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Estudos sobre o efeito da condição sexual sobre o desempenho, características de carcaça e qualidade de carne de bovinos Nelore.

Pirassununga

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BELINE, M. **Estudos sobre o efeito da condição sexual sobre o desempenho, características de carcaça e qualidade de carne de bovinos Nelore**. 2022. 98 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2022.

Resumo: Dois estudos foram realizados para avaliar o efeito da condição sexual sobre o desempenho, características e carcaça e qualidade de carne de bovinos Nelore. No primeiro experimento (Capítulo 2), foram utilizados 120 Nelores machos castrados (CA) e 120 não-castrados (NC). Foram obtidos dados de desempenho, características de carcaça e rendimento de cortes além de imagens de ultrassom durante a recria e terminação para determinar deposição de músculo e gordura. Além disso, as características de qualidade da carne de amostras maturadas por 0, 7 e 14 dias foram avaliadas. O ganho de peso, deposição de músculo e gordura em função da idade aumentaram de forma quadrática ao longo do tempo. Os animais NC apresentaram maior ganho de peso, eficiência alimentar durante o período de terminação, e consequentemente, apresentaram carcaças mais pesadas, com menor acabamento e com maiores pesos de cortes comerciais que CA. Foi observado um efeito do tratamento para todas as características de qualidade da carne com maiores valores de cor e menor força de cisalhamento em carne dos animais CA. No segundo experimento (Capítulo 3), foram utilizados 10 Nelores machos CA e 10 NC. Valores de pH 24h, e aproximadamente 10g de músculo *Longissimus thoracis* foi coletado para determinação da concentração de glicogênio, análise de lipidômica e metabolômica além de uma amostra de 2,5cm de espessura para posterior análise de cor e maciez da carne. Menores concentrações de glicogênio, menores valores de cor e maior força de cisalhamento foram observadas nos animais NC. Os perfis metabólitos e lipídicos e suas vias metabólicas foram relacionados principalmente ao metabolismo energético muscular, como o ciclo TCA,

glicólise/gliconeogênese, piruvato, beta-oxidação mitocondrial de ácidos graxos de cadeia longa e metabolismo de ácidos graxos. Músculo de animais CA apresentaram um perfil mais glicolítico que NC, consequentemente influenciando positivamente a qualidade da carne de animais CA.

Palavras-chave: castração, cor, lipidômica, maciez, metabolômica, rendimento de cortes

BELINE, M. **Studies on the effect of sexual condition on performance, carcass traits, and meat quality of Nellore cattle**. 2022. 98 p. PhD Thesis – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2022.

Abstract: Two studies were carried out to evaluate the effect of sexual condition on performance, carcass, and meat quality traits of Nellore cattle. In the first experiment (Chapter 2), 120 castrated Nellore males (CA) and 120 non-castrated (NC) were used. Performance data, carcass traits and cutting yield were obtained, as well as ultrasound images during rearing and finishing to determine muscle and fat deposition. In addition, the meat quality characteristics of samples aged for 0, 7 and 14 days were evaluated. Weight gain, muscle, and fat deposition as a function of age increased quadratically over time. The NC animals showed higher weight gain and feed efficiency during the finishing period, and consequently, presented heavier and leaner carcasses with higher commercial cuts weights than CA. A treatment effect was observed for all meat quality traits with higher color values and lower shear force in meat from CA animals. In the second experiment (Chapter 3), 10 male Nelores CA and 10 NC were used. pH values 24h, and approximately 10g of Longissimus thoracis muscle was collected for determination of glycogen concentration, lipidomics and metabolomics analysis, in addition to a 2.5cm thick sample for further analysis of meat color and tenderness. Lower glycogen concentrations, higher pH, lower color values and higher shear force were observed in NC animals. Metabolite and lipid profiles and their metabolic pathways were mainly related to muscle energy metabolism, such as the TCA cycle, glycolysis/gluconeogenesis, pyruvate, mitochondrial beta-oxidation of long-chain fatty acids and fatty acid metabolism. Muscle from CA animals showed a more glycolytic profile than NC, consequently positively influencing the meat quality of CA animals.

Key words: castration, color, cutting yield, gender, lipidomics, metabolomic, tenderness

SUMÁRIO

1. Introdução

É consenso entre os especialistas que a população mundial chegará a 9,7 bilhões de habitantes até 2050 (UN, 2019) e para atender esta demanda, a produção global de carne deve aumentar entre 60 e 95%. Também é consenso que o aumento da produção será obtido principalmente pelo aumento da eficiência da produção e não pelo aumento do número de animais e/ou área utilizada.

Aumentar a eficiência produtiva da pecuária de corte envolve estratégias que consideram fatores como raça, melhoramento genético, condição sexual, manejo reprodutivo e nutricional, além de práticas que promovam o bem-estar animal. Dentre elas, a condição de gênero é de grande relevância, pois está intimamente relacionada com a eficiência da produção, rendimento e qualidade da carne, práticas de manejo e bem-estar dos animais.

Gerrard e Grant, (2006) afirmam que uma das maneiras mais fáceis de aumentar a eficiência, em termos de eficiência alimentar e de crescimento, é eliminar a castração. Segundo Field (1971), animais não-castrados são mais eficientes na conversão de alimentos em proteína de alto valor biológico (músculo/carne magra) em cerca de 13-15% em relação aos castrados e, segundo Steen (1995), bovinos não-castrados podem apresentar ganho de peso diário 19% maior quando comparados aos animais castrados, com aumento de apenas 3% no consumo de ração. Além disso, animais não-castrados apresentam maior peso de abate e cortes comerciais em comparação aos castrados (BERG; BUTTERFIELD, 1976; HEDRICK; THOMPSON; KRAUSE, 1969). Portanto, em um cenário em que aumentar a eficiência da produção de carne é um dos principais objetivos, a utilização de machos não-castrados é uma opção relevante e deve ser considerada pela cadeia produtiva.

A condição sexual dos animais tem grande efeito sobre os padrões de crescimento dos tecidos. O crescimento muscular e suas propriedades intrínsecas determinam, pelo menos em parte, a quantidade e a qualidade da carne produzida. A massa muscular é determinada principalmente pelo número e tamanho das fibras (REHFELDT et al., 2000). Ao nascimento, o número de fibras musculares já está estabelecido (DU; YIN; ZHU, 2010) e o desenvolvimento do músculo esquelético pós-natal se dá principalmente pela hipertrofia das fibras musculares (REHFELDT et al., 2000). A hipertrofia das fibras pode se dar pela síntese de proteínas, que envolvem processos moleculares, desde a transcrição do DNA até o posicionamento em lugares específicos das proteínas produzidas (GERRARD; GRANT, 2006). O recrutamento de células satélites também auxilia na hipertrofia, uma vez que essas células se fundem com as fibras existentes aumentando o conteúdo de DNA e a capacidade sintética das proteínas (GERRARD; GRANT, 2006; REHFELDT et al., 2000).

Entretanto, o músculo esquelético pode não ser o primeiro ponto de síntese proteica, pois de acordo com Jones (2014), a ordem relativa de desenvolvimento dos órgãos se inicia pelo sistema nervoso, seguido pelos ossos, músculo e por último a gordura. De acordo com o mesmo autor, a maioria dos animais apresentam padrões de crescimento similares, variando somente no tempo em que levam para atingirem a maturidade. De acordo com Brody (1964), Owens, Dubeski e Hanson (1993), o animal atinge sua maturidade quando o acréscimo de proteína cessa e a massa muscular atinge seu máximo. Animais com rápida taxa de crescimento atingem a maturidade mais rapidamente do que animais de baixa taxa de crescimento. Animais de maturidade tardia são mais magros enquanto animais de maturidade precoce apresentam mais gordura e se encontram mais avançados na curva de crescimento (GERRARD; GRANT, 2006).

A curva de crescimento bem como a composição corporal do animal pode ser influenciada por diferentes fatores intrínsecos do animal tais como idade e maturidade, frame ou porte do animal, genética, raça e sexo. Essas mudanças na curva de crescimento e na composição do ganho têm como base mudanças na estrutura muscular e em todos os tecidos corporais (OWENS et al., 1995; OWENS; DUBESKI; HANSON, 1993).

1.1. Efeito da condição sexual sobre o crescimento dos tecidos muscular e adiposo

A condição sexual tem um grande impacto no padrão de crescimento de músculo e gordura, uma vez que o sexo influencia na maturação das gônadas levando a um aumento na síntese e secreção de testosterona nos machos (BEERMANN, 2014). A testosterona, principal hormônio androgênico, é sintetizada pelas células Leydig dos testículos e é responsável pela maturação do trato reprodutivo dos machos, comportamento sexual, espermatogênese e pelo crescimento corporal (GERRARD; GRANT, 2006).

A testosterona se liga nos receptores de andrógenos e entram nas células estimulando vias de sinalização (KRAEMER et al., 2020) que podem alterar a expressão de genes envolvidos na estrutura, tipo de fibras (DUBOIS et al., 2014) e metabolismo muscular (WHITE et al., 2013). A testosterona aumenta a massa muscular e de acordo com Sinha-Hikim et al. (2003), o aumento de massa muscular está associado a hipertrofia das fibras musculares e incorporação das células satélites. O exato mecanismo em que a testosterona regula a massa muscular ainda não é completamente compreendido, tendo como uma possível explicação que a testosterona ativa ou age de forma sinérgica com outros efetores de crescimento muscular, como GH e IGF-I (SERRA et al., 2011).

Estudos mostraram que a testosterona aumentou GH e IGF-1 circulante, a expressão de IGF-1, e aumentou os efeitos anabólico destes hormônios, e essas interações

positivas são importantes para aumentar o crescimento em animais (GIBNEY et al., 2005; SERRA et al., 2011). Uma das principais ações do GH é a produção do IGF-1, aumento da massa magra devido ao aumento da síntese proteica e redução da degradação além de estar relacionado com aumento da atividade das células satélites que fornecem DNA para acréscimo de proteína, por outro lado, GH aumenta a lipólise por estimular a mobilização de ácidos graxos dos adipócitos (GERRARD; GRANT, 2006).

Por outro lado, a testosterona apresenta efeito lipolítico que é mediado pelo aumento no número de receptores β-adrenoreceptores e aumento da atividade da adenilato ciclase, proteína-quinase A e lipase (DICKER et al., 2004; XU; DE PERGOLAF; BJORNTORP, 1990). Além disso, a testosterona supressa o desenvolvimento, proliferação e maturação de pré-adipócitos em adipócitos (REN et al., 2017). De acordo com Gentile et al. (2010) e Oh et al. (2005) a testosterona previne a diferenciação adipogênica e acumulo de lipídeos nas células musculares de bovinos através da inibição da atividade da enzima glicerol-3-fosfato desidrogenase como também bloqueia a interação do receptor de andrógeno com a β-catenina.

Após a castração, ocorre uma rápida diminuição nos níveis de testosterona (ÁLVAREZ-RODRÍGUEZ et al., 2017; GÓMEZ et al., 2017), já caindo pela metade sua concentração sérica uma hora após da castração (LIN et al., 1994). A redução nos níveis de testosterona após a castração desregula a sinalização dos receptores de andrógeno e com isso, reduz a massa muscular e expressão dos receptores de andrógeno (BAIK et al., 2020). A baixa concentração de testosterona diminui os efeitos fisiológicos desse andrógeno como redução de lipídeos plasmáticos, aumento da lipólise nos adipócitos e a estimulação dos receptores de andrógeno, resultando em um aumento dos lipídeos plasmáticos e gordura intramuscular (XU; DE PERGOLAF; BJORNTORP, 1990; ZHANG et al., 2017). Além disso, Jeong et al. (2012) demonstraram que a castração

alterou a expressão de genes relacionados ao metabolismo lipídico, favorecendo a deposição de gordura intramuscular através do aumento da adipogênese, lipogênese e síntese de triglicerídeos (JEONG et al., 2013).

1.2. Tecido muscular esquelético

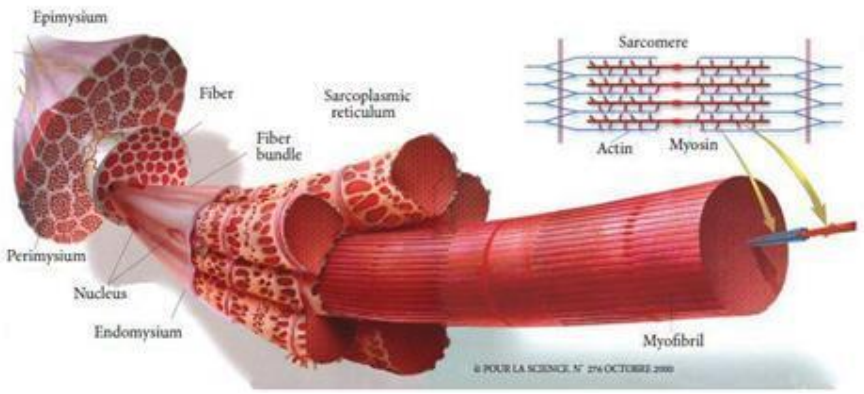
O principal componente da carne é o tecido muscular, o qual é dividido em tecido muscular liso (principalmente como componente dos vasos sanguíneos), tecido muscular estriado cardíaco (restrito apenas ao coração) e tecido muscular estriado esquelético, que representa a maior parte (35 – 65%) do peso da carcaça de animais de corte (ABERLE et al., 2012). O músculo esquelético é composto principalmente por água (75%), proteína (20%), e outras substâncias incluindo sais inorgânicos, minerais, gordura e carboidratos (5%) (FRONTERA; OCHALA, 2015).

Os músculos esqueléticos são constituídos de fibras musculares multinucleadas compostas de feixes de miofibrilas alongadas em uma configuração paralela. As fibras musculares, por sua vez, são constituídas de uma membrana externa (sarcolema), de um citoplasma diferenciado (sarcoplasma), que está praticamente tomado pelas miofibrilas (LISTRAT et al., 2016). De acordo com os mesmos autores, o sarcômero constitui a menor unidade contrátil estrutural repetitiva da miofibrila (Figura 1), apresentando um papel importante no ciclo de contração e relaxamento muscular.

O músculo tem influência sobre o metabolismo energético basal, pode ser lugar de armazenagem de substratos importantes como aminoácidos e carboidratos, produção de calor para a manutenção da temperatura e o consumo da maioria do oxigênio e energia usada durante a atividade física (LISTRAT et al., 2016). Do ponto de vista mecânico, a principal função do músculo esquelético é converter energia química em energia

mecânica para gerar força, manter a postura e produzir movimento que possibilita a atividade física (FRONTERA; OCHALA, 2015).

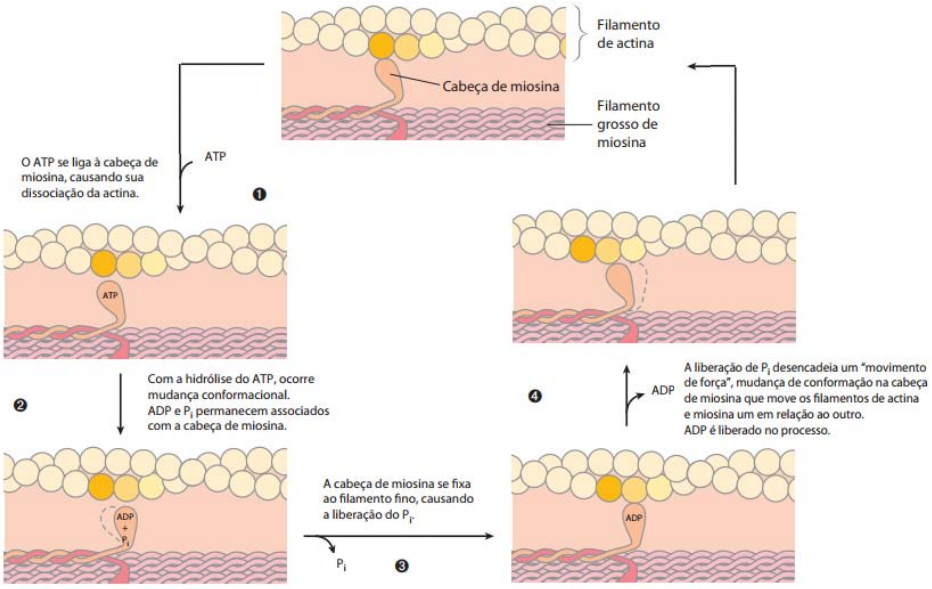
Figura 1. Estrutura geral dos músculos esqueléticos.



Fonte: LISTRAT et al., 2016. How muscle structure and composition influence meat and flesh quality. Scientific World Journal. http://doi.org/10.1155/2016/3182746.

A principal função do músculo esquelético é a contração. Portanto, é necessário que haja o desenvolvimento de um aparelho específico que permita as células musculares estarem completamente desenvolvidas antes do músculo iniciar o processo de contração (GERRARD; GRANT, 2006). O músculo se contrai em respostas a estímulos neurais que irá liberar cálcio no sarcoplasma, e quando este excede limiar regulatório, ocorre a interação entre miosina e a actina criando movimento através do consumo de Adenosina Trifosfato (ATP), via ATPase miofibrilar, cuja hidrólise converte energia química em energia mecânica (FERGUSON; GERRARD, 2014). Ou seja, as pontes de miosina durante interações transitórias com ligações em um filamento de actina, agem como transdutor biológico de energia (BARCLAY, 2015). De acordo com Lodish et al. (2000) e Purslow (2017), o ciclo das pontes cruzadas possui quatro etapas (Figura 2), na qual se inicia com a ligação do ATP à cabeça da miosina fazendo com que ela se desassocie da actina (etapa 1).

Figura 2. Mecanismo molecular da contração muscular.



Fonte: Adaptado de NELSON, David L.; COX, Michael M. Princípios de bioquímica de Lehninger. Artmed Editora, 2014.

Posteriormente, o ATP é hidrolisado à ADP e o músculo volta ao repouso, com a actina sendo bloqueada de se ligar a miosina devido ao complexo troponina-tropomiosina. A partir de um estímulo elétrico, o cálcio é liberado pelo retículo sarcoplasmático, se liga ao complexo da troponina, movendo a tropomiosina e permitindo a ligação da cabeça da miosina com a actina (etapa 2), que libera o fosfato inorgânico (Pi) e promove uma mudança na conformação da cabeça, desencadeando um movimento de força (etapa 3). Finalmente, o ADP é liberado (etapa 4) e, caso haja ATP disponível, a cabeça da miosina pode se ligar ao ATP, começando novamente o ciclo. A contração é terminada devido ao sequestro de cálcio pelo retículo sarcoplasmático, o que leva o complexo tropomina-tropomiosina a bloquear a ligação miosina-actina.

No músculo pós-morte, os níveis baixos de ATP e altos níveis de cálcio devido à falha das bombas de ATP dependente de cálcio fazem com que as pontes cruzadas de

miosina formadas entre as cabeças de miosina e actina fiquem ligadas, estabelecendo, desse modo, o rigor mortis (LODISH et al., 2000; PURSLOW, 2017).

1.2.1. Fibras musculares

Um dos atributos únicos que o músculo esquelético apresenta é seu numeroso tipo de fibra e suas distintas características funcionais e de composição (PETTE; STARON, 2000). Fibras musculares são formadas por células multinucleadas que apresentam diâmetro de 10 a 100 µm e comprimento que podem variar de milímetros a até mais que 30 centímetros, e padrão de estrias da miofibrila se repete com uma periodicidade de aproximadamente 2 a 3 µm e no músculo vertebrado, o sarcômero é uma estrutura complexa contendo pelo menos 28 proteínas diferentes (CRAIG; PADRÓN, 2004). Os tipos de fibra diferem de acordo com suas propriedades moleculares, metabólicas e contrateis e podem ser agrupados de acordo com várias características, incluindo isoformas de proteína miofibrilar, perfil metabólico enzimático, e propriedades contrateis e estruturais (Tabela 1; (BOTTINELLI; REGGIANI, 2000).

Tabela 1. Características biológicas individuais dos tipos de fibras.

	I	IIa	IIx	IIb
Contraction speed	+	+++	++++	+++++
Myofibrillar ATPase	+	+++	++++	+++++
Oxidative metabolism	+++++	++++, +++++	+, ++	+
Glycolytic metabolism	+	++++	++++	+++++
Hexokinase	+++++	+++	+	+
GLUT-4	+++++	+++	+	+
Phosphocreatine	+	+++++	+++++	+++++
Glycogen	+	+++++	++++	+++++
Triglycérides	+++++	++	+	+
Vascularization	+++++	+++	+, ++	+
Myoglobine	+++++	++++	++	+
Buffering capacity	+	++++	+++++	+++++
Diameter	++	+, ++	++++	+++++
Fatigue resistance	+++++	++++	++	+

Fonte: LEFAUCHEUR, L. A second look into fibre typing - Relation to meat quality. Meat Science, v. 84, n. 2, p. 257–270, 2010. DOI: 10.1016/j.meatsci.2009.05.004.

Diferentes músculos variam na atividade da ATPase, que reflete suas características contráteis, bem como atividades enzimáticas envolvidas em suas vias metabólicas (BOLAND et al., 2019). Com base nas diferenças da atividade da ATPase da actomiosina à pré-incubação do pH, três tipos principais de fibras são convencionalmente determinados por histoquímica no músculo esquelético adulto, isto é, fibras dos tipos I, IIA e IIB (BROOKE; KAISER, 1970). A velocidade de contração aumenta na ordem de classificação I < IIa < IIx < IIb (SCHIAFFINO; REGGIANI, 1994).

Em relação a atividade enzimática, os tipos de fibras podem ser identificados de acordo com as enzimas que permitem a realização do metabolismo oxidativo ou glicolítico (GERRARD; GRANT, 2006). Enzimas presentes na via glicolítica, tais como fosfofrutoquinase (PFK), gliceraldeído-3-fosfato desidrogenase (GAPDH) e lactato desidrogenase (LDH) são mais ativas em fibras musculares brancas, enquanto enzimas envolvidas na via oxidativa, tais como citrato sintase (CS), isocitrato desidrogenase (ICDH), malato desidrogenase (MDH) prevalecem em fibras musculares vermelhas (PURSLOW, 2017).

As fibras do tipo l, ou fibras de contração lenta, geram energia predominantemente pela via aeróbica, possuem um baixo nível de atividade da ATPase e uma capacidade oxidativa mais desenvolvida que em fibras de contração rápida (CHOI; KIM, 2009). Possuem maior vascularização, o que facilita a transferência de nutrientes e oxigênio a partir dos vasos sanguíneos, maior quantidade de mioglobina e mitocôndrias, consequentemente, apresentam metabolismo mais oxidativo (LEFAUCHEUR, 2010). A contração desse tipo de fibra é lenta e prolongada, se fadigam menos e geram tensão

muscular relativamente menor que as fibras de contração rápida (BANKS, 1991; FORREST et al., 1979).

Por outro lado, as fibras do tipo II, ou fibras de contração rápida, são maiores e ricas em enzimas glicolíticas e apresentam maior quantidade de glicogênio. As mitocôndrias e mioglobinas estão presentes em pequena quantidade, e consequentemente, uma menor vascularização (LEFAUCHEUR, 2010). O metabolismo glicolítico que predomina nas fibras do Tipo IIx e IIB acontece tanto na presença como na ausência de oxigênio. Portanto, elas se contraem rapidamente, gerando grandes picos de tensão muscular, porém se fadigam com facilidade (BANKS, 1991; FORREST et al., 1979). As fibras do Tipo IIa, ou fibras de contração intermediária, se contraem mais rapidamente que as do Tipo I e são mais resistentes à fadiga que as do Tipo IIx e IIb devido a quantidade intermediaria de mioglobina e mitocôndria (LEFAUCHEUR, 2010). Desse modo, apresentam ambos os metabolismos oxidativo e glicolítico (BANKS, 1991)

1.2.2. Metabolismo energético

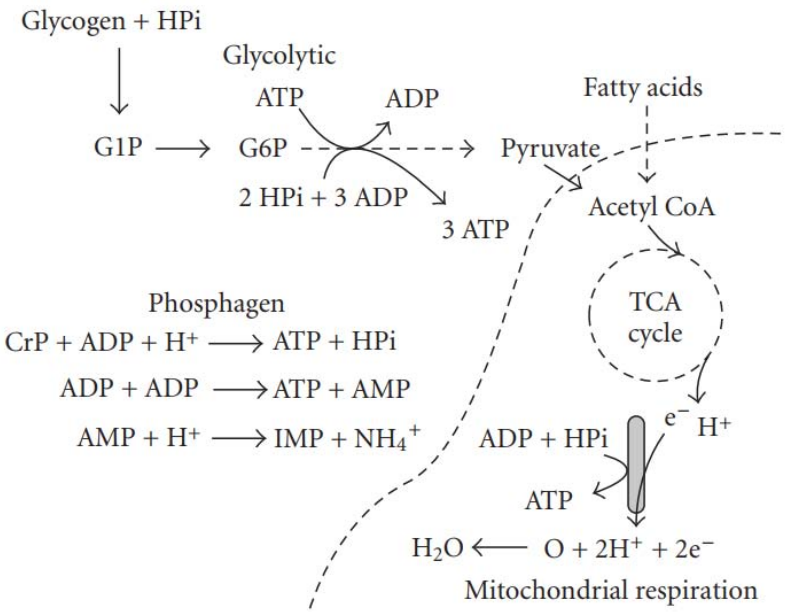
A hidrólise de ATP fornece energia que pode ser utilizada para trabalho mecânico (contração muscular), químico (biossíntese e anabolismo) e osmótico (transporte ativo) (SCHEFFLER; PARK; GERRARD, 2011). De acordo com os mesmos autores, a quebra de ATP é balanceada pelas vias produtoras de energia que catabolizam nutrientes estocados, como glicogênio ou gordura, para gerar ATP, mantendo assim a célula viva e permitindo manter-se em homeostase. O tecido muscular é único, pois pode variar sua taxa metabólica em maior extensão do que qualquer outro tecido, dependendo das exigências impostas a ele (GLAISTER, 2005).

A concentração de ATP muscular não se encontra armazenada como um estoque de energia, mas atua coletivamente com ADP, AMP e Pi, sendo o ATP um substrato

essencial para a função celular ideal (BAKER; MCCORMICK; ROBERGS, 2010). Além disso, qualquer redução na concentração de ATP muscular coincide com as condições celulares associadas ao rápido desenvolvimento da fadiga, definido como uma redução na capacidade de um músculo produzir força ou potência, ou uma redução na renovação do músculo esquelético do ATP (BIGLAND-RITCHIE; WOODS, 1984; SØGAARD et al., 2006).

Existem três principais sistemas de energia que são responsáveis pela ressíntese de ATP (Figura 3), o sistema fosfágeno, o sistema glicolítico seguido pelo Ciclo de Krebs e a respiração mitocondrial.

Figura 3. Os três sistemas de energia de regeneração muscular de ATP.



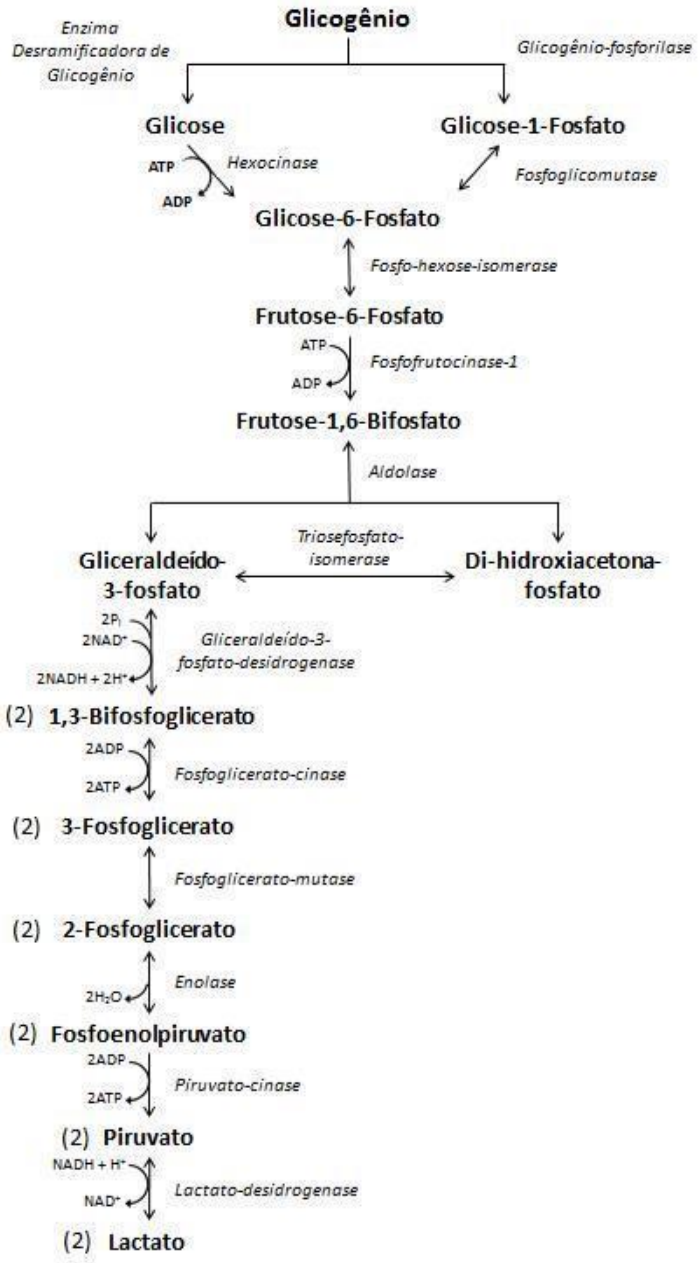
Fonte: BAKER, J. S.; MCCORMICK, Marie C.; ROBERGS, R.A. Interaction among skeletal muscle metabolic energy systems during intense exercise. Journal of Nutrition and Metabolism, 2010. DOI: 10.1155/2010/905612.

O sistema fosfágeno consiste em três reações (Figura 4) que foram explicadas por Baker; Mccormick e Robergs (2010). As duas primeiras reações da creatina quinase (CK) e adenilato quinase (AK) produzem ATP, embora a primeira apresentar maior capacidade

de regeneração de ATP. De acordo com os mesmos autores, o consumo de ATP e o início da acidificação muscular ativa a adenosina monofosfato deaminase que irá consumir a adenosina monofosfato (AMP), produzida pela segunda reação, junto com H+ produzindo inosina monofosfato (IMP) e amônia. Outro fator importante é a produção de AMP, que é um ativador alosterico da enzima glicogênio fosforilase, que aumenta a quebra de glicogênio e com isso aumenta as concentrações de G6P servindo de substrato para glicólise, e consequentemente, aumenta as taxas de geração de ATP (BAKER; MCCORMICK; ROBERGS, 2010).

A glicólise envolve muito mais reações do que qualquer componente do sistema fosfágeno, diminuindo ligeiramente a taxa máxima de regeneração de ATP (Figura 4). No entanto, a glicólise continua sendo um meio muito rápido para regenerar o ATP em comparação com a respiração mitocondrial (GREENHAFF et al., 1994). A glicólise consiste em uma serie de 10 reações bioquímicas que metabolizam uma molécula de seis átomos de carbono em duas moléculas de piruvato, cada uma com três carbonos, e durante esse processo ocorre geração de ATP (NELSON; COX, 2014). De acordo com os mesmos autores, as cinco primeiras reações constituem a fase preparatória, na qual a glicose é metabolizada a gliceraldeído-3-fosfato com gasto de ATP e o ganho de energia vem da fase de pagamento, constituída das cinco reações finais, mantendo as duas moléculas de três carbonos em forma de piruvato.

Figura 4. Enzimas e metabolitos intermediários da glicólise.

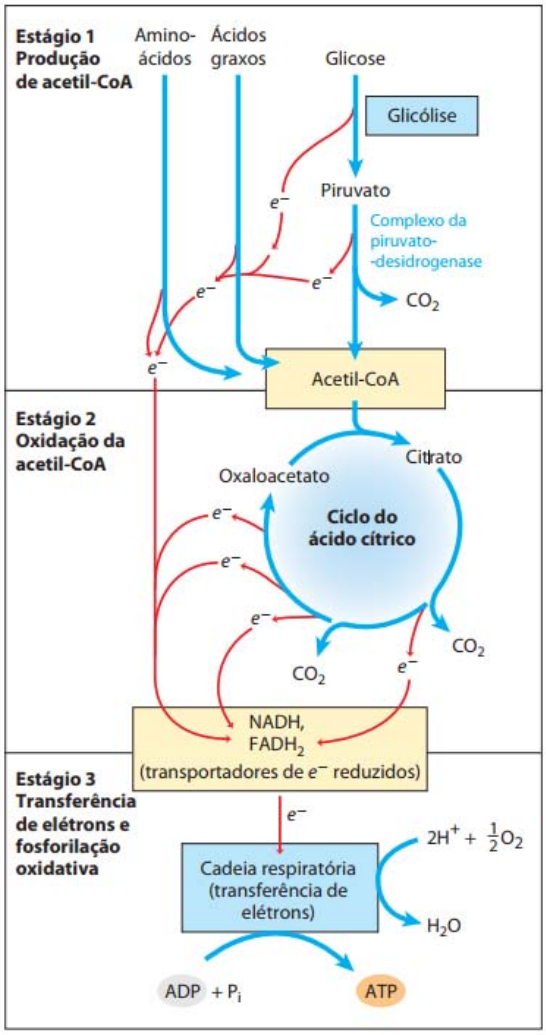


Fonte: Adaptado de SCHEFFLER, T. L.; GERRARD, D. E. Mechanisms controlling pork quality development: The biochemistry controlling pós-morte energy metabolism. Meat science, v. 77, n. 1, p. 7-16, 2007.

A respiração celular acontece em três estágios principais (Figura 5). No primeiro, moléculas combustíveis orgânicas – glicose, ácidos graxos e alguns aminoácidos – são oxidadas para produzirem fragmentos de dois carbonos, na forma do grupo acetil da acetilcoenzima A, em seguida no segundo estágio, os grupos acetil entram no ciclo do

ácido cítrico, que os oxida enzimaticamente a CO2; a energia liberada é conservada nos transportadores de elétrons reduzidos NADH e FADH2; já no terceiro estágio da respiração, estas coenzimas reduzidas são oxidadas, doando prótons (H+) e elétrons, que são transferidos ao O2 por meio de uma cadeia de moléculas transportadoras de elétrons, sendo a grande quantidade de energia liberada é conservada na forma de ATP, por um processo chamado de fosforilação oxidativa.

Figura 5. Catabolismo de proteínas, gorduras e carboidratos durante os três estágios da respiração celular.



Fonte: NELSON, D. L.; COX, M. M. Princípios de bioquímica de Lehninger. Artmed Editora, 2014.

O sistema anaeróbico é capaz de responder imediatamente às demandas de energia do exercício e é capaz de suportar a aplicação de força muscular extremamente alta e a produção de energia. O sistema de energia aeróbica responde rapidamente às demandas de exercícios intensos, mas devido a uma taxa relativamente baixa de rotatividade de ATP, é incapaz de atender às demandas de energia no início do exercício, independentemente da intensidade do exercício ou exercício intenso (BAKER; MCCORMICK; ROBERGS, 2010).

1.2.3. Efeito da condição sexual sobre o perfil de fibras musculares e metabolismo energético muscular ante e pós-morte

Seideman, Crouse e Cross (1986) mostraram que a condição sexual afeta a composição do tipo de fibra e suas características e com isso, a composição da carcaça e a qualidade da carne. O efeito de andrógenos já foi amplamente descrito indicando uma maior hipertrofia das fibras musculares uma vez que machos não-castrados apresentam maiores áreas transversais das fibras quando comparados com animais castrados (JIANG; GE, 2014; WEGNER et al., 2000).

Estudos também mostram diferenças no perfil das fibras entre animais de diferentes condições sexuais, como descrito por (BRANDSTETTER; PICARD; GEAY, 1998) os quais mostraram que a partir dos 12 meses de idade, animais inteiros apresentaram maiores proporções de fibras do tipo I e menores proporções de fibras do tipo IIx. No mesmo sentido, Moran et al. (2017) e Picard, Robelin e Geay (1995) observaram maiores proporções de fibras glicolíticas em machos inteiros quando comparados com castrados.

Essa alteração no perfil de fibras no músculo consequentemente irá alterar o metabolismo energético, uma vez que músculos com maiores proporções de fibras do tipo

I irão depender de um metabolismo mais oxidativo para geração de energia do que músculos com maiores proporções de fibras do tipo II. Menor atividade oxidativa e maior atividade glicolítica foi observado em músculos de bovinos castrados (BRANDSTETTER; PICARD; GEAY, 1998). Moran et al. (2017) observaram maiores atividades da enzima fosfofrutoquinase (PFK), uma das principais enzimas reguladoras do fluxo glicolítico, em animais castrados embora não tenham observado alteração da atividade de enzimas da via oxidativa.

O metabolismo energético predominante no músculo na hora do abate irá influenciar fortemente os processos bioquímicos da conversão do músculo em carne. Durante o período pós-morte, muitos mecanismos homeostáticos do animal são interrompidos, o que leva a uma série de mudanças energéticas, bioquímicas e físicas que ocorrem rapidamente no músculo, resultando na sua conversão em carne (MATARNEH et al., 2017a). A partir da sangria, o animal perde a habilidade de distribuir oxigênio para os tecidos, entrando em um estado de anoxia. Do mesmo modo, o músculo esquelético continua a sintetizar e utilizar ATP através do glicogênio e compostos de fosfato de alta energia armazenados no músculo (SCHEFFLER; PARK; GERRARD, 2011). Devido ao metabolismo anaeróbico ser menos eficiente do que o metabolismo aeróbico, o consumo de ATP excede a sua produção e consequentemente desencadeia o início do rigor mortis (NELSON; COX, 2014).

O rigor mortis, também conhecido por rigidez cadavérica, é um processo irreversível de contração muscular caracterizado pela inextensibilidade e rigidez do músculo (MATARNEH et al., 2017a). O rigor mortis apresenta três fases: na fase de pré-rigor, os níveis de ATP e glicogênio muscular ainda são elevados e o músculo ainda é extensível; na fase de rigor ocorre um aumento gradativo da contração muscular até que atinja seu grau máximo (PRATES, 2000), quando as cabeças de miosina permanecem

aderidas aos sítios de ligação da actina devido à deficiência de ATP para sua liberação (NELSON; COX, 2014); e a pós-rigor que é caracterizada pela diminuição da dureza da carne até que se atinjam valores semelhantes aos encontrados antes do abate através das calpaínas (PRATES, 2000). O término do rigor mortis ocorre entre 6-12 horas pós-morte, dependendo principalmente da espécie, tipo de fibra muscular e condições ante e pós-morte (MATARNEH et al., 2017a).

A taxa de declínio do pH durante a conversão do músculo em carne reflete a intensidade de metabolismo pós-morte. Durante as primeiras alterações pós-morte nos músculos de animais abatidos, o pH no músculo cai de aproximadamente 7,0 a 7,3 e para 5,5 a 5,8 após 24 horas (HONIKEL, 2014; PEARSON; YOUNG, 1989). Scopes (1974) concluiu que a taxa de hidrólise de ATP pelas ATPases musculares impulsiona a taxa de metabolismo pós-morte. Concomitante com a hidrólise do ATP ocorre a produção de íons H+, e o acúmulo de íons H+ acidifica os músculos devido à falta de mecanismos de eliminação da célula (ENGLAND et al., 2017).

A quantidade de enzimas e atividade enzimática, tipo de fibra, e o status energético contribuem para a manutenção do turnover de ATP, e consequentemente o andamento e extensão do metabolismo pós-morte (SCHEFFLER; PARK; GERRARD, 2011). Músculos oxidativos possuem menor atividade e abundância de enzimas glicolíticas (menor capacidade glicolítica) e, portanto, uma taxa de metabolismo pós-morte mais lenta quando comparado com músculos glicolíticos (MATARNEH et al., 2017b). No entanto, a taxa de declínio do pH, o pH final no qual esse processo termina e a temperatura muscular contribuem para a determinação das características de qualidade da carne, como cor, capacidade de retenção de água e textura.

A resolução do pH final coincide com a terminação da glicólise, e isso tem sido atribuído pela falta de substrato disponível (FERGUSON; GERRARD, 2014). Henckel et al. (2002) encontraram que a concentração mínima de glicogênio para que haja uma queda normal de pH é de 53 μmol/g de músculo. De acordo com Van Laack, Stevens e Stalder (2001), o aumento nos níveis de glicogênio acima de 53 μml/g de músculo não está associado com maior queda de pH.

A resolução do pH final tem sido atribuída também pela inatividade de uma ou mais enzimas glicolíticas devido a condições acidas (FERGUSON; GERRARD, 2014). England et al. (2014) indicaram que a PFK começa a perder atividade com o pH em torno de 5,9 e se torna completamente inativa em pH de 5,5. Além disso, a mitocôndria contribui significantemente para o metabolismo pós-morte aumentando a degradação de glicogênio, o acúmulo de lactato e declínio do pH (MATARNEH et al., 2018). No entanto, esses fatores não são suficientes para predizer mais do que 50% da variação do pH final (VAN LAACK; STEVENS; STALDER, 2001). Portanto, entender os fatores que determinam o pH é o ponto chave para minimizar as variações na qualidade da carne (MATARNEH et al., 2017a).

1.3. Características de qualidade de carne

As características de qualidade importantes para a carne são cor, capacidade de retenção de água, textura e conteúdo de gordura (intramuscular/intermuscular /subcutânea), enquanto as características importantes para a qualidade da carne cozida são maciez, sabor e suculência (JOO et al., 2013). Em geral, os consumidores classificam a cor como a característica de qualidade mais importante para carne fresca, enquanto a maciez é classificada como a característica de palatabilidade mais importante para carne cozida, seguida de sabor e suculência (GLITSCH, 2000).

Entretanto existe grande variabilidade da qualidade da carne, gerando dificuldade para indústria comercializar seus produtos, e apesar de muito trabalho para entender a base científica dos atributos de qualidade, sua avaliação, previsão e controle ainda permanecem falhos (MULLEN, 2002). De acordo com a mesma autora, as razões para a variabilidade da qualidade da carne são numerosas, mas elas decorrem do fato de que esses atributos de qualidade são alterados por fatores ao longo da cadeia produtiva, pós-abate, e unidade de processamento.

A maciez é a mais importante característica de qualidade alimentar, pois influencia fortemente a percepção de aceitação do consumidor (KIM et al., 2013; MILLER et al., 2001). Embora seja o atributo de qualidade mais importante no momento do consumo, 25% das carnes não atendem às expectativas dos consumidores em relação à maciez (HENDRIX, 2016). A grande inconsistência da maciez é a principal razão para a insatisfação do consumidor, levando a um grande problema para a indústria de carne bovina.

A maciez da carne bovina é uma característica altamente variável e músculo-específica, pois diferentes músculos da mesma carcaça podem exibir variações consideráveis (SEGGERN et al., 2005). Existe grande variação na maciez da carne, isso se dá devido ao grande número de vias que exercem influência sobre essa característica (HUFF LONERGAN; ZHANG; LONERGAN, 2010). As características bioquímicas distintas de diferentes músculos, como suscetibilidade de proteínas estruturais à proteólise (WHEELER; KOOHMARAIE, 1994), conteúdo do tecido conjuntivo (SEIDEMAN et al., 1984) e comprimento de sarcômero (KOOHMARAIE et al., 1988) contribuem para as variações intermusculares da maciez da carne bovina.

A maciez da carne é atribuída ao enfraquecimento da estrutura miofibrilar altamente organizada devido à degradação proteolítica das principais proteínas pós-

morte, tendo os sistemas enzimáticos das calpaínas e catepsinas importantes na proteólise pós-morte (LOMIWES et al., 2014b). Resumidamente, a falha das bombas iônicas no pós-morte com o esgotamento de ATP resulta no acúmulo de Ca2+ sarcoplasmático a concentrações necessárias para ativar isoformas μ e m da calpaína que, por sua vez, hidrolisam proteínas miofibrilares (GOLL et al., 2003; KOOHMARAIE, 1994). Além disso, o enfraquecimento da membrana lisossômica à medida que o pH do músculo diminui durante o período pós-morte, libera catepsinas dos lisossomos tornando-as disponíveis para hidrolisar proteínas miofibrilares (SANCHO et al., 1997; SENTANDREU; COULIS; OUALI, 2002).

A heterogeneidade das fibras musculares presente nos músculos também exerce influência sobre a maciez (MALTIN et al., 2003), entretanto, a relação das fibras musculares com a maciez ainda é controversa. Durante o período pós-morte o comprimento dos sarcômeros variam porque cada fibra muscular entra em rigor em momentos diferentes, e consequentemente, dependendo do tipo de músculo, haverá variação na taxa de glicólise, no início do rigor e na extensão da glicólise (ERTBJERG; PUOLANNE, 2017). Maiores comprimentos de sarcômeros indicam menor sobreposição dos filamentos de actina e miosina e, consequentemente, menor número de pontes cruzadas de actomiosina e um produto mais macio (LONERGAN; TOPEL; MARPLE, 2019).

A cor é uma característica importante da carne que influencia as decisões de compra, uma vez que os consumidores usam a cor da carne como uma indicação de frescor e qualidade (FAUSTMAN; CASSENS, 1990; KIM et al., 2013; MANCINI; HUNT, 2005; PONNAMPALAM et al., 2013). Uma cor vermelha cereja é um dos pré-requisitos para que a carne seja considerada normal (MANCINI; HUNT, 2005), enquanto a descoloração da carne influencia criticamente as decisões de compra dos consumidores

e, simultaneamente, o preço da carne (VILJOEN; DE KOCK; WEBB, 2002) podendo levar à rejeição do produto e perdas econômicas. Nos Estados Unidos, a indústria de carne incorre em uma perda anual estimada de mais de US$ 1 bilhão devido a descontos nos preços induzidos pela descoloração (SMITH et al., 2000).

Os consumidores consideram a cor da carne um dos atributos mais importantes na compra (SMITH et al., 2000). No entanto, 15% de todos os cortes de carne bovina no varejo não atendem às expectativas associadas à designação magra de vermelho cereja (SMITH et al., 2000; KILLINGER et al., 2004). Variações na qualidade da carne ocorrem por vários aspectos fisiológicos inerentes ao animal, incluindo idade, sexo, raça, taxa de crescimento e nutrição (SUMAN; JOSEPH, 2014).

Entretanto, a cor da carne está relacionada principalmente à concentração de pigmentos (principalmente mioglobina e seu estado químico), ao potencial antioxidante da carne, à estrutura das fibras, tipo de músculo, além de vários fatores de manejo e processamento de animais que se manifestam amplamente em alterações no metabolismo energético pós-morte (ABRIL et al., 2001; SUMAN et al., 2014).

1.3.1. Efeito da condição sexual sobre a maciez e cor da carne

Existem na literatura diversos trabalhos que estudaram o efeito da castração sobre características de qualidade de carne onde foram encontrados diferença nos resultados de cor (GÓMEZ et al., 2017; MARTI et al., 2013; MONTEIRO; NAVAS; LEMOS, 2014; PANJONO et al., 2009) e maciez (ANARUMA et al., 2020; MARTI et al., 2013; PURCHAS; BURNHAM; MORRIS, 2002) na carne de animais castrados quando comparados com inteiros. Além disso, estudos mostram também que animais inteiros apresentam maiores valores de pH final quando comparado com castrados (GÓMEZ et al., 2017; MARTI et al., 2013).

A taxa e extensão do declínio do pH é um dos fatores mais importantes que alteram a qualidade da carne, pois tem influência sobre a cor e a maciez da carne. Quando o músculo apresenta uma queda do pH gradual e sutil, com o pH final ficando acima de 5,8, ocorre o aumento da incidência de carnes escuras, firmes e secas (DFD). Uma queda reduzida no pH está, geralmente, associada com as reservas limitadas de glicogênio, restringindo assim a capacidade muscular para realizar a glicólise pós-morte (SCHEFFLER; PARK; GERRARD, 2011). Em geral, a carne de touros apresenta pH final mais alto, o que geralmente está associado à carne DFD devido às menores concentrações de glicogênio, uma vez que esses animais são mais susceptíveis ao estresse pré-abate.

A carne DFD é tipicamente rejeitada pela maioria dos consumidores devido à sua aparência; além disso, a carne de alto pH é mais propensa ao crescimento de microrganismos, o que pode reduzir bastante a vida útil da carne (GILL; NEWTON, 1979). Em relação a maciez, carnes com um pH final alto (> 6,2) geralmente apresentam maciez semelhante ou maior em comparação com carnes com um pH final de cerca de 5,5 (GRAYSON et al., 2016; LOMIWES et al., 2014; WATANABE; DEVINE, 1996). Estudos mostram que pH final na faixa de 5,8 a 6,2, encontra-se uma menor quantidade de carnes macias (JEREMIAH; TONG; GIBSON, 1991; PURCHAS, 1990).

Além disso, o pH final elevado de carnes DFD influencia a cor devido a maior retenção de água nas fibras gerando menor espaço entre as mesmas, e com isso uma menor dispersão da luz deixando a carne mais translucida, e com isso, com uma cor mais escura (PONNAMPALAM et al., 2017). O aumento da capacidade de retenção de água e a estrutura fechada de miofibrilas em carnes DFD resistem à difusão de O2, e juntamente com alta taxa de consumo de oxigênio pela mitocôndria e baixa taxa de oxidação, torna a desoximioglobina o pigmento dominante (BEKHIT; FAUSTMAN, 2005).

O pH da carne pode ser afetado também pelo rápido resfriamento das carcaças, uma vez que animais inteiros, geralmente, apresentam menor cobertura de gordura subcutânea (BLANCO et al., 2020; CANCIAN et al., 2013; KIRKLAND et al., 2006) e com isso as carcaças resfriam mais rapidamente fazendo com que haja uma redução na queda do pH além de poder ocasionar o encurtamento das fibras pelo frio, reduzindo a maciez da carne (HUFF LONERGAN; ZHANG; LONERGAN, 2010).

Outra possível alternativa para diferença na queda de pH entre diferentes condições sexuais, independentemente das concentrações de glicogênio no momento do abate e acabamento de carcaça, é o metabolismo energético predominante no músculo. Conforme já citado anteriormente, animais inteiros apresentam metabolismo mais oxidativo que animais castrados e de acordo com England et al. (2016), músculos oxidativos param o metabolismo pós-morte prematuramente quando comparados com glicolíticos, mesmo na presença de glicogênio, uma vez que apresentam menor capacidade glicolítica. Apaoblaza et al. (2020) mostraram que em função do metabolismo mais oxidativo, animais podem apresentar carnes mais escuras. Por outro lado, músculos mais glicolíticos são mais susceptíveis a uma taxa de glicólise pós-morte mais rápida contribuindo para maior maciez (PICARD; GAGAOUA, 2020) .

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2. Effect of sexual condition on growth, performance, carcass traits, cuttability and meat quality of Nellore cattle

Resumo: o objetivo deste trabalho foi avaliar curvas de crescimento, desempenho, características de carcaça, rendimento de cortes e qualidade da carne de bovinos Nelore castrados e não castrados durante os períodos de recria e terminação. Para isto, 240 machos Nelore foram divididos em dois tratamentos, castrados (CA; n = 120) e não-castrados (NC; n = 120). A deposição de músculo e gordura foi avaliada por ultrassonografia de carcaças durante as fases de crescimento e terminação. Foram obtidos dados de desempenho, características de carcaça e rendimento de cortes. Além disso, as características de qualidade da carne de amostras maturadas por 0, 7 e 14 dias foram avaliadas. O ganho de peso, músculo e deposição de gordura em função da idade apresentaram um crescimento quadrático, e o padrão de deposição foi semelhante entre os tratamentos. Os animais NC apresentaram maior ganho de peso médio diário (1,8 vs. 1,5kg/d), eficiência alimentar (168,2 vs. 149,0), e 43,2 kg mais pesados que animais CA durante o período de terminação e, consequentemente, apresentaram carcaças 26kg mais pesadas e mais magras. Além disso, quartos e cortes comerciais do traseiro mais pesados foram observados em animais NC. Foi observado um efeito do tratamento para todas as características de qualidade da carne com maiores valores de L*(38,8 vs. 33,2), a* (19,2 vs. 17,8), e b*(16,5 vs. 15,1) e menor força de cisalhamento (7,7 vs. 8,1 kg) na carne de animais CA. A castração diminui o peso corporal e a deposição de massa magra e aumenta a deposição de gordura. Os animais NC apresentam melhor desempenho no confinamento resultando em maiores carcaças com maior rendimento de corte. A castração reduziu a incidência de cortes escuros e melhorou a cor e maciez da carne.

Palavras-chave: castração, crescimento, cor, maciez, rendimento de cortes, sexo.

Abstract: the aim of this study was to evaluate growth curves, performance, carcass traits, cuttability yield, and meat quality of castrated and non-castrated Nellore cattle during growing and finishing periods. For that, 240 Nellore males were split into two treatments, castrated (CA; n = 120) and non-castrated (NC; n = 120). Muscle and fat deposition were evaluated by ultrasound during the growing and finishing phases. Performance, carcass traits, and cutting yield data were obtained. Also, meat quality traits of samples aged for 0, 7, and 14 days were measured. The growth, muscle, and fat deposition as a function of age showed a quadratic effect, and the pattern of deposition was similar between treatments. NC animals showed higher average daily weight gain (1.8 vs. 1.5 kg/d), feed efficiency (168.2 vs. 149.0 g/kg), and 43.2 kg heavier than CA animals during the finishing period and, consequently, presented carcasses 26 kg heavier and leaner. Also, higher cutting yield and hindquarter primal cuts were observed in NC animals. A treatment effect was observed for all meat quality traits with higher values of L* (38.8 vs. 33.2), a* (19.2 vs. 17.8), and b* (16.5 vs. 15.1) and lower shear force (7.7 vs. 8.1 kg) in meat from CA animals. Castration decreases BW and lean deposition and increases fat deposition. NC animals perform better in the feedlot resulting in greater carcasses with higher cutting yield. Castration improved beef ultimate pH, meat color, and tenderness.

Key words: castration, cutting yield, gender, growth, meat color, meat tenderness.

2.1. Introduction

Meat is a high nutritional quality food, indispensable for a well-balanced and healthy diet. According to experts, the projections show that meat demand will increase between 40-60% by 2050 due to population and income growth, and along with the

increase in pressure on the environment, as stated by Greenwood (2021), it underlies the need for improvements in productivity and efficiency of beef production.

Improving the productive efficiency of beef cattle can be achieved by encompassing different strategies such as animal breeding, genetic improvement, gender status, reproductive and nutritional management, besides practices that promote animal welfare.

Gerrard and Grant (2006) stated that one of the easiest ways to increase efficiency, in terms of feed and growth efficiency, is to eliminate castration. Several previous studies have shown that intact males are more efficient in converting food into high biological value protein (muscle/lean meat) in about 13-15% compared to castrated ones (Field et al., 1971) and, according to Steen (1995) non-castrated cattle may show 19% greater daily weight gain when compared to castrated animals.

In the meantime, the use of intact males still faces resistance in the different segments of the productive sector, since non-castrated males are more reactive, which difficult the management, and produce leaner and less tender meat, impairing quality. In addition, a lack in uniformity for tenderness, higher collagen maturation, and darker color have been reported in meat from non-castrated animals, in addition to a tendency of higher dark cutting incidence (ABERLE et al., 2012; GÓMEZ et al., 2017).

In Brazil, raising intact males for beef production is a well-stablished practice, where animals are raised on pasture up to about 24 months and then feedlot finished for short periods (70-90 days). According to Dias (2022 – personal communication) about 67% of the cattle slaughtered in Brazil in 2021 by JBS slaughterhouses was from intact males.

Despite previous studies reporting differences in performance, carcass and meat quality traits in a variety of breeds, few studies have described detailed information on

growing and finishing phases of Nellore breed and, given the relevance of this product worldwide, this information can contribute to better evaluate the impact of this production system on meat yield and quality.

Therefore, the aim of this study was to evaluate growth curves, performance, carcass traits, cuttability yield and meat quality of castrated and non-castrated Nellore cattle during growing and finishing periods.

2.2. Material and methods

All procedures used were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines (protocol #2008.04.005) and approved by the committees of the College of Animal Science and Food Engineering – University of Sao Paulo.

2.2.1. Animals and feeding management

Two hundred and forty Nellore purebred males were used in a three-year (80 animal/year) study. Each year, half of the animals were castrated at weaning, with approximately 8–10 mo. old and 219 ± 22 kg of body weight (BW), while the other half remained intact.

For the surgical castration, animals were restrained in a chute, and an anesthesia was induced 5 min before castration with 1% lidocaine injected above the skin incision line. A scalpel was used for scrotal incision and the testicles were removed by traction. Intramuscular penicillin (10,000 IU/kg BW) and flunixin meglumine (1.1 mg/kg BW; Banamine®; Schering-Plough, Brazil) were given after the procedure. All the castration procedures were performed by a veterinarian.

After weaning animals were kept together in pasture (Brachiaria brizantha Cv Marandu grass) and supplemented with a mineral mixture up to 20 mo. old, when they

were moved to the feedlot facilities. Animals were randomly housed either in individual (n = 32) or collective (n = 48) pens equipped with electronic gates (Calan Gates, American Calan Inc., Northwood, NH, USA). The animals were subjected to a 21-d adaptation period and then fed a common diet (Table 1). Orts were weighed daily and sampled for dry matter determination to calculate the daily dry matter intake (DMI).

Table 1. Composition and analyzed nutrient content (DM basis) of the experimental diet.

Item	% of DM
Ingredients	
Sugarcane bagasse	15.0
Corn grain	50.0
Soybean meal	11.8
Corn gluten feed	23.0
Mineral mix[1]	0.2
Chemical composition, %	
Dry matter	
Crude Protein	14.3
TDN[2]	25.4
Ether extract	2.4

[1] The trace mineral mixture contained (minimum per kilogram): calcium, 120 g; cobalt, 18 mg; phosphorus, 15 g; magnesium, 12 g; iron, 400 mg; sulfur, 22 g; copper, 297 mg; manganese, 846 mg; 2.5 mg Cr; selenium, 5 mg; zinc, 1100 mg; iodine, 22 mg; vitamin A, 110,000 UI; vitamin D 15,000 UI; 100 UI Vit. D; monensin sodium,1000 mg. [2] Total digestible nutrients calculated according to [13].

2.2.2. Live animal measurements

The BW and real-time ultrasound measurements of Longissimus muscle area (ULMA) and backfat thickness (UBFT) between the 12/13th ribs, and rump fat thickness (URFT) over the Biceps femoris muscle between ilium and ischium, were taken as described by Silva et al. (2012).

Data was collected from weaning until the end of finishing phase, every approximately 28 days. Ultrasound images were acquired using an Aloka® equipment,

model SSD 500 Micrus (Aloka Co. Ltd., Zug, Switzerland), with a linear probe 3.5 mHz and 172 mm. Images were saved in a portable computer and interpreted using an image analysis software (Lince, M&S Consultoria Agropecuaria Ltda, Pirassununga, Brazil). Average daily gain (ADG) and gain to feed ratio were calculated based on DMI data and BW measurements.

2.2.3. Carcass traits and muscle sampling

When reached a 5mm average UBFT, animals were harvested at the University of Sao Paulo abattoir, located about 200 m from the feedlot facilities, in accordance with human practices and commercial harvesting procedures. After evisceration weights of kidney, pelvic and inguinal (udder and cod) fats (KPIF) and hot carcass (HCW) were collected.

After 24h of chilling (0 – 2 ºC) cold carcass weight (CCW) and pH (pH24; Hanna Instruments model HI8314, Sao Paulo, Brazil) were registered. Then the right-side of each carcass was ribbed between the 12/13th ribs and digital images of the Longissimus thoracis surface were obtained, to determine carcass loin muscle area (CLMA) and carcass backfat thickness (CBFT). Following, three 2.5cm LT samples were collected, individually identified and vacuum packaged, and aged (0-2ºC) for 0, 7 and 14 days for further meat quality analyzes.

The left side of each carcass was boned and primal forequarter, hindquarter and combined plate, flank, and short ribs, total saleable meat, bones, fat trim, were weighed. The evaluated cuts were striploin (# 2140), tenderloin (# 2150), rump (# 2090), rump cap (# 2091), inside (# 2010), outside flat (# 2050), eye of round (# 2040), knuckle (# 2070; UNECE, 2004).

2.2.4. Color, cooking loss and shear force

At the end of each aging period, samples were removed from vacuum packages and allowed to bloom at 4 °C for 30 min. Subsequently, an objective evaluation of meat color was performed using the CIELab system (CIE, 1986) using a portable spectrophotometer, model CM2500d (Konica Minolta Sensing Inc., Osaka, Japan) with standard D65 illuminant, 10° observation angle and 30 mm shutter aperture. L* (luminosity), a* (red intensity) and b* (yellow intensity) values of each sample were obtained through the average of three random readings.

After color measurements, tenderness was determined using guidelines outlined by the American Meat Science Association (AMSA, 2015). Briefly, 2.5 cm thick LT samples were weighed and roasted in an industrial electric oven (Model F130 / L – Forno de Ouro, Ltda, Sao Paulo, Brazil) set to 170°C. When samples reached an internal temperature of 40°C, samples were turned and remained in the oven until reaching the internal temperature of 71°C. Samples were then held at room temperature and cooled to ± 25°C, when they were re-weighed to determine the cooking loss (CL). Samples were then wrapped in plastic and kept in a refrigerator (4°C to 6°C) for 12 h. Six core samples, 1.27 cm in diameter, parallel to the longitudinal direction of the muscle fibers were removed for Warner-Bratzler shear force (WBSF) assessment through WBSF equipment (G-R Manufacturing Co., Manhattan, KS, USA).

2.2.5. Statistical analysis

Performance, carcass traits and cutting yield data were analyzed by analysis of variance using the MIXED procedure of SAS software (SAS Institute Inc., Cary, NC, USA) as a completely randomized design with 120 replicates per treatment. Sex condition was considered as fixed effect, time on feed within year as a random effect and animal was the experimental unit. Meat quality traits of aged meat were analyzed as repeated

measures. Covariance structures were tested, and the best fitted was used (CROWDER; HAND, 1990). Differences were considered statistically significant when $P \leq 0.05$. The equations to estimate live BW and ultrasound traits from grazing and feedlot phases were obtained by the Multiple Regression procedure of SAS software, considering sex condition and age (days) as fixed effects and year as random effect.

2.3. Results

2.3.1. Growth curves

A quadratic association was observed for BW, ULMA, UBFT, and URFT as a function of age (days) during growing phase ($P < 0.001$; Figure 1). The increase in BW and ULMA were similar between treatments during the growing phase (Figures 1-A and 1-B, respectively). Also, a greater UBFT and URFT deposition were observed in CA as animal aged (Figures 1-C and 1-D, respectively).

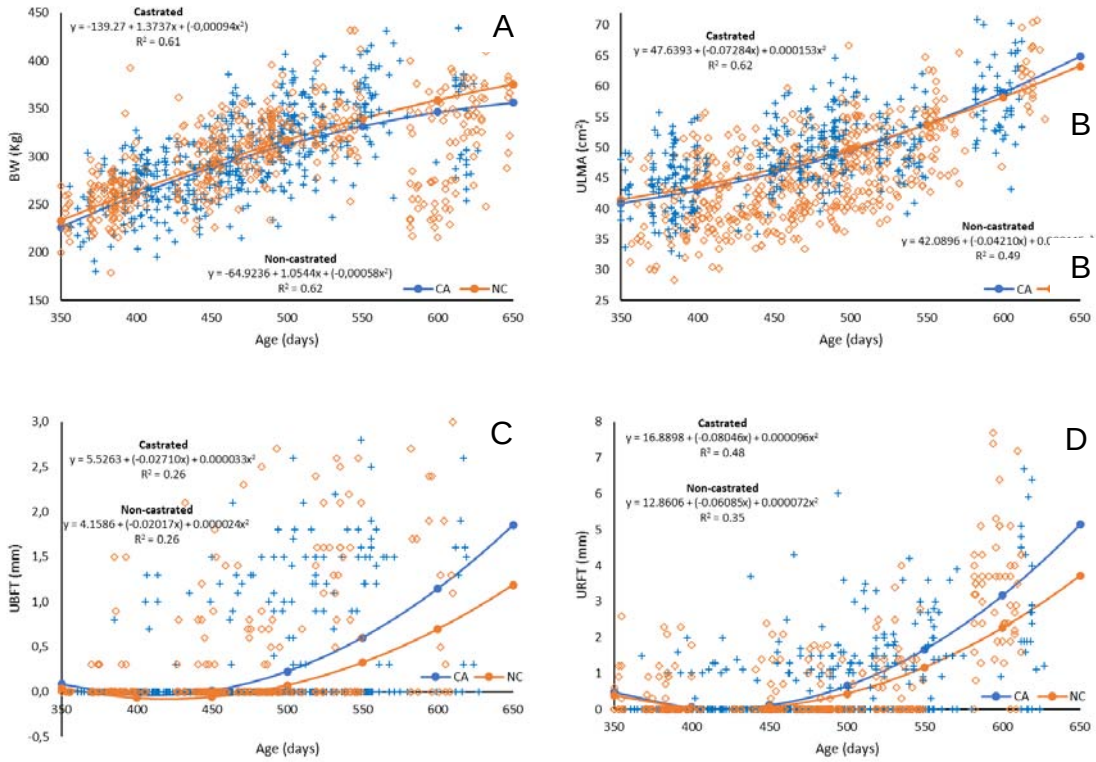

Figure 1. Body weight (BW; A) and ultrasound Longissimus muscle area (ULMA; B) and backfat thickness (UBFT; C) at 12nd rib level, and rump fat thickness (URFT; D) distribution as a function of age (days) during the growing phase of castrated (CA) and non-castrated (NC) animals.

In addition, a quadratic association was observed for BW (P < 0.001), ULMA (P < 0.001), UBFT (P = 0.028), and URFT (P = 0.008) as a function of age (days) during the finishing phase (Figure 2). The NC animals were heavier and had a higher growth rate in all ages than CA (Figure 2-A). Muscle deposition was higher in NC animals at the during finishing phase (Figure 2-B). On the other hand, the CA group showed higher UBFT and URFT deposition during the finishing phase (Figure 2-D and 2-E, respectively).

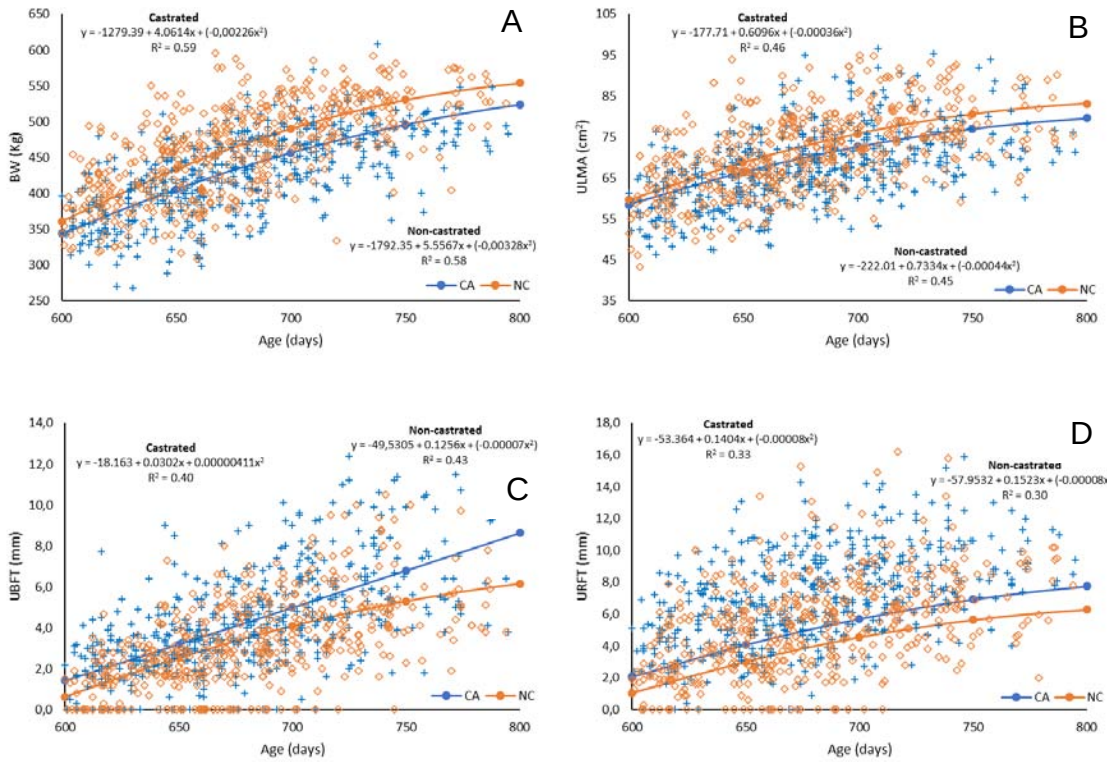

Figure 2. Body weight (BW; A) and ultrasound Longissimus muscle area (ULMA; B) and backfat thickness (UBFT; C) at 12nd rib level, and rump fat thickness (URFT; D) distribution as a function of age (days) during the finishing phase of castrated (CA) and non-castrated (NC) animals.

2.3.2. Performance and carcass traits

The NC animals started the finishing period heavier (19.0 kg; P < 0.001), had greater ADG (P < 0.001) and, consequently were heavier at slaughter (43.2 kg; P < 0.001) compared to the CA group (Table 2). The NC animals had higher DMI (P <0.001) and were 11,5% more efficient (P < 0.001) than CA group.

The NC males showed heavier HCW (26.7 kg) and CCW (26.3 kg) as well as greater CLMA (5 cm^2; P< 0.001) than CA group. Conversely, the CA animals showed higher KPIF and CBFT (P < 0.001) then the NC group, while no difference was observed found for dressing percentage between treatments. A higher pH24 (P < 0.001) was observed in NC animals.

Table 2. Effects of sexual condition on performance and carcass traits of Nellore male.

Traits	Sexual condition		SEM	P-value
	Castrated	Non-castrated		
Performance				
Initial BW, kg	353.5	372.5	8.33	<0.001
Final BW, kg	485.9	529.1	5.94	<0.001
ADG, kg/d	1.5	1.8	0.08	<0.001
DMI, kg/d	10.0	10.4	0.32	0.002
Gain:Feed, g/kg	149.0	168.2	4.23	<0.001
Carcass traits				
HCW, kg	287.8	314.5	3.66	<0.001
Dressing percentage, %	59.2	59.5	0.24	0.311
CCW, kg	283.6	309.9	3.49	<0.001
KPIF, %	3.9	3.1	0.11	<0.001
CLMA, cm2	71.5	77.4	1.05	<0.001
CBFT, mm	6.1	4.9	0.41	<0.001
pH 24h	5.54	5.68	0.04	<0.001

BW: body weight; ADG: average daily gain; DMI: dry matter intake; HCW: hot carcass weight; CCW: cold carcass weight; KPIF: kidney, pelvic, and inguinal fat; CLMA: Carcass loin muscle area; CBFT: carcass backfat thickness.

2.3.3. Retail cuts

The NC group showed higher primal FQ, HQ and combined plate, flank and short ribs, saleable meat and bone weights (P < 0.001) and lower fat trimmings (P = 0.005) than CA group (Table 3). In addition, all the HQ primal cuts analyzed were heavier (P < 0.05) in NC compared to CA animals (Table 4).

Table 3. Effects of sexual condition on cutting yield of Nellore male.

Traits (kg)	Sexual condition		SEM	P-value
	Castrated	Non-castrated		
Forequarter (5 ribs)				
Total wholesale	50.3	57.2	0.61	<0.001
Saleable meat	33.7	40.2	0.51	<0.001
Fat trimmings	6.3	5.8	0.20	0.005
Bones	10.2	11.1	0.10	<0.001
Hindquarter				
Total wholesale	65.6	70.0	0.79	<0.001
Saleable meat	47.2	51.2	0.59	<0.001
Fat trimmings	5.9	5.4	0.19	0.002
Bones	12.5	13.3	0.14	<0.001
Plate, flank, and short ribs				
Total wholesale	21.9	22.5	0.27	0.039
Saleable meat	15.6	16.3	0.30	<0.001
Fat trimmings	2.7	2.3	0.15	<0.001
Bones	3.7	3.9	0.07	0.024

Table 4. Effects of sexual condition on hindquarter primal cuts of Nellore male.

Traits (kg)	Sexual condition		SEM	P-value
	Castrated	Non-castrated		
Striploin	7.3	7.9	0.17	<0.001
Tenderloin	1.8	1.9	0.03	<0.001
Rump cap	1.6	1.7	0.04	0.003
Rump	5.0	5.5	0.07	<0.001
Knuckle	5.2	5.5	0.05	<0.001
Eye of round	2.4	2.8	0.04	<0.001
Inside	9.2	9.8	0.12	<0.001
Outside flat	5.5	6.1	0.12	<0.001

2.3.4. Meat quality

There was no sexual condition x aging time interaction for meat quality traits (Table 5). The NC animals presented a darker meat, with lower L*, a* and b* values (P < 0.001) than CA, while the latter showed lower WBSF (0.4 kg; P < 0.001) than the NC, with no difference for cooking loss between treatments (P = 0.497). The color (L*, a*, and b*; P < 0.001) and cooking loss (P = 0.003) increased quadratically with ageing time, while WBSF decreased linearly (P < 0.001) over aging period.

Table 5. Effects of sexual condition and aging time on meat quality of Nellore male.

Traits	Sexual condition		Aging (days)			SEM	P-value		
	CA	NC	0	7	14		SC	Aging	SC*Aging
L*	34.8	33.2	32.1	35.2	34.6	1.55	<0.001	<0.001	0.623
a*	19.2	17.8	18.5	18.0	19.1	0.83	<0.001	<0.001	0.103
b*	16.5	15.1	15.2	16.1	16.0	1.13	<0.001	<0.001	0.092
WBSF, kg	7.7	8.1	9.2	7.9	6.6	0.28	<0.001	<0.001	0.061
CL, %	24.5	24.3	23.6	25.1	24.5	0.59	0.497	0.003	0.264

CA: castrated; NC: non-castrated; SC: sexual condition; L*: luminosity; a*: redness; b*: yellowness; WBSF: Warner-Bratzler shear force; CL: cooking loss.

2.4. Discussion

Under favorable conditions, bulls grow 10-20% faster than steers (PURCHAS, 1991), difference which could be observed during the finishing phase, where NC animals showed a higher growth rate for BW and ULMA than castrated animals. Intact animals present higher testosterone concentrations (GÓMEZ et al., 2017), and this hormone is a driver for BW gain and also promotes lean muscle growth (SEIDEMAN et al., 1982). However, the growth rates for BW and ULMA during the growing phase (pasture) were quite similar between treatments. It can be a result of a higher muscle protein synthesis stimulated by testosterone, which comes with a significant increase in energy expenditure (LOBLEY et al., 1990), promoting a higher maintenance requirement for bulls

(GRIFFITHS, 1980). Consequently, when animals are under less favorable conditions, the differences in growth rate are less distinct (HARTE, 1984).

Although some animals showed UBFT and URFT earlier, a considerably increase in subcutaneous fat deposition began to be observed around 15-16 months of age for both treatments during growing phase. In general, CA animals were fatter than NC animals at the same age for UBFT and URFT in both stock ang feedlot period. It can be due to castrated animals reach maturity earlier than NC, and according to Jones (2014), because fat is a later developing tissue than muscle, early mature animals have an increased fat deposition compared to NC.

Even though a higher increase in BW of NC animals was observed only at the end of growing phase, it was sufficient for them to be significantly heavier at the beginning of the feedlot. In addition, NC animals gained more weight and were more efficient during the feedlot phase, hence were heavier at harvest, showing higher hot and cold carcass weight, and CLMA than castrated animals. Moreover, the higher performance of NC animals also has extended to the carcass cutability, once primal cuts and saleable meat were higher in NC animals. On the other hand, as expected, fat trimming was higher in CA animals. Similar results were found by Costa et al. (2019) for finishing performance, and by Gómez et al. (2017) for feedlot performance and carcass traits. The higher performance, better carcass traits and carcass cutability observed in NC animals may be due to a higher muscle protein synthesis caused by testosterone, which increases intracellular amino acid reutilization (FERRANDO et al., 1998) and decreases muscle protein breakdown (FERRANDO et al., 2002). Also, testosterone facilitates the incorporation of the satellite cells within the muscle fiber, thus facilitating muscle growth by muscle fiber hypertrophy (JOHNSON; BECKETT, 2014). On the other hand, the development, proliferation, and maturation of adipocytes is suppressed by testosterone

(REN et al., 2017), which can explain the lower KPIF percentage and CBFT, also lower fat trimming percentage in NC animals.

The gender also has shown to affect meat quality in this study. Although NC animals showed higher carcass pH than CA, both treatments were considered normal (APAOBLAZA et al., 2015) showing an average ultimate pH below 5.8. However, intact animals are more susceptible to present meat with higher ultimate pH due to pre slaughter stress (MOREIRA et al., 2018), increasing the incidence of dark cutting. Alternatively, as proposed by Apaoblaza et al. (2020), dark beef is not always a function of attenuated pH decline. In this study, meat from NC animals was darker even with a normal ultimate pH, suggesting that the darker meat in NC males might be a consequence of differences in muscle energy metabolism. Moran et al. (2017) found lower phosphofructokinase activity, higher proportions of MyHC I and IIa, and lower proportions of MyHC IIx in bulls compared with steers when slaughtered at the same age, suggesting a more oxidative metabolism in these animals. In addition, Brandstetter, Picard and Geay (1998), showed a higher glycolytic activity in steers compared with bulls. Muscles with lower proportions of type I fibers showed lower lightness (GAGAOUA et al., 2015). In addition, oxidative muscles have greater amounts of mitochondria (PICARD; GAGAOUA, 2020), which competes with myoglobin for oxygen, resulting in higher oxygen consumption rate (KLONT; BROCKS; EIKELENBOOM, 1998), decreasing the formation of oxymyoglobin (TANG et al., 2005), consequently developing a dark red color.

In this study, it was not found effect of gender on aging period, but in general, steaks from steers were tender than from bulls. On the other hand, Gómez et al. (2017) observed that fresh beef from steers were tender than from bulls, but no differences were observed after aging for 14 days. In addition, Silva et al. (2019) and Blanco et al. (2020) found higher tenderness in beef from steers aged for 14 days compared to bulls. As

described earlier, NC animals have more testosterone than CA, and a higher fiber hypertrophy, promoted by testosterone, is expected in these animals, and might have caused a reduction in tenderness of meat from NC. Results found by Maltin et al. (1998), Renand et al. (2001), and Beline et al. (2021), showed a lower tenderness in meat from muscles with larger fiber sizes. In addition, a higher muscle hypertrophy in intact males supported by an increase calpastatin activity may reduce postmortem tenderization (Morgan et al., 1993), resulting in less tender beef. Moreover, pH plays a role in proteolysis (GAGAOUA et al., 2021) and muscles that contain higher proportions of glycolytic fibers are more susceptible to rapid postmortem glycolysis and proteolytic degradation (OUALI, 1990), resulting in a more tender meat (PICARD; GAGAOUA, 2020). Alternatively, higher testosterone concentrations found in bulls is likely to stimulate collagen synthesis (CROSS et al., 1984), hence a greater amount of intramuscular collagen can be found in bulls than in steers (DESTEFANIS et al., 2003; GERRARD et al., 1987). According to Gerrard et al. (1987), the thermal stability of collagen increases faster in bulls, suggesting that maturation of collagen may be affected by testosterone, which decreases collagen degradation rate.

2.5. Conclusion

Growth curves are affected by gender status, especially in favorable conditions. Castration decreases BW and lean deposition and increases fat deposition. NC animals perform better in the feedlot resulting in greater carcasses with higher cutting yield. Castration reduced beef ultimate pH value, improved meat color and tenderness.

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3. Integrated metabolomic and lipidomic approach for determination of compounds and pathways related to beef quality traits of castrated and non-castrated Nellore cattle

Resumo: o objetivo deste estudo foi combinar abordagens metabolômicas e lipidômicas para explicar as diferenças nas características de qualidade da carne de bovinos Nelore castrados e não-castrados. Foram utilizados 20 machos Nelores em um delineamento inteiramente casualizado com dois tratamentos: 1) castrados (CA; n = 10) e 2) não-castrados (NC; n = 10). Vinte e quatro horas após o abate, o pH foi medido no nível da 12ª costela, e aproximadamente 10g de músculo *Longissimus thoracis* foi coletado para determinação da concentração de glicogênio, análise de lipidômica e metabolômica e uma amostra de 2,5cm de espessura foi coletada para posterior análise de cor e maciez da carne. Menores concentrações de glicogênio (7,6 vs. 18,5 µmol/g), maior valor de pH (5,73 vs. 5,60), menores valores de L* (37,4 vs. 39,1), a* (14,6 vs. 16,2), e b* (12,0 vs. 13,8) e maior força de cisalhamento (12,0 vs. 9,9 kg) foram observadas em carnes dos animais NC. Os perfis metabólitos e lipídicos e suas vias metabólicas foram relacionados principalmente ao metabolismo energético muscular, como o ciclo TCA, glicólise/gliconeogênese, piruvato, beta-oxidação mitocondrial de ácidos graxos de cadeia longa e metabolismo de ácidos graxos. Betaína, colina e piruvato foram encontrados em maiores concentrações em músculos de animais CA, enquanto creatina e xantina foram encontradas em maiores concentrações em músculos de animais NC. A maioria dos lipídeos superregulados em músculos de animai NC encontrados pertencem a classe das acilcarnitinas. Características de qualidade da carne e metaboloma e lipidoma da carne são afetados pelo status de gênero. As diferentes concentrações de metabólitos e lipídios e suas vias metabólicas relacionadas são úteis para explicar as diferenças nas

características de qualidade da carne entre animais castrados e não-castrados. Os perfis metabólitos e lipídicos mostram que o músculo de animais castrados é mais glicolítico e apresenta mais metabólitos com propriedades antioxidantes, e essas características podem influenciar no desenvolvimento da qualidade da carne.

Palavras-chave: castração, cor, lipidoma, maciez, metaboloma, sexo

Abstract: the aim of this study was to combine non-targeted metabolomic and lipidomic approaches to explain differences in meat quality traits from Nellore bull vs. steer cattle. A total of 20 Nellore males were used in a completely randomized design with two treatments: 1) castrated (n = 10) and 2) non-castrated (n = 10). Twenty-four hours after slaughter, pH was measured at the 12th rib level, and approximately 10g of Longissimus thoracis muscle was sampled for determination of glycogen concentration, lipidomic and metabolomic analysis and, one 2.5cm thick sample was collected for further meat color and tenderness analysis. Lower concentrations of glycogen (7.6 vs. 18.5 µmol/g), higher pH value (5.73 vs. 5.60), lower L* values (37.4 vs. 39.1), the * (14.6 vs. 16.2), and b* (12.0 vs. 13.8) and higher shear force (12.0 vs. 9.9 kg) were observed in meat from NC animals. The metabolite and lipid profiles and their pathways were mainly related to muscle energy metabolisms such as the TCA cycle, glycolysis/gluconeogenesis, pyruvate, mitochondrial beta-oxidation of long-chain fatty acids, and fatty acid metabolisms. Betaine, choline, and pyruvate were found in higher concentrations in muscles of CA animals, while creatine and xanthine were found in higher concentrations in muscles of NC animals. Most of the upregulated lipids found in muscles from NC belong to the acylcarnitine class. Beef quality traits and meat metabolome and lipidome are affected by gender status. The different metabolites and lipids concentrations and their related metabolic pathways are useful to explain differences in meat quality traits between

castrated and non-castrated animals. The metabolite and lipid profiles show that muscle from castrated animals is more glycolytic and presents more metabolites with antioxidant properties, and these characteristics may influence meat quality development.

Key words: castration, gender, lipidome, meat color, meat tenderness, metabolome

3.1. Introduction

Meat industry is constantly looking for methods to increase production and meat quality aiming to satisfy consumer demands. Consumers are the endpoint of the beef industry chain and meeting their expectations and satisfactions is important for future purchasing behavior (BOITO et al., 2021). Appearance and eating quality traits such as color, marbling, and tenderness contribute to consumer's perceptions of a high-quality meat (JOO et al., 2013), which can be influenced by factors such as sex, age, growth, and muscle characteristics.

The use of intact animals for beef production is efficient once bulls have shown higher gains than steers which can be a consequence of the anabolic effects of testosterone (BLANCO et al., 2020). Moreover, bulls utilize feed more efficiently (MARTI et al., 2011) and produce heavier carcasses (MOREIRA et al., 2017). However, bulls have more aggressive behavior, leaner carcasses, lower meat tenderness, and darker meat color (SEIDEMAN et al., 1982; 2017; SILVA et al., 2019). Moreover, higher DFD incidence is observed in bulls compared to steers (GÓMEZ et al., 2017) usually due to the lower glycogen concentrations usually associated with pre-slaughter stress.

Castration is a widely used practice in beef production because it facilitates handling, reduces animal aggressive behavior, stress susceptibility and carcasses injuries (GÓMEZ et al., 2017; KATZ, 2007; BONNEAU; ENRIGHT, 1995). Moreover,

castration is used to improve meat quality once it can increase fat deposition, improve meat tenderness and color, and decrease dark cutting incidence (MOREIRA et al., 2018; PANOJO et al., 2009; SEIDEMAN et al., 1982). These quality traits are affected by muscle characteristics and post-mortem metabolic processes (OUALI, 1990), and according to Jurie et al. (2006), gender affects muscle characteristics. Brandstetter et al. (1998), showed a shift towards a more glycolytic type in muscle from steers. Moreover, according to Silva et al. (2019), castration affected the conversion of muscle into meat and post-mortem proteolysis.

Thus, a comprehensive understanding of muscle biochemical processes could provide information of pathways responsible for the development of meat quality traits in bull and steer. Metabolomics is a powerful tool to characterize small molecules (metabolites) in biological samples resulting from metabolic activity (OLIVEVRONA, 2020). The characterization and quantification of low molecular weight metabolites in small samples have been extensively obtained by one-dimensional proton nuclear magnetic resonance (1D 1H NMR; EMWAS et al., 2019). 1D 1H NMR has shown to be an efficient tool to obtain meat metabolite profiles associated with meat quality traits (AKHTAR et al., 2021; ANTONELO et al., 2020b; JEONG et al., 2020; KODANI et al., 2017).

Moreover, according to Sun et al. (2020), lipidomic has emerged and developed into a valuable area of metabolomics, and its study comprehends the type and functions of lipids in biological metabolism (GRIFFITHS; WANG, 2009). Antonelo et al. (2021) observed that lipidomics was a useful tool to understand the effect of feeding regimes on beef color. Also, differentiation of meat from animals prevenient from distinct finishing system was possible by combining metabolomics and lipidomics profiling (WANG et al., 2021). Therefore, the aim of this study was to combine non-targeted metabolomic and

lipidomic approaches to explain differences in meat quality traits from Nellore bull vs. steer cattle.

3.2. Material and methods

All procedures used were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines (protocol #2008.04.005) and approved by the committees of the College of Animal Science and Food Engineering – University of Sao Paulo.

3.2.1. Animals and sampling

Animals. A total of 20 Nellore males obtained from the University of Sao Paulo research herd, located in Pirassununga/Brazil, were used in a completely randomized design with two treatments: 1) Steer (n = 10) and 2) Bulls (n = 10). Steers were surgically castrated at weaning (8–10 mo. old). Animals were grazed in Brachiaria brizantha Cv Marandu grass and mineral mixtures until 20 mo. old, when were transported to the feedlot facilities. Animals were randomly housed in individual pens and fed a standard feedlot diet for approximately 88 days.

Sampling. After feeding period, animals were harvested at the University of Sao Paulo abattoir, located about 200 m from the feedlot facility, in accordance with commercial practices and harvesting procedures. After 24h of chilling, carcasses were ribbed between the 12/13th ribs and LT muscle pH was measured at 12th rib level, using a digital pH meter (Hanna Instruments model HI8314, Sao Paulo, Brazil). Subsequently, approximately 10g of LT muscle was sampled for glycogen, lipidomic and metabolomic analysis and one 2.5cm thick sample was collected, individually identified and vacuum packaged for further meat quality analysis.

3.2.2. Meat quality analysis

Color. Samples were removed from vacuum packages and allowed to equilibrate at 4 °C for 30 min. Subsequently, an objective evaluation of meat color was performed using the CIELab system (CIE, 1986) using a portable spectrophotometer, model Minolta (CM2500d, Konica Minolta Sensing Inc., Osaka, Japan) with standard D65 illuminant, 10° observation angle and 30 mm shutter aperture. L*(luminosity), a* (red intensity) and b* (yellow intensity) of each sample were obtained through the average of three random readings.

Warner-Braztler Shear Force. Tenderness was determined using guidelines outlined by the American Meat Science Association (AMSA, 2015). Briefly, 2.5 cm thick LT samples were roasted in an industrial electric oven (Model F130 / L – Forno de Ouro, Ltda, Sao Paulo, Brazil) set to 170°C. When samples reached an internal temperature of 40°C, samples were turned and remained in the oven until reaching the internal temperature of 71°C. Then were held at room temperature and cooled to ± 25°C, wrapped in plastic and kept in a refrigerator (4°C to 6°C) for 12 h. Six core samples, 1.27 cm in diameter, parallel to the longitudinal direction of the muscle fibers were removed for Warner-Bratzler shear force (WBSF) assessment through WBSF equipment (G-R Manufacturing Co., Manhattan, KS, USA).

Glycogen. Glycogen concentration of 24h samples were obtained according to Scheffler et al. (2013). Briefly, frozen samples were homogenized at a ratio of 1:10 (w/v) with 1.25 M HCl solution, heated at 90 °C for 2 hours and centrifuged at 13,000×g for 5 minutes. Subsequently, the supernatant was neutralized with 1.25 M KOH (BERGMEYER, 1984). Glycogen analyses were performed according to the methodology proposed by Bergmeyer (1984) and modified for 96-well plates (HAMMELMAN et al., 2003).

3.2.3. Metabolome analysis

Polar metabolites extraction. Samples were powdered in liquid nitrogen and 500 mg were homogenized with 1mL methanol and 213 µL nanopure water using an Ultra-Turrax (MA102/B model, Marconi Laboratory Equipment, Piracicaba, Brazil). Next, 1mL of methanol and 500 µL of chloroform were added and homogenate was vortexed, as described by Beckonert et al. (2007). Samples were kept on ice for 15 min and then centrifuged for 10 min at 10,000×g at 4°C and 500 µL of supernatant was transferred to 1.5 mL centrifuge tubes and freeze dried.

The pellet was reconstituted in 600 µL of 100 mM phosphate buffer (containing 10% D2O and 90% H2O [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 1H NMR spectroscopy was used for metabolite profiling, and the analyses were performed at EMBRAPA Instrumentation (São Carlos, São Paulo, Brazil). The 1H 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 1H, 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 1H. Standard 1D 1H 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, 256 scans, 3.89-s acquisition time, and spectral width of 14.03 parts per million [ppm].

Spectral processing and metabolite quantitation. The 1D 1H 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.

Twenty-two metabolites were quantified in the 1D 1H 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 (22 metabolites × 9 samples per sexual condition) was exported to Microsoft Excel, where sample identifiers were added.

3.2.4. Lipidome analysis

Lipidome extraction. Approximately 50 mg of each sample (n = 20; 10 per sexual condition) was weighed and ground in liquid nitrogen for lipid extraction using a method reported by Bligh and Dyer (1959). Tissue homogenate (300 µL in ultrapure water) was transferred to a new microtube and mixed with 250 µL of chloroform and 450 µL of methanol. This solution was incubated at 4 °C for 15 min prior to the addition of 250 µL of chloroform and 250 µL of water and centrifugation for 10 min at 16,000 × g, forming a 2-phase solution where the bottom phase contained the lipids (organic phase). The organic phase was transferred to a new tube and dried using a centrifugal vacuum

concentrator (GenevacTM miVac, Genevac LTD., Ipswich, UK), and samples were stored at -80 °C until further analysis.

Multiple reaction monitoring (MRM)-profiling. Targeted lipid profiling was performed using discovery MRM-profiling methods and instrumentation as recently reviewed by Xie et al. (2021). Specifically, dried lipid extracts were diluted in 50 µL of methanol/chloroform 3:1 (v/v) and 250 µL of injection solvent (acetonitrile/methanol/ammonium acetate 300 mM 3:6.65:0.35 [v/v/v]) to obtain a stock solution. Mass spectrometry data were acquired by flow-injection (no chromatographic separation) from 8 µL of stock solution that was diluted 300x in injection solvent that was spiked with EquiSPLASH™ LIPIDOMIX® Quantitative Mass Spec Internal Standard (0.1 ng/µL of each of the internal standards) prior to being delivered using a micro-autosampler (G1377A) to the ESI source of an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). A capillary pump was connected to the autosampler and operated at a flow rate of 7 µL/min and pressure of 100 bar. Capillary voltage of the instrument was 4 kV, and the gas flow 5.1 L/min at 300 °C.

The MRM profiling method was used to profile 1,366 MRMs related to lipids, from 11 lipid classes. The MRM set included 152 phosphatidylethanolamines (PE), 62 acyl-carnitines (AC), 57 cholesteryl esters (CE), 121 phosphatidylcholines (PC), 27 sphingomyelins (SM), 148 phosphatidylinositols (PI), 152 phosphatidylglycerols (PG), 148 phosphatidylserines (PS), 36 free fatty acids (FFA), 80 ceramides (CER), and 383 triacylglycerols (TAG). TAGs were profiled using parent ions and a product ion related to the presence of specific fatty acyl residues (C16:0, C16:1, C18:0, C18:1, C18:2, and C20:4). The assigned short hand identity, with TAG 16:0_36:1 as an example, starts with class (TAG) followed by the fatty acyl chain related to the product ion (16:0) and ends with the sum of the carbon:unsaturation number related to the other two fatty acyl chains

at the TAG (e.g. 36:1), as recommended by the Lipid Maps nomenclature group (LIEBISCH et al., 2020). Phospholipids were identified by their class (PG, PS, PI, PE, or PC), the number of carbon atoms between both esterified fatty acids, and the number of carbon-carbon double bonds present in the molecule, e.g. PE (34:4). Ion intensity data of each MRM per sample was obtained using in-house scripts that were used for subsequent analysis.

3.2.5. Statistical analysis

Meat quality. Phenotypic data were evaluated as a completely randomized design using 10 replications per sexual condition. Sexual condition was considered as fixed effects, and time on feed were considered as random effect. Data were analyzed by analysis of variance 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 $P \leq 0.05$.

Metabolome. Metabolomic data were analyzed using MetaboAnalyst 5.0 (Chong et al., 2018). The metabolite concentration table was uploaded to MetaboAnalyst, and the data were Pareto-scaled prior to analysis. Treatment differentiation was obtained by using partial least square discriminant analysis (PLS-DA). PLS-DA validation was performed using a 10-fold cross validation method, and the values for R2 (cumulative interpretation ability of model; 0.73) and Q2 (predictive ability of model; 0.54) 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. 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, 6 metabolic pathways related to the identified metabolites (n=22) were mapped based on P values ($P \leq 0.05$) and pathway impact ($PI > 0$).

Lipidome. To avoid noisy MRMs, ion intensities of 1.3-fold or higher than the ion intensity for a blank sample were considered for statistical analysis. Relative ion intensity was calculated for each MRM by dividing its ion intensity by the sum of all ion intensities across the sample. MRMs were then assigned to one of two lipid classifications: 1) DG, TAG, CE, and AC; and 2) CER, PC, PE, PG, PI, PS, and SM. The effect of treatments on TAG distribution in samples was analyzed using three distinct groupings for Student's t-test analysis: 1) total number of carbons [e.g. TAG (54)]; 2) total number of unsaturation [e.g. (:4)]; and 3) total number of unsaturations grouped into 0, up to 2 and more than 3 unsaturations. The relative ion intensities were uploaded to Metaboanalyst 5.0 (https://www.metaboanalyst.ca/) (CHONG et al., 2019). Relative ion intensity data were normalized by auto-scaling and statistical analysis was performed using Student's t-test. Moreover, the information of the internal standard was used to obtain the relative quantification of the total TAG, total phospholipid, and phospholipid profile according to its class. Differences were considered statistically significant when $P \leq 0.05$.

Treatment differentiation was obtained by using PLS-DA. PLS-DA validation was performed using a 10-fold cross validation method, and the values for R2 (cumulative interpretation ability of model; 0.79 and 0.97 for method 1 and 2, respectively) and Q2 (predictive ability of model; 0.21 and 0.48 for method 1 and 2, respectively) were employed as initial indicators for evaluating the goodness of fit. In the PLS-DA model, a VIP plot was used to rank the lipids based on their relative importance of variation in discriminating groups. In addition, quantitative enrichment analysis was performed using lipid quantification data sets for each treatment. The compound name was standardized

according to KEGG, HMDB or PubChem ID and the library chosen was the small molecule pathway database (SMPDB).

3.3. Results

Meat quality. Glycogen concentration was higher in castrated animals (P = 0.030; Table 1), while meat ultimate pH was higher in non-castrated (P < 0.001). Steaks from non-castrated group had lower L* (P = 0.040; darker appearance), a* (P = 0.001; decreased redness), and b* (P = 0.002; decreased yellowness), and higher WBSF (P = 0.005; less tender) than those from castrated.

Table 1. Ultimate pH, color (L*, a*, and b*), and WBSF of Longissimus thoracis muscle from castrated and non-castrated Nellore males.

Traits	Sexual Condition		SEM	*P*-value
	Castrated (n = 10)	Non-castrated (n = 10)		
Glycogen, µmol/g	18.5	7.6	5.14	0.030
Ultimate pH	5.60	5.73	0.02	<0.001
*L**	39.1	37.4	0.77	0.040
*a**	16.2	14.6	0.46	0.001
*b**	13.8	12.0	0.44	0.002
WBSF, kg	9.9	12.0	0.67	0.005

L*: luminosity; a*: red intensity; b*: yellow intensity; WBSF: Warner-Bratzler shear force.

Metabolome. Twenty-two compounds were identified in Nellore meat based on 1D 1H NMR analysis (Table S1, supplementary data). The PLS-DA revealed distinct clusters between castrated and non-castrated Nellore animals for the metabolite concentrations (Figure 1). Based on VIP scores, compounds that presented higher percentage of explained residual in PLS-DA plot between castrated and non-castrated animals, in order of importance, were creatine, pyruvate, glycine, choline, betaine, o-

acetylcarnitine, alanine, n-nitrosodimethylamine, inosine monophosphate (IMP), methionine, xanthine, carnitine, 3-hydroxybutyrate, adenosine monophosphate (AMP), and valine, respectively (Figure 1).

Steaks from castrated animals presented higher concentrations of betaine (P = 0.024), choline (P = 0.045), pyruvate (P < 0.001), and xanthine (P = 0.002), and lower concentrations of creatine (P = 0.002) than those from non-castrated animals (Table 2).

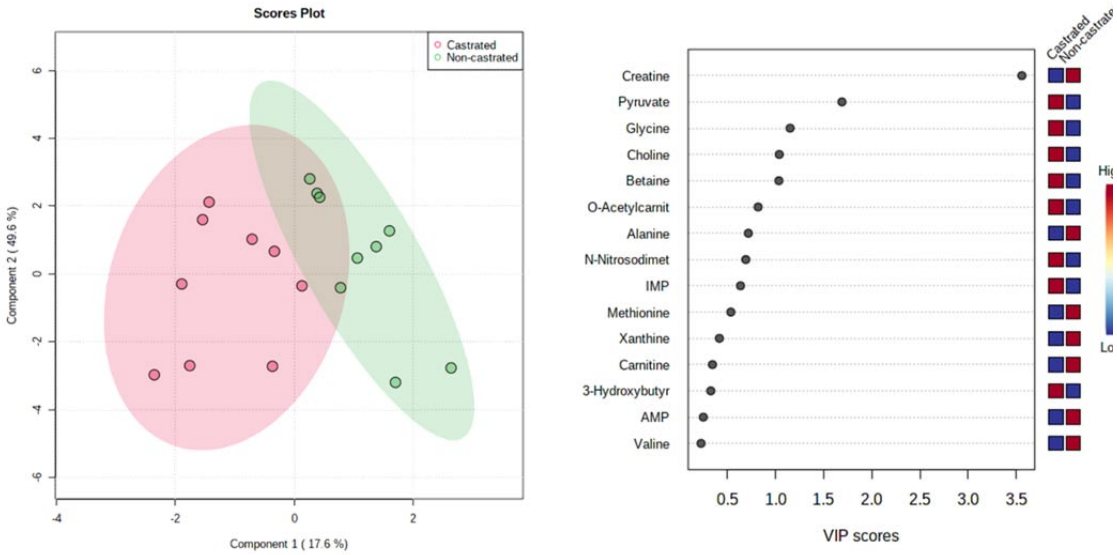

Figure 1. Partial least square discrimination analysis (PLS-DA) scores plot (left) and variable importance in projection (VIP) scores (right) of Longissimus thoracis metabolite distribution from castrated and non-castrated Nellore males. AMP: adenosine monophosphate; IMP: inosine monophosphate.

Table 2. Metabolite concentrations of Longissimus thoracis muscle from castrated and non-castrated Nellore males.

Metabolites, µmol/g meat	Sexual Condition		SEM	*P*-value
	Castrated (n = 9)	Non-castrated (n = 9)		
3-Hydroxybutyrate	0.28	0.25	0.015	0.120
ADP	1.63	1.68	0.075	0.644
AMP	1.49	1.54	0.068	0.579
Alanine	1.52	1.70	0.085	0.155
Betaine	**0.85**	**0.60**	**0.070**	**0.024**
Carnitine	2.24	2.35	0.144	0.599
Choline	**1.35**	**1.08**	**0.089**	**0.045**
Creatine	**15.99**	**18.25**	**0.431**	**0.002**
Glucose-6-phosphate	0.08	0.08	0.005	0.969
Glycine	3.10	2.47	0.424	0.308
IMP	0.57	0.46	0.042	0.072
Inosine	1.65	1.70	0.078	0.648
Isoleucine	2.44	2.44	0.023	0.919
Lactate	0.33	0.32	0.060	0.918
Leucine	0.08	0.09	0.007	0.678
Methionine	1.04	1.26	0.550	0.786
N-Nitrosodimethylamine	42.62	41.85	1.790	0.766
NADH	0.14	0.13	0.011	0.829
O-Acetylcarnitine	1.19	0.99	0.079	0.094
Pyruvate	**0.58**	**0.17**	**0.047**	**<0.001**
Valine	0.98	1.03	0.083	0.649
Xanthine	**0.06**	**0.09**	**0.006**	**0.002**

ADP: adenosine diphosphate; AMP: adenosine monophosphate; IMP: inosine monophosphate; NADH: nicotinamide adenine dinucleotide.

The most relevant pathways associated with the identified metabolites were glycine, serine, and threonine metabolism (P < 0.001; PI = 0.32), pyruvate metabolism (P < 0.0001; PI = 0.21), glycolysis/gluconeogenesis (P < 0.001; PI = 0.10), citrate cycle (TCA cycle; P < 0.001; PI = 0.05), arginine and proline metabolism (P < 0.001; PI = 0.01), and glycerophospholipid metabolism (P = 0.0446; PI = 0.03; Figure 2).

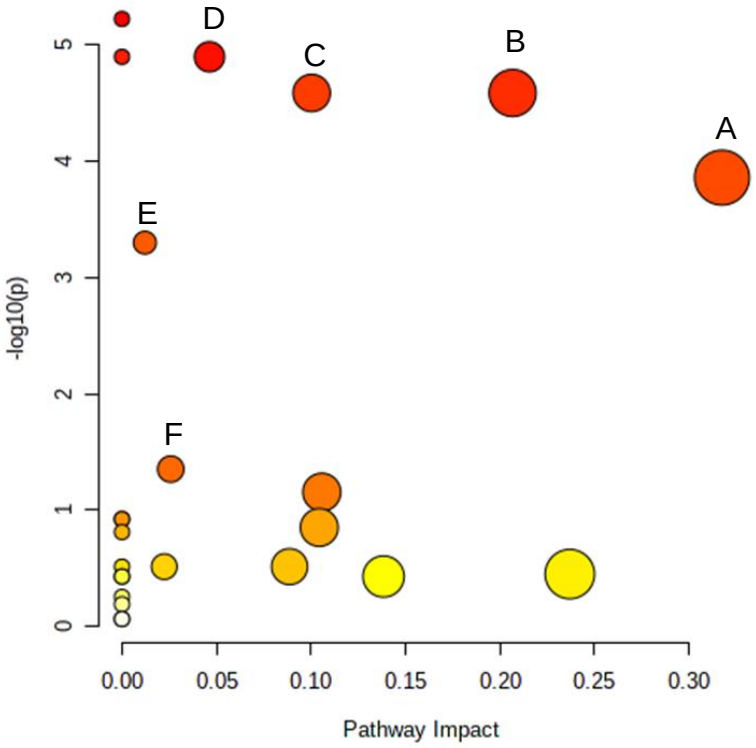

Figure 2. Metabolome view map of Longissimus thoracis metabolites from castrated and non-castrated Nellore males. Larger sizes and darker colors represent higher pathway enrichment and higher pathway impact values, respectively. All the pathways described had $P \leq 0.05$ and pathway impact > 0. A: glycine, serine, and threonine metabolism; B: pyruvate metabolism; C: Glycolysis/Gluconeogenesis; D: Citrate cycle (TCA cycle); E: Arginine and proline metabolism; F: Glycerophospholipid metabolism.

Lipidome. Of the 1,366 ion transitions (MRMs) scanned, 360 were found to have an intensity of at least 1.3-fold higher than the blank sample (injection solvent) (Table S2, supplementary data). These were related mainly to TAG (112), PC (89), AC (62) and PE (44) lipids. The PLS-DA revealed distinct clusters between castrated and non-castrated Nellore animals for the lipid methods 1 and 2 (Figure 3).

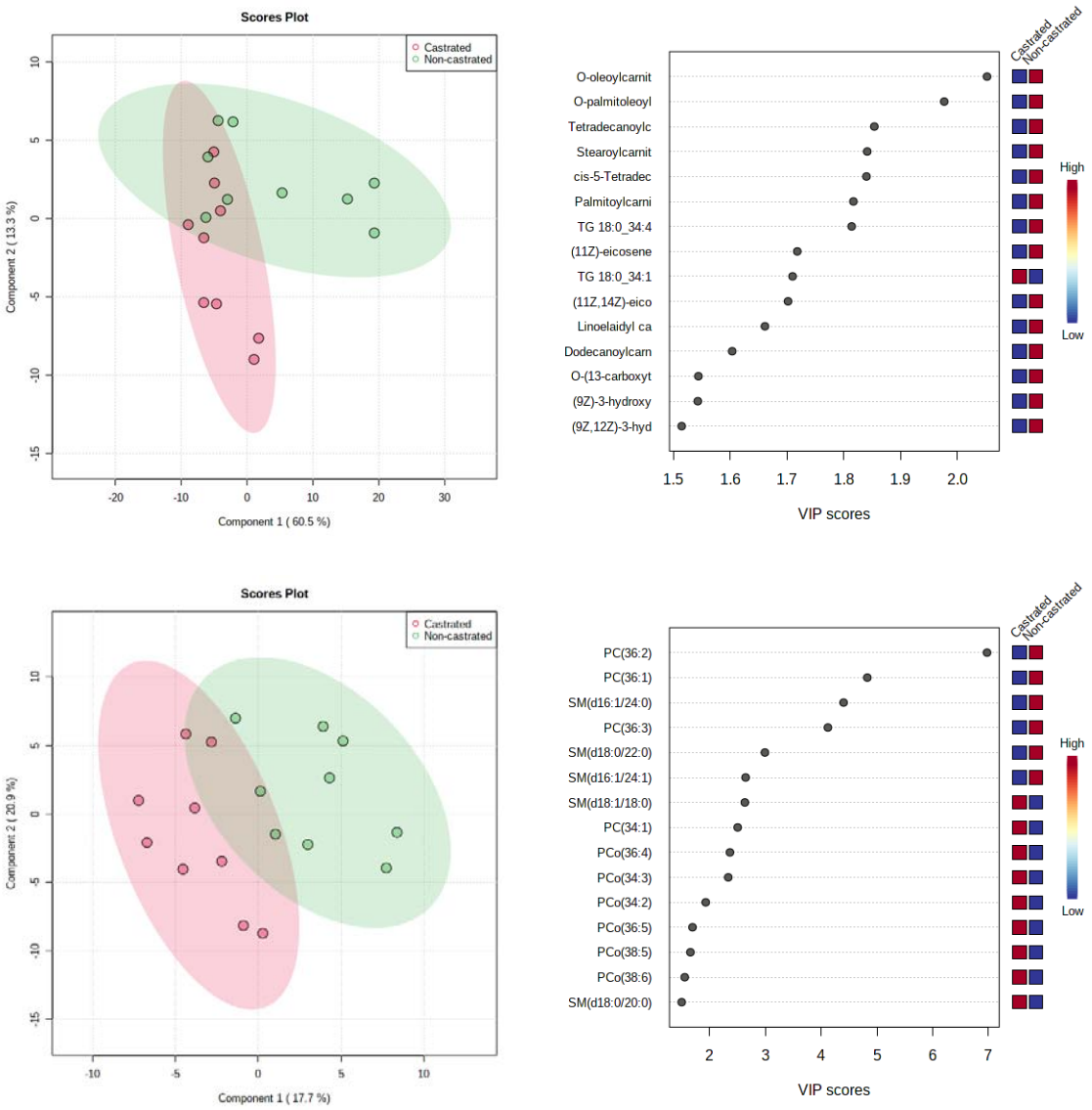

Figure 3. Partial least square discrimination analysis (PLS-DA) scores plot (left) and variable importance in projection (VIP) scores (right) of lipidomic distribution based on: Method 1 (upper) – diglyceride (DG), triglyceride (TG), cholesteryl ester and acyl-carnitine; and Method 2 (lower) – ceramides, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS) and sphingomyelin (SM) in the Longissimus thoracis muscle from castrated and non-castrated Nellore males.

Based on VIP analysis, the 15 most important compounds differing Method 1 between castrated and non-castrated Nellore animals included (in order of importance) O-oleoylcarnitine, O-palmitoleoylcarnitine, Tetradecanoylcarnitine, Stearoylcarnitine,

cis-5-Tetradecenoylcarnitine, Palmitoylcarnitine, TG 18:0_34:4, (11Z)-eicoseneoylcarnitine, TG 18:0_34:1, (11Z,14Z)-eicosadienoylcarnitine, Linoelaidyl carnitine, Dodecanoylcarnitine, O-(13-carboxytridecanoyl)carnitine, (9Z)-3-hydroxyoctadecenoylcarnitine, and (9Z,12Z)-3-hydroxyhexadecadienoylcarnitine. Moreover, the 15 most important compounds differing Method 2 between castrated and non-castrated Nellore animals included (in order of importance) PC(36:2), PC(36:1), SM(d16:1/24:0), PC(36:3), SM(d18:0/22:0), SM(d16:1/24:1), SM(d18:1/18:0), PC(34:1), PCo(36:4), PCo(34:3), PCo(34:2), PCo(36:5), PCo(38:5), PCo(38:6), and SM(d18:0/20:0).

Relative ion intensity data showed that 21 AC, 3 TAG, 4 PC and 2 SM differed ($P \leq 0.05$) between castrated and non-castrated animals (Table 3). However, there were no differences ($P \geq 0.05$) for total TAG and TAG profile according to carbon chain length and unsaturation degree between castrated and non-castrated animals (Table 4). Furthermore, no differences were observed ($P \geq 0.05$) for total phospholipids and phospholipid classes between castrated and non-castrated animals (Table 5).

Table 3. Lipids that differed ($P \leq 0.05$) in the Longissimus thoracis muscle from castrated and non-castrated Nellore males.

Lipid	Upregulation	Fold ratio[a]	*P*-value
	Method 1		
(11Z)-eicoseneoylcarnitine	Non-castrated	3.18	0.017
(11Z, 14Z)-eicosadienoylcarnitine	Non-castrated	2.50	0.018
(5Z)-13-carboxytridec-5-enoylcarnitine	Non-castrated	3.50	0.011
(9Z)-3-hydroxyoctadecenoylcarnitine	Non-castrated	2.33	0.036
(9Z,12Z)-3-hydroxyhexadecadienoylcarnitine	Non-castrated	2.00	0.040
9,12-Hexadecadienylcarnitine	Non-castrated	2.25	0.022
cis-5-Tetradecenoylcarnitine	Non-castrated	2.33	0.009
Decanoylcarnitine	Non-castrated	2.33	0.040
Dodecanoylcarnitine	Non-castrated	3.00	0.028
Elaidic carnitine	Non-castrated	5.00	0.003

Hexadecanedioic acid mono-L-carnitine ester	Non-castrated	3.16	0.009
Linoelaidyl carnitine	Non-castrated	2.25	0.022
O-(13-carboxytridecanoyl)carnitine	Non-castrated	2.00	0.036
O-dodecanoylcarnitine	Non-castrated	3.00	0.028
O-linoleoylcarnitine	Non-castrated	2.25	0.022
O-oleoylcarnitine	Non-castrated	5.00	0.003
O-palmitoleoylcarnitine	Non-castrated	4.75	0.004
O-tetradecanoylcarnitine	Non-castrated	4.00	0.009
Palmitoylcarnitine	Non-castrated	3.50	0.011
Stearoylcarnitine	Non-castrated	3.16	0.009
Tetradecanoylcarnitine	Non-castrated	4.00	0.009
trans-Hexadec-2-enoyl carnitine	Non-castrated	4.75	0.004
TG 18:0_32:2	Castrated	0.88	0.043
TG 18:0_34:1	Castrated	0.89	0.018
TG 18:0_34:4	Non-castrated	1.22	0.011
Method 2			
PC (36:1)	Non-castrated	1.23	<0.001
PC (36:2)	Non-castrated	1.31	<0.001
PC (36:3)	Non-castrated	1.27	0.012
PCo (40:5)	Castrated	0.89	0.018
SM (d16:1/24:0)	Non-castrated	1.23	<0.001
SM (d16:1/24:1)	Non-castrated	1.25	0.012

Table 4. Triglyceride profile of Longissimus thoracis muscle from castrated and non-castrated Nellore males.

Item	Sexual condition		SEM	*P*-value
	Castrated (n = 10)	Non-castrated (n = 10)		
Total triglycerides, ng/µg muscle tissue	14.6	18.0	4.40	0.593
Carbon number, %				
48	6.2	6.1	0.29	0.879
50	20.2	19.4	0.56	0.372
52	50.2	49.4	0.62	0.416
54	22.7	24.2	0.93	0.284
56	0.8	0.8	0.13	0.732
Unsaturation number, %				
0	1.9	1.8	0.16	0.686
1	15.9	15.2	0.60	0.469
2	43.2	43.0	0.34	0.768

3	33.0	33.4	0.74	0.692
4	4.1	4.3	0.18	0.314
5	0.4	0.4	0.08	0.858
6	0.3	0.3	0.05	0.840
7	0.2	0.2	0.03	0.761
8	1.1	1.2	0.09	0.389
9	0.1	0.1	0.01	0.582
Grouped unsaturation, %				
Up to 2	59.0	58.3	0.69	0.445
More than 3	39.1	39.9	0.78	0.448

Table 5. Phospholipid profile of Longissimus thoracis muscle from castrated and non-castrated Nellore males.

Class, ng/µg muscle tissue	Sexual condition		SEM	*P*-value
	Castrated (n = 10)	Non-castrated (n = 10)		
Total phospholipids	5.2	5.1	0.64	0.887
Phosphatidylcholine	3.6	3.6	0.45	0.941
Phosphatidylethanolamine	0.69	0.65	0.084	0.752
Phosphatidylglycerol	0.02	0.03	0.003	0.431
Phosphatidylinositol	0.05	0.05	0.006	0.759
Phosphatidylserine	0.04	0.05	0.004	0.579
Sphingomyelin	0.84	0.79	0.101	0.719

Quantitative enrichment analysis (Figure 4) revealed that the main metabolic pathways that were affected by lipid profile from castrated and non-castrated Nellore males were mitochondrial beta-oxidation of long chain saturated fatty acids (P = 0.009) and fatty acid metabolism (P = 0.010).

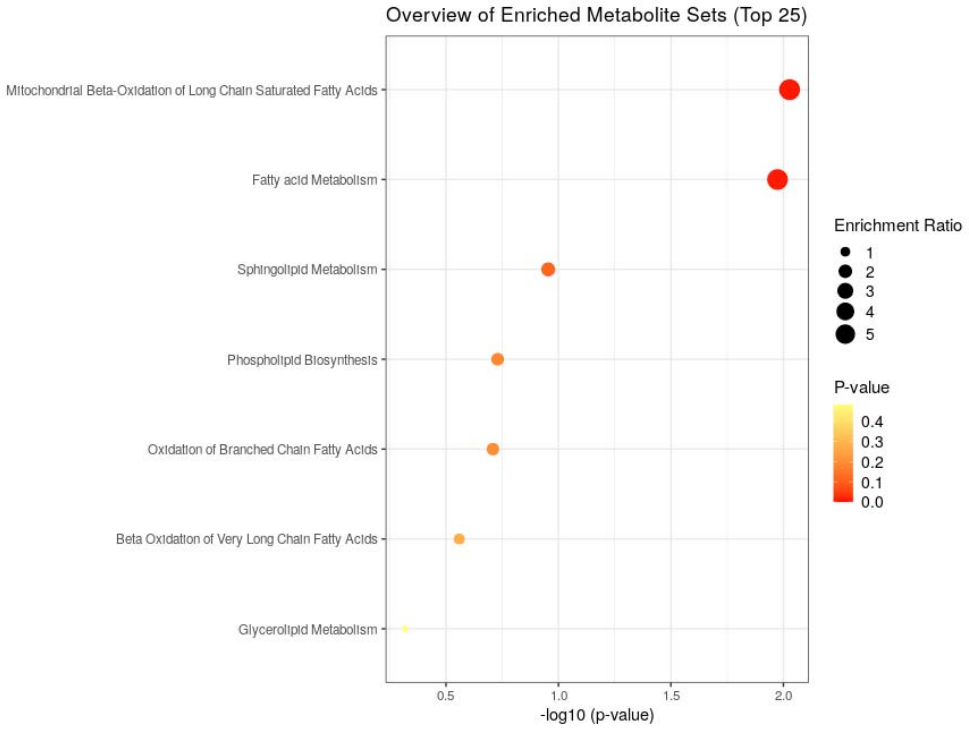

Figure 4. Enrichment overview of Longissimus thoracis muscle lipidome from castrated and non-castrated Nellore males.

3.4. Discussion

Differences in meat quality traits between bulls and steers have been widely established with tender meat and bright color values observed in steers. The rate and extent of pH decline is one of the most important factors altering meat quality as it influences meat color and tenderness and water holding capacity. In general, meat from bulls have higher ultimate pH, which is usually associated with DFD meat because of the lower glycogen concentrations caused by pre-slaughter stress. In this study, glycogen concentrations in LT were in fact lower in bulls than in steers, however, it was enough for carcasses to reach normal pH values 24h post-mortem once both treatments presented pH below 5.8. Though, even with residual glycogen available in both treatments, differences in pH, tenderness and color remained, suggesting that other factors may be responsible for these differences.

Muscle characteristics influence the conversion of muscle into meat and pos-mortem energy metabolism, thus meat quality traits. Based on metabolome view map, the carbohydrate metabolism was the one with the most relevant pathways associated with the identified metabolites in this study. The TCA cycle, pyruvate and glycolysis/gluconeogenesis were the pathways that had higher impact and it suggest that the major processes that took place in meat from bulls and steers aimed to restore ATP homeostasis.

Meat from bulls showed higher concentration of creatine, a nitrogenous organic acid. Creatine and its phosphorylated form play important role in early postmortem energy metabolism. Phosphocreatine and ADP are converted to ATP and creatine by the enzyme creatine kinase (CK) through the phosphagen system (SHEFFLER et al., 2011). In addition, creatine activates the mitochondrial CK, which has higher activity in oxidative muscles, and can effectively promote mitochondrial respiration (KAY et al., 2000). In addition to participating in the phosphagen system and mitochondrial respiration, Barbieri et al. (2016) demonstrated that creatine preserves mitochondrial structure, morphology, and function.

The positive effect of creatine on mitochondria function and structure might explain the lower pyruvate concentrations in bulls, once pyruvate, the end-product of glycolysis, can be oxidized in mitochondria into acetyl-CoA for energy production through TCA cycle (ZANGARI et al., 2020). Lower pyruvate concentrations were observed in meat from bulls, and according to Scholz et al. (1978), at lower concentrations of pyruvate, fatty acids stimulate the transport of pyruvate into the mitochondria, which could contribute even more for the lower concentrations observed in meat from bulls.

According to Picard et al. (2019), Lefaucheur and Gerrard (2011), Young and Bass (1998), and Dreyer et al. (1977), a higher percentage of slow-twitch oxidative fiber resulting in a more oxidative and less glycolytic metabolism is observed in bulls (BRANDSTETTER et al., 1998), and along with creatine favoring mitochondria function and an increased pyruvate oxidation, it can be suggested a more oxidative metabolism in bulls than steers in this study. Oxidative muscles rely more on mitochondria to produce energy once they present lower glycogen concentration and lower glycolytic capacity. Accordingly, the lower glycogen concentration in bulls suggests that other mechanisms had to be activated previously in order to muscle obtain energy, which corroborates with the enriched mitochondrial beta-oxidation of long chain saturated fatty acids (mFAβO) and fatty acid metabolisms observed on lipidome overview.

According to Bonen et al. (1998), higher oxidation rates of long-chain fatty acids is observed in muscle with higher amounts of oxidative fibers. The majority of lipid upregulated in bulls belong to acylcarnitine, which is formed by acyl-CoA though the enzyme carnitine palmitoyl-transferase 1 and undergoes through to beta-oxidation for energy supply (HÉNIQUE et al., 2015). On the other hand, betaine and choline, which were found in higher concentrations in steers in this study, are associated with lipid metabolism. Choline is utilized for the synthesis of membrane phospholipids, especially phosphatidylcholine, that accounts for 50% of membrane phospholipids (ZEISEL, 2006). Choline belongs to the glycerophospholipid metabolism and increase in choline concentrations could indicate an increase in energy supply compound in glycolytic metabolism (MUROYA et al., 2019).

While betaine, a methyl group donor, can decrease the requirements of other methyl donors, such as choline, and participates in lipid metabolism by promoting the fatty acid uptake in muscle and regulating FAT/CD36, FATP1, LPL and FABP3

expression (LI et al., 2017). Moreover, betaine increases expression of PPARy in skeletal muscle, which is a transcriptional factor that controls lipid metabolism and adipogenesis (MORI et al., 2005; Wu et al., 2018), thus corroborating with Gagaoua et al. (2016), who showed that meat from steers contains more intramuscular fat (IMF). An increase in lipid uptake and lipogenesis, and a reduction in lipolysis can explain the higher IMF in steers (BONG et al., 2012). According to Kim and Lee (2003), beef with increased marbling score shows lower WBSF. Because IMF decreases muscle density, dilutes the effects of tougher myofibrillar, and reduces de rigidity of muscle structure, it can promote an increase in tenderness (KARLSSON et al., 1999; WARRIS, 2010), as observed in meat from steer in this study.

Studies have shown that betaine has antioxidant properties such as ability to scavenge free radicals (ALIREZAEI et al., 2012), increase antioxidase activity (ALIREZAEI et al., 2015), upregulate endogenous nonenzymatic antioxidant, and isolating cells from the oxidative stressor, thus preventing ROS generation (ZHANG et al., 2016). Additionally, betaine is a precursor of S-adenosylmethionine, which enhances the supply of cysteine needed for the synthesis of glutathione, the major antioxidant compound in tissues (LIEBER, 2002). The increased concentrations of betaine observed in steers may have reduced the ROS production, protecting proteases from oxidative damage thus promoting meat tenderness (LONERGAN et al., 2010). Moreover, reducing the oxidative environment of the muscle cell makes μ-calpain more active (ROWE et al., 2004), hence increasing tenderness in meat from steers.

In addition to betaine, pyruvate also presents antioxidant properties. According with Bassenge et al. (2000), pyruvate has a ketoenol group which scavenge ROS by superoxide-generating enzymes. Pyruvate decreased lipid oxidation in beef loin (RAMANATHAN et al., 2011), in ground beef patties (RAMANATHAN et al., 2012),

and in a non-meat model system (PUNTEL et al., 2007). The products of lipid oxidation can accelerate myoglobin oxidation, thus enhancing meat discoloration (FAUSTMAN et al., 2010). Therefore, increased muscle antioxidant capacity increases color stability (SUMAN et al., 2014; FAUSTMAN et al., 1998; RAMANATHAN et al., 2012) and raw beef color (RAMANATHAN et al., 2011), corroborating with the brighter color values in meat from steers and higher concentrations of metabolites with antioxidant properties in these animals.

The lower antioxidant capacity in bulls may have favored the ROS production prevenient from mFAβO, xanthine production and preslaughter stress. Xanthine, a purine base compound, was higher in meat from bulls. The main precursors of xanthine are guanine and hypoxanthine, and during metabolic processes, the xanthine oxidase converts hypoxanthine to xanthine and H2O2, and the guanine deaminase converts guanine to xanthine and NH3 (MARRO et al., 1997; KHAN, AHOMMED; DAIZY et al., 2020; LANZNASTER et al., 2016). Xanthine production comes along with reactive oxygen species (ROS) production, resulting in oxidative stress. According to Xing et al. (2018), preslaughter stress also leads to oxidative stress, and due to the lower glycogen concentrations observed in bulls in this study, we can hypothesize that these animals were under stressor conditions preslaughter, in addition to the higher mFAβO which also increases ROS production, thus entering in oxidative stress earlier than steers. Under oxidative stress conditions, μ-calpain is inactivated because of the formation of disulfide bond (Cys115 and Cys108) reducing proteolysis in beef, and consequently negatively affecting beef tenderness (XING et al., 2018; LAMETSCH, LONERGAN; HUFF-LONERGAN, 2008), which agrees with the lower tenderness observed in met from bulls in this study.

Muscle intrinsic characteristics are also associated with proteolysis, and according to Ramos et al. (2021), glycolytic muscle exhibits higher proteolysis during the first 24h postmortem, which may explain the higher tenderness observed in meat from steers in this study. Moreover, an increase in creatine concentrations was observed to reduce the pH decline in porcine meat (PETTIGREW; ESNAOLA, 2001) and in poultry meat (NISSEN; YOUNG, 2006). Similarly, meat from bulls containing higher creatine concentrations showed higher pH values than steers in this study. Low ultimate pH alters muscle microstructure promoting a transverse shrinkage of muscle fibers and consequently increases the extracellular space enhancing light scattering (HUGHES et al., 2019), thus increasing lightness, which corroborates with the higher L* values observed in meat from steers. In addition, meat color is highly affected by myoglobin and its concentration depends on the muscle fiber type, with oxidative muscles containing higher myoglobin concentrations (RAMANATHAN; MANCINI, 2018). Once bulls are more oxidative and thus present more mitochondria, the competition for oxygen between myoglobin and mitochondria might have limited the development of bright cherry-red color in meat from bulls.

3.5. Conclusion

Beef quality traits and metabolome and lipidome are affected by gender status. The different metabolites and lipids concentrations and their related metabolic pathways are useful to explain differences in meat quality traits between bulls and steers. The metabolite and lipid profile show that muscle from steers is more glycolytic and presents more metabolites with antioxidant properties, and these characteristics may influence meat quality development.

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