Meat and Muscle Biology™

Color and Tenderness Proteomic Biomarkers of Beef From Steers Fed Sorghum Substituted for Maize

Yonela Z. Njisane[1], Farouk Semwogerere[1], Gadija Mohamed[2,3], Bongani K. Ndimba[2,3], and Cletos Mapiye[1]*

[1]Department of Animal Sciences, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa
[2]Post-Harvest and Agro-Processing Technologies (PHATs), Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa
[3]Department of Biotechnology, Faculty of Natural Sciences, University of the Western Cape, Private Bag X17, Bellville, 7535, South Africa
*Corresponding author. Email: cmapiye@sun.ac.za (Cletos Mapiye)

Abstract: The objective of this study was to identify proteins and biochemical pathways associated with beef color and tenderness from Angus steers fed graded levels of sorghum-based finisher diets. Twenty-one 7-month-old (230 ± 28 kg average initial weight) Angus steers were individually housed in pens and randomly allocated to one of the 3 dietary treatments ($n = 3$) for 90 d of feeding. The pellet diets were formulated by replacing white maize in the basal diet with either 0 (control, SGD-0), 200 (SGD-200), or 400 (SGD-400) g/kg dry matter of sorghum grain. The *longissimus thoracis et lumborum* (LTL) muscle was harvested 24 h post-slaughter for physical (pHu, color, and tenderness) and proteomic quality analyses. Proteomic profiling was done using a combination of Bradford assay, SDS-PAGE, and liquid chromatography-tandem mass spectrometry methods. The inclusion of sorghum in beef finisher diets showed a tendency ($P = 0.083$) to increase ultimate pH and linearly increased ($P < 0.05$) Warner-Bratzler shear force values without affecting ($P > 0.05$) color. Of the 11 differentially expressed proteins (false discovery rate < 0.05), sorghum diets downregulated ($P < 0.05$) MYH1, MYH8, GYS1, HSPA8, HSP90AA1, and HSPB6, while PEBP1 was upregulated ($P < 0.05$). Two (MYL3 and YWHAE) and 3 (HSPA9, PDIA3, and ANKRD2) tenderness-regulating proteins were uniquely expressed in SGD-200 and SGD-400, respectively. With respect to color regulation, 4 (MYH2, PDHX, LAP3, and P4HB) and 2 (MYH1 and HSPA9) proteins were correspondingly expressed in SGD-200 and SGD-400. Several differentially and uniquely expressed (DUE) structural proteins and glycolytic enzymes suggested that SGD could produce less tender beef while heat shock proteins indicated its association with beef tenderness. However, there was an indication from most DUE proteins that SGD could increase beef redness. Overall, DUE proteins suggested that diets containing sorghum up to 400 g/kg yield beef of less desirable tenderness.

Key words: beef, color, proteomics, sorghum, tenderness
Meat and Muscle Biology 8(1): 17305, 1–16 (2024) **doi:10.22175/mmb.17305**
Submitted 7 December 2023 Accepted 11 June 2024

The abstract of this article was corrected on October 1, 2024. In the second sentence of the abstract, it was erroneously stated that "Nine 8-month-old (230 ± 28 kg average initial weight) Angus steers . . ." This was corrected to "Twenty-one, 7-month-old (230 ± 28 kg average initial weight) Angus steers . . ."

Introduction

Color and tenderness are the main quality attributes that determine consumers' decisions to purchase and consume beef; thus, they play a significant role in the economics and sustainability of the beef industry (Suman and Joseph, 2013; Suman et al., 2023). Color influences consumers' willingness to purchase beef at retail, whereas tenderness is determined at the eating time with more tender meat resulting in repetitive purchases (Picard and Gagaoua, 2020; Suman et al., 2023). Numerous studies have

explored the proteome of beef to understand the underlying biochemical processes that determine color and tenderness (Nair et al., 2018; Bonnet et al., 2020; Gagaoua et al., 2020a; Antonelo et al., 2022). Notably, meat color and tenderness share several proteins and biological pathways in the skeletal muscle proteome, but the degree to which they are expressed and utilized in various functions differs (Gagaoua et al., 2020b; Picard and Gagaoua, 2020; Suman et al., 2023). The quantity of these proteins in the skeletal muscle proteome undergoes substantial and continuous changes throughout ante-, peri-, and postmortem meat production and processing phases (Carvalho et al., 2019; Picard and Gagaoua, 2020; Antonelo et al., 2022).

Dietary composition has been reported as a major determinant of meat proteome profile during the antemortem phase (Picard and Gagaoua, 2020; Antonelo et al., 2022; Suman et al., 2023). Dietary protein and energy contents dictate growth rate and glycogen reserves which are the main factors that influence changes in meat color and tenderness postmortem (Picard and Gagaoua, 2020; Antonelo et al., 2022). More so, dietary bioactive phytochemicals such as polyphenolic compounds can potentially be assimilated into the meat (Zhong et al., 2016; Orzuna-Orzuna et al., 2021) and tend to retard/inhibit enzyme activities involved in glycogen metabolism (i.e., glycogen phosphorylase and lactate dehydrogenase) (Kamiyama et al., 2010; Han et al., 2023) and myofibrillar protein degradation (i.e., calpains) (Louis et al., 2014), consequently altering meat color and tenderness postmortem (Purslow et al., 2021; Suman et al., 2023). Thus, assessing variations in the meat proteome profile of beef fed diets containing bioactive phytochemicals is paramount for high product quality.

Sorghum contains up to 30 g/kg dry matter (DM) tannins and it has been widely utilized in beef feedlot finisher diets to curb the ever-increasing prices of maize as an energy source (Mccuistion et al., 2019; Osman et al., 2022). Studies have reported variable color changes and increased shear force values when beef is finished with sorghum-based diets and attribute the changes to the effects of tannins on myofibrillar degradation, fat deposition, and oxidation (Zhong et al., 2016; Sun et al., 2018; Orzuna-Orzuna et al., 2021). However, little is still known about the biochemistry of underlying processes and the major proteins involved in meat color and tenderness changes when finisher diets containing sorghum are fed.

On one hand, meat color variations on the surface are a product of myoglobin redox forms (i.e., deoxymyoglobin, oxymyoglobin, and metmyoglobin) that are strongly linked to oxygen consumption and reductive enzyme activity which are influenced by pH in the postmortem muscle (Nair et al., 2018; Gagaoua et al., 2020b; Antonelo et al., 2022). On the other hand, meat tenderness is mostly influenced by structural and metabolic activities, reflecting muscle restructuring in response to proteolysis of muscle myofibrillar and cytoskeletal proteins, regulated by endogenous calcium-dependent calpain system postmortem (Zamaratskaia and Li, 2017; Picard and Gagaoua, 2020). To the authors' knowledge, no study has explored the proteome changes resulting from finishing steers with sorghum-based diets. Thus, the current study objective was to identify proteins and biochemical pathways associated with beef color and tenderness from Angus steers fed graded levels of sorghum-based finisher diets.

Materials and Methods

Between February and June 2022, a feeding trial was executed at Mariendahl Research Farm, Cape Town, South Africa. Ethical approval for the care and use of animals was permitted by Stellenbosch University (ACU-2020-17090) as guided by South African National Standards (SANS 10386:2008).

Experimental diets, animal management, and design

Whole red grain sorghum was sourced from a commercial producer (AGT, Cape Town, South Africa) and milled through a 0.5 mm sieve. Three pelleted (5 mm × 30 mm; 45°C) maize-lucerne-based complete diets containing either 0 (SGD-0), 200 (SGD-200), or 400 (SGD-400) g/kg DM of sorghum grain substituting white maize grain sourced from Perdigon Proprietary Limited, Paarl, South Africa as a primary energy source were formulated by commercial feed producer. The diets were isoenergetic and met the nutritional requirements of growing steers (Tables 1 and 2; National Research Council, 2016). Though the diets were not isonitrogenous, findings from a companion study (Njisane et al., submitted) shows that protein intake was similar ($P > 0.05$) across diets. Twenty-one Angus steers (7 mo; 230 ± 28 kg) were purchased from a single commercial producer, and each steer was housed in an individual concrete floor pen (2 m × 4 m) covered with straw. The steers were adapted for 21 d followed by 90 d of feeding.

Table 1. Feed ingredient proportions in experimental diets (g/kg DM)

	Sorghum inclusion level in the diet		
Ingredients (g/kg DM)	0	200	400
White maize	400	200	0
Sorghum	0	200	400
Wheat bran	235.5	235.5	235.5
Lucerne hay	110	110	110
Wheat straw	90	90	90
Soybean meal	75.6	75.6	75t.6
Molasses	70	70	70
Feed lime	13	13	13
Coarse salt	2.7	2.7	2.7
Urea	2	2	2
Vitamin-mineral premix*	0.6	0.6	0.6
Ammonium sulfate	0.5	0.5	0.5
Zinc amino acid complex	0.1	0.1	0.1

*The composition of the vitamin/premix was not included because of a non-disclosure agreement with the feed manufacturer.

Slaughter procedures and meat sampling

The steers were slaughtered at a commercial abattoir, 64 km from the experimental farm. During slaughter, steers were stunned using a non-penetrating captive-bolt and exsanguinated according to South African meat law (South African Government Gazette, 2000). Twenty-four h postmortem, the pH of all carcasses was measured using a mobile pH meter with a built-in Pt1000 temperature sensor for automatic temperature compensation (CrisonTM pH meter PH 25+, Lasec, South Africa) in the 12th and 13th right rib region. Then, the left *longissimus thoracis et lumborum* (LTL) muscle was removed from each carcass from the 9th to 13th rib. Thereafter, a two-gram cube was sampled from 3 loins per treatment, dipped in liquid nitrogen, and stored (−80 °C) in 15 ml tubes until proteomic analysis.

Color and shear force assays

Muscle color samples were allowed to bloom for 30 min, and 3 readings were taken per sample. Color

Table 2. Analyzed chemical composition of dietary ingredients and experimental diets (g/kg DM)

Chemical composition (g/kg DM unless stated)	Maize	Sorghum	SEM[1]	Inclusion level (g/kg DM) 0	200	400	SEM[1]	*P*-value Diet
Dry matter (DM)	875.3	871.2	1.84	887.7	890.7	891.0	0.65	0.001
Ash	8.6	11.4	0.45	53.0	52.8	52.4	0.39	0.512
Crude protein (CP)	49.3	69.6	0.90	97.6	106.7	111.9	0.80	0.001
Ether extract (EE)	27.8	24.1	0.22	23.3	23.1	23.0	0.35	0.793
Neutral detergent fiber (aNDFom)[2]	76.6	94.7	7.53	182.6	194.2	195.6	3.63	0.045
Acid detergent fiber (ADFom)[3]	22.1	24.9	1.44	90.8	94.5	93.0	1.61	0.284
Lignin (sa)[4]	3.4	6.1	0.58	18.2	18.3	18.3	0.73	0.997
Metabolizable energy (MJ/kg)[5]	12.8	13.0	0.11	12.1	12.1	12.1	0.02	0.477
Non-fibrous carbohydrates (NFC)[6]	794.0	791.4	8.28	643.5	623.2	617.3	3.88	0.001
Total phenols (g GAE/kg DM)[7]	0.7	1.0	0.02	1.6	1.6	1.9	0.03	0.001
Tannins (g GAE/kg DM)[7]	0.5	0.9	0.03	1.1	1.1	1.2	0.03	0.007
Proanthocyanidin (g GAE/kg DM)[7]	0.1	0.3	0.01	0.5	0.7	1.0	0.08	0.001
Fatty acids (g/100 g total FA)								
C16:0	13.9	15.1	0.11	17.7	18.2	22.0	0.13	0.001
C18:0	2.5	1.4	0.03	2.9	2.8	2.5	0.03	0.001
C18:1n-9	30.4	28.7	0.08	25.5	23.3	22.4	0.12	0.001
C18:2n-6	50.8	51.7	0.12	50.7	50.8	49.5	0.11	0.001
C18:3n-3	0.8	1.6	0.02	2.5	2.9	3.1	0.03	0.001

All chemical analyses were analyzed in triplicate with 5 replicates per sample.

[1]SEM: Standard error of means.

[2]aNDFom: Neutral detergent fiber analyzed with a heat-stable amylase and reported without ash.

[3]ADFom: Acid detergent fiber reported without ash.

[4]Lignin (sa.): Lignin analyzed by solubilization of cellulose with sulfuric acid.

[5]Calculated according to (Freer et al., 2019).

[6]Non-fibrous carbohydrates: calculated as: 1000 − (aNDFom + crude protein + ether extract + ash; g/kg).

[7]GAE: Gallic acid equivalent.

The table components listed mirror the proportions reported by Njisane et al. (Submitted).

coordinates (L^*, lightness; a^*, redness; b^*, yellowness) were measured using a BYK-209 Gardner GmbH (Gerestried, Germany) colorimeter set to sample mode and calibrated with D65/10° against black and white tiles using observer settings and an 11-mm diameter aperture (AMSA, 2012). Warner-Bratzler shear force (WBSF) was determined using a ≈100 g meat sample cooked in a water bath at 80°C to an internal temperature of 75°C (AMSA, 2015). A thermocouple probe affixed onto a digital monitor (Testo 176T4, South Africa) was injected into the center of an individual sample ($n = 5$) to monitor internal temperature throughout the cooking process.

Proteomic characterization

Sample preparation, protein extraction and quantification. Frozen samples were ground into a fine powder using liquid nitrogen in a stainless-steel blender. The ground material (0.5 g) was resuspended in 1 mL extraction buffer (4 M Urea, 2 M Thiourea; 2.5 mM Ethylenediaminetetraacetic acid [EDTA]; 5 mM 1,4 Dithiothreitol [DTT]; 2% glycerol), thoroughly vortexed for 30 s, incubated 40 min at −20°C, and centrifuged at 15,000 × g for 20 min at 4°C. The supernatant containing the protein extract was transferred to clean tubes, and protein concentration was quantified using the Bradford assay with bovine serum albumin as a standard (Kruger, 2009). All the samples were extracted in triplicate.

SDS-PAGE protein visualization and quantitation. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol was deployed to determine the quality and purity of the extracted proteins. Briefly, proteins (30 µg) were prepared and incubated as described in Mahlare et al. (2023). Proteins were heated at 70°C for 10 min and resolved as described by Mahlare et al. (2023). Following a 12% SDS-PAGE gel electrophoresis, proteins were visualized using Coomassie brilliant blue stain, and the gels were also processed as defined by Mahlare et al. (2023). Protein expression densitometric analysis of bands was performed using AlphaEase FC software (AlphaImager™ IS-2200 for Windows 2000/XP; Version 4), and default background subtraction for relative intensity correction was activated to only record analyte signals. The protein band intensities were expressed as integrated density values (IDV), presented as averages of 3 technical replicates.

In-gel digest, peptide extraction, and clean-up. Protein bands of interest were excised excluding strongly stained bands (Figure 1), and gel lanes of interest were uniformly cut into smaller gel cubes (≈ 1.0 mm × 1.0 mm) using a surgical blade and an A4 cutting board with a 20 cm aluminum ruler, and destained using 50% acetonitrile in 100 mM Ammonium Bicarbonate (NH_4HCO_3) until clear, and dehydrated with 100% acetonitrile for 5 min, with shaking. Dehydrated gel cubes were then reduced in 2 mM tris 2-carboxyethyl phosphine (TCEP) in 25 mM NH_4HCO_3 at room temperature (RT) for 15 min. Alkylation was conducted with 20 mM iodoacetamide in 25 mM NH_4HCO_3 for 30 min in the dark at RT. Following this, the gel cubes were dehydrated with 100% acetonitrile before rehydration with trypsin solution (10 ng/µl in 25 mM NH_4HCO_3) for 45 min at 4°C. After rehydration, excess trypsin solution was aspirated, and the gel cubes incubated in 50 µl 25 mM NH_4HCO_3 to enable overnight digestion at 37°C. Peptides were extracted from the gel cubes with 100 µl milli Q water by vortexing for 45 min. The supernatant was transferred to 1.5 ml Lo-bind Eppendorf tubes and the extracts dried down in a Centrivap Benchtop Vacuum Concentrator (Centrivap; Labconco Corporations, Kansas City,

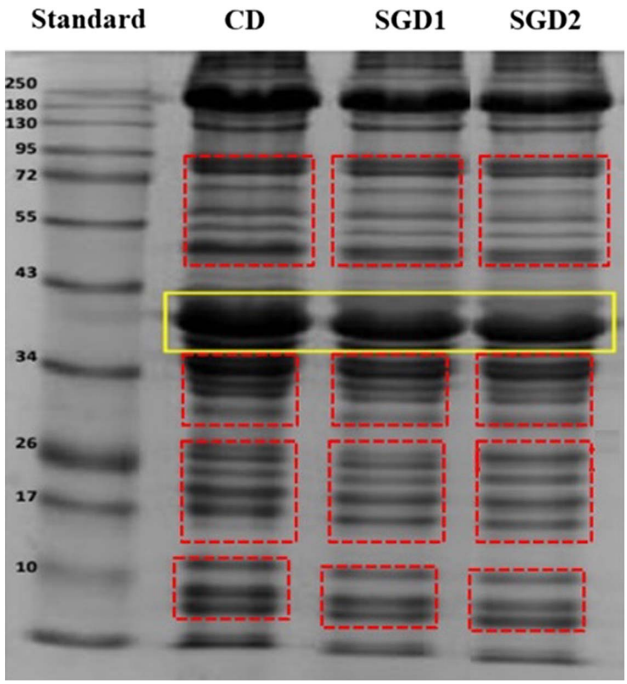

Figure 1. A representative SDS-PAGE gel of myofibrillar filaments of LTL beef from steers finished with sorghum-based diets. About 30 µg protein was loaded on to 12% SDS-PAGE gel. Standard represents the molecular weight marker; CD represents the control diet (SGD-0); SGD1 represents 200 g/kg DM of sorghum diet; SGD2 represents 400 g/kg DM of sorghum diet; the yellow box represents actin; the red box represents marked area of changes in protein intensity. The mean protein band intensities are expressed as integrated density values (IDV; SGD-0: 31864, SGD-200: 28538, SGD-400: 26107; Standard error of means: 5158.9) and area (SGD-0: 1246, SGD-200: 1208; SGD-400: 1119; Standard error of means: 67.6).

MO, USA) at RT before resuspension in 0.1% formic acid (FA). Samples were further purified and desalted as described by Mahlare et al. (2023) with slight modification. StageTips were conditioned with 10 µL of acetonitrile and equilibrated using 10 µL Buffer A (2% ACN/0.1% FA). Samples were then loaded onto and passed through the StageTip once before the tip was washed using 10 µL Buffer A. Peptides were eluted in 10 µL Buffer B (50% ACN/0.1% FA) and the peptide extracts pooled before the eluates were evaporated in a Centrivap at RT. Before liquid chromatography analysis, the dried peptide eluates were reconstituted in 15 µl Buffer A for liquid chromatography analysis.

Liquid chromatography with tandem mass spectrometry. The applied liquid chromatography with tandem mass spectrometry (LC-MS/MS) proteomic investigation was adapted from Hooijberg et al. (2018). The detailed procedures employed for peptide separation, protein quantification and characterizing, respectively achieved by liquid chromatography and mass spectrometer, are described in Mahlare et al. (2023). Briefly, peptide separation was initiated using loading solvents (Solvent A: 2% acetonitrile: water, 0.1% FA; Solvent B:100 acetonitrile: water, respectively) to deposit the samples into the trap column then onto the analytical column, and chromatography was performed at 40°C (Thermo Scientific UltiMate 3000 HPLC; Thermo Scientific, Rockford, IL, USA). The outflow was emitted onto the MS (Thermo Scientific Fusion MS:Nanospray Flex ionization source; Thermo Scientific, Rockford, IL, USA) for quantifying and characterizing, and the data attained as a weighted average of the mass peak (centroid mode).

Data processing, protein identification, and bioinformatics analysis. The raw data files were exported using Thermo Proteome Discoverer 1.4 (2012 Thermo Fisher Scientific Inc., Thermo Scientific, United States), and the Sequest and Amanda algorithms were applied for further processing. The resultant files were interrogated with reference to the Universal Protein (uniport) "Bos Taurus reviewed" concatenated database as detailed in Mahlare et al. (2023). Further validation was achieved in Scaffold Q+ (Settings: 95% Protein identification probability, 1% false discovery rate [FDR] protein threshold, 2 minimum number of peptides; www.proteomesoftware.com; accessed 14 August 2023). The functions of the identified proteins were assessed by mapping to Uniprot Resource (https://www.uniprot.org/id-mapping; accessed 14 August 2023). The bioinformatics webtool was used to visualize the common proteins identified in each diet (http://bioinformatics.psb.ugent.be/webtools/Venn/; accessed 14 August 2023).

Gene ontology (GO) and annotation enrichment analysis were done using the GO resource (http://geneontology.org/; accessed 14 August 2023), Panther classification system (https://pantherdb.org/; accessed 14 August 2023), String (https://string-db.org/; accessed 14 August 2023), and Kyoto Encyclopedia of Genes and Genomes (KEGG). Fisher's exact test was applied to determine the significance level of the protein enrichment of a specified GO term (FDR; $P < 0.05$). For protein-protein interactions (PPI), default settings (i.e., medium confidence of 0.4 and active interaction sources: text-mining, experiments, databases, co-expression, neighborhood, gene fusion, and co-occurrence) were used. The PPI networks were further clustered using the Markov cluster algorithm (MCL), with the inflation parameter set at 2 and PPI enrichment value at $P < 0.05$, while also retaining the floating proteins. To categorize the proteins intrinsic in beef tenderness and color development, the identified protein gene names were compared with published literature data for tenderness and color elaborated by Picard and Gagaoua (2020) and Gagaoua et al. (2020b), respectively.

Statistical analysis

The GLIMMIX procedure of SAS (version 9.4; SAS Institute Inc. Cary, NC, USA) was used to analyze physical attribute data with diet as fixed effect and animal a random factor. All data were subjected to the Shapiro-Wilk test (Shapiro & Wilk, 1965) for normality. A Tukey's test was used to qualify the least-squares means as significantly different at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Results

Physical attributes, proteomic bioinformatics, and protein expression

The inclusion of sorghum in beef finisher diets exhibited a tendency ($P = 0.083$) to increase ultimate pH and linearly increased ($P < 0.05$) WBSF values but had no effect ($P > 0.05$) on color (Table 3). Protein loading appeared consistently even, with no visible protein streaking in the meat protein extracts (Figure 1). The explored data incorporate details of the molecular weight and the quantity of protein extract of each treatment. The molecular weight of the protein

Table 3. Effects of feeding increasing levels of sorghum on physical attributes of *longissimus thoracis* meat from steers ($n = 7$)

	Inclusion level (g/kg DM)				
Parameters	0	200	400	SEM	*P*-value
Ultimate pH	5.7	5.9	5.8	0.04	0.082
Lightness (L^*)	41.6	41.1	40.6	0.58	0.510
Redness (a^*)	14.6	14.3	14.6	0.47	0.931
Yellowness (b^*)	12.4	12.0	12.4	0.34	0.592
WBSF (N)	55.8[b]	60.6[ab]	65.2[a]	2.18	0.013

SEM, standard error of means; WBSF, Warner-Bratzler shear force.

[a–b]Means within a row with different superscripts are different ($P < 0.05$).

bands ranged between 10 to 250 kDa for all the treatment extracts. Myosin heavy chain (MHC; ≈230 kDa) and actin (≈42 kDa) were the most dominant bands across all treatments. The intensity protein bands were similar ($P > 0.05$) across diets as shown by the IDV and area (Figure 1).

A total of 692 bovine proteins were identified, and 108 were common to all diets, while 74, 60, and 56 were respectively unique to treatments SGD-0, SGD-200, and SGD-400 (Figure 2). Of 108 common proteins, 11 were differentially expressed of which 7 edit to 8 (PYGM, PYGM, MYH1, MYH8, HSPA8, CAPZB, HSPB6, and PEBP1) are associated with both tenderness and color while 4 edit to 3 (PARP6, HSPOAA1, and GYS1) are only associated with color (Table 4). A total of 8 tenderness-regulating proteins were identified to be unique to diets, with 3 for SGD-0 (RABGGTA, HSPA5, and APOBEC2), 2 for SGD-200 (MYL3 and YWHAE), and 3 for SGD-400 (HSPA9, PDIA3, and ANKRD2). A sum of 9 color-regulating proteins were unique to diets, with 3 in SGD-0 (OXCT1, HSPA5, and MYH7), 4 in SGD-200 (MYH2, PDHX, LAP3, and P4HB), and 2 in SGD-400 (MYH1 and HSPA9). Of the 11 differentially expressed proteins (Table 4), 6 (MYH8, MYH1, HSP90AA1, GSY1, HSPB6, and HSPA8) were downregulated ($P < 0.05$) by sorghum diets (SGD-200 and SGD-400). Proteins PARP6 and PYGM were only downregulated ($P < 0.05$) in SGD-400. The CAPZB was only upregulated ($P < 0.05$) in SGD-400, while PEBP1 was upregulated in sorghum diets.

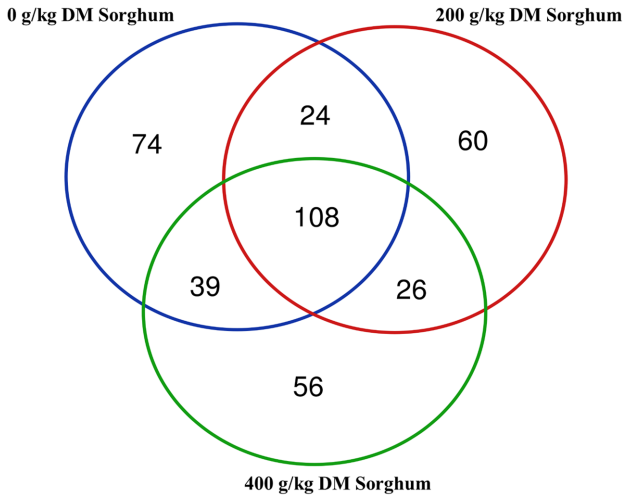

Figure 2. Number of proteins identified in each of the treatments visualized by bioinformatics webtool (http://bioinformatics.psb.ugent.be/webtools/Venn/, accessed 14 August 2023).

Gene ontology functional and pathway enrichment analyses

The differentially expressed as well as diet-unique proteins for tenderness and color were clustered into biological processes, molecular function, and cellular component (Figure 3). The cellular anatomical entity, cellular process, catalytic activity, and binding activities dominated the biological function, molecular function, and cellular component GO clusters, in that order (Figure 3). The GO clustering of diet-specific proteins revealed that the biological process function group was dominated by cellular process with SGD-0, SGD-200, and SGD-400 having 38%, 36%, and 38%, respectively (Figure 4). The binding (48%, 44%, and 53%, respectively) and catalytic activities (40%, 42%, and 37%, respectively) were highly represented in the molecular functions (Figure 4). Cellular anatomical entity (72%, 71%, and 81%, respectively) predominated the cellular component category (Figure 4). The pathways were dominated by HSPA8, HSPA9, HSPA5, and YWHAE clustered under Parkinson disease (Figure 5). Chaperones contributed 36% of the protein classes with differentially expressed HSPB6, HSP90AA1, HSPA8, and diet-specific HSPA5 (SGD-0), P4HB (SGD-200), PDIA3, and HSPA9 (SGD-400; Figure 5). Cytoskeletal proteins (32%) included differentially expressed MYH1 and CAPZB, as well as MYH7 (SGD-0), MYH2, and MYL3 (SGD-200; Figure 5). Metabolic interconversion enzymes (18%) included differentially expressed PYGM along with RABGGTA and OXCT1 (SGD-0), and PDHX (SGD-200; Figure 5). The extensive pathways were cytoskeletal regulation by Rho GTPase (16%), signaling pathway (16%), inflammation mediated by chemokine and cytokine signaling pathway (16%), and nicotinic acetylcholine receptor signaling pathway (16%).

Table 4. Differentially regulated and unique proteins associated with tenderness and/or color in LTL from beef fed graded levels of sorghum

Accessions	Entry	Gene names	Protein names	Mass (kDa)	Inclusion level (g/kg DM)		
					0	200	400
Differentially expressed proteins associated with tenderness and color							
A0A4W2ERW4_BOBOX	A0A4W2ERW4	PARP6	Multifunctional fusion protein (polymerase & pyruvate kinase)	102.5	Up	Up	Down
B0JYK6_BOVIN	B0JYK6	PYGM	Alpha-1,4 glucan phosphorylase	97.3	Up	Up	Down
PYGM_BOVIN	P79334	PYGM	Glycogen phosphorylase, muscle form (myophosphorylase)	97.3	Up	Up	Down
A0A3Q1LYQ9_BOVIN	A0A3Q1LYQ9	MYH1	Myosin heavy chain 1	220.8	Up	Down	Down
A0A4W2C7P3_BOBOX	A0A4W2C7P3	MYH8	Myosin-8	222.8	Up	Down	Down
A0A4W2FK20_BOBOX	A0A4W2FK20	HSPA8	Heat shock protein family A (Hsp70) member 8	72.3	Up	Down	Down
A0A4W2C816_BOBOX	A0A4W2C816	HSP90AA1	Heat shock protein 90 alpha family class A member 1	89.1	Up	Down	Down
PEBP1_BOVIN	P13696	PEBP1	Phosphatidylethanolamine-binding protein 1 (PEBP-1)	21.0	Down	Up	Up
A0A4W2CJ22_BOBOX	A0A4W2CJ22	GYS1	Glycogen synthase	76.5	Up	Down	Down
A0A3Q1LZP7_BOVIN	A0A3Q1LZP7	CAPZB	F-actin-capping protein subunit beta	32.2	Down	Down	Up
A0A0U2YDA7_BOBOX	A0A0U2YDA7	HSPB6	Heat shock protein	17.5	Up	Down	Down
Uniquely expressed proteins associated with beef tenderness							
PGTA_BOVIN	Q5EA80	RABGGTA	Geranylgeranyl transferase type-2 subunit alpha	64.9	Unique		
A0A4W2EIV7_BOBOX	A0A4W2EIV7	HSPA5	Heat shock protein 70 family protein 5	72.4	Unique		
ABEC2_BOVIN	Q3SYR3	APOBEC2	Probable C->U-editing enzyme APOBEC-2 (mRNA cytosine deaminase 2)	26.0	Unique		
A0A4W2IAK2_BOBOX	A0A4W2IAK2	MYL3	Myosin light chain 3	27.2		Unique	
1433E_BOVIN	P62261	YWHAE	14-3-3 protein epsilon (14-3-3E)	29.2		Unique	
A0A4W2FQ02_BOBOX	A0A4W2FQ02	HSPA9	Heat shock 70 kDa protein 9	71.2			Unique
A0A4W2CAQ4_BOBOX	A0A4W2CAQ4	PDIA3	Protein disulfide-isomerase	51.9			Unique
A0A4W2FD13_BOBOX	A0A4W2FD13	ANKRD2	Ankyrin repeat domain 2	39.3			Unique
Uniquely expressed proteins associated with beef color							
A0A3Q1LIX4_BOVIN	A0A3Q1LIX4	OXCT1	Succinyl-CoA:3-ketoacid-coenzyme A transferase	56.8	Unique		
A0A4W2EIV7_BOBOX	A0A4W2EIV7	HSPA5	78 kDa glucose-regulated protein	72.4	Unique		
F1MM07_BOVIN	F1MM07	MYH7	Myosin heavy chain 7	223.2	Unique		
A0A4W2HXW2_BOBOX	A0A4W2HXW2	MYH2	Myosin-2	223.3		Unique	
A0A3Q1LKL1_BOVIN	A0A3Q1LKL1	PDHX	Dihydrolipoamide acetyltransferase	50.8		Unique	
AMPL_BOVIN	P00727	LAP3	Cytosol aminopeptidase (leucine aminopeptidase 3; LAP-3)	56.3		Unique	
A0A4W2FY74_BOBOX	A0A4W2FY74	P4HB	Prolyl 4-hydroxylase	63.3		Unique	
MYH1_BOVIN	Q9BE40	MYH1	Myosin-1 (myosin heavy chain 1)	223.0			Unique
A0A4W2FQ02_BOBOX	A0A4W2FQ02	HSPA9	Stress-70 protein (heat shock 70 kDa protein 9)	71.2			Unique

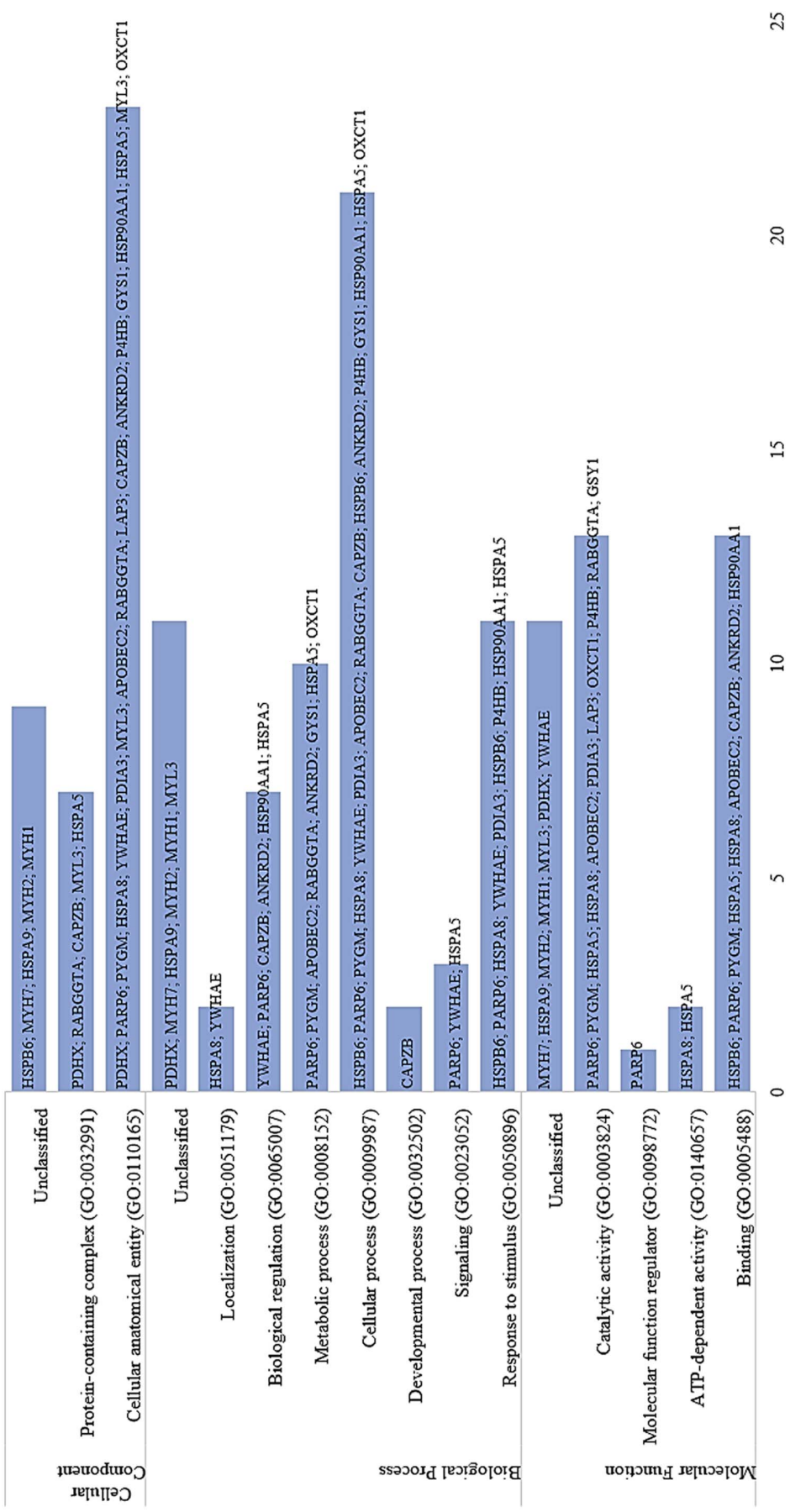

Figure 3. Gene ontology of differentially regulated and unique proteins associated with color and/or tenderness of beef LTL from steers fed graded levels of sorghum analyzed by Panther (http://www.pantherdb.org, accessed on 14 August 2023).

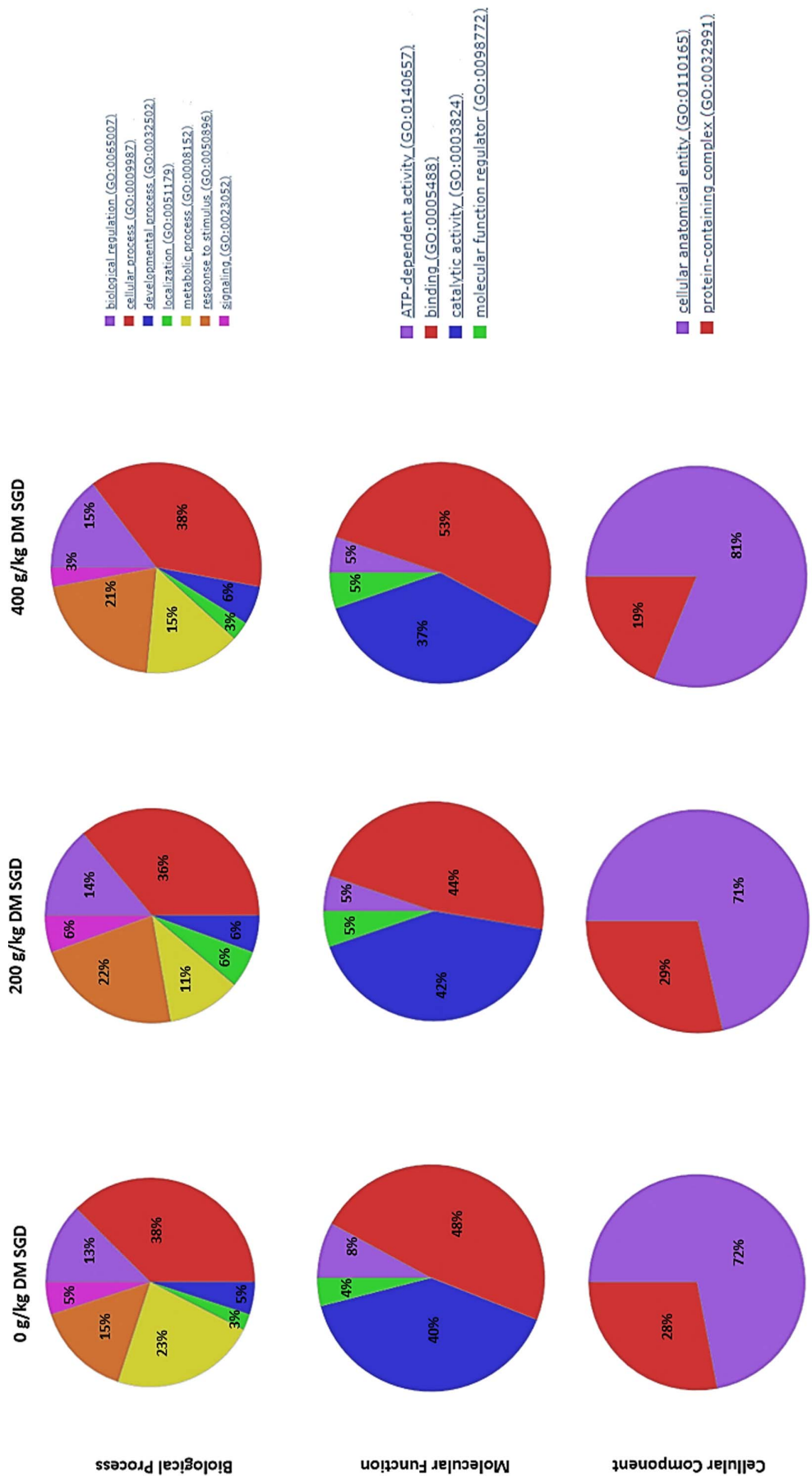

Figure 4. Functional distribution of differentially and uniquely expressed protein associated with color and/or tenderness of beef LTL from steers fed finisher diets containing increasing levels of sorghum; 0 g/kg DM SGD (number of proteins = 17), 200 g/kg DM SGD (number of proteins = 17), 400 g/kg DM SGD (number of proteins = 15) (http://www.pantherdb.org, accessed on 14 August 2023).

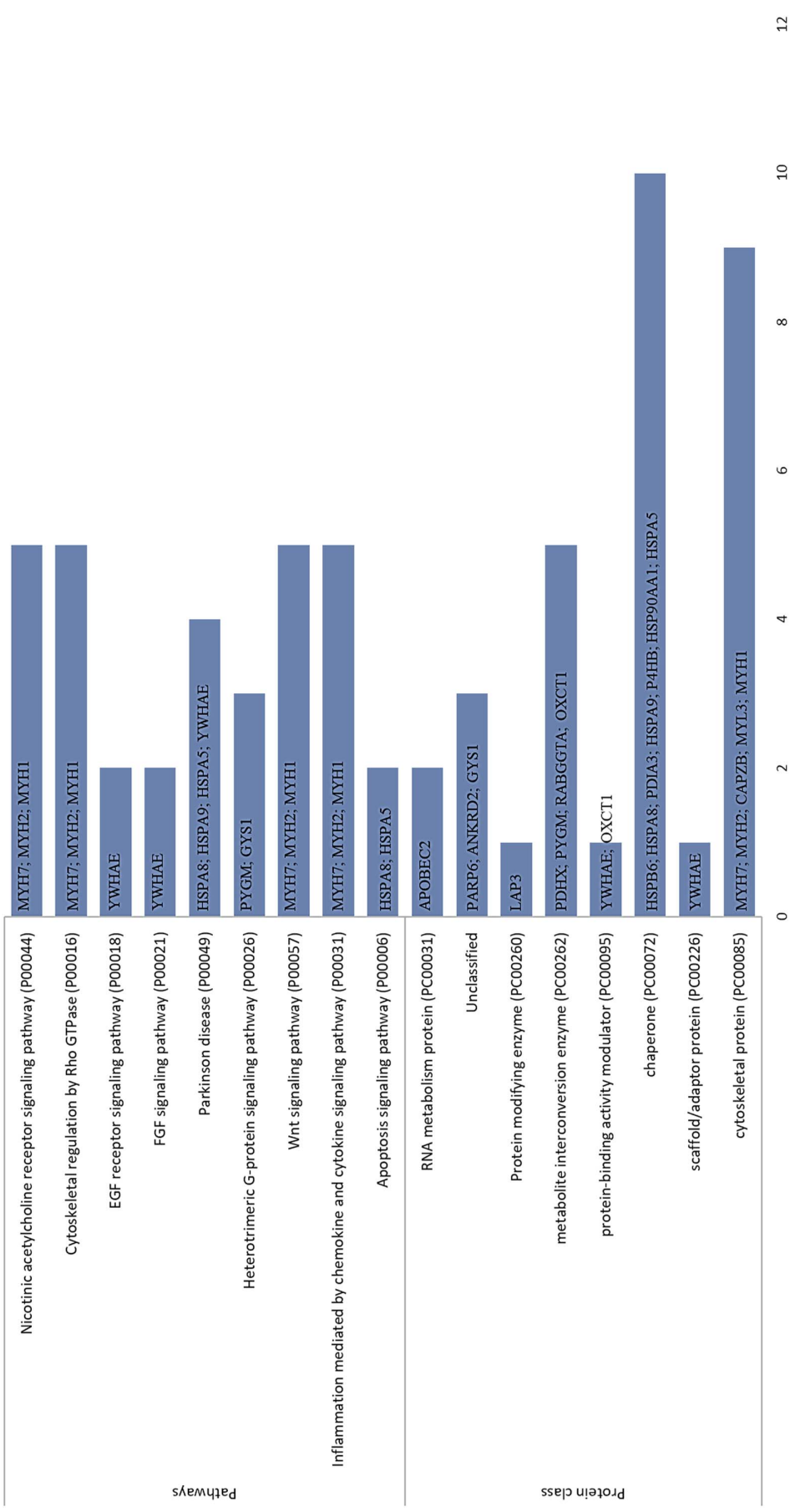

Figure 5. Metabolic pathways and protein classes linked to differentially regulated and unique proteins associated with color and/or tenderness of beef LTL from steers fed finisher diets containing increasing levels of sorghum by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (http://www.pantherdb.org, accessed on 14 August 2023). EGF, Epidermal Growth Factor; FGF, Fibroblast Growth Factor.

Protein-protein interaction networks and KEGG pathways

The PPI revealed that GYS1, PYGM, MYH1 and MYH8 were interconnected within the energy metabolism and muscle activity pathway, while HSPB6, HSPA8, HSP90AA1, and CAPZB were interlinked in the cellular response to stress and modification pathways (Figure 6A). The aforementioned pathways remained distinct when PPI of differentially expressed and diet-unique proteins were analyzed (Figure 6B, 6C, and 6D) with only 2 extra pathways (muscle contraction: MYH2, MYL3, MYH1, and MYH8; uncategorized network: PARP6 and PDHX) in the SGD-200 diet. Three enzymes (PYGM, GYS1, and PARP6) were identified to be linked to the glycolytic pathway, with PYGM and GYS1 being involved in glucose metabolism (Figure 7A) and PARP6 in the pyruvate pathway (Figure 7B). Two enzymes are involved in protein degradation; LAP3 breaks down peptides to proline (Figure 7C) and OXCT1 is linked to the interconversion of acetoacetate to acetoacetyl-Coa in the degradation of valine, leucine, and isoleucine (Figure 7D).

Discussion

The downregulation of structural proteins such as heavy chain myosin (MYH1 and MYH8) in SGD could be ascribed to the over-expression of CAPZB which provides binding sites for μ-calpain that breaks down myosin chains (Picard and Gagaoua, 2017; Bhat et al., 2018; Gagaoua et al., 2021). The over-expressed CAPZB in sorghum diets might be accredited to the slightly lower calcium content in sorghum (9.9 g/100g) vs maize (10.7 g/100g) that reduces the abundance of phosphatidylinositol (4,5) bisphosphate, which inhibits CAPZB capping activity (Maiti and Bamburg, 2013; Jocelyne et al., 2020; Katan and Cockcroft, 2020). Thus, the downregulation of structural proteins (MYH1 and MYH8) in sorghum diets could be indicative of less tender LTL beef. Specifically, MYH1 was described as a good biomarker for tenderness in the more glycolytic muscles such as the LTL from Angus cattle and young bulls (Gagaoua et al., 2020a; Picard and Gagaoua, 2020; Gagaoua et al., 2021). Structural proteins in general have been identified as the key contributors to beef tenderness development through activating proteolysis, weakening and/or loosening the myofibrillar structure and cytoskeletal proteins (Gagaoua et al., 2020a; Gagaoua et al., 2021; Ding et al., 2022). Structural proteins are interlinked through tropomyosin-actin and actomyosin interactions in striated muscle. Thus, denaturing myosin heads or troponin destroys the PPI, breaking the thin filaments in the sarcomeric I band, where proteolysis is initiated (Gagaoua et al., 2020a; Ding et al., 2022). MYL3 contractile protein in SGD-200 could be associated with less tender beef as it indicates less myosin head enzymatic degradation (Franco et al., 2015; Ding et al., 2022).

Glycolytic enzymes have been identified as the second major contributor to beef tenderness and color (Gobert et al., 2014; Gagaoua et al., 2021; Suman et al., 2023). Glycolytic enzymes are involved in the generation of ATP and lactic acid from glycogen thus affecting the rate of protein phosphorylation and pH decline, which directly or indirectly influence meat tenderness and color (Gagaoua et al., 2021; Suman et al., 2023). Generally, the differentially expressed glycolytic enzymes (i.e., PYGM: Glycogen phosphorylase; PARP6: polymerase and Pyruvate kinase; PEBP1: Phosphatidylethanolamine-binding protein 1; and GYS1: Glycogen synthase) are involved in the guanine nucleotide-binding protein (G-protein) pathway catalyzing and regulating metabolic processes. Thus, the lowered expression of GYS1 in both sorghum-based beef diets and PYGM and PARP6 in SGD-400 beef could be attributed to dietary tannins' inhibitory effects on glycolytic protein expression and replenishment of glycogen reserves (Chung et al., 1998; Antonelo et al., 2022; Fang et al., 2022; Xu et al., 2022), which may result in less tender and darker meat. More so, the downregulation of PYGM reduces cell antioxidant capacity by limiting energy, which promotes the conversion of more glycolytic to slow glycolytic skeletal muscles, thereby reducing meat tenderness (Severino et al., 2022; Xu et al., 2022). The GYS1 protein has been positively associated with high pH, dark and less tender pork loin (Zuo et al., 2007; Lei et al., 2015), but little is known regarding its association with beef quality, and this merits investigation. The abundance of PEBP1, a serine protease inhibitor and calpain substrate in SGD-400, suggests a decrease in tenderness, lightness, and redness of beef though the mechanism of action is still unexplored (Mahmood et al., 2018; Gagaoua et al., 2020b; Severino et al., 2022). Of importance, PPI networks did not show any interaction between PEBP1 and other proteins and this could limit its influence on either color or tenderness of beef.

The PARP6 metabolic enzyme, associated with the irreversible conversion of phosphoenolpyruvate to pyruvate, was downregulated in the SGD-400 diet.

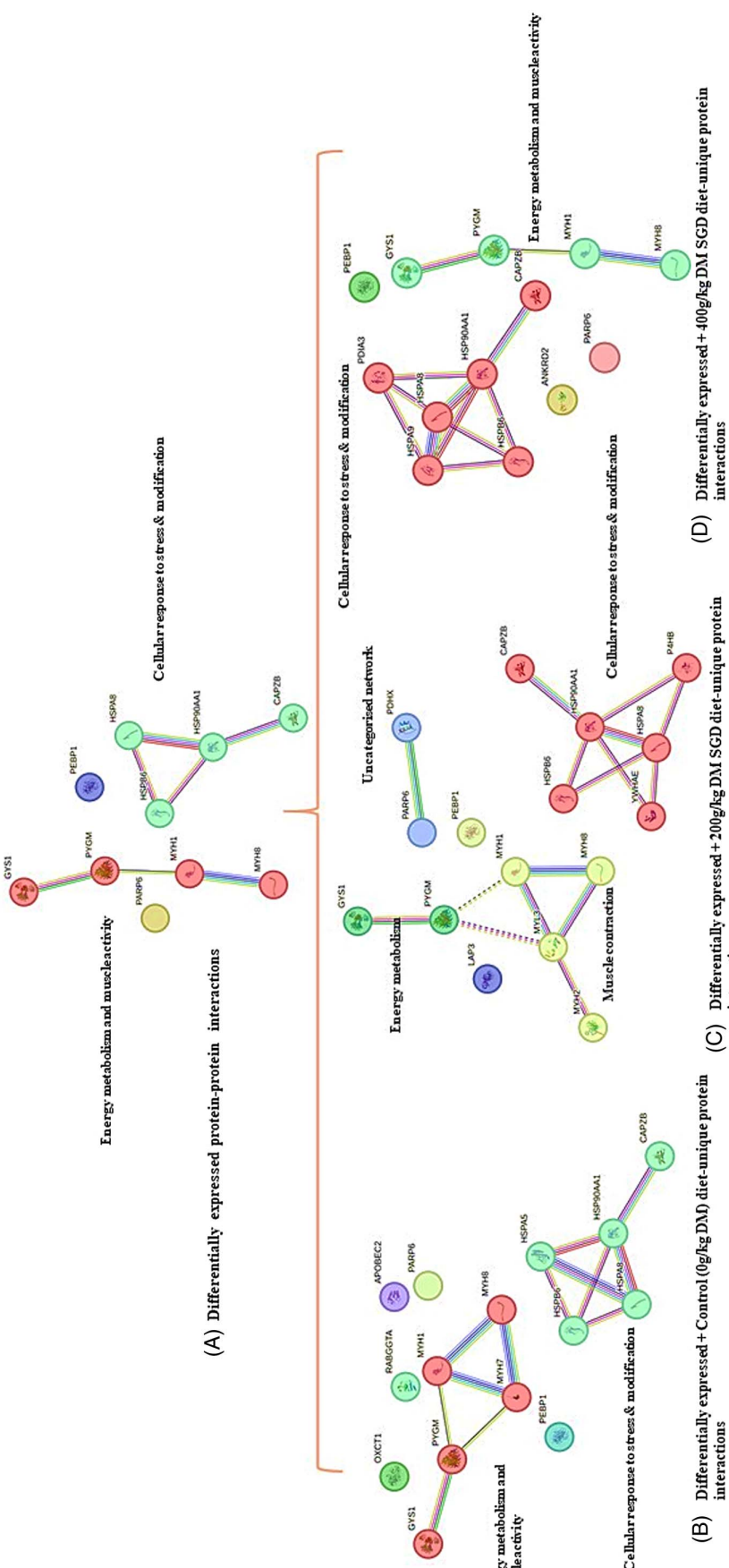

Figure 6. Protein–protein interaction networks of differentially regulated and unique proteins associated with color and/or tenderness of beef LTL from steers fed finisher diets containing increasing levels of sorghum as analyzed by string 12.0. The nodes were proteins from *Bos Taurus* database, and the lines were the predicted interactions with light blue line – curated databases; purple line – experimentally determined; green line – gene neighborhood; red line – gene fusions; dark blue line – gene co-occurrence; light green – textmining; black line – co-expression; light purple line – protein homology (https://string-db.org/ accessed 14 August 2023).

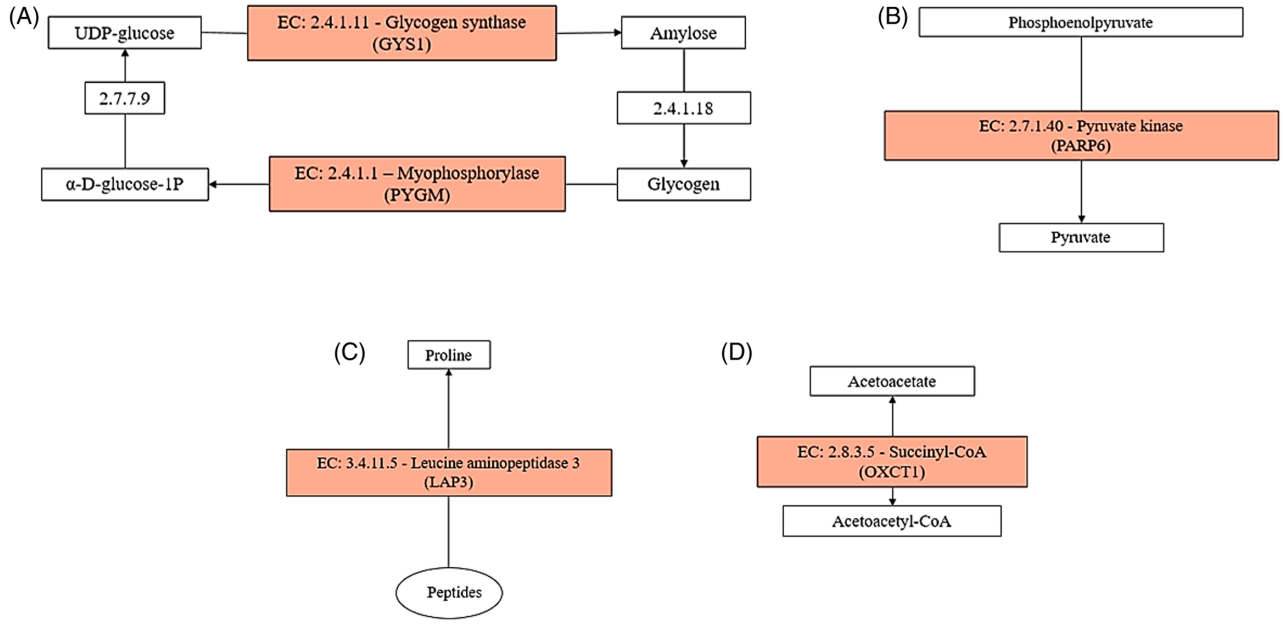

Figure 7. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis linked to differentially regulated and unique proteins associated with color and/or tenderness of beef LTL from steers fed finisher diets containing increasing levels of sorghum showing enzymes linked to **A** glucose metabolism by converting UDP-glucose to amylose and glycogen to α-D-glucose-1P; **B** converting phosphoenolpyruvate to pyruvate; **C** arginine and proline metabolism; and **D** valine, leucine, and isoleucine degradation. Shaded area is the position in the pathway occupied by the enzyme identified in beef LTL fed graded levels of sorghum. EC, enzyme commission number.

The reduction in PARP6 could indicate reduced pyruvate accumulation, which is positively correlated with redness stability in beef (Ramanathan et al., 2012; Yang and Liu, 2021). Pyruvate achieves this by increasing meat acidity thus limiting accumulation of metmyoglobin and stabilizing myoglobin redox forms (Ramanathan et al., 2011; Yang and Liu, 2021). However, accumulation of pyruvate postmortem is limited as it is rapidly converted to lactic acid (Zelentsova et al., 2016). Hence, the association of PARP6 to beef color is doubtable and this could further be explained by its lack of connection with other proteins in the PPI network reported in the current study. Dihydrolipoamide acetyltransferase (PDHX) and proteolytic enzyme (LAP3) unique to SGD-200 diet, which degrades and hydrolyses peptides to proline, is positively associated with beef tenderness and redness (Wu et al., 2015; Wu et al., 2016; Malheiros et al., 2021).

The downregulation of Hsp70 (HSPA8), Hsp90 (HSP90AA1), and small heat shock protein (sHsp: HSPB6) in the sorghum-based beef and the presence of Hsp70 (HSPA9) and co-chaperones (PDIA3, YWHAE, and P4HB) in the SGD-400 could be associated with tender meat. This is due to the ability of HSP to inhibit myofibrillar protein degradation by limiting chaperone activities, thus reducing tenderness (Picard et al., 2014; Oh et al., 2019; Gagaoua et al., 2020a). Numerous studies have reported an increase in meat tenderness when HSP declined (Carvalho et al., 2019; Malheiros et al., 2021; Sentandreu et al., 2021). The downregulation of HSP in sorghum diets could be attributed to the enhanced antioxidative ability provided by tannins, thus reducing oxidative stress, which is a major precursor for HSP (Hu et al., 2022). All the differentially expressed HSP interacted with CAPZB in all diets, a structural protein abundant in the Angus breed, responsible for regulating actin myofilament contractility and thus tenderizing beef (Picard et al., 2014; Gagaoua et al., 2021). HSPA8 and HSPA9 are positively correlated with beef loin color traits such as redness and yellowness (Gagaoua et al., 2020b; Suman et al., 2023). However, HSPA8 and HSPA9 have a negative influence on beef lightness (L^*) as they provide a protective activity on structural proteins including myosin (MYH and MYL) thereby reducing available disintegrated protein aggregates and free myowater (Gagaoua et al., 2020b; Pearce et al., 2011; Purslow et al., 2020). Hence, less light is allowed to scatter and reflect on the muscle.

Based on the present findings, differentially and uniquely expressed (DUE) structural proteins and glycolytic enzymes suggest that the inclusion of sorghum in beef finisher diets could be associated with less tender beef. Interestingly, DUE structural proteins and

glycolytic enzymes results align with instrumental tenderness (i.e., WBSF values) results, confirming their role as key proteomic biomarkers of beef tenderness. The increase in instrumental tenderness with addition of sorghum to the diet could be linked to dietary polyphenols which exhibited the same trend. Polyphenols have been reported to inhibit calpains which are responsible myofibrillar protein degradation (Louis et al., 2014). However, downregulation of the DUE HSP and co-chaperones in the sorghum diets did not correspond with the instrumental tenderness, which could suggest that the expression of the former proteins was not high enough to elicit significant changes in beef tenderness. Of importance, the cytoprotective role of HSP in myofibrillar degradation generally relies on ATP generated by the glycolytic pathway (Grubbs et al., 2014; Picard et al., 2018; Carvalho et al., 2019). This further confirms that structural and glycolytic proteins are the main contributors to beef tenderness and HSP are not a reliable proteomic biomarker for tenderness. With regards to color, DUE structural proteins, glycolytic enzymes, and HSP all indicate that feeding sorghum diets could increase beef redness, but their expression was not strong enough to prompt changes in instrumental redness. Thus, it may not influence beef purchase decisions at the point of purchase.

Conclusions

DUE structural proteins, glycolytic enzymes, and HSP suggest that the inclusion of sorghum beef finisher diets could produce beef of less desirable tenderness. The study findings could be applied to cattle nutritional programs to produce beef of desirable tenderness. Further research is important to determine if the changes in DUE proteomic biomarkers of beef color and tenderness found when feeding sorghum diets would positively influence consumers' purchase and repurchase decisions.

Acknowledgments

Research funds and author Yonela Z. Njisane's postdoctoral fellowship were funded by the Department of Trade, Industry and Competition (DTIC)'s Technology and Human Resources for Industry Programme (THRIP) administered by Chumani Water Solutions. Dr. L. Husselmann and Mr. Xola Bomela are acknowledged for their assistance with proteomics bioinformatics training and procurement of feed resources, respectively.

Conflict of interest statement: The authors have no conflicts of interest to declare.

Literature Cited

American Meat Science Association (AMSA). 2012. Meat color measurement guidelines. 2nd ed. Am. Meat Sci. Assoc. Champaign, IL.

American Meat Science Association (AMSA). 2015. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. 2nd ed. Am. Meat Sci. Assoc. Champaign, IL.

Antonelo, D. S., J. F. M. Gómez, S. L. Silva, M. Beline, X. Zhang, Y. Wang, B. Pavan, L. A. Koulicoff, A. F. Rosa, R. S. Goulart, S. Li, D. E. Gerrard, S. P. Suman, M. Wes Schilling, and J. C. C. Balieiro. 2022. Proteome basis for the biological variations in color and tenderness of longissimus thoracis muscle from beef cattle differing in growth rate and feeding regime. Food Res. Int. 153:110947. https://doi.org/10.1016/j.foodres.2022.110947

Bhat, Z. F., J. D. Morton, S. L. Mason, and A. E. D. A. Bekhit. 2018. Role of calpain system in meat tenderness: A review. Food Science and Human Wellness 7:196–204. https://doi.org/10.1016/j.fshw.2018.08.002

Bonnet, M., J. Soulat, J. Bons, S. Léger, L. De Koning, C. Carapito, and B. Picard. 2020. Quantification of biomarkers for beef meat qualities using a combination of Parallel Reaction Monitoring- and antibody-based proteomics. Food Chem. 317:126376. https://doi.org/10.1016/j.foodchem.2020.126376

Carvalho, E. B., M. P. Gionbelli, R. T. S. Rodrigues, S. F. M. Bonilha, C. J. Newbold, S. E. F. Guimarães, W. Silva, L. L. Verardo, F. F. Silva, E. Detmann, and M. S. Duarte. 2019. Differentially expressed mRNAs, proteins and miRNAs associated to energy metabolism in skeletal muscle of beef cattle identified for low and high residual feed intake. BMC Genomics 20:1–12. https://doi.org/10.1186/s12864-019-5890-z

Chung, K. T., C. I. Wei, and M. G. Johnson. 1998. Are tannins a double-edged sword in biology and health? Trends Food Sci. Tech. 9:168–175. https://doi.org/10.1016/S0924-2244(98)00028-4

Ding, Z., Q. Wei, C. Liu, H. Zhang, and F. Huang. 2022. The quality changes and proteomic analysis of cattle muscle postmortem during rigor mortis. Foods 11:217. https://doi.org/10.3390/foods11020217

Fang, J., L. Zeng, Y. He, X. Liu, T. Zhang, and Q. Wang. 2022. Effects of dietary tannic acid on obesity and gut microbiota in C57BL/6J mice fed with high-fat diet. Foods 11:3325. https://doi.org/10.3390/foods11213325

Franco, D., A. Mato, F. J. Salgado, M. López-Pedrouso, M. Carrera, S. Bravo, M. Parrado, J. M. Gallardo, and C. Zapata. 2015. Tackling proteome changes in the longissimus thoracis bovine muscle in response to pre-slaughter stress. J. Proteomics 122:73–85. https://doi.org/10.1016/j.jprot.2015.03.029

Freer, M., H. Dove, and J. V. Nolan. 2019. Nutrient Requirements of Domesticated Ruminants, Nutrient Requirements of Domesticated Ruminants. CSIRO publishing, Collingwood, Australia. https://doi.org/10.1071/9780643095106

Gagaoua, M., M. Bonnet, and B. Picard. 2020a. Protein array-based approach to evaluate biomarkers of beef tenderness and marbling in cows: Understanding of the underlying mechanisms and prediction. Foods 9:1180. https://doi.org/10.3390/foods9091180

Gagaoua, M., J. Hughes, E. M. C. Terlouw, R. D. Warner, P. P. Purslow, J. M. Lorenzo, and B. Picard. 2020b. Proteomic biomarkers of beef color. Trends Food Sci. Tech. 101:234–252. https://doi.org/10.1016/j.tifs.2020.05.005

Gagaoua, M., E. M. C. Terlouw, A. M. Mullen, D. Franco, R. D. Warner, J. M. Lorenzo, P. P. Purslow, D. Gerrard, D. L. Hopkins, D. Troy, and B. Picard. 2021. Molecular signatures of beef tenderness: Underlying mechanisms based on integromics of protein biomarkers from multi-platform proteomics studies. Meat Sci. 172:108311. https://doi.org/10.1016/j.meatsci.2020.108311

Gobert, M., T. Sayd, P. Gatellier, and V. Santé-Lhoutellier. 2014. Application to proteomics to understand and modify meat quality. Meat Sci. 98:539–543. https://doi.org/10.1016/j.meatsci.2014.06.035

Grubbs, J. K., E. Huff-Lonergan, N. K. Gabler, J. C. M. Dekkers, and S. M. Lonergan. 2014. Liver and skeletal muscle mitochondria proteomes are altered in pigs divergently selected for residual feed intake. J. Anim. Sci. 92:1995–2007. https://doi.org/10.2527/jas.2013-7391

Han, J. H., E. J. Lee, W. Park, K. T. Ha, and H. S.Chung. 2023. Natural compounds as lactate dehydrogenase inhibitors: Potential therapeutics for lactate dehydrogenase inhibitors-related diseases. Front. Pharmacol. 14:1275000. https://doi:10.3389/fphar.2023.1275000

Hooijberg, E. H., M. Miller, C., Cray, P. Buss, G. Steenkamp, and A. Goddard. 2018. Serum protein electrophoresis in healthy and injured southern white rhinoceros (Ceratotherium simum simum). PLoS One 13:1–17. https://doi.org/10.1371/journal.pone.0200347

Hu, C., J. Yang, Z. Qi, H. Wu, B. Wang, F. Zou, H. Mei, J. Liu, W. Wang, and Q Liu. 2022. Heat shock proteins: Biological functions, pathological roles, and therapeutic opportunities. MedComm 3:1–39. https://doi.org/10.1002/mco2.161

Jocelyne, R. E., K. Béhiblo, and A. K. Ernest. 2020. Comparative study of nutritional value of wheat, maize, sorghum, millet, and fonio: Some cereals commonly consumed in Côte d'Ivoire. European Scientific Journal 16:118–131. https://doi.org/10.19044/esj.2020.v16n21p118

Kamiyama, O., F. Sanae, K. Ikeda, Y. Higashi, Y. Minami, N. Asano, I. Adachi, and A. Kato. 2010. In vitro inhibition of α-glucosidases and glycogen phosphorylase by catechin gallates in green tea. Food Chem. 122:1061–1066. https://doi:10.1016/j.foodchem.2010.03.075

Katan, M., and S. Cockcroft. 2020. Phosphatidylinositol (4,5) bisphosphate: Diverse functions at the plasma membrane. Essays Biochem. 0:13–531. https://doi.org/10.1042/EBC20200041

Kruger, N. J. 2009. The Bradford method for protein quantitation. In: J. M. Walker, editor, The protein protocols handbook. Springer Protocols Handbooks series. Humana Press, Totowa, NJ. pp. 17–24. https://doi.org/10.1007/978-1-59745-198-7_4

Lei, H. G., L. Y. Shen, S. H. Zhang, Z. H. Wu, J. Shen, G. Q. Tang, Y. Z. Jiang, M. Z. Li, L. Bai, X. W. Li, and L. Zhu. 2015. Comparison of the meat quality, post-mortem muscle energy metabolism, and the expression of glycogen synthesis-related genes in three pig crossbreeds. Anim. Prod. Sci. 55:501–507. https://doi.org/10.1071/AN13484

Louis, X. L, S. J. Thandapilly, W. Kalt, M. Vinqvist-Tymchuk, B. M. Aloud, P. Raj, L. Yu, H. Le, and T. Netticadan. 2014. Blueberry polyphenols prevent cardiomyocyte death by preventing calpain activation and oxidative stress. Food Funct. 5:1785–94. https://doi:10.1039/c3fo60588d

Mahlare, M. J. S., L. Husselmann, M. N. Lewu, C. Bester, F. B. Lewu, and O. J. Caleb. 2023. Analysis of the differentially expressed proteins and metabolic pathways of honeybush (Cyclopia subternata) in response to water deficit stress. Plants 12:2181. https://doi.org/10.3390/plants12112181

Mahmood, S., N. Turchinsky, F. Paradis, W. T. Dixon, and H. L. Bruce. 2018. Proteomics of dark cutting longissimus thoracis muscle from heifer and steer carcasses. Meat Sci. 137:47–57. https://doi.org/10.1016/j.meatsci.2017.11.014

Maiti, S., and J. R. Bamburg. 2013. Actin-capping and -severing proteins. In: W. J. Lennarz and M. D. Lane, editors, Encyclopedia of biological chemistry. Academic Press. pp. 18–26. https://doi.org/10.1016/B978-0-12-378630-2.00415-1

Malheiros, J. M., C. E. Enríquez-Valencia, C. P. Braga, J. C. S. Vieira, D. S. Vieira, G. L. Pereira, R. A. Curi, O. R. M. Neto, H. N. Oliveira, P. M. Padilha, and L. A. L. Chardulo. 2021. Application of proteomic to investigate the different degrees of meat tenderness in Nellore breed. J. Proteomics 248:104331. https://doi.org/10.1016/j.jprot.2021.104331

Mccuistion, K. C., P. H. Selle, S. Y. Liu, and R. D. Goodband. 2019. Sorghum as a feed grain for animal production. In: J. R. N. Taylor and K. G. Duodu, editors, Sorghum and millets. AACC International Press. pp. 355–391. https://doi.org/10.1016/B978-0-12-811527-5.00012-5

Nair, M. N., S. Li, C. M. Beach, G. Rentfrow, and S. P. Suman. 2018. Changes in the sarcoplasmic proteome of beef muscles with differential color stability during postmortem aging. Meat Muscle Biol. 2. https://doi.org/10.22175/mmb2017.07.0037

National Research Council. 2016. Nutrient requirements of beef cattle. In: The National Academies Press. 8th Revised Edition. https://doi.org/10.17226/19014

Oh, E., B. Lee, and Y. M. Choi. 2019. Associations of heat-shock protein expression with meat quality and sensory quality characteristics in highly marbled longissimus thoracis muscle from Hanwoo steers categorized by Warner–Bratzler shear force value. Foods 8:638. https://doi.org/10.3390/foods8120638

Orzuna-Orzuna, J. F., G. Dorantes-Iturbide, A. Lara-Bueno, G. D. Mendoza-Martínez, L. A. Miranda-Romero, and H. A. Lee-Rangel. 2021. Growth performance, meat quality and antioxidant status of sheep supplemented with tannins: A meta-analysis. Animals 11:1–26. https://doi.org/10.3390/ani11113184

Osman, A., A. A. El-wahab, M. Fawzy, E. Ahmed, M. Buschmann, C. Visscher, C. B. Hartung, and J. B. Lingens. 2022. Nutrient composition and in vitro fermentation characteristics of sorghum depending on variety and year of cultivation in northern Italy. Foods 11:1–14. https://doi.org/10.3390/foods11203255

Pearce, K. L., K. Rosenvold, H. J. Andersen, and D. L. Hopkins. 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes - A review. Meat Sci. 89:111–124. https://doi.org/10.1016/j.meatsci.2011.04.007

Picard, B., and M. Gagaoua. 2017. Proteomic investigations of beef tenderness. In: M. L. Colgrave, editor, Proteomics in food science: From farm to fork. Academic Press. pp. 177–197. http://dx.doi.org/10.1016/B978-0-12-804007-2.00011-4

Picard, B., and M. Gagaoua. 2020. Meta-proteomics for the discovery of protein biomarkers of beef tenderness: An overview of integrated studies. Food Res. Int. 127:108739. https://doi.org/10.1016/j.foodres.2019.108739

Picard, B., M. Gagaoua, M. Al-Jammas, L. De Koning, A. Valais, and M. Bonnet. 2018. Beef tenderness and intramuscular fat proteomic biomarkers: Muscle type effect. PeerJ 6:e4891. https://doi.org/10.7717/peerj.4891

Picard, B., M. Gagaoua, D. Micol, I. Cassar-Malek, and C. E. M. Terlouw. 2014. Inverse relationships between biomarkers and beef tenderness according to contractile and metabolic properties of the muscle. J. Agr. Food Chem. 62:9808–9818. https://doi.org/10.1021/jf501528s

Purslow, P. P., M. Gagaoua, and R. D. Warner. 2021. Insights on meat quality from combining traditional studies and proteomics. Meat Sci. 174:108423. https://doi.org/10.1016/j.meatsci.2020.108423

Purslow, P. P., R. D. Warner, F. M. Clarke, and J. M. Hughes. 2020. Variations in meat colour due to factors other than myoglobin chemistry; a synthesis of recent findings (invited review). Meat Sci. 159:107941. https://doi.org/10.1016/j.meatsci.2019.107941

Ramanathan, R., R. A. Mancini, and G. A. Dady. 2011. Effects of pyruvate, succinate, and lactate enhancement on beef longissimus raw color. Meat Sci. 88:424–428. https://doi.org/10.1016/j.meatsci.2011.01.021

Ramanathan, R., R. A. Mancini, C. B. Van Buiten, S. P. Suman, and C. M. Beach. 2012. Effects of pyruvate on lipid oxidation and ground beef color. J. Food Sci. 77:886–892. https://doi.org/10.1111/j.1750-3841.2012.02814.x

Sentandreu, E., C. Fuente-García, O. Pardo, M. Oliván, N. León, N. Aldai, V. Yusà, and M. A. Sentandreu. 2021. Protein biomarkers of bovine defective meats at a glance: Gel-free hybrid quadrupole-orbitrap analysis for rapid screening. J. Agr. Food Chem. 69:7478–7487. https://doi.org/10.1021/acs.jafc.1c02016

Severino, M., M. Gagaoua, W. Baldassini, R. Ribeiro, J. Torrecilhas, G. Pereira, R. Curi, L. A. Chardulo, P. Padilha, and O. M. Neto. 2022. Proteomics unveils post-mortem changes in beef muscle proteins and provides insight into variations in meat quality traits of crossbred young steers and heifers raised in feedlot. Int. J. Mol. Sci. 23:2259. https://doi.org/10.3390/ijms232012259

South African Government Gazzette. 2000. Meat safety act. Parliament of the republic of South Africa, Pretoria, South Africa.

Shapiro, S. S., and M. B. Wilk. 1965. An Analysis of Variance Test for Normality (Complete Samples). Biometrika 52, 591–611.

Suman, S. P., and P. Joseph. 2013. Myoglobin chemistry and meat color. Annu. Rev. Food Sci. T. 4:79–99. https://doi.org/10.1146/annurev-food-030212-182623

Suman, S. P., Y. Wang, M. Gagaoua, F. Kiyimba, and R. Ramanathan. 2023. Proteomic approaches to characterize biochemistry of fresh beef color. J. Proteomics 281:104893. https://doi.org/10.1016/j.jprot.2023.104893

Sun, H. X., T. S. Gao, R. Z. Zhong, Y. Fang, G. L. Di, and D. W. Zhou. 2018. Effects of corn replacement by sorghum in diets on performance, nutrient utilization, blood parameters, antioxidant status, and meat colour stability in lambs. Can. J. Anim. Sci. 98:723–731. https://doi.org/10.1139/cjas-2017-0136

Wu, W., X. G. Gao, Y. Dai, Y. Fu, X. M. Li, and R. T. Dai. 2015. Post-mortem changes in sarcoplasmic proteome and its relationship to meat color traits in M. semitendinosus of Chinese Luxi yellow cattle. Food Res. Int. 72:98–105. https://doi.org/10.1016/j.foodres.2015.03.030

Wu, W., Q. Q. Yu, Y. Fu, X. J. Tian, F. Jia, X. M. Liand R. T. Dai. 2016. Towards muscle-specific meat color stability of Chinese Luxi yellow cattle: A proteomic insight into post-mortem storage. Journal of Proteomics, 147:108–118. https://doi.org/10.1016/j.jprot.2015.10.027

Xu, M., X. Chen, Z. Huang, D. Chen, M. Li, J. He, H. Chen, P. Zheng, J. Yu, Y. Luo, and B. Yu. 2022. Effects of dietary grape seed proanthocyanidin extract supplementation on meat quality, muscle fiber characteristics and antioxidant capacity of finishing pigs. Food Chem. 367:130781. https://doi.org/10.1016/j.foodchem.2021.130781

Yang, B., and X. Liu. 2021. Application of proteomics to understand the molecular mechanisms determining meat quality of beef muscles during postmortem aging. PLoS One 16: 1–13. https://doi.org/10.1371/journal.pone.0246955

Zamaratskaia, G., and S. Li. 2017. Proteomics in meat science—Current status and future perspective. Theory Practice of Meat Processing 2:18–26. https://doi.org/10.21323/2414-438x-2017-2-1-18-26

Zelentsova, E. A., L. V. Yanshole, O. A. Snytnikova, V. V. Yanshole, Y. P. Tsentalovich, and R. Z. Sagdeev. 2016. Post-mortem changes in the metabolomic compositions of rabbit blood, aqueous and vitreous humors. Metabolomics 12:1–11. https://doi.org/10.1007/s11306-016-1118-2

Zhong, R. Z., Y. Fang, Y. Q. Wang, H. X. Sun, and D. W. Zhou. 2016. Effects of substituting finely ground sorghum for finely ground corn on feed digestion and meat quality in lambs infected with *Haemonchus contortus*. Anim. Feed Sci. Tech. 211:31–40. https://doi.org/10.1016/j.anifeedsci.2015.08.007

Zuo, B., H. Yang, M. G. Lei, F. E. Li, C. Y. Deng, S. W. Jiang, and Y. Z. Xiong. 2007. Association of the polymorphism in GYS1 and ACOX1 genes with meat quality traits in pigs. Animal 1:1243–1248. https://doi.org/10.1017/S1751731107000523