat may contribute to improved other meat quality traits. One of the main factors influencing meat quality is pH [9], which can be affected by several causes such as pre-slaughter stress, muscle fiber composition and sex class [7]. Pre-

slaughter stress can reduce muscle glycogen levels, limiting pH decline and resulting in higher final pH values in the meat [39]. The higher pH values of the meat of bulls in the present study may be associated with the fact that this sex class has greater susceptibility to pre-slaughter stress when compared to castrated animals [40], even considering the short transport distance (approximately 10 km) of animals from farm to slaughterhouse. Other studies in the literature also observed higher values of final pH in the meat of bulls versus steers finished in feedlot [6,41].

In agreement with the results of the present study, researchers found higher pH values in the meat of bulls crossbred animals, compared to steers [10]. Additionally, the L* and a* values decreased when the final pH increased, which agrees with the results found for the meat color of bulls (lower redness). On the other hand, the lower b* values, probably occurred due to differences in intramuscular fat (IMF) deposition between steers and bulls, as these parameters (b* × intramuscular fat) are positively correlated in other studies [42,43]. The current results of marbling degree of steers compared to bull confirm this hypothesis.

Cooking losses can be an important factor in the sensory quality of meat. According to researchers [44], sex class affects the water-holding capacity (WHC) of beef, and lower cooking losses were observed in steers compared to bulls in other studies in the literature [8,45], similarly to the results of the present study. In addition, the higher cooking losses for bulls can be explained by differences in moisture and IMF between sex classes. The WHC of meat is influenced by the IMF and marbling degree. As the IMF increases, the moisture content decreases, consequently, water losses during cooking are lower in meats with high fat content [46].

*Muscle proteome*

Proteomics is the study of proteins and their functions, interactions and dynamics in

biological systems. In the context of meat research, proteomics can be used to better understand the molecular mechanisms underlying meat quality and processing, including tenderness, flavor, and texture. This can be achieved through the analysis of the protein composition and changes in protein expression levels in meat tissues during different stages of production and processing. Thus, proteomics revealed that several proteins are responsible for defining meat color [47], mainly those involved in muscle contraction, energy metabolism, signaling pathways, chaperones and key proteins of apoptosis [48]. Dark cutting beef occurs due to higher pH, which prevents the decrease in mitochondrial activity that normally occurs as muscle pH decreases after the animal is slaughtered. As a result, an increase in mitochondrial activity promotes oxygen consumption and reduction of oxygen partial pressure [49]. Sophisticated interacting pathways has been reported in the integromics meta-analysis of Gagaoua et al. [49]. The earlier studies gathered in these meta-analyses [48, 49] support our finding with F1 Angus-Nellore cattle, where bulls showed positive regulation for proteins with different functions from the mitochondria. Proteins such as HSP60, ATP5F1B (ATP producer from ADP in the presence of a proton gradient across the membrane that is generated by electron transport complexes of the respiratory chain) and PDHB (responsible for catalyzing the global conversion of pyruvate to acetyl-CoA and $CO_2$, binding to glycolytic pathway to the tricarboxylic cycle) were up regulated in bulls. In agreement, researchers also reported heat shock protein beta 1 (HSPB1) were up-regulated in bulls [50], whereas mitochondrial ATP5F1B, involved in oxidative phosphorylation, was down-regulated [4].

Moreover, MB has the function of reserving and facilitating the movement of oxygen within the muscles. The myoglobin (less expressed in LT muscle of steers) is part of the sarcoplasmic proteome and regulates different biochemical processes that influence meat color stability [51], which help to explain the differences observed in meat color between

steers and bulls.

A previous study on beef [52] found a positive correlation between redness and several sarcoplasmic proteins, including GAPDH. In the current study, greater abundance of this protein was observed in castrated animals. In another study, Gagaoua et al. [53] observed a positive correlation of the MDH1 protein with redness of LT of Blonde d'Aquitaine animals. The higher abundance of MDH1 help to explain the differences in meat color (and tenderness) observed in the present study (greater abundance in bulls compared to steers). According to Gagaoua et al. [12], among enzymes of oxidative pathway, MDH1 plays a key role in Krebs cycle (malate–aspartate shuttle), which may be indicative of increased oxidative phosphorylation capacity of the muscle in response to cellular stress. The study by Picard et al. [34] compared the muscles of cows and steers. The authors revealed differences in the abundances of 8 proteins belonging to energy metabolism, contraction, and cellular stress pathways: including MDH1. This suggests that MDH1 is an interesting biomarker that can be used for gender discrimination [2].

Among the differently regulated proteins, in tender meat, a greater abundance of glycolytic enzymes (ENO1, PGM1, TPI) was observed [54], diverging from the results observed in the present study. In contrast, a previous study on beef [4], observed that the abundance of PGM1 in the unaged meat sample was correlated with WBSF, suggesting that this could be a biomarker to predict meat tenderness before slaughter. This protein was also described as a robust biomarker of beef tenderness in another study [12].

According to other studies in the literature [24,47] proteins from the HSPs family are potential biomarkers of proteolysis, thus, the highest abundances of HSP60 and HSPA8 proteins in bulls impaired tenderness (WBSF) of the meat of this group of animals. In this sense, heat shock proteins have diverse functions and anti-apoptotic properties and may contribute to delaying the postmortem apoptosis [55] and, therefore, also influencing the

tenderness and conversion of muscle to meat [12].

Castration was also responsible for increasing the abundance of CKM protein in another study in the literature [4]. This molecules plays a central role in energy transformation in energy-demanding tissues such as skeletal muscle [56]. Similarly, the greater abundance of CKM in steers versus bulls may have contributed to the differences observed in WBSF (meat tenderness). Moreover, the relationship between tenderness and abundance of GAPDH may be related to differences in sensitivity to insulin or sex hormones between sexes. Estrogens and their receptors regulate energy metabolism pathways, including glucose transport, glycolysis, the Krebs cycle, mitochondrial respiratory chain, and fatty acid ß-oxidation [13].

The enzyme GAPDH also reversibly converts glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. Researchers reported higher abundance of the enzymes GAPDH and ALDOA in the proteome of steers, with higher IMF content, when compared to Nellore bulls [4]. We recently also found these enzymes more abundant in muscle tissue proteome of crossbred heifers compared to steers due to the higher IMF content in the meat [16]. Therefore, GAPDH and ALDOA were highly and significant expressed in the crossbred steers of the current study, which explain the greater carcass fatness and marbling degree found in these animals.

Moreover, ADSL and ENO1 were more abundant in muscle steer samples (more tender meat). Similar results were described for ENO1 [12] and ADSL [13]. Alpha-enolase and Adenylosuccinate lyase plays a central role in post-mortem cellular events. These enzymes catalyze the formation of phosphoenolpyruvate from 2-phosphoglycerate and synthesis of inosine monophosphate, respectively. In steers such differences in protein abundance (greater ENO1 and ADSL) could be explained mainly by differences in sex hormones between and carcass fatness, affecting meat tenderness and color parameters.

## Conclusion

Castration reduced animal performance and increased carcass fatness in crossbred cattle feedlot finished. Crossbred Angus-Nellore steers produced meat with better pH, color variables and objective tenderness (WBSF) compared to bulls. These improvements resulted from observed differences in protein abundance, which plays a pivotal role in metabolic pathways including ATP production, energy metabolism and oxidative stress and cell redox. Bulls had greater abundance of proteins associated with primary and catabolic processes; oxidative stress; and muscle contraction, which can be associated with the inferior meat quality traits of these animals.

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**Figure captions**

**Figure 1.** Protein spots selected for characterization by mass spectrometry (ESI-MS) after image analysis. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE): 12.5% (w/v) and pH gradient 3–10. Muscle tissue samples (*Longissimus thoracis*) from F1 Angus-Nellore bulls (**A**) or steers (**B**) feedlot finished.

**Figure 2.** Bioinformatics analyses based on the differentially expressed proteins identified in this experiment. (**A**) Enriched ontology clusters based on the significantly enriched gene ontology (GO) terms obtained using the protein lists of bulls (n = 14) and steers (n = 6) identified in *Longissimus thoracis* (LT) muscle tissue of feedlot-finished F1 Angus-Nellore. The graphs highlight all the enriched terms across the protein lists with the importance of energy metabolism, muscle structure and contraction, response to stress, and transport and binding according to −Log *P*-values. (**B**) Analysis of protein-protein interactions using the differentially expressed proteins in LT muscle tissue. (**C, D**) Proteomaps analyses visualisations resulting from the comparative proteome profiling of the changing proteins between bulls and steers. Every polygon or circle represents a protein, the size of which is given by the number of identifications. The proteins are grouped in functional categories based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The proteomap shows that glycolysis and amino acid metabolism are the main hierarchy levels. (**E**) Enriched network related to the previous terms of (A) highlighting the degree of interconnectedness.

**Figure 3.** Hierarchical heatmap clustering comparing the enriched gene ontology (GO) terms within bulls and steers as well the main biological process in each condition. The

heatmaps colored by the *P*-values are indicated by color, where grey cells indicate the lack of significant enrichment, palest brown indicates a low p-value and darkest brown indicates a high *P* -value.

**Figure 4**. Classification of proteins identified in tissue samples (*Longissimus thoracis*) from F1 Angus-Nellore bulls feedlot finished. The OMICSBOX software was used to classify the proteins according to biological process (BP), molecular function (MF), and cellular component (CC).

**Figure 5** Classification of proteins identified in tissue samples (*Longissimus thoracis*) from F1 Angus-Nellore steers feedlot finished. The OMICSBOX software was used to classify the proteins according to biological process (BP), molecular function (MF), and cellular component (CC).

**Author contributions**

Conceptualization, W.B. and O.M.N.; Methodology, M.G., B.S., P.P., and R.T.; Software, M.G., W.B., and R.T.; Validation, W.B., M.G., P.P., and L.A.C.; Formal analysis, W.B., M.G., B.S., M.C. L.A., and G.P.; Investigation, W.B., M.G.; Resources, O.M.N.; Data curation, W.B. and M.G.; Writing—original draft preparation, B.S. and W.B.; Wwriting—review and editing, W.B., M.G., R.C., R.T.; Visualization, W.B.; M.C.; L.A. and M.G.; Supervision, L.A.C.; Project administration, O.M.N.; Funding acquisition, O.M.N.

**Conflict of Interest**

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non financial interest in the subject matter or materials discussed in this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Table 1. Performance and carcass traits of crossbred bulls and steers feedlot finished.

| Variables[1] | Bulls | Steers | SEM[2] | *P-value* |
|---|---|---|---|---|
| IBW, kg | 296.91 | 299.38 | 4.52 | 0.773 |
| ADG, kg/day | 1.60 | 1.38 | 0.05 | 0.004 |
| FBW, kg | 585.13 | 547.44 | 9.30 | 0.037 |
| HCW, kg | 333.71 | 298.39 | 7.76 | 0.009 |
| CCW, kg | 326.17 | 290.05 | 7.93 | 0.009 |
| CY, % | 57.04 | 54.51 | 0.50 | <0.001 |
| REA, cm² | 81.03 | 68.61 | 2.56 | 0.003 |
| BFT, mm | 12.41 | 18.87 | 1.54 | 0.001 |
| Marbling, 1 to 9 | 2.22 | 6.10 | 0.06 | <0.001 |

[1]IBW = initial body weight; ADG = average daily gain; FBW = final body weight; HCW = hot carcass weight; CCW = cold carcass weight; REA = ribeye area; BFT = Backfat thickness; Marbling = visual marbling score categories (1 = devoid, 2 = practically devoid, 3 = traces, 4 = slight, 5 = small, 6 = modest, 7 = moderate, 8 = slightly abundant, and 9 = moderately abundant).

[2]Standard error of mean.

Table 2. Meat quality traits of crossbred bulls and steers feedlot finished.

| Variables[1] | Bulls | Steers | Bulls | Steers | SEM[2] | *P*-value[3] | | |
|---|---|---|---|---|---|---|---|---|
| | Aging time | | | | | SC | Day | S × D |
| | 3 days | | 10 days | | | | | |
| L* | 29.82 | 34.58 | 31.19 | 35.86 | 0.24 | <0.001 | <0.001 | 0.893 |
| a* | 14.17 | 15.90 | 14.33 | 16.88 | 0.16 | <0.001 | 0.029 | 0.105 |
| b* | 5.30 | 6.83 | 5.39 | 6.99 | 0.09 | <0.001 | 0.319 | 0.642 |
| Chroma | 15.14 | 17.32 | 15.33 | 18.28 | 0.18 | <0.001 | 0.035 | 0.122 |
| Hue | 20.16 | 23.41 | 20.33 | 22.52 | 0.19 | <0.001 | 0.446 | 0.182 |
| pH | 5.78 | 5.59 | 5.82 | 5.65 | 0.02 | <0.001 | 0.034 | 0.706 |
| EL, % | 24.93 | 23.01 | 23.80 | 20.66 | 0.29 | <0.001 | <0.001 | 0.178 |
| DL, % | 5.11 | 6.59 | 5.18 | 6.39 | 0.13 | <0.001 | 0.405 | 0.315 |
| TL, % | 30.04 | 29.61 | 28.98 | 27.05 | 0.33 | 0.045 | 0.004 | 0.202 |
| WBSF, kg | 4.97 | 3.68 | 4.08 | 3.19 | 0.08 | <0.001 | <0.001 | 0.588 |

[1] *Longissimus thoracis* samples. L* = lightness; a* = redness; b* = yellowness; EL = Evaporation losses; DL = Drip losses; TL = Total cooking losses; WBSF = Warner-Bratzler shear force.

[2]Standar error of the mean.

[3]SC = effect of sex class; Day = effect of aging time; S × D = interaction.

Table 3. Proteins separated by two-dimensional electrophoresis (2D-PAGE) and identified by mass spectrometry (ESI-MS/MS) in samples of *Longissimus thoracis* muscle from F1 Angus-Nellore bulls and steers feedlot finished.

| SPOT ID | Full protein names | Gene symbol | Uniprot accession number | pI experimental/ theoretical | MW experimental/ theoretical | Matched peptides | Sequence coverage (%) | Score | Fold-change (steers/ bulls)[1] |
|---|---|---|---|---|---|---|---|---|---|
| *Energy metabolism* | | | | | | | | | |
| 17 | Adenylosuccinate lyase | ADSL | A3KN12 | 6.45/ 6.81 | 55.48/ 55.09 | 30 | 13.06 | 2311.33 | 1.16 (UP in steers) * |
| 31 | Alpha-1_4 glucan phosphorylase | PYGM | B0JYK6 | 7.03/ 6.67 | 90.56/ 97,28 | 58 | 60.33 | 3653 6.30 | 1.50 (UP in steers) * |
| 29 | Alpha-enolase | ENO1 | Q9XSJ4 | 6.37/ 6.57 | 47.33/ 50.54 | 24 | 48.20 | 3745 4.42 | 1.55 (UP in steers) ** |
| 10 | Creatine kinase M-type | CKM | Q9XSC6 | 6.63/ 7.22 | 42.99/ 36.02 | 18 | 40.94 | 3322 6.53 | 1.76 (UP in steers) ** |
| 20 | Creatine kinase M-type | CKM | Q9XSC3 | 6.63/ 7.22 | 42.99/ 42.06 | 50 | 75.07 | 2239 59.20 | 1.68 (UP in steers) ** |
| 21 | Creatine kinase M-type | CKM | Q9XSC6 | 6.63/ 7.11 | 42.99/ 42.06 | 44 | 71.13 | 1263 23.20 | 1.42 (UP in steers) ** |
| 22 | Creatine kinase M-type | CKM | Q9XSC6 | 6.63/ 7.02 | 42.99/ 43.76 | 33 | 70.34 | 8179 4.26 | 1.32 (UP in steers) ** |
| 23 | Creatine kinase M-type | CKM | Q9XSC6 | 6.63/ 6.85 | 42.99/ 36.74 | 7 | 20.21 | 3226 2.01 | 1.64 (UP in steers) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | ** |
| 11 | Fructose-bisfosfate aldolase | ALDOA | A6QLL8 | 8.45/7.94 | 39.44/38.38 | 34 | 88.46 | 154143.40 | 1.58 (UP in steers)** |
| 41 | Glyceraldehyde-3-phosphate dehydrogenase | GAPDH | P10096 | 8.51/8.97 | 35.87/30.83 | 13 | 36.64 | 164369.40 | 1.56 (UP in steers)** |
| 44 | Glyceraldehyde-3-phosphate dehydrogenase | GAPDH | P10096 | 8.51/8.90 | 35.87/32.61 | 39 | 60.66 | 592238.60 | 1.75 (UP in steers)** |
| 45 | Glyceraldehyde-3-phosphate dehydrogenase | GAPDH | P10096 | 8.51/8.34 | 35.87/37.04 | 13 | 38.74 | 57299.81 | 1.55 (UP in steers)* |
| 13 | ATP synthase subunit beta, mitochondrial | ATP5F1B | P00829 | 5.15/5.29 | 56.28/50.94 | 34 | 49.43 | 144259.90 | 0.84 (UP in bulls)** |
| 32 | Phosphoglucomutase-1 | PGM1 | Q28DP0 | 6.36/7.21 | 61.59/67.26 | 48 | 76.51 | 134508.60 | 0.59 (UP in bulls)* |
| 33 | Phosphoglucomutase-1 | PGM1 | Q08DP0 | 6.65/6.36 | 68.98/61+.59 | 37 | 61.03 | 24598.74 | 0.71 (UP in bulls)* |
| 8 | Pyruvate dehydrogenase E1 component subunit beta, mitochondrial | PDHB | P11966 | 6.21/5.77 | 39.13/30.97 | 22 | 69.36 | 81559.12 | 0.78 (UP in bulls)** |
| 7 | Triosephosphate isomerase | TPI1 | Q5E956 | 6.45/7.27 | 26.69/24.23 | 27 | 90.36 | 273848.10 | 0.72 (UP in bulls)** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 9 | Malate dehydrogenase | MDH1 | A0A452DIW4 | 6.15/6.53 | 35.86/32.35 | 14 | 44.38 | 56966.64 | 0.57 (UP in bulls)* |
| *Transport and binding* | | | | | | | | | |
| 2 | Hemoglobin subunit alpha | HBA | P01966 | 9.03/8.07 | 12.84/15.18 | 6 | 42.96 | 28263.91 | 0.57 (UP in bulls)* |
| 1 | Hemoglobin subunit beta | HBB | P02070 | 7.87/7.02 | 12.69/15.95 | 17 | 91.03 | 159600.20 | 0.53 (UP in bulls)* |
| 3 | Myoglobin | MB | A0A1K0FUF3 | 6.90/7.80 | 17.08/14.20 | 20 | 82.47 | 391701.30 | 0.79 (UP in bulls)** |
| 4 | Myoglobin | MB | A0A1K0FUF3 | 6.90/7.69 | 17.08/16.25 | 3 | 58.44 | 109942.50 | 0.30 (UP in bulls)* |
| *Responses to stress* | | | | | | | | | |
| 6 | Peroxiredoxin-2 | PRDX2 | Q9BGI3 | 5.50/5.37 | 20.42/21.95 | 8 | 38.19 | 48098.32 | 0.76 (UP in bulls)** |
| 19 | Glutathione S-transferase | GSTP1 | P28801 | 7.80/6.89 | 22.19/23.61 | 18 | 70.48 | 164519.10 | 0.55 (UP in bulls)* |
| 30 | Heat shock cognate 71 kDa protein | HSPA8 | P19120 | 5.37/5.67 | 71.24/75.83 | 52 | 64.15 | 380574.90 | 0.84 (UP in bulls)** |
| 14 | 60 kDa heat shock protein, mitochondrial | HSP60 | P31081 | 5.71/5.61 | 60.68/63.21 | 42 | 62.48 | 138291.30 | 0.54 (UP in bulls)* |
| *Muscle structure* | | | | | | | | | |
| 18 | Troponin I2, fast skeletal type | TNNI2 | F6QIC1 | 9.30/9.78 | 20.97/20.70 | 24 | 59.55 | 40427.62 | 0.62 (UP in bulls)* |
| 23 | Troponin T, fast skeletal muscle | TNNT3 | Q8MKI4 | 5.93/6.85 | 32.12/36.74 | 13 | 28.78 | 11939.12 | 0.64 (UP in bulls)* |

[1]Protein spots abundance in experimental groups (bulls and steers). For 2D gel image comparisons, a reference gel per treatment was listed, which contained the highest number and best definition of spots, and the reference gel of a treatment was contrasted with each gel of another treatment, totaling 12 comparisons. Value greater than on represents upregulation in steers.

$^{**}P < 0.01$

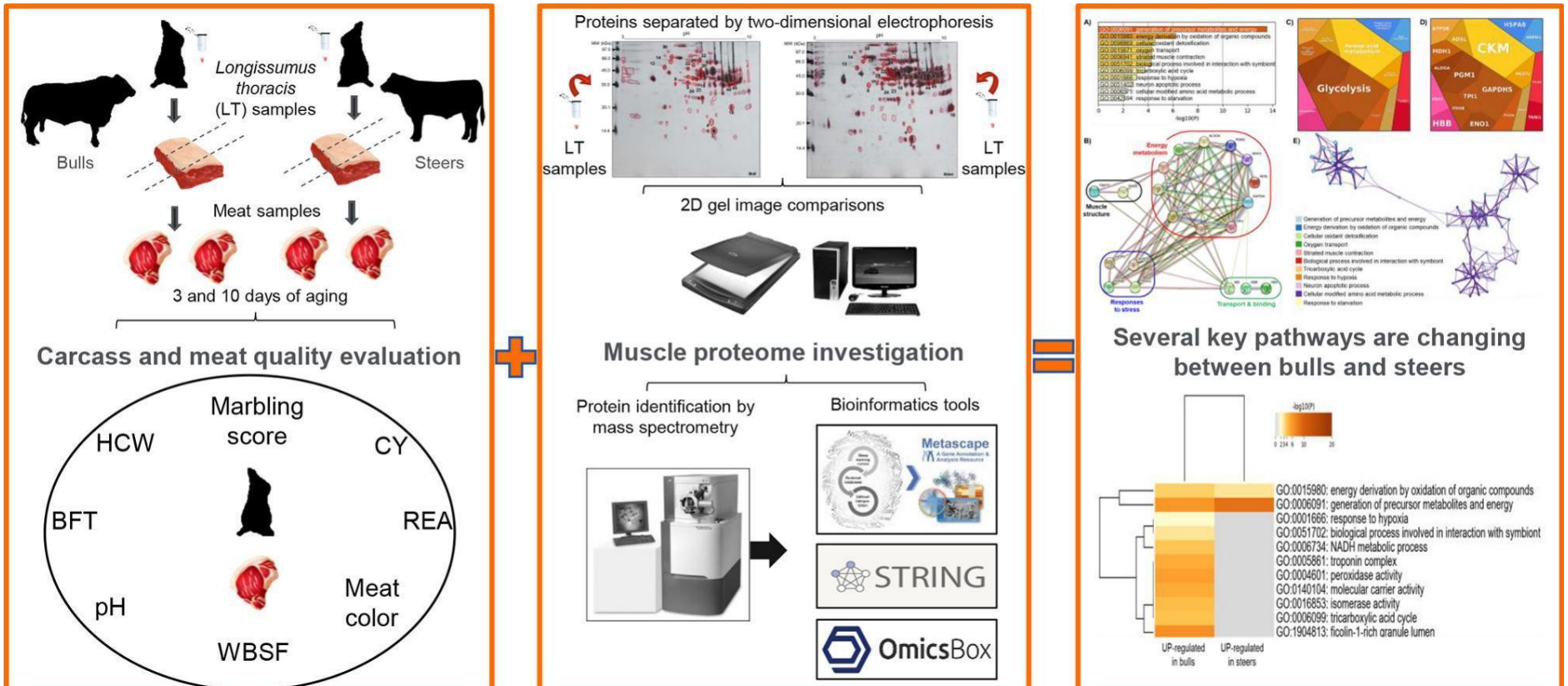
$^{*}P < 0.05$

**Significance**

Sexual condition of cattle is known to be an important factor affecting animal performances and growth as well as the carcass and meat quality traits. The investigation of skeletal muscle proteome help a better understanding of the origin of the differences in quality traits between bulls and steers. The inferior meat quality of bulls was found to be due to the greater expression of proteins associated with primary and catabolic processes, oxidative stress, and muscle contraction. Steers had greater expression of proteins, from which several are known biomarkers of beef quality (mainly tenderness).

**Highlights**

- Muscle proteome, carcass and meat quality traits of crossbred bulls and steers were described.
- Investigation of proteome help to understanding differences in carcass and beef quality.
- Bulls had more key proteins of catabolic processes, oxidative stress, and muscle contraction.
- Proteins known as biomarkers of tenderness and marbling degree were up-regulated in steers.
- Protein interactions in steers decreases muscle growth, but improves tenderness and marbling.




Graphics Abstract

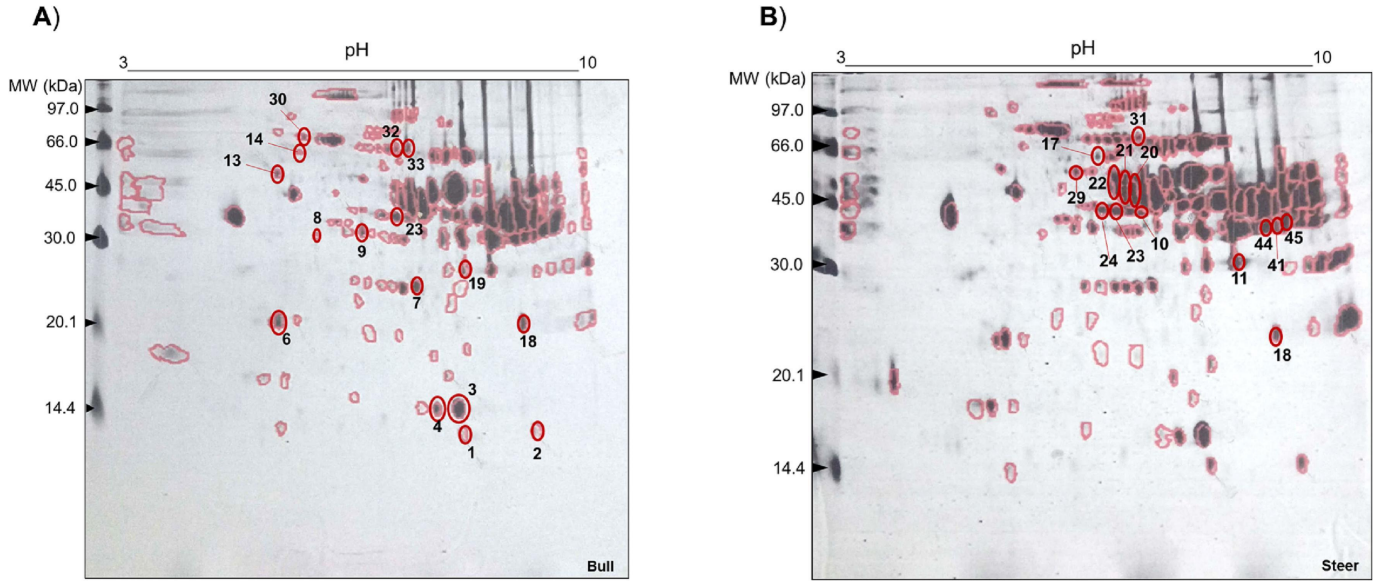

A)
pH
3
10
MW (kDa)
97.0
66.0
45.0
30.0
20.1
14.4
30
14
13
32
33
8
23
9
19
7
6
18
3
4
1
2
Bull
B)
pH
3
10
MW (kDa)
97.0
66.0
45.0
30.0
20.1
14.4
31
17
21
20
22
29
10
24
23
44
45
11
41
18
Steer

Figure 1

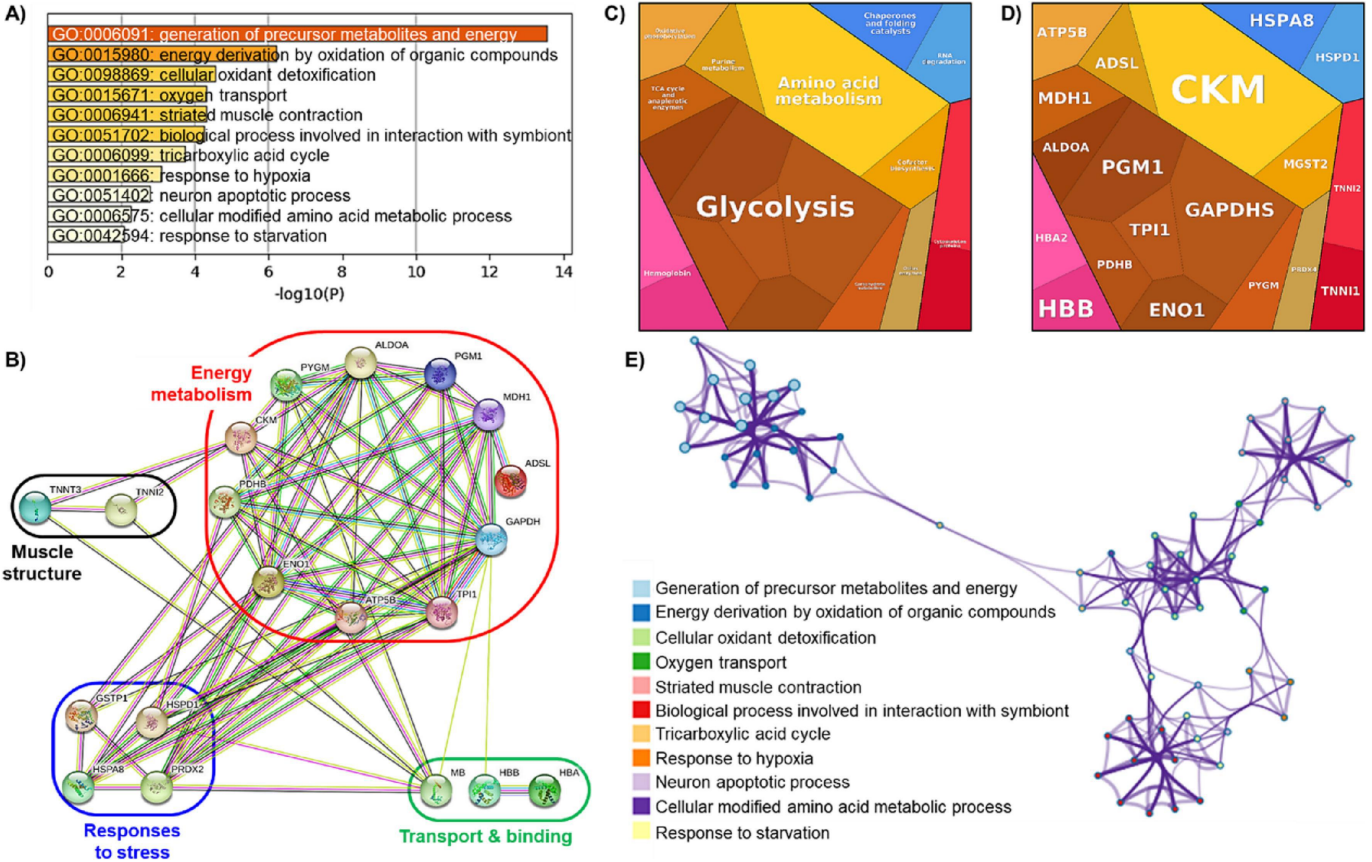

A)
GO:0006091: generation of precursor metabolites and energy
GO:0015980: energy derivation by oxidation of organic compounds
GO:0098869: cellular oxidant detoxification
GO:0015671: oxygen transport
GO:0006941: striated muscle contraction
GO:0051702: biological process involved in interaction with symbiont
GO:0006099: tricarboxylic acid cycle
GO:0001666: response to hypoxia
GO:0051402: neuron apoptotic process
GO:0006575: cellular modified amino acid metabolic process
GO:0042594: response to starvation
0
2
4
6
8
10
12
14
-log10(P)
B)
Energy metabolism
ALDOA
PYGM
PGM1
MDH1
CKM
ADSL
PDHB
GAPDH
ENO1
ATP5B
TPI1
TNNT3
TNNI2
Muscle structure
GSTP1
HSPD1
HSPA8
PRDX2
Responses to stress
MB
HBB
HBA
Transport & binding
C)
Chaperones and folding catalysts
Amino acid metabolism
Glycolysis
D)
ATP5B
HSPA8
ADSL
HSPD1
MDH1
CKM
ALDOA
PGM1
MGST2
TNNI2
GAPDHS
TPI1
HBA2
PDHB
PRDX4
PYGM
TNNI1
HBB
ENO1
E)
Generation of precursor metabolites and energy
Energy derivation by oxidation of organic compounds
Cellular oxidant detoxification
Oxygen transport
Striated muscle contraction
Biological process involved in interaction with symbiont
Tricarboxylic acid cycle
Response to hypoxia
Neuron apoptotic process
Cellular modified amino acid metabolic process
Response to starvation

Figure 2

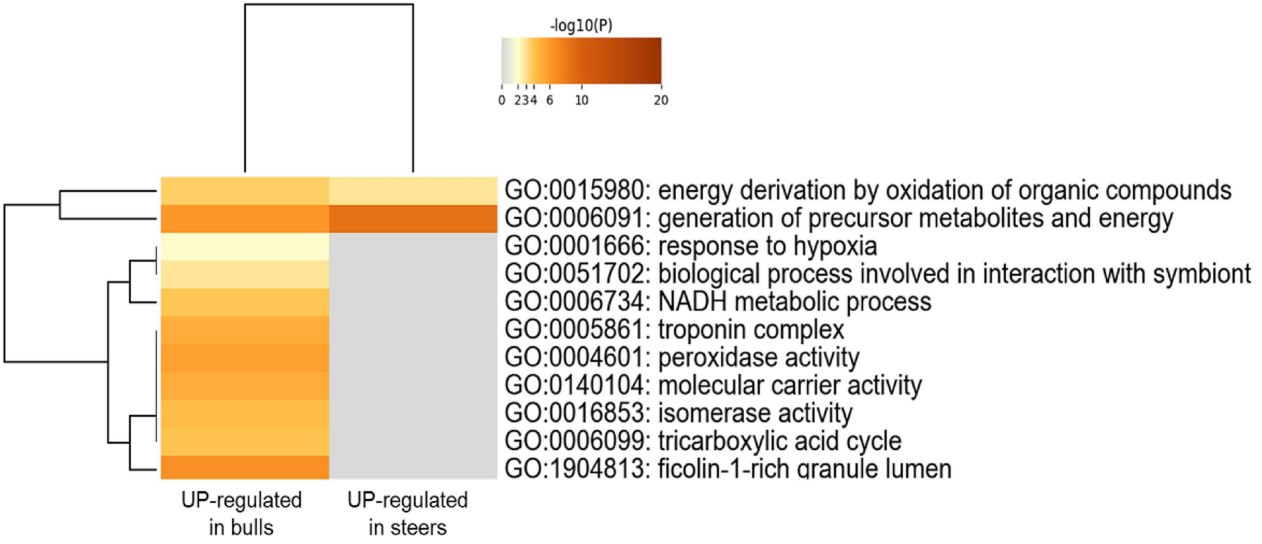

-log10(P)
0 2 3 4 6 10 20
GO:0015980: energy derivation by oxidation of organic compounds
GO:0006091: generation of precursor metabolites and energy
GO:0001666: response to hypoxia
GO:0051702: biological process involved in interaction with symbiont
GO:0006734: NADH metabolic process
GO:0005861: troponin complex
GO:0004601: peroxidase activity
GO:0140104: molecular carrier activity
GO:0016853: isomerase activity
GO:0006099: tricarboxylic acid cycle
GO:1904813: ficolin-1-rich granule lumen
UP-regulated in bulls
UP-regulated in steers

Figure 3

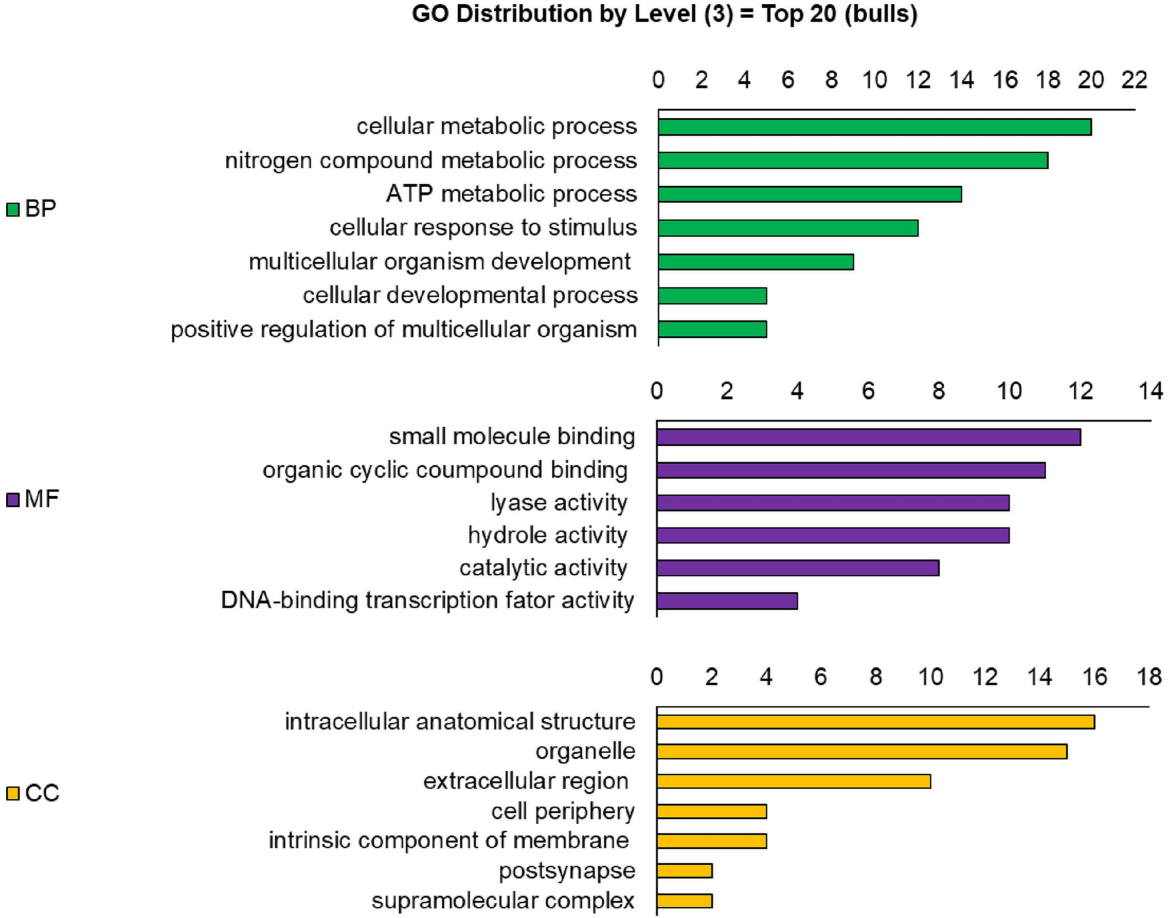

GO Distribution by Level (3) = Top 20 (bulls)
BP
0 2 4 6 8 10 12 14 16 18 20 22
cellular metabolic process
nitrogen compound metabolic process
ATP metabolic process
cellular response to stimulus
multicellular organism development
cellular developmental process
positive regulation of multicellular organism
MF
0 2 4 6 8 10 12 14
small molecule binding
organic cyclic coumpound binding
lyase activity
hydrole activity
catalytic activity
DNA-binding transcription fator activity
CC
0 2 4 6 8 10 12 14 16 18
intracellular anatomical structure
organelle
extracellular region
cell periphery
intrinsic component of membrane
postsynapse
supramolecular complex

Figure 4

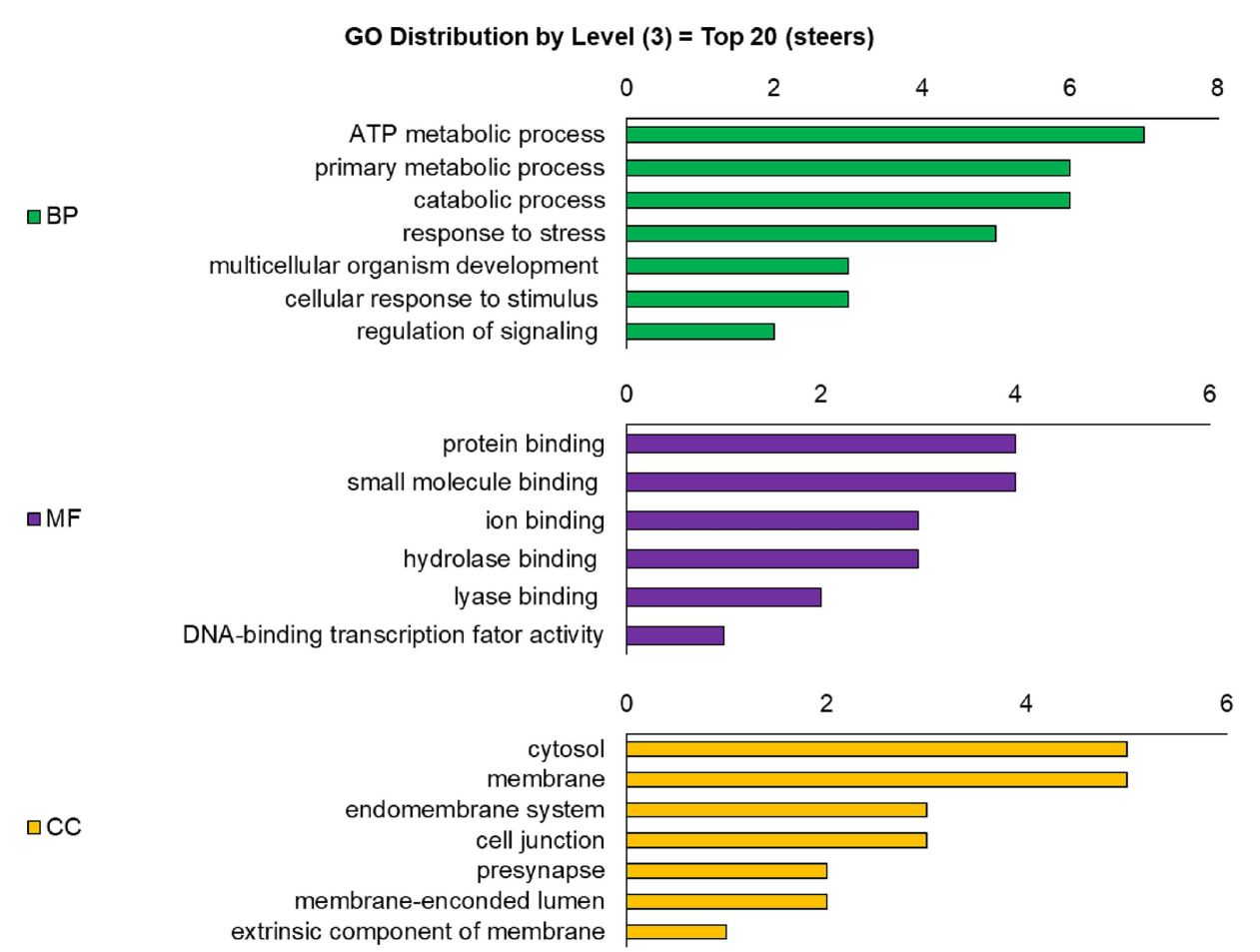

GO Distribution by Level (3) = Top 20 (steers)
BP
0
2
4
6
8
ATP metabolic process
primary metabolic process
catabolic process
response to stress
multicellular organism development
cellular response to stimulus
regulation of signaling
MF
0
2
4
6
protein binding
small molecule binding
ion binding
hydrolase binding
lyase binding
DNA-binding transcription fator activity
CC
0
2
4
6
cytosol
membrane
endomembrane system
cell junction
presynapse
membrane-enconded lumen
extrinsic component of membrane

Figure 5