Meat and Muscle Biology™

Metabolites and Metabolic Pathways Correlated With Beef Tenderness

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Abstract: Metabolite profile has been used to understand the causes of variability in beef tenderness, but still little is known about how metabolites contribute to beef quality. Therefore, this study was carried out to evaluate how meat metabolites and their metabolic pathways correlate to variability in beef tenderness. Carcasses from 60 noncastrated male cattle were selected, and three 2.5-cm-thick *longissimus thoracis* steaks were obtained and aged (0°C to 4°C) for 7 d. Warner-Bratzler shear force (WBSF) was performed (steak 1). Based on WBSF data, 2 tenderness classes ($n = 30$; 15 per class [tender and tough]) were created to perform sarcomere length (steak 2) and metabolomic analysis (steak 3). Meat ultimate pH did not differ between tenderness classes. However, steaks classified as tender had greater sarcomere length ($P = 0.019$) than those classified as tough. Acetyl-carnitine ($P = 0.026$), adenine ($P = 0.026$), beta-alanine ($P = 0.005$), fumarate ($P = 0.022$), glutamine ($P = 0.043$), and valine ($P = 0.030$) concentration were higher in tender beef compared with tough beef. The 4 most important compounds differing between tender and tough beef were lactate, glucose, creatine, and glutamine, which may indicate that metabolic pathways such as D-glutamine and D-glutamate metabolism, beta-alanine metabolism, purine metabolism, and tricarboxylic acid cycle affected the tenderness classes. Beta-alanine ($r = -0.45$), acetyl-carnitine ($r = -0.40$), fumarate ($r = -0.38$), valine ($r = -0.34$), glucose ($r = -0.32$), glutamine ($r = -0.31$), and adenine ($r = -0.31$) were negatively correlated with WBSF values. Metabolite profile in tender beef indicated a greater oxidative metabolism, which promoted modifications in the muscle structure and proteolysis, favoring its tenderization.

Key words: meat quality, metabolomics, oxidative metabolism, oxidative stress, shear force
Meat and Muscle Biology 4(1): 19, 1–9 (2020) **doi:10.22175/mmb.10854**
Submitted 26 March 2020 Accepted 21 May 2020

Introduction

Tenderness is one of the most important traits related to meat quality. While consumers are able to discriminate among tenderness variations and are willing to pay a premium for tender beef (Ouali et al., 2013), there is a significant inconsistency in this trait. According to Hendrix (2016), approximately 25% of commercial beef does not meet consumer expectations regarding tenderness. Beef tenderness is a complex trait and may be affected by several intrinsic and

extrinsic factors and their interaction (Lomiwes et al., 2014; Gagaoua et al., 2015). Several studies have shown that while postmortem factors—including meat pH, sarcomere length, and endogenous proteolytic systems—affect meat tenderness (Huff Lonergan et al., 2010; D'Alessandro et al., 2012; Ouali et al., 2013; Ramos et al., 2020b), they do not fully explain variability in meat tenderness (King et al., 2019), and in some cases, they are not sufficient to provide insight into observed treatment differences in tenderness (Shackelford et al., 2012). Thus, genomic (Rubio Lozano et al., 2016; Carvalho et al., 2017), proteomic (Gagaoua et al., 2015; Bonnet et al., 2020; Picard and Gagaoua, 2017, 2020), and metabolomic (Kodani et al., 2017; King et al., 2019; Muroya et al., 2019) approaches have been studied to better understand the causes of variability in tenderness and the biochemical mechanisms underlying tenderization.

One-dimensional proton nuclear magnetic resonance (1D ^{1}H NMR) spectroscopy has been widely used to characterize and quantify low–molecular-weight metabolites in small samples (Emwas et al., 2019). 1D ^{1}H NMR has been successfully used to obtain meat metabolic profiles that have been related to genetic selection (Cônsolo et al., 2020), breeds (Straadt et al., 2014; Antonelo et al., 2020a), diets (Zawadzki et al., 2017; Antonelo et al., 2020a), and aging (Graham et al., 2010; Kodani et al., 2017).

Previous studies reported that some metabolites are correlated with beef tenderness (Kodani et al., 2017; King et al., 2019; Muroya et al., 2019), but little is known about how metabolites are related to beef quality. Biological pathways related to meat tenderness usually include proteolysis, muscular structure and contraction, oxidative stress, heat shock proteins, and apoptosis (Bonnet et al., 2020; Picard and Gagaoua, 2020). In this sense, understanding the relationship between metabolites, metabolic pathways, and beef tenderness may help facilitate the creation of management schemes to mitigate limitations in this beef quality trait. Thus, the aims of this work were to evaluate which and how meat metabolites are correlated to variability in beef tenderness.

Materials and Methods

All procedures used in this study were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines (protocol 7294130616) and were approved by the committees of the College of Animal Science and Food Engineering of the University of Sao Paulo.

Meat sampling and handling

Sixty 24-month-old noncastrated male cattle, 30 each Nellore ("NEL") and crossbred Angus × Nellore ("AxN"), with average initial body weight of 363 ± 28 kg, were fed 2 different diets: (1) "CON," a standard feedlot diet containing no soybean oil, and (2) "SBO," a control diet containing 3.5% soybean oil replacing corn grain. After 133 d on feed, animals were transported to a federal inspected commercial slaughterhouse (Frigorifico Dom Glutao, Ibitinga, SP, Brazil) located 184 km from the experimental site. Upon arrival, animals were in lairage for 10 h with free access to water and then were slaughtered according to humane slaughter procedures as required by Brazilian law. Briefly, animals were restrained in a stunning box and stunned using a penetrative captive bolt, followed by bleeding through the jugular vein and carotid artery.

After 48 h of chilling (0°C to 2°C), muscle pH was measured in the *longissimus thoracis* (LT) between the 12th and 13th ribs using a digital pH meter (Hanna Instruments model HI99163, São Paulo, Brazil). Afterward, the left side of each carcass was ribbed between the 12th and 13th ribs, and a 7.5-cm section from the LT muscle was sampled, vacuum packed, and aged (0°C to 4°C) for 7 d (9 d post harvest). After aging, three 2.5-cm-thick LT samples of each carcass were obtained for Warner-Bratzler shear force (WBSF), sarcomere length, and metabolomic analysis.

Steaks sampled for WBSF were roasted in an oven at 170°C (Model F130/L, Electric Furnaces Golden Arrow Industry and Commerce Ltda., Sao Paulo, Brazil). The internal temperature of each steak was monitored using individual thermometers until an internal temperature of 40°C was attained, at which point steaks were flipped and cooked to an internal temperature of 71°C, as recommended by the American Meat Science Association (AMSA, 2015). Steaks were cooled further to 4°C for 12 h, and 6 core (1.27-cm diameter) samples were taken parallel to muscle with muscle fiber orientation using a Drill Brench (Model FG-13B, Caracol Trading of Machinery and Tools LTDA, Sao Paulo, Brazil). Shear force was determined on cores using the TMS-PRO texture analyzer (Food Technology Corporation, Sterling, VA) coupled with a Warner-Bratzler shear device set at a speed of 200 mm/min (AMSA, 2015). Shear force values were determined by averaging 6 replicates per sample.

There was no difference in WBSF (Antonelo et al., 2020b) between diets (40.7 vs. 44.6 N for CON and SBO, respectively) and breeds (41.8 vs. 43.5 N for NEL and AxN, respectively), even though some meat

metabolites were different between them (Antonelo et al., 2020a). In this sense, the WBSF values from all samples were utilized to identify the carcasses with the lowest (most tender) and highest (toughest) WBSF to create 2 tenderness classes ($n = 15$ per class [one-quarter of the total carcasses to accurately distinguish tender and tough meat]; tender [minimum = 26.0 N; maximum = 34.0 N; SD = 2.39] and tough [minimum = 48.5 N; maximum = 66.0 N; SD = 5.40]). Breed and diet treatments were evenly distributed across classes, such that 4 NEL-SBO, 4 NEL-CON, 4 AxN-SBO, and 3 AxN-CON were sourced for the tough class and 3 NEL-SBO, 4 NEL-CON, 4 AxN-SBO, and 4 AxN-CON were sourced for the tender class.

Sarcomere length

Six cores (1.27-cm diameter) of each fresh sample were taken, placed into a plastic container containing 0.2 M sucrose solution, and fixed overnight at 4°C to determine sarcomere length by laser diffraction using the Helium Neon Laser 05-LHR-021 model (Melles Griot, Carlsbad, CA), according to the methodology proposed by Cross et al. (1981). Six measurements were obtained from each core, totaling 36 sarcomere length measurements from each sample. Sarcomere length values were determined by averaging 36 measurements per sample.

Extraction of polar metabolites from meat

A total of 0.5 g of each meat sample ($n = 15$ per tenderness class) was macerated and homogenized using an Ultra-Turrax (MA102/B model, Marconi Laboratory Equipment, Piracicaba, Brazil), and metabolites were extracted with 3.5 mL of a cold methanol/water solution (4:3 v/v) while vortexing for 1 min, as previously described by Beckonert et al. (2007). Samples were stored on ice for 15 min and then centrifuged for 15 min at 10,000*g* at 4°C to remove protein precipitate and connective tissue. Supernatants were transferred to 1.5 mL centrifuge tubes and freeze dried. The remaining residue was reconstituted in 600 µL of 100 mM phosphate buffer (containing 10% D_2O and 90% H_2O [pH 7.0]) and 60 µL internal standard solution (containing 5 mM 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt [DSS] as a quantitation standard and chemical shift reference and 100 mM imidazole as a pH indicator). Samples were centrifuged at 10,000*g* for 3 min at 4°C to remove any precipitate. The supernatant (600 µL) was transferred to standard 5×178-mm thin-walled nuclear magnetic resonance (NMR) tubes (VWR International, Radnor, PA).

NMR spectroscopy

1D ^{1}H NMR spectroscopy was used for metabolite profiling, and the analyses were performed at EMBRAPA Instrumentation (São Carlos, São Paulo, Brazil). The ^{1}H NMR spectra were acquired at 300 K on a Bruker Avance 14.1 T spectrometer (Bruker Corporation, Karlsruhe, Baden-Württemberg, Germany) at 600.13 MHz for ^{1}H, using a 5-mm Broadband Observe probe. Deuterium oxide was used as a lock solvent, and DSS was used as the chemical shift reference for ^{1}H. Standard 1D ^{1}H NMR spectra were acquired using a single 90° pulse experiment. Water suppression was performed using the BRUKER "zgesgp" pulse sequence (excitation sculpting with gradients), and the following acquisition parameters were used: 13.05 µs for the 90° pulse, 5-s relaxation delay, 64-K data points, 64 scans, 3.89-s acquisition time, and spectral width of 14.03 parts per million [ppm].

Spectral processing and metabolite quantitation

The 1D ^{1}H NMR spectra were processed using Chenomx NMR Suite Professional 7.7 software (Chenomx Inc., Edmonton, Canada). Phasing and baseline correction were performed, and the pH was calibrated using the resonances from imidazole. The spectra were referenced to the DSS methyl peak at 0.00 ppm, which was used as an internal standard for quantitation.

Thirty-one metabolites were quantified in the 1D ^{1}H NMR spectra of meat extracts using the Profiler module on the Chenomx NMR Suite Professional software with an in-built one-dimensional spectral library. Quantitation was based on comparing the area of selected metabolite peaks with the area under the DSS methyl peak, which corresponds to a known concentration of 0.5 mM in each sample. The resulting metabolite concentration table (31 metabolites × 15 samples per each tenderness class) was exported to Microsoft Excel, where sample identifiers were added.

Statistical analysis

Univariate and multivariate data analysis were performed. Data were evaluated as a completely randomized design using 15 replications (extremes of WBSF) per tenderness class (animal was the experimental unit). Tenderness classes were considered as fixed effects, and breed and diet of animals were considered as random effect. All the data were analyzed using the Mixed procedure of SAS version 9.4 software (SAS Institute Inc., Cary, NC). The least-squares means

("LSMEANS") statement was used to calculate the adjusted means for treatment, and the means were compared by Student *t* test. Differences were considered statistically significant when $P \leq 0.05$.

Metabolomic data were also processed statistically using MetaboAnalyst version 4.0 (Chong et al., 2018). The metabolite concentration table was uploaded to MetaboAnalyst, and the data were log-transformed and Pareto-scaled prior to analysis. Unsupervised (principal component analysis [PCA]) and supervised (partial least square discriminant analysis [PLS-DA]) were performed. PLS-DA validation was performed using a 10-fold cross validation method, and the values for R^2 (cumulative interpretation ability of model; 0.96) and Q^2 (predictive ability of model; 0.90) were employed as initial indicators for evaluating the goodness of fit. In the PLS-DA model, a variable importance in projection (VIP) plot was used to rank the metabolites based on their relative importance of variation in discriminating groups. Correlation analysis was performed between WBSF and metabolites using the PatternHunter method on MetaboAnalyst, in which Pearson's correlation was applied as a distance measure. In addition, pathway analysis was performed to identify the most relevant pathways associated with the identified metabolites using a web-based analysis module (http://metaboanalyst.ca) that is based on the Kyoto Encyclopedia of Genes and Genomes database. Through pathway analysis, 9 metabolic pathways related to the identified metabolites ($n = 31$) were mapped based on *P* values ($P < 0.10$) and pathway impact (PI; >0).

Results

Meat pH did not differ between the tender and tough steak classifications. However, steaks classified as tender had greater sarcomere length ($P = 0.019$) than those classified as tough (Table 1). Based on 1D ^{1}H NMR analyses, 31 compounds were identified in meat.

Table 1. Ultimate pH, WBSF, and sarcomere length of *longissimus thoracis* steaks classified as tender or tough after 7 d of aging

	Tenderness class			
Trait	Tender ($n = 15$)	Tough ($n = 15$)	SEM	*P* value
Ultimate pH	5.65	5.70	0.061	0.538
WBSF (N)	28.7	54.7	1.078	<0.001
Sarcomere length (μm)	1.79	1.73	0.017	0.019

WBSF = Warner-Bratzler shear force.

Compounds were segregated according to tenderness class using PCA score plot (Figure 1). Based on VIP scores, compounds that contributed a higher percentage of the explained residuals in the PLS-DA plot between tenderness classes (in descending order) were lactate, glucose, creatine, glutamine, acetyl-carnitine, adenine, and anserine (Figure 2).

Concentration of acetyl-carnitine ($P = 0.026$), adenine ($P = 0.026$), beta-alanine ($P = 0.005$), fumarate ($P = 0.022$), glutamine ($P = 0.043$), and valine ($P = 0.030$) were higher in tender meat compared with tough meat (Table 2). Moreover, D-glutamine and D-glutamate metabolism ($P = 0.056$; PI = 1.00); beta-alanine metabolism ($P = 0.050$; PI = 0.46); starch and sucrose metabolism ($P = 0.06$; PI = 0.42); glycerolipid metabolism ($P = 0.085$; PI = 0.03); alanine, aspartate, and glutamate metabolism ($P = 0.052$; PI = 0.42); purine metabolism ($P = 0.067$; PI = 0.14); arginine biosynthesis ($P = 0.032$; PI = 0.12); tricarboxylic acid (TCA) cycle ($P = 0.045$; PI = 0.06); tyrosine metabolism; and pantothenate and coenzyme A biosynthesis ($P = 0.045$; PI = 0.02) were the most relevant pathways associated with the identified metabolites (Figure 3).

In general, most metabolites had low correlations ($-0.3 < r < 0.3$) with WBSF values (Figure 4). However, beta-alanine ($r = -0.45$), acetyl-carnitine ($r = -0.40$), fumarate ($r = -0.38$), valine ($r = -0.34$), glucose ($r = -0.32$), glutamine ($r = -0.31$), and adenine

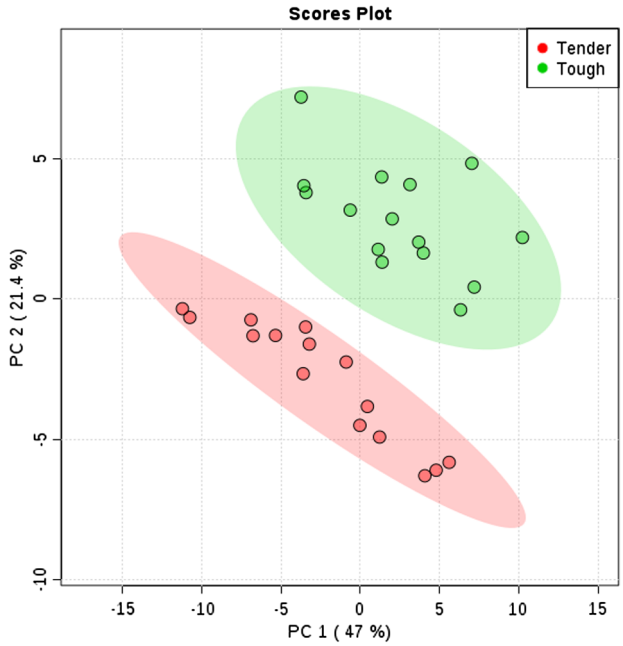

Figure 1. Principal component analysis (PCA) scores plot of metabolome distribution of *longissimus thoracis* steaks classified as tender or tough after 7 d of aging.

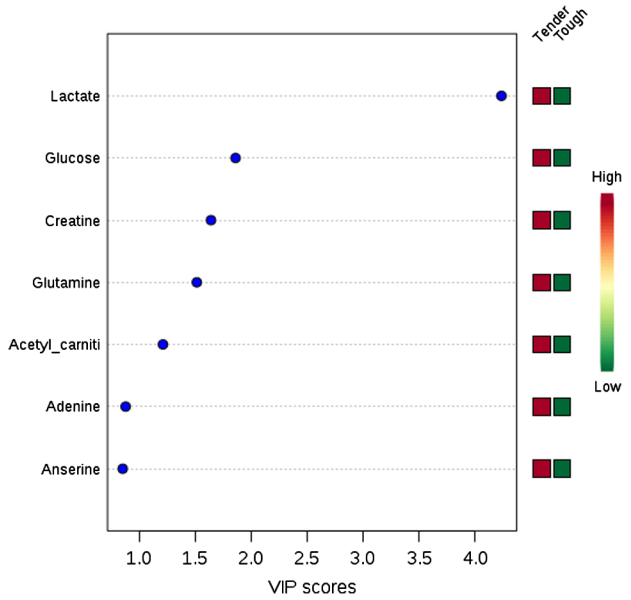

Figure 2. Variable importance in projection (VIP) plot of metabolites of *longissimus thoracis* steaks classified as tender or tough after 7 d of aging.

(r = −0.31) were moderately correlated (−0.5 < r ≥ −0.3) with WBSF values.

Discussion

In general, meat pH is associated with meat tenderness because of its influence on proteolytic activity (Lomiwes et al., 2014); however, in the present study, meat pH did not differ between tender and tough meat. According to Huff Lonergan et al. (2010), meat pH provides an environment for proteolytic enzymes to degrade myofibrillar proteins that determine the sarcomere length and the shear force. In the present study, sarcomere length was greater in steaks that were classified as tender, which may indicate a greater amount of myofibrillar protein degradation, contributing to a lower shear force in this group. Nonetheless, King et al. (2019) observed no difference in sarcomere length between tender and tough steaks; on the other hand, candidate biomarkers related to meat tenderness were identified as intermediates of glycolysis and the TCA cycle and are part of energy-producing shuttles associated with these pathways, such as glucose, glucose-6-phosphate, and malic acid. Likewise, Straadt et al. (2014) reported that carnosine and choline are indicators of tenderness. In the present study, as the difference in WBSF between tenderness classes was not pH dependent, variability in meat metabolite profiles and their pathways can be suggested as a partial contributor to explain variability in tenderness.

Table 2. Metabolites of *longissimus thoracis* steaks classified as tender or tough after 7 d of aging

Meat metabolites, µmol/g meat	Tenderness class		SEM	*P* value
	Tender (*n* = 15)	Tough (*n* = 15)		
Acetate	0.13	0.15	0.025	0.699
Acetyl-carnitine	0.99	0.53	0.137	**0.026**
Adenine	0.60	0.36	0.073	**0.026**
Alanine	1.49	1.64	0.244	0.673
Anserine	0.76	0.45	0.129	0.106
ATP	0.58	0.43	0.084	0.214
Beta-alanine	0.18	0.11	0.015	**0.005**
Betaine	1.06	0.93	0.145	0.530
Carnitine	1.72	1.76	0.255	0.918
Carnosine	11.42	11.44	1.320	0.989
Choline	0.10	0.73	0.113	0.110
Creatine	22.46	20.58	1.375	0.340
Creatinine	0.65	0.55	0.081	0.368
Fucose	0.34	0.30	0.057	0.598
Fumarate	0.12	0.06	0.018	**0.022**
Glucose	4.11	2.83	0.471	0.650
Glutamate	0.40	0.33	0.048	0.327
Glutamine	2.27	1.44	0.292	**0.043**
Glycerate	2.06	2.39	0.251	0.361
Glycerol	2.24	2.34	0.325	0.830
Glycine	1.05	0.98	0.118	0.684
Inosine monophosphate	0.81	0.64	0.147	0.414
Inosine	0.47	0.46	0.052	0.849
Isoleucine	0.18	0.13	0.024	0.175
Lactate	52.54	42.83	5.401	0.214
Methionine	1.00	1.02	0.142	0.890
NADH	0.35	0.25	0.046	0.146
Proline	0.10	0.08	0.019	0.425
Succinate	0.06	0.04	0.007	0.107
Threonine	0.65	0.65	0.075	0.964
Valine	0.32	0.19	0.041	**0.030**

In bold $P < 0.05$.

ATP = adenosine triphosphate; NADH = nicotinamide adenine dinucleotide.

Based on 1D ^{1}H NMR analyses, 31 metabolites were identified in the meat from animals in this study. Metabolites were segregated according to tenderness classification using a PCA score plot, which suggested differences in metabolite concentrations between tender and tough meat. Lactate, glucose, creatine, glutamine, acetyl-carnitine, adenine, and anserine were the most associated compounds with differences in tenderness, based on VIP scores. Therefore, the metabolite profile of tender and tough beef was different and may suggest altered metabolic pathways associated with tenderness variability, including D-glutamine and D-glutamate metabolism, beta-alanine metabolism, purine metabolism, and the TCA cycle.

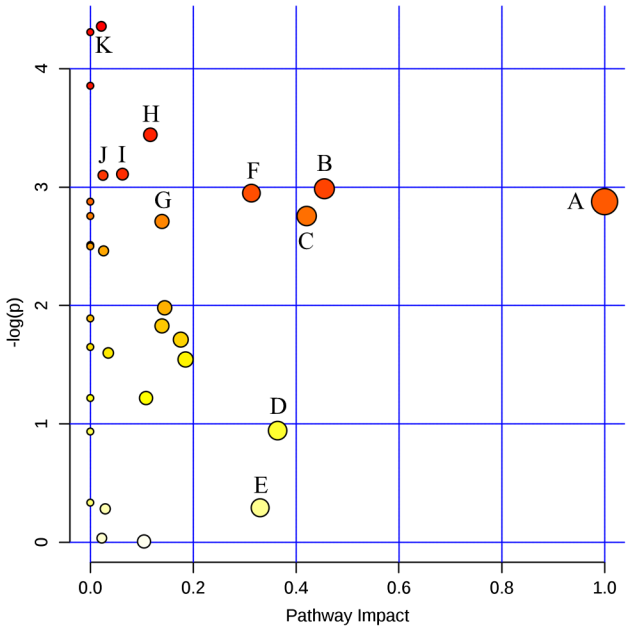

Figure 3. Pathway analysis using metabolites from *longissimus thoracis* steaks classified as tender or tough after 7 d of aging. The "metabolome view" presents pathways arranged according to the scores based on enrichment analysis (y-axis) and topology analysis (x-axis). The color and size of each circle are based on *P* values and pathway impact values, respectively. All the pathways described had $P < 0.10$ and pathway impact > 0. (A) D-glutamine and D-glutamate metabolism; (B) beta-alanine metabolism; (C) starch and sucrose metabolism; (D) glycerolipid metabolism; (E) alanine, aspartate, and glutamate metabolism; (F) purine metabolism; (G) arginine biosynthesis; (H) tricarboxylic acid (TCA) cycle; (I) tyrosine metabolism; (J) pantothenate and coenzyme A (CoA) biosynthesis.

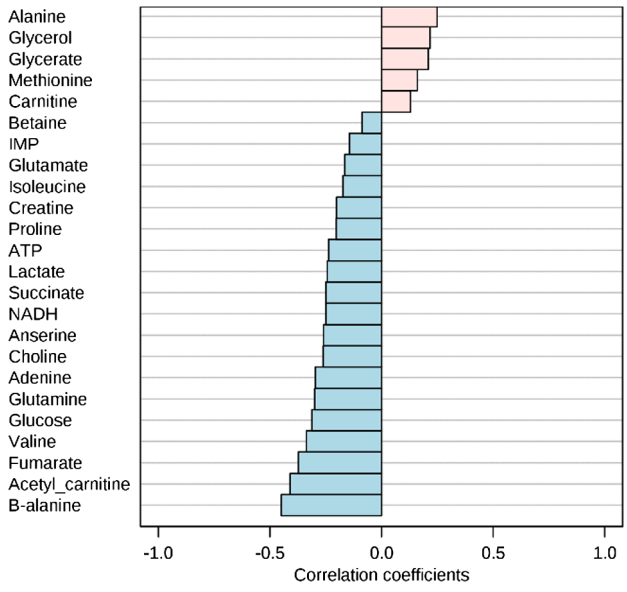

Figure 4. Top 24 metabolites that were associated with shear force using Pearson correlation as a distance measure.

In this study, tender beef had a greater concentration of valine, a branch chain amino acid (BCAA) which may regulate the rate of protein synthesis and degradation in skeletal muscle and provide nonspecific carbon source of oxidation for production of energy (Monirujjaman and Ferdouse, 2014). In general, amino acids are an important source of energy in early postmortem muscle because all of them can be degraded to a component of the TCA cycle or an intermediate compound that can be converted to a component of this cycle (Ouali et al., 2013). In this sense, BCAA degradation may indicate that early postmortem differences in energy status impacted proteolysis and favored meat tenderization, as observed in this study by the greater sarcomere length in tender beef. Similarly, King et al. (2019) reported that BCAA were greater in concentration in tender beef compared with tough beef after 7-d aging. In this study, as a likely consequence of BCAA degradation, tender beef had a greater amount of acetylcarnitine (fifth most important metabolite differing between tenderness classes), which is responsible for transferring acetyl groups such long-chain fatty acids into the mitochondrial matrix for beta-oxidation to be used in the TCA cycle (Reda et al., 2003). In addition, Monirujjaman and Ferdouse (2014) reported that BCAA degradation pathway promotes an increase in glucose metabolism and fatty acid oxidation in order to generate energy.

In this study, tender beef contained more glutamine (fourth most important metabolite differing between tenderness classes), which is a nonessential amino acid that impacts glutamine-glutamate metabolism (Tapiero et al., 2002) and alanine, aspartate, and glutamine metabolism (Graham et al., 2010). Kodani et al. (2017) also reported that glutamine and alanine were more concentrated in tender beef during postmortem aging. The synthesis of glutamine from glutamate occurs post mortem because glutamate is abundant and displaces the adenosine triphosphate (ATP)-dependent reaction towards the formation of glutamine (Ouali et al., 2013). In addition, tender beef contained more adenine (sixth most important metabolite differing between tenderness classes) and fumarate, which affect purine metabolism and are essential for organisms as the precursors for generation of coenzymes such as ATP, nicotinamide adenine dinucleotide (NAD+), and nicotinamide adenine dinucleotide phosphate (NADP+) that are crucial to many metabolic pathways, including the TCA cycle (Nelson and Cox, 2013). Similarly, Muroya et al. (2019) reported an increase in purine metabolism in tender beef during postmortem aging.

Ramos et al. (2020a, 2020b) reported that early postmortem differences in energy status could be related to resistance to cellular stress post mortem

and, consequently, meat tenderization. According to Yu et al. (2019), the content of metabolites involved in the TCA cycle change during the postmortem period. In this study, the TCA cycle pathway was associated with tenderness class, specifically due to the higher concentrations of adenine, fumarate, glutamine, and valine observed in tender beef, which were negatively correlated with WBSF. Those metabolites are directly related to oxidative metabolism, which provides substrates to the electron transport chain and to the ATP synthase that is responsible for ATP production. In addition, some specific muscle metabolites have been identified to differentiate muscles that differ in muscle fiber types (Berri et al., 2019). Yu et al. (2019) stated that muscles with different fiber type composition that also differ in postmortem energy metabolism pattern result in distinct quality characteristics during storage and retail.

Although the relationship between fiber type and meat tenderness is not completely understood, some studies have indicated that muscles with greater oxidative metabolism are more tender than muscles that are more prone to glycolytic metabolism (Hwang et al., 2010; Kim et al., 2018). Moreover, oxidative muscles undergo apoptosis earlier than those from glycolytic muscles, due to cellular stress (Rønning et al., 2017). Hamelin et al. (2007) observed that the majority of the oxidative metabolism-related proteins that were detected may contribute to the regulation of stress. In addition, as muscle is subjected to various reactive oxygen species under oxidative stress, increased antioxidant activities may regulate apoptosis and affect meat tenderness development (Picard and Gagaoua, 2017).

It is well documented that oxidative stress induces damage to cellular proteins compromising their functions and leading to cellular dysfunction and finally cell death (Ouali et al., 2013; Lomiwes et al., 2014; Picard and Gagaoua, 2017). It has been suggested that the onset of apoptosis (prior to rigor mortis) is the very first phase in the muscle-to-meat conversion process (Kemp et al., 2010; Ouali et al., 2013). A set of pro- and anti-apoptotic proteins are released from mitochondria, and their ratio defines the rate and extent of apoptosis development (Ouali et al., 2013; Lomiwes et al., 2014). According to Ramos et al. (2020a), mitochondria could be a contributing factor to delayed calpain-mediated proteolysis and tenderization. Pro-apoptotic proteins such as cytochrome C can induce or enhance caspase activation, which has been suggested to contribute to postmortem proteolysis and meat tenderization via their critical roles in apoptosis (Kemp et al., 2010; Ouali et al., 2013; Lomiwes et al., 2014). Smuder et al. (2018) proposed that oxidative modification of myofibrillar proteins makes them susceptible to degradation by caspases, thus promoting tenderization. In contrast, anti-apoptotic proteins, such as heat shock proteins, increase their activity/expression to preserve cellular proteins from denaturation and/or oxidative-stress–related damage (Kemp et al., 2010; Lomiwes et al., 2014; Picard and Gagaoua, 2017, 2020). In general, heat shock proteins inhibit the onset of apoptosis by binding to cytochrome c and/or preventing the activation of caspase-3, thereby delaying meat tenderization (Picard and Gagaoua, 2017, 2020; Bonnet et al., 2020).

In the present study, compounds of the oxidative metabolism such as beta-alanine, acetyl-carnitine, fumarate, valine, glutamine, and adenine were negatively correlated with WBSF. Muroya et al. (2019) and Yu et al. (2019) reported that some TCA cycle metabolites are correlated with aged beef quality traits. Similarly, King et al. (2019) reported that intermediates of glycolysis and the TCA cycle were correlated with beef tenderness. Thus, results of the present study suggest that, due to increased oxidative metabolism and/or decreased antioxidant properties, tender beef underwent oxidative stress earlier post mortem than tough beef so that structural proteins remained more vulnerable to myofibrillar proteolysis, which positively contributed to meat tenderization in this group. Similarly, D'Alessandro et al. (2012) observed greater oxidative stress in tender than in tough beef, suggesting that oxidative stress may be associated with meat tenderness.

This study provided a more in-depth view of the metabolomics of beef LT differing in tenderness. Some candidate biomarker metabolites such as acetyl-carnitine, adenine, beta-alanine, fumarate, glutamine, and valine had greater concentration in tender beef. Predominant metabolic pathways such as D-glutamine and D-glutamate metabolism, beta-alanine metabolism, purine metabolism, and the TCA cycle were affected by tenderness class. Thus, greater oxidative metabolism of muscle followed by amino acid degradation in the tender group promoted modifications in the muscle structure and proteolysis, likely due to the increase of oxidative stress and apoptosis, favoring its tenderization.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES) (Finance Code 001).

The authors declare no conflicts of interest associated with this research.

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