UNIVERSIDADE ESTADUAL PAULISTA - UNESP CÂMPUS DE JABOTICABAL

UMA ABORDAGEM TRANSCRIPTÔMICA E PROTEÔMICA DO MÚSCULO *LONGISSIMUS THORACI*S DE BOVINOS DE CORTE CONFINADOS, COM USO DE DIFERENTES PRÁTICAS DE MANEJO

Irene Alexandre Reis

Zootecnista

2025

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Discente: Irene Alexandre Reis

Orientador: Prof. Dr. Otávio Rodrigues Machado Neto

Coorientador: Prof. Dr. Welder Angelo Baldassini

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Ciência Animal.

2025

R375a

Reis, Irene Alexandre
Uma abordagem transcriptômica e proteômica do músculo longissimus thoracis de bovinos de corte confinados, com uso de diferentes práticas de manejo / Irene Alexandre Reis. -- , 2025
71 p.

Tese (doutorado) - Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal,
Orientador: Otávio Rodrigues Machado Neto
Coorientador: Welder Angelo Baldassini

1. Abundância proteica. 2. Classe sexual. 3. Crescimento muscular. 4. Músculo esquelético. 5. Sequenciamento de RNA. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Dados fornecidos pelo autor(a).

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: UMA ABORDAGEM TRANSCRIPTÔMICA E PROTEÔMICA DO MÚSCULO Longissimus thoracis DE BOVINOS DE CORTE CONFINADOS, COM USO DE DIFERENTES PRÁTICAS DE MANEJO

AUTORA: IRENE ALEXANDRE REIS
ORIENTADOR: OTAVIO RODRIGUES MACHADO NETOCOORIENTADOR: WELDER ANGELO BALDASSINI

Aprovada como parte das exigências para obtenção do Título de Doutora em Ciência Animal, área: Nutrição Animal pela Comissão Examinadora:

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Prof. Assoc. OTAVIO RODRIGUES MACHADO NETO (Participaçao Virtual)
Departamento de Produção Animal e Medicina Veterinária Preventiva / FMVZ UNESP Botucatu

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Pós-Doutorando SÉRGIO PEREIRA JUNIOR (Participaçao Virtual)
Departamento de Genética e Biotecnologia / Universidade Federal de São Carlos (UFSCar) - São Carlos/SP

Jaboticabal, 09 de dezembro de 2025.

DADOS CURRICULARES DO AUTOR

Irene Alexandre Reis, nascida em 14 de fevereiro de 1997, Porto Nacional, Tocantins. Filha de Maria Tereza Alexandre Reis e Raimundo da Cunha Reis. Possui graduação em Zootecnia (2015 – 2019), pelo Centro Universitário Católica do Tocantins. Atuando em projetos de extensões sob a orientação do professor Dr. Iberê Pereira Parente (2016) e professora Dra. Angélica Pedrico (2017- 2018) e em iniciações científicas (2018 – 2019) na Embrapa Pesca e Aquicultura (Núcleo Temático de Sistemas Agrícolas), sob a orientação do Dr. Rodrigo Estevam Munhoz de Almeida. Em fevereiro de 2020 ingressou no mestrado pela Faculdade de Ciências Agrárias e Veterinárias - UNESP, Jaboticabal, São Paulo, no programa de pós-graduação em Zootecnia sob a orientação do Dr. Flávio Dutra de Resende. Em março de 2022, ingressou no doutorado pela mesma universidade, sob a orientação do professor Dr. Otávio Rodrigues Machado Neto. Entre o período de dezembro de 2023 a julho de 2025, realizou o doutorado sanduíche na Colorado State University, sob a orientação do Dr. Pedro Carvalho no departamento de Animal Science – AgNext, Fort Collins, Colorado.

"Quem persiste aprende que as dificuldades não são o fim, mas sim degraus para alcançar alturas que antes pareciam inalcançáveis."

Autor desconhecido

Dedico,

Aos meus pais, que não mediram esforços para que eu chegasse até aqui, em especial à minha mãe, que sempre me apoiou e incentivou em todos os momentos.

Aos meus irmãos e a todos aqueles que estiveram e caminharam comigo durante essa jornada.

AGRADECIMENTOS

A Deus pelo dom da vida, por sempre estar comigo, iluminando e guiando todos os passos ao longo desta jornada, dando força e sabedoria para enfrentar todos os desafios.

À minha família. Aos meus pais, Maria Tereza e Raimundo pelo amor, incentivo e apoio incondicional. Em especial a minha mãe, pelo carinho, compreensão, por estar presente em todos os momentos da minha vida, por acreditar em mim e não medir esforços para ajudar durante esta caminhada, meu grande exemplo de força, educação, fé, coragem e determinação. Aos meus irmãos, Anna Inez e Thiago, pelo apoio, carinho, incentivo e companheirismo.

À minha amiga Mailza, pela cumplicidade, amizade, ensinamentos, por toda a ajuda durante esta jornada. Obrigada por sempre estar ao meu lado em todos os momentos, pelas conversas e pelo apoio. Gratidão pela sua amizade.

À Juliana e à Iasmin, por todo o apoio, incentivo, amizade, pelas boas conversas, cafés e ensinamentos, vocês foram muito importantes durante esta caminhada. Obrigada por todos os momentos compartilhados.

Ao meu orientador, Prof. Dr. Otavio Machado, pelo apoio, ensinamentos, paciência, oportunidade e confiança na realização deste trabalho.

Ao meu coorientador, Prof. Dr. Welder Baldassini, por toda a ajuda, confiança, incentivo, paciência (principalmente com as análises e interpretação dos dados), pela disponibilidade e por todas as contribuições durante esse tempo.

Aos amigos de pós-graduação, Daniela, Richard, Rafaela, Murilo, Jose Breno e Paloma, pelo apoio, parceria, convivência e pelos valiosos ensinamentos e momentos compartilhados ao longo dessa jornada.

Às minhas amigas de longa data, Taiana, Juliana Arruda, Rannyelle e Ana Cecília, que, mesmo a quilômetros de distância estiveram presentes em todos os momentos, apoiando e incentivando o meu crescimento. Obrigada por todo o companheirismo, amizade e apoio.

Ao Programa de Pós-Graduação em Ciência Animal da UNESP de Jaboticabal e seus docentes pelos ensinamentos e oportunidade de realização do doutorado.

Aos membros da banca de qualificação e defesa da tese, Prof. Dr. Luis Chardulo, Dr. Pedro Carvalho, Dra. Juliana Akamine, Dr. Sérgio Pereira e Prof. Dr. Miguel Santana, pelos ensinamentos, comentários e pelas valiosas contribuições a este trabalho.

Ao Dr. Pedro Carvalho, pela oportunidade de realizar o período de intercâmbio sob sua supervisão na Colorado State University, pelo apoio, incentivo e por acreditar no meu potencial. Agradeço por todas as contribuições e orientação durante esse período, pelos desafios propostos que me ajudaram a evoluir e por todos os conhecimentos compartilhados, que foram fundamentais para o meu crescimento pessoal e profissional. Pela disponibilidade em sempre ajudar, pela colaboração e parceria com o projeto para o segundo capítulo da tese. Sou extremamente grata pela oportunidade, acolhimento e amizade.

A todos os membros do grupo AgNext - Colorado State University, pelo apoio, oportunidades e convívio, vocês fizeram com que essa experiência de intercâmbio se tornasse muito especial.

A todos que contribuíram e incentivaram de alguma forma. MUITO OBRIGADA!

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

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UMA ABORDAGEM TRANSCRIPTÔMICA E PROTEÔMICA DO MÚSCULO *LONGISSIMUS THORACI*S DE BOVINOS DE CORTE CONFINADOS, COM USO DE DIFERENTES PRÁTICAS DE MANEJO

RESUMO GERAL - Dois estudos foram conduzidos utilizando abordagens ômicas para avaliar a expressão de genes e proteínas relacionadas ao músculo *Longissimus thoracis* de bovinos de corte submetidos a diferentes práticas de manejo e tecnologias de crescimento. No primeiro estudo, 640 touros (não castrados) e novilhos (castrados) F1 Angus-Nelore com peso inicial de 298,15 kg (n = 320/grupo) foram submetidos à terminação em confinamento por 180 dias. 60 amostras do *Longissimus thoracis* foram coletadas aleatoriamente de cada grupo na carcaça quente (no abate) e 48 h post-mortem (na desossa), entre a 12ª e a 13ª vértebras torácicas. Após esse processo, 3 amostras do músculo de cada grupo foram selecionadas aleatoriamente para análise de RNA-Seq, enquanto as amostras de carne pós-desossa foram submetidas à determinação do conteúdo de gordura intramuscular (GIM). Os novilhos apresentaram um conteúdo de GIM 2,7 vezes maior do que os touros (5,59 vs. 2,07%; $P < 0,01$). Um total de 921 genes diferencialmente expressos (DEGs; FDR < 0,05) foram identificados em contraste entre touros e novilhos; destes, 371 foram up-regulated e 550 foram down-regulated. A análise de enriquecimento do transcriptoma funcional revelou diferenças em processos biológicos e nas vias metabólicas relacionadas à adipogênese e lipogênese, como resistência à insulina, AMPK, AMPc, regulação da lipólise em adipócitos e vias de sinalização PI3K-Akt. Genes candidatos como *FOXO1, PPARG, PCK2, CALM1, LEP, ADIPOQ, FASN, FABP4, PLIN1, PIK3R3, ROCK2, ADCY5* e *ADORA1* foram down-regulated em novilhos, o que pode explicar a diferença expressiva no conteúdo de GIM quando comparados aos touros. Os resultados sugerem a importância dessas vias e genes para o metabolismo lipídico em novilhos com maior GIM. Notavelmente, este estudo revela pela primeira vez o envolvimento da via PI3K-Akt e genes associados na regulação da deposição de GIM em bovinos F1 Angus-Nelore. A castração influenciou os DEGs ligados ao metabolismo energético e à biossíntese de lipídios, destacando os principais fatores moleculares responsáveis pelo acúmulo de GIM pós-castração em bovinos de corte. No segundo estudo, foram utilizados 200 novilhos (castrados), 100 Brahman (*Bos indicus*) e 100 Angus (*Bos taurus*), em um delineamento fatorial 2×2 para avaliar os efeitos das tecnologias promotoras de crescimento (TPC), sobre o desempenho de crescimento e as características da carcaça de bovinos confinados. Os novilhos receberam TPC (implantes hormonais de crescimento, ionóforo, antibiótico e agonista β-adrenérgico na ração), designados para o TRT (tratamento) ou não receberam TPC, designados para CON (controle). Todos os animais passaram por um período de adaptação de 21 dias antes de serem terminados com uma dieta à base de grãos por 159 dias (total de 180 dias de confinamento). Ao final do período de engorda, os animais foram abatidos e amostras do *Longissimus thoracis* (5 por tratamento) foram analisadas por proteômica baseada em espectrometria de massa para avaliar as diferenças na expressão proteica relacionadas à raça e ao uso de TPC. Novilhos Angus apresentaram maior ($P < 0,01$) ganho médio diário (GMD) e peso corporal final (PCF) em comparação com novilhos Brahman. Novilhos que receberam TPC apresentaram maior ($P < 0,01$) GMD, PCF, peso da carcaça quente (PCQ) e área do músculo *Longissimus* (AOL) do que novilhos manejados sem TPC. Um total de 87 proteínas diferencialmente abundantes (PDAs) foram mais abundantes no músculo

de novilhos Angus TRT, enquanto 27 PDAs foram mais abundantes no Angus CON. Além disso, 23 PDAs foram mais abundantes no músculo dos novilhos Brahman TRT, enquanto 17 PDAs foram mais abundantes no Brahman CON. Independentemente da raça, os novilhos do grupo TRT apresentaram maior abundância de PDAs relacionados ao metabolismo energético celular, hipertrofia muscular e proliferação celular (NDUFS, NDUFB, CKMT2, HSPB, HSPA9, PREP, MYOF, MYH6, TPM2), enquanto os novilhos do grupo CON apresentaram maior abundância de PDAs associadas a processos catabólicos e estresse oxidativo (FKBP4, MAPK1, GALM, LMO7, CALAR, UBE2M, PLIN1). As PDAs mais abundantes nos novilhos TRT em comparação com os novilhos CON foram associadas ao metabolismo celular mais eficiente, favorecendo o crescimento muscular. O presente estudo demonstrou que o uso de TPC melhora o metabolismo muscular por meio da ativação de vias anabólicas. Consequentemente, as alterações na via da respiração celular resultaram em melhor desempenho de crescimento em bovinos que utilizaram TPC em comparação com bovinos do grupo CON.

Palavras-chave: Abundância proteica, classe sexual, crescimento muscular, marmoreio, músculo esquelético, sequenciamento de RNA

A TRANSCRIPTOMIC AND PROTEOMIC APPROACH TO THE LONGISSIMUS THORACIS MUSCLE OF FEEDLOT BEEF CATTLE USING DIFFERENT MANAGEMENT PRACTICES

GENERAL ABSTRACT - Two studies were conducted using omics approaches to evaluate the expression of genes and proteins related to the *Longissimus thoracis* muscle of beef cattle subjected to different management practices and growth technologies. In the first study, 640 F1 Angus-Nellore bulls and steers with initial body weight of 298.15 kg (n = 320/group) were submitted to feedlot finishing for 180 days. 60 *Longissimus thoracis* samples were randomly collected from each group in the hot carcass (at slaughter) and 48 h post-mortem (at deboning), between 12th and 13th thoracic vertebrae. Subsequently, 3 muscle samples per group were randomly selected for RNA-Seq analysis, while the post-deboning meat samples were submitted to determination of IMF content. Steers had a 2.7- fold greater IMF content than bulls (5.59 vs. 2.07%; $P < 0.01$). A total of 921 DEGs (differentially expressed genes) (FDR < 0.05) were identified in the comparison between bulls and steers; of these, 371 were up-regulated, and 550 were down-regulated. Functional transcriptome enrichment analysis revealed differences in biological processes and metabolic pathways related to adipogenesis and lipogenesis, such as insulin resistance, AMPK, cAMP, regulation of lipolysis in adipocytes, and PI3K-Akt signaling pathways. Candidate genes such as *FOXO1*, *PPARG*, *PCK2*, *CALM1*, *LEP*, *ADIPOQ*, *FASN*, *FABP4*, *PLIN1*, *PIK3R3*, *ROCK2*, *ADCY5*, and *ADORA1* were down-regulated in steers, which may explain the marked difference difference in IMF content when compared to bulls. The current findings suggest the importance of these pathways and genes for lipid metabolism in steers with greater IMF. Notably, this study reveals for the first time the involvement of the PI3K-Akt pathway and associated genes in regulating IMF deposition in F1 Angus-Nellore cattle. Castration influenced DEGs linked to energy metabolism and lipid biosynthesis, highlighting key molecular players responsible for IMF accumulation post-castration in beef cattle. In the second study, 200 steers, 100 Brahman (*Bos indicus*) and 100 Angus (*Bos taurus*), were used in a 2×2 factorial design to evaluate the effects of GPT on feedlot growth performance and carcass traits. Steers received either GPT (anabolic growth-hormonal implants, and an in-feed ionophore, antibiotic, and β-adrenergic agonist), designated as TRT (treatment) or no GPT designated as CON (control). All animals underwent a 21-day diet adaptation before being finished on a grain-based diet for 159 days (total of 180 days on feed). At the end of the feeding period, animals were harvested and *Longissimus thoracis* samples (five per treatment within breed) were analyzed by mass spectrometry–based proteomics to assess protein expression differences related to breed and GPT use. Angus steers had greater ($P < 0.01$) average daily gain (ADG) and final body weight (FBW) compared to Brahman steers. Steers that receive GPT exhibited greater ($P < 0.01$) ADG, FBW, hot carcass weight (HCW) and *longissimus* muscle area (LMA) than steers managed without GPT. A total of 87 differentially abundant proteins (DAPs) were more abundant in the muscle of steers in the Angus TRT group, while 27 DAPs were more abundant in the Angus CON group; moreover, 23 DAPs were more abundant in the muscle of steers in the Brahman TRT group, while 17 DAPs were more abundant in the Brahman CON group. Regardless of breed, steers in the TRT group had greater abundance of DAPs related to cellular energy metabolism, muscle hypertrophy and cell proliferation (NDUFS, NDUFB, CKMT2, HSPB, HSPA9, PREP, MYOF, MYH6, TPM2), while CON

steers had more DAPs associated with catabolic processes and oxidative stress (FKBP4, MAPK1, GALM, LMO7, CALAR, UBE2M, PLIN1). The DAPs expressed in the TRT steers compared to CON cattle were related to more efficient cellular metabolism, favoring muscle growth. The study demonstrated that GPT enhances muscle metabolism by activating anabolic pathways. Consequently, changes in cellular respiration pathway led to improved growth performance in cattle that use GPT compared to CON cattle.

Keywords: Protein abundance, sex class, muscle growth, marbling, skeletal muscle, RNA sequencing

CAPÍTULO 1 - Considerações gerais

1. Introdução

Estratégias para entender e modificar características de qualidade da carne bovina têm sido amplamente estudadas na nutrição e seleção animal, devido à grande importância desse alimento e seu impacto no mercado mundial. O Brasil se destaca como o segundo maior produtor mundial de carne bovina, atingindo em 2024 à marca de 11,8 milhões de toneladas equivalente de carcaça (TEC), e como o maior exportador, com volume de exportações de 3.8 milhões de toneladas (ABIEC, 2025). A maior parte dessa carne é proveniente de bovinos Nelore (zebuínos) (Utsunomiya et al. 2022), em razão da predominância dessa raça nos sistemas de produção nas condições tropicais e de suas características marcantes de adaptação.

Em contrapartida, Estados Unidos é o maior produtor mundial de carne bovina, com aproximadamente 12,3 milhões de TEC, e o quarto maior exportador, com volume de 1.2 milhões de toneladas (USDA, 2025). Nos sistemas de produção de carne americana, a genética Angus exerce grande influência devido à sua qualidade superior de carcaça, maturidade precoce, marmoreio e rápido crescimento (Feuz et al., 2022; Scheffler, 2022). Além da raça, o uso de tecnologias promotoras de crescimento, como implantes hormonais, beta-agonistas e ionóforos tem impacto significativo na produção de carne. Essas tecnologias influenciam diretamente na eficiência alimentar, no ganho médio diário e no peso da carcaça (Drouillard, 2018).

Os cruzamentos entre essas raças abrigam naturalmente conjuntos de haplótipos zebuínos e taurinos muito diferentes, os quais podem contribuir para a variação fenotípica, especialmente quando presentes em alta frequência (Rodrigues et al., 2017; Utsunomiya et al. 2022). Alguns desses genes estão relacionados a respostas ao estresse, imunidade, crescimento e reprodução (Edea et al., 2018; Utsunomiya et al. 2021).

Considerando que a melhoria da qualidade da carne é certamente uma forma de agregar valor ao produto, a maciez é um dos atributos sensoriais de maior importância para avaliar a qualidade e a preferência do consumidor (Puente et al., 2019; Baldassini et al., 2021). Dessa forma, além da raça, fatores como classe sexual e o uso de tecnologias promotoras de crescimento exercem forte influência sobre a qualidade da carne, afetando diretamente o crescimento e a deposição de gordura na carcaça. Portanto, o objetivo desse estudo é avaliar como as práticas de manejo

influenciam os mecanismos moleculares envolvidos na deposição de gordura intramuscular e no crescimento muscular de bovinos de corte submetidos a diferentes práticas de manejo, por meio de abordagens transcriptômica e proteômica aplicadas ao músculo *Longissimus thoracis*.

2. Revisão de literatura

2.1 Cruzamentos entre *Bos taurus* × *Bos indicus*

No Brasil há grande predominância de animais zebuínos, principalmente a raça Nelore, que são animais bem adaptados às condições de clima e manejo no país. A carne de animais zebuínos apresenta qualidade inferior, menor maciez e menor marmoreio em comparação aos animais taurinos (Font-i-Furnols e Guerrero, 2014; Rodrigues et al., 2017).

Nesse contexto, raças taurinas passaram a contribuir com o objetivo de combinar características de melhor eficiência produtiva e qualidade da carne (Rodrigues et al., 2017; Piccoli et al., 2020). O cruzamento entre as raças promove melhorias adicionais no mérito genético das características de eficiência produtiva nessas populações, e são de extrema importância para manter a competitividade da cadeia produtiva da carne (Piccoli et al., 2020).

As características de qualidade da carcaça e da carne são de grande interesse econômico, e são influenciadas pelas práticas alimentares, manejo pré e pós abate e métodos de processamento e armazenamento da carne (Guerrero et al., 2013; Grigoletto et al., 2020). Dessa forma, o cruzamento entre raças taurinas e zebuínas produz animais com maior peso de abate, melhor rendimento e conformação de carcaça, contribuindo para a competitividade e eficiência econômica da produção de carne bovina.

2.2 Castração, desempenho e qualidade da carne bovina

A classe sexual do animal afeta significativamente a qualidade da carcaça e da carne, sabe-se que a castração tem sido usada como forma de melhorar características como, deposição de gordura intramuscular, maciez e suculência (Seideman et al. 1982; Freitas et al. 2015). A castração diminui a produção de hormônios sexuais, o que reduz a assimilação de proteínas e crescimento muscular,

porém, traz benefícios no aumento da eficiência do metabolismo da gordura, e carboidratos, melhor acabamento de carcaça e maior docilidade dos animais, facilitando o manejo e refletindo em menores danos à carcaça e melhor bem-estar animal (Wang et al., 2017; Li et al., 2022),

No entanto, animais inteiros apresentam melhor eficiência produtiva, produzindo carcaças com maior musculosidade e menor teor de gordura (Mach et al., 2009), isso está relacionado com os hormônios sexuais, especialmente a testosterona, que leva a maior deposição de massa muscular (Anaruma et al., 2020). Segundo Prior et al. (1983), a testosterona inibe as atividades das enzimas lipogênicas presentes no tecido adiposo, induzindo a altas taxas lipolíticas basais. A carne com coloração mais escura, característica em animais inteiros, pode ser explicada pela síntese e atividade de hormônios androgênicos, que afetam também o complexo calpaína-calpastatina resultando no aumento da atividade de calpastatina e bloqueando a ação da calpaína, responsável pela maciez da carne (Koohmaraie, 1990).

No estudo de Na et al. (2020), foi relatado que a castração regulou positivamente genes relacionados a lipogênese e vias de sinalização de ácidos graxos, e alterações na expressão gênica de fatores imunológicos e via de transdução intracelular. Bong et al. (2012), estudando a expressão de genes associados ao metabolismo lipídico de animais castrados e inteiros, observaram a regulação positiva do gene lipogênico da acetil-CoA carboxilase, ácido graxo síntase e monoglicerídeo lipase no músculo *longissimus dorsi*. Esses resultados demonstraram que a castração contribuiu para melhorar atributos de qualidade, como o marmoreio pelo maior acúmulo de gordura.

2.3 Tecnologias promotoras de crescimento para bovinos de corte nos EUA

Historicamente, a produção de carne americana é fortemente influenciada pelo uso de tecnologias promotoras de crescimento, sejam elas, implantes hormonais, beta-agonistas, ionóforos e antibióticos (Drouillard, 2018). Aproximadamente, 90% dos animais confinados nos EUA, recebem algum tipo de promotor de crescimento durante o seu ciclo de produção (Johnson et al., 2013; NAHMS-USDA, 2013). Isso se deve, porque ao longo dos anos a indústria da carne bovina tem evoluído, principalmente, pela necessidade de atender às demandas do mercado e o objetivo

de aumentar a eficiência do crescimento animal, bem como às preocupações com a sustentabilidade ambiental da produção pecuária (Crawford et al., 2022).

O uso de tecnologias de crescimento também tem efeitos significativos no aspecto econômico. Capper e Hayes (2012) relataram que a remoção de tecnologias dos sistemas de produção de carne bovina reduz a taxa de crescimento e o peso de abate dos animais, além de aumentar os custos totais de produção de carne bovina em 9,1%. Ou seja, essas tecnologias influenciam diretamente no aumento da massa muscular magra, ganho médio diário, e consequentemente, aumento da produção e melhora na eficiência alimentar do gado (Johnson et al., 2013).

Os implantes hormonais utilizam componentes estrogênicos (estradiol) e androgênicos (testosterona ou acetato de trembolona), ou combinações destes. Os implantes estimulam a ingestão de ração e a deposição de proteína, tendo um impacto significativo na eficiência alimentar, desempenho e no ganho médio diário de bovinos confinados (Duckett e Pratt, 2014; Torrentera et al., 2017). Segundo Duckett e Pratt (2014), o uso de implantes hormonais em bovinos de corte aumentou em 18% o ganho médio diário (GMD), 6% o consumo de matéria seca (CMS), 8% a eficiência alimentar, 5% o peso de carcaça e em 4% a área de olho de lombo em comparação com bovinos não implantados.

Além dos implantes, outra categoria amplamente utilizada em dietas de bovinos confinados são os agonistas β-adrenérgicos, utilizados para estimular a acreção muscular, aumentando a síntese proteica e diminuindo o catabolismo proteico (Drouillard, 2018). Os beta-agonistas mais utilizados em bovinos de corte são a ractopamina e o zilpaterol, administrados na fase final de terminação, esses compostos são fornecidos tipicamente entre 20 e 42 dias antes do abate e têm como objetivo aumentar o GMD, eficiência alimentar, rendimento da carcaça, e a área de olho de lombo (Baxa et al., 2010; Johnson et al., 2013). As melhorias que ocorrem na hipertrofia do músculo esquelético são resultado de alterações nas taxas de síntese e degradação de proteínas que o uso de beta-agonista promove no músculo (Johnson et al., 2013).

De forma complementar, os ionóforos e antibióticos são incluídos na dieta de bovinos na fase de terminação para melhorar a saúde animal e a eficiência alimentar. Os ionóforos são compostos antimicrobianos que alteram a fermentação ruminal para melhorar a eficiência alimentar e o desempenho de crescimento em bovinos (Thompson et al., 2016). De acordo com Samuelson et al. (2016), a monensina é o

principal ionóforo incluído nas dietas de confinamento nos EUA, sendo conhecida por reduzir o CMS, especialmente em dietas com alta proporção de forragem, devido ao aumento da proporção molar de propionato e à diminuição da proporção molar de acetato e butirato (Baggio et al., 2023).

Em uma meta-análise conduzida por Duffield et al. (2012), a monensina reduziu o CMS em 3%, melhorou a eficiência alimentar de bovinos de corte em terminação em 2,5 a 3,5%. Esse efeito positivo do uso de monensina em bovinos é frequentemente atribuído à maior eficiência do metabolismo energético, como resultado do aumento da produção de propionato no rúmen (Russell e Strobel, 1989). A administração de monensina reduz a variância do pH ruminal, o que pode contribuir para a prevenção da acidose ruminal em dietas de terminação com alto teor de grãos (Erickson et al., 2003).

Bovinos que são submetidos a dietas ricas em grãos por longos períodos podem apresentar algum distúrbio metabólico, aumentando assim a incidência de abscessos hepáticos (Nagaraja e Chengappa,1998). O uso de antibióticos é uma prática comum em operações intensivas de alimentação animal, utilizados para prevenir distúrbios específicos associados ao ambiente de confinamento (Cazer et al., 2018). A tilosina é um antibiótico comumente utilizado na dieta de bovinos, pois ajuda na redução da incidência de abscessos hepáticos, que são uma sequela da acidose ruminal, que ocorre em animais que são alimentados com altas quantidades de carboidratos fermentáveis, e constitui a principal causa de condenação do fígado (Amachawadi e Nagaraja, 2016).

Segundo Potter et al. (1985), a inclusão de tilosina na dieta de bovinos confinados reduziu a incidência de abscessos hepáticos de 27% para 9%. De acordo com Wileman et al. (2009), a alimentação de bovinos confinados com tilosina também reduziu de 30% para 8% os riscos de abcessos hepáticos. Esse conjunto de tecnologias são mais frequentemente utilizadas em combinação para maximizar a produtividade animal, ou seja, à medida que as tecnologias são integradas aos sistemas de alimentação, seus efeitos resultam no aumento da produtividade de carne bovina por animal e uma produção de forma mais eficiente e econômica (Wileman et al., 2009; Maxwell et al., 2015).

2.4 Ferramentas moleculares na produção animal e qualidade da carcaça

A busca pela produção de alta qualidade tem sido cada vez mais demandada dentro da cadeia da carne. Dessa forma, diversas estratégias e pesquisas passaram a estudar fatores que são cruciais para entregar um produto de melhor qualidade, raça, classe sexual, idade, uniformidade carcaça e teor de gordura intramuscular (Magalhães et al., 2016; Muniz et al., 2022).

Com isso, estudos moleculares se tornaram essenciais para o conhecimento de características complexas ligadas qualidade da carcaça e da carne, indicando genes, proteínas e mecanismos relacionados a esse processo (Ramalingam e Hwang, 2021; Muniz et al., 2022). Uma ferramenta que pode colaborar para aumentar o conhecimento sobre a expressão gênica dessas características é a transcriptômica, que é caracterizada pelo entendimento dos mecanismos genéticos por trás dos perfis de transcrição envolvidos na variação de características de qualidade da carne, (por exemplo, maciez e marmoreio) (D'Alessandro et al., 2012; Magalhães et al., 2016; Muniz et al., 2022; Gagaoua et al., 2022).

O sequenciamento de alto rendimento tem sido cada vez mais utilizado para elucidar mecanismos moleculares e recebe cada vez mais a atenção de pesquisadores. O sequenciamento de RNA (RNA-Seq) pode descrever e investigar quantitativamente o transcriptoma mais amplamente, na classificação dos genes diferencialmente expressos em células ou tecidos (Ramalingam, e Hwang, 2021). Apesar de crescente a compreensão molecular relacionada à carne, pouco se sabe sobre os genes que determinam atributos de qualidade e sua função precisa, nesse contexto, a compreensão dos genes expressos, vias regulatórias e redes envolvidas nesses fatores importantes em bovinos, fornecerá o entendimento para melhorar a qualidade do produto.

Outra ferramenta que tem sido utilizada para desvendar a complexidade do músculo, é a proteômica que representa uma caracterização abrangente de proteínas expressas presentes em uma amostra, fornecendo detalhes significativos sobre as mudanças no padrão de expressão proteica em uma condição fisiológica específica (Gagaoua e Zhu et al., 2022). Na produção animal, a proteômica tem sido usada principalmente para investigar os mecanismos moleculares envolvidos na variação das características da carcaça e características de qualidade da carne bovina, como maciez, cor, marmoreio, declínio do pH, entre outras características de qualidade

(Almeida et al., 2017; Rosa et al., 2018; Gagaoua et al., 2021; Gagaoua e Zhu et al., 2022). Isso se deve, porque o estudo do proteoma permite compreender os mecanismos que sustentam os fenótipos de interesse na produção animal, por ser o principal constituinte do tecido muscular e tem papéis importantes na regulação das rotas metabólicas envolvidas na transformação do músculo em carne (D'Alessandro et al., 2012).

Características complexas como as que influenciam a qualidade da carne são pouco exploradas, em virtude de serem expressas tardiamente, sendo difíceis e caras de mensurar ou mensuradas somente após o abate (Magalhães et al., 2016). Portanto, é essencial avançar na compreensão dos mecanismos moleculares que afetam características complexas de qualidade da carne, assim como, elucidar as alterações moleculares associadas a castração e ao uso de tecnologias promotoras de crescimento, de forma a melhorar a qualidade da carcaça e atender às demandas do mercado consumidor.

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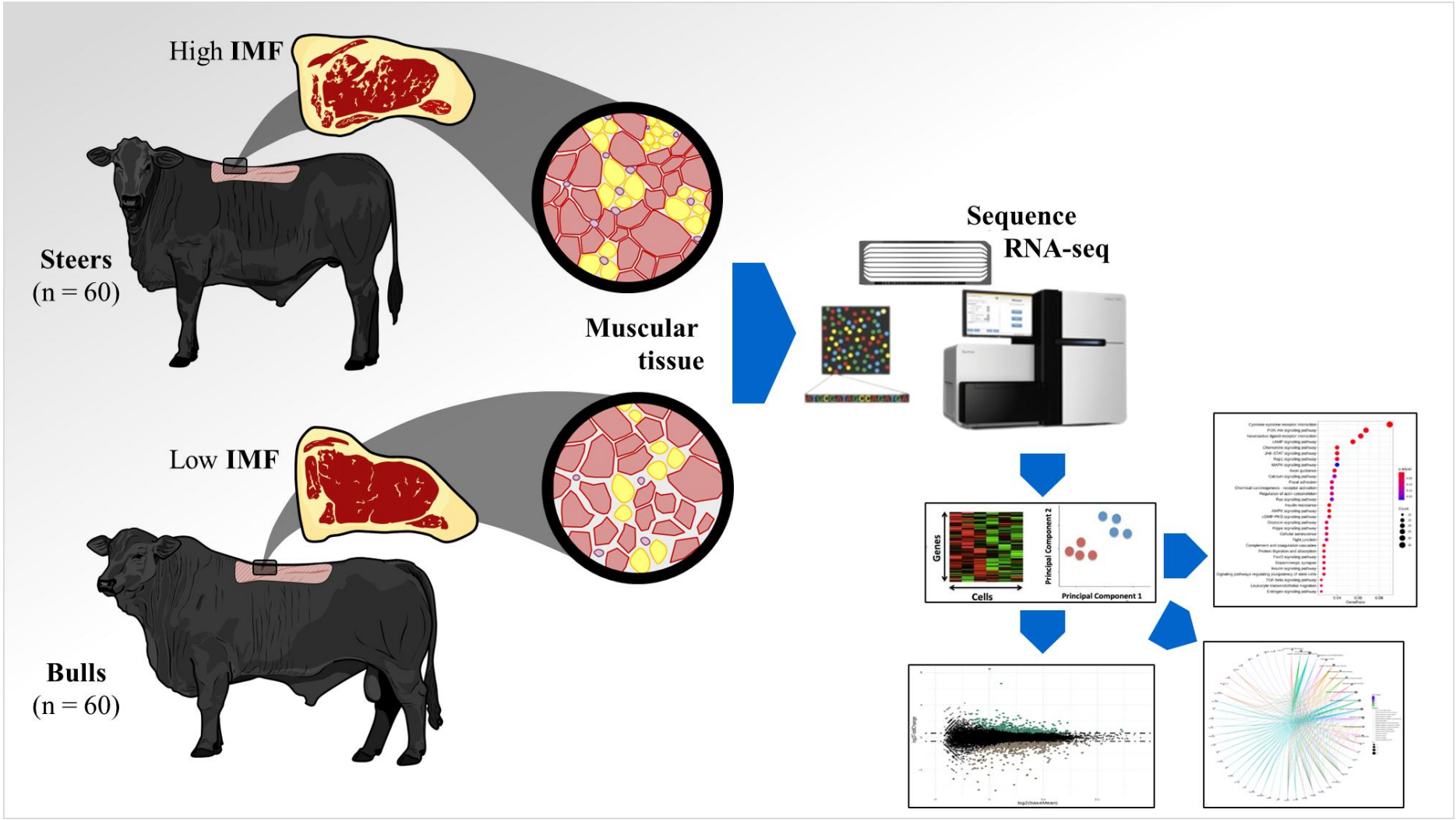
CHAPTER 2 - Muscle tissue transcriptome of F1 Angus- Nellore bulls and steers feedlot finished: impacts on intramuscular fat deposition[1]

[1]Este capítulo corresponde ao artigo científico publicado na revista *BMC Genomics* 25:1178, 2024

Abstract: Castration is a common practice in beef cattle production systems to manage breeding and enhance meat quality by promoting intramuscular fat (IMF) deposition, known as marbling. However, the molecular mechanisms that are influenced by castration in beef cattle are poorly understood. The objective of this study was to identify differentially expressed genes (DEGs) and metabolic pathways that regulate IMF deposition in crossbred cattle by RNA sequencing (RNA-Seq) of skeletal muscle tissue. Six hundred and forty F1 Angus-Nellore bulls and steers (*n* = 320/group) were submitted to feedlot finishing for 180 days. Sixty *Longissimus thoracis* muscle samples were collected randomly from each group in the hot carcass (at slaughter) and 48 h post-mortem (at deboning), at between 12th and 13th thoracic vertebrae. Three muscle samples of each group were randomly selected for RNA-Seq analysis, while the post-deboning meat samples were submitted to determination of IMF content. Steers had a 2.7-fold greater IMF content than bulls (5.59 vs. 2.07%; $P < 0.01$). A total of 921 DEGs (FDR < 0.05) were identified in contrast between Bulls *versus* Steers; of these, 371 were up-regulated, and 550 were down-regulated. Functional transcriptome enrichment analysis revealed differences in biological processes and metabolic pathways related to adipogenesis and lipogenesis, such as insulin resistance, AMPK, cAMP, regulation of lipolysis in adipocytes, and PI3K-Akt signaling pathways. Candidate genes such as *FOXO1*, *PPARG*, *PCK2*, *CALM1*, *LEP*, *ADIPOQ*, *FASN*, *FABP4*, *PLIN1*, *PIK3R3*, *ROCK2*, *ADCY5*, and *ADORA1* were regulated in steers, which explains the expressive difference in IMF content when compared to bulls. The current findings suggest the importance of these pathways and genes for lipid metabolism in steers with greater IMF. Notably, this study reveals for the first time the involvement of the PI3K-Akt pathway and associated genes in regulating IMF deposition in F1 Angus-Nellore cattle. Castration influenced DEGs linked to energy metabolism and lipid biosynthesis, highlighting key molecular players responsible for IMF accumulation post-castration in beef cattle.

Keywords Sex class, *Longissimus thoracis*, Marbling, RNA sequencing

1 **Graphical abstract**



1. Introduction

In beef cattle production systems, castration improves traits such as meat color, flavor, tenderness, and juiciness, which are related to the degree of carcass finishing and deposition of intramuscular fat (IMF) in meat cuts [1]. Bulls exhibit better growth performance characterized by greater feed-to-gain conversion, producing more muscle tissue than fat compared to castrated animals [2, 3], therefore improving profitability in production systems where exogenous anabolic steroids are prohibited [4]. However, the meat of bulls animals is usually tougher, darker, and contains less IMF. Castration is known to modify the animal's body composition [5], improving IMF deposition, tenderness, and juiciness of meat compared to non-castrated animals [3, 6, 7]. These traits are determined by many genes and are regulated by transcription factors that directly or indirectly control different metabolic pathways, particularly lipid metabolism and biosynthesis [8, 9].

The advancement of molecular biology techniques such as RNA sequencing (RNA-Seq) has made it possible to identify thousands of expressed genes, allowing us to elucidate important molecular mechanisms that regulate traits of interest, such as IMF [10]. Recently, researchers have investigated in depth the transcriptional events that regulate the expression of genes and their effects on traits associated with beef quality [6, 9, 11]. RNA-Seq has been used to identify differentially expressed genes (DEGs) in muscle tissue of different species, including zebu cattle that are divergent for meat tenderness [12] and IMF [13]. However, studies on crossbred *Bos taurus* × *Bos indicus* cattle finished in feedlot grain-based diets are limited. Considering the economic importance of marbling and the increasing use of cross-bred cattle as a biological model in tropical production systems, studying the effect of castration on the regulation of the profile of DEGs can help identify genes that control the molecular mechanisms responsible for the associated phenotypic variation in IMF of these animals.

Gene expression profiling is a powerful tool for identifying changes in gene expression associated with production traits like marbling. It also aids in discovering genes that contribute to quantitative variation between different sexes or breeds. Recent research has primarily focused on physiological and genetic mechanisms related to intramuscular fat (IMF), as well as the patterns of gene expression and their interactions during muscle development. Understanding the complex molecular

mechanisms that govern traits such as IMF and tenderness in beef cattle is essential for enhancing carcass quality. Therefore, we hypothesize that castration changes the expression of key genes involved in adipogenesis, lipogenesis, and energy metabolism pathways, ultimately leading to increased IMF content in skeletal muscle tissue. Specifically, we predict that steers will exhibit down-regulation of genes associated with energy expenditure and up-regulation of genes promoting lipid accumulation, contributing to the observed differences in IMF content compared to bulls. In this context, the objective of the current study was to identify DEGs in feedlot-finished *Bos taurus* × *Bos indicus* bulls versus steers by investigating the skeletal muscle transcriptome.

2. Material and Methods

2.1 Animals and samples

The experiment was conducted in the experimental feedlot facilities of Fazenda Turbilhão, Estrela D'Oeste, São Paulo, Brazil. The study evaluated 640 feedlot-finished F1 Angus-Nellore cattle with an initial body weight of 298.15 ± 28.58 kg and an age of 18 months. Animals were divided into two groups (320 bulls and 320 steers) and randomly allocated to eight pens (n = 4 pens/treatment; 80 animals/pen) equipped with a concrete feed bunk and water trough. All steers were castrated after weaning (7 ± 8months old) using a standard surgical procedure as described by [14] Silva et al. During the first two weeks after castration, steers were monitored daily, and appropriate medications (containing silver sulfadiazine and zinc oxide) were applied until complete healing.

The experiment lasted 180 days, including 14 days of adaptation of the animals to the diet and facilities. The experimental diet was the same for both treatments and consisted of 14% forage and 86% corn-based concentrate (Supplementary Table S1). The diet was provided with *ad libitum* twice a day, at 9 am and 3 pm. All animals were slaughtered in a commercial slaughterhouse following routine federal inspection procedures. During slaughter, hot carcass samples were randomly collected from 60 steers and 60 bulls. On the same occasion, a sample (approximately 100 mg) of *Longissimus thoracis* (LT) muscle was collected from the right half carcass between the 12th and 13th thoracic vertebrae and kept in RNA stabilizing solution (RNAlater,

Sigma-Aldrich, St. Louis, MO, USA). These samples were then transported to the laboratory and stored in a freezer at -80^{o}C until the time of analysis.

During deboning (48 h post-mortem), a meat sample of the LT muscle was collected between the 12th and 13th thoracic vertebrae from the left half carcass of each animal (*n* = 120). Samples were then taken to the laboratory, where 2.54-cm thick steaks were cut, vacuum packed individually and stored in a refrigerator at 2^{o}C for 10-day aging process. After this period, meat quality was analyzed as described by Santiago et al. [15]. Based on the meat quality results, three hot carcass samples were selected randomly for RNA-Seq analysis from each experimental group.

2.2 RNA sequencing

Total RNA was extracted from the samples using the RNeasy Mini Kit and the RNAse-Free DNase Set (both from Qiagen). Sequencing libraries were prepared with the TruSeq Stranded mRNA Kit (Illumina) using 500 ng total RNA. Briefly, RNA integrity was checked in a bioanalyzer using the RNA 6000 Nano Kit (Agilent). RNAs with poly-A tails were captured using poly-T oligos adhered to magnetic beads. The RNA with a poly-A tail was then fragmented, and the fragments were subjected to double-stranded cDNA synthesis, ligated to dual-index adapters, enriched by PR, and purified with magnetic beads to obtain the cDNA library. The size and purity of the libraries were checked in a bioanalyzer using the High Sensitivity DNA Kit (Agilent). Finally, the libraries were quantified in Qubit using the High Sensitivity dsDNA Assay Kit (Thermo Fisher) and pooled in equimolar amounts. The final concentration of the pool was adjusted to 4 nM. Sequencing was carried out on the Illumina HiSeq 2500 platform, considering 2 × 100 bp reads.

Quality control of the reads (fastp) was performed by individual analysis using FastQC v.0.11.9 [16]. Based on the results obtained, parameters were adopted to clean the reads in terms of the reading quality of each nucleotide, minimum read size (≥ 25), and presence of Illumina adapters and indexes using the FastP v.0.20.0 program [17]. After quality control, the reads were mapped to the bovine reference genome (Bos taurus UMD 3.1.1, http://www.ensembl.org/Bos_taurus/Info/Index/) with STAR v.2.7.10a [18]. Mapping was performed independently for each sample, allowing up to two mismatches per read. The sequencing quality was evaluated using the Quali-

Map v.2.2.1 [19] and v.1.12 programs [20]. Finally, a raw count matrix was constructed using featureCounts v.2.0.0 [21].

The expression of each gene was calculated as the average expression obtained for all samples of each group/ treatment and expressed as the log2 count per million (logCPM). Based on the logCPM ratio between the two treatments, the log2 fold-change was obtained (log2FC). The Benjamini and Hochberg [22] procedure was used to indicate the expected proportion of erroneously rejected null hypotheses, which permits to control of the false discovery rate (FDR). Significance values adjusted for FDR < 0.05 and log2FC thresholds < -0.5 and > 0.5 were used to identify DEGs. The alignment rate of the reads was obtained for all samples according to the group of animals (Supplementary Table S2).

2.3 Gene ontology analysis and identification of metabolic pathways

Inferences on the functional role of the DEGs were made using contrasts. For this purpose, overrepresentation analysis was performed by a hypergeometric test using the clusterProfiler v.4.2 package [23] of the R program. Biological pathways and processes were considered, with annotation of Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways and Gene Ontology (GO) terms. Genes that were differentially expressed in the two groups (Bulls *versus* Steers) were identified according to their biological function and then categorized and hierarchized into functional groups. This procedure permitted us to investigate expression patterns of functional groups and to identify cellular processes, cellular components, molecular functions, and differentially regulated biological pathways.

2.4 Co-expression and transcription factors analysis

The genes were submitted to co-expression and transcription factor analysis by CeTF package [24], for identify hub genes of study. This analysis employs regulatory impact factors (RIF) and partial correlation and information theory (PCIT) described by Reverter et al. [25] and Reverter and Chan [26]. Additionally, the genes found in CeTF analysis together with other DEG were used to correlate with ribeye area (REA) and IMF content in meat in the same animals, as described by Santiago et al. [3, 27].

2.5 Statistical analysis

Data was analyzed for residual normality by the Shapiro- Wilk test using PROC UNIVARIATE of the SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The analysis of variance (ANOVA) was applied to evaluate the effects of sex class on the performance variables and carcass traits using PROC GLM (SAS 9.4). The DEGs were obtained using the edgeR v.3.38.4 package [28] of the R software, in which normalization factors were estimated by trimmed mean of M-values (TMM) for all samples (pairwise). The hyperdispersion parameter was estimated by the robust empirical Bayes method, which was subsequently applied to obtain quasi-likelihood estimates of the count distribution parameters for each gene (in each group) using a generalized linear model and assuming a negative binomial distribution.

Functional enrichment of GO terms and KEGG metabolic pathways was performed using the clusterProfiler v.4.4.4 package [23] of R software v.4.2.1 (R Core Team, 2021). Biological processes and KEGG pathways were enriched by overrepresentation analysis using a hypergeometric test, with the level of significance set at 5%. The correlation graphic was plotted using corrplot function of the corrplot package [29] using the RStudio software, version 09.1.

3. Results

According to Santiago et al. [3, 27], castrated animals from the current experiment had a decrease in cattle growth performance and carcass weight; however, castrated animals improved meat quality traits of the animals ($P < 0.01$), according to previous studies using the same animals [3, 27]. An exorbitant difference ($P < 0.01$) in IMF content was found between the subgroups of steers versus bulls sampled for RNA-Seq analysis (5.59% versus 2.07%) and Ribeye area (Fig. 1).

3.1 Differentially expressed genes

The current study detected 16,173 different RNAs in the samples analyzed. Of these, 921 genes were differentially expressed, including 550 genes that were down-regulated and 371 that were up-regulated in the muscle of bulls versus steers (FDR < 0.05; Table S3). Principal component analysis presents a significant difference

between bulls and steers animals, with the first two principles components explaining more than 85% of the variation between samples (Fig. 2).

The MD plot (Fig. 3 illustrates the mRNAs that were differentially expressed in Bulls *versus* Steers. The heatmap in Fig. 4 lists the top 50 DEGs.

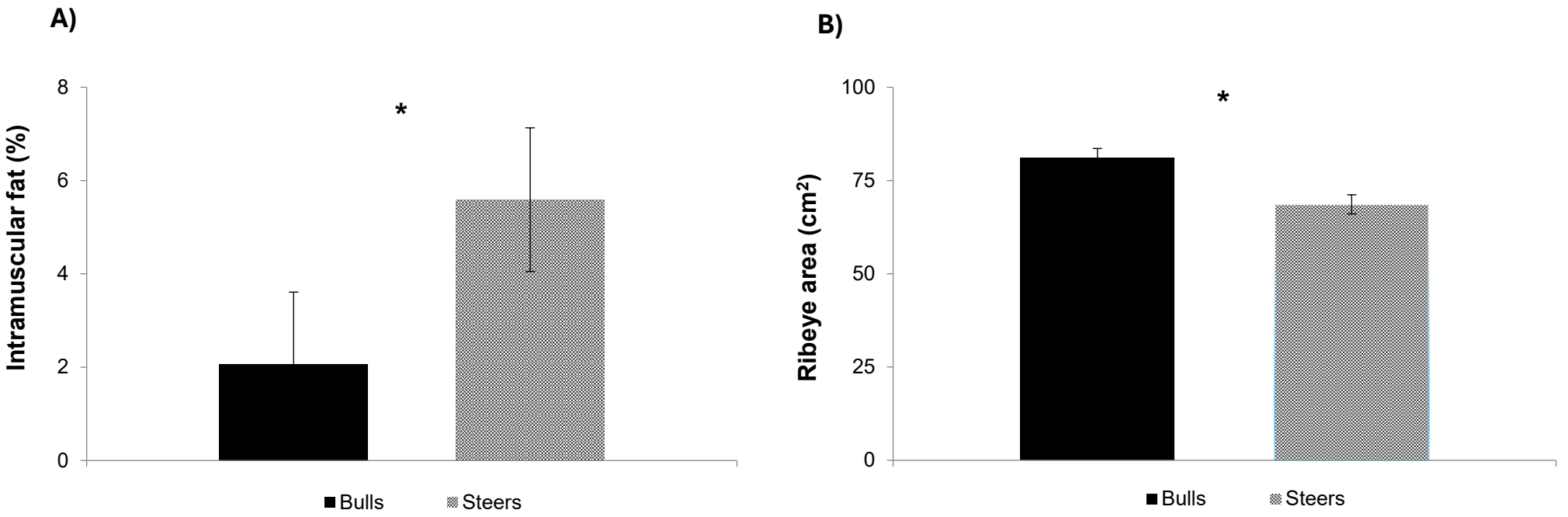


Fig 1. (A) Intramuscular fat content **(B)** Ribeye area in *Longissimus thoracis* muscle of feedlot-finished F1 Angus-Nellore bulls (n = 60) and steers (n = 60). * Significant difference ($P < 0.05$).

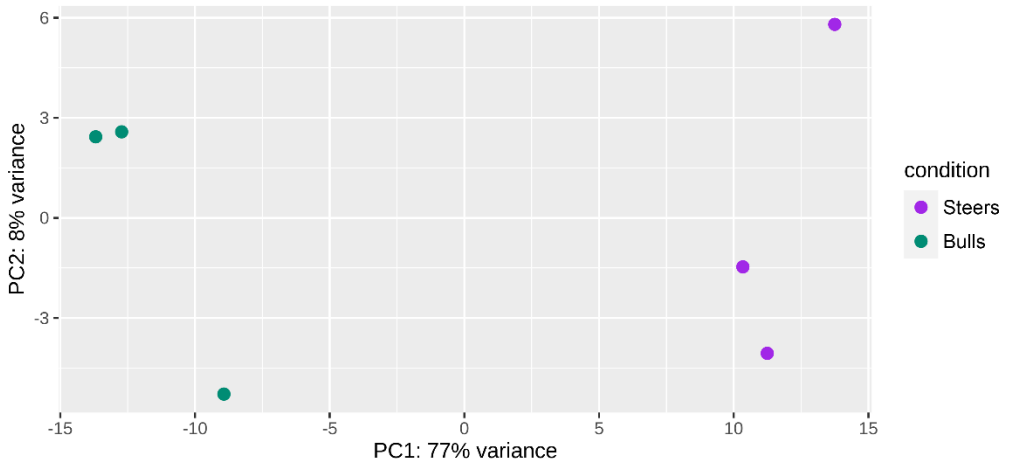


Figure 2. Principal component (PC) analysis of transcripts detected in the groups of animals (bulls versus steers).

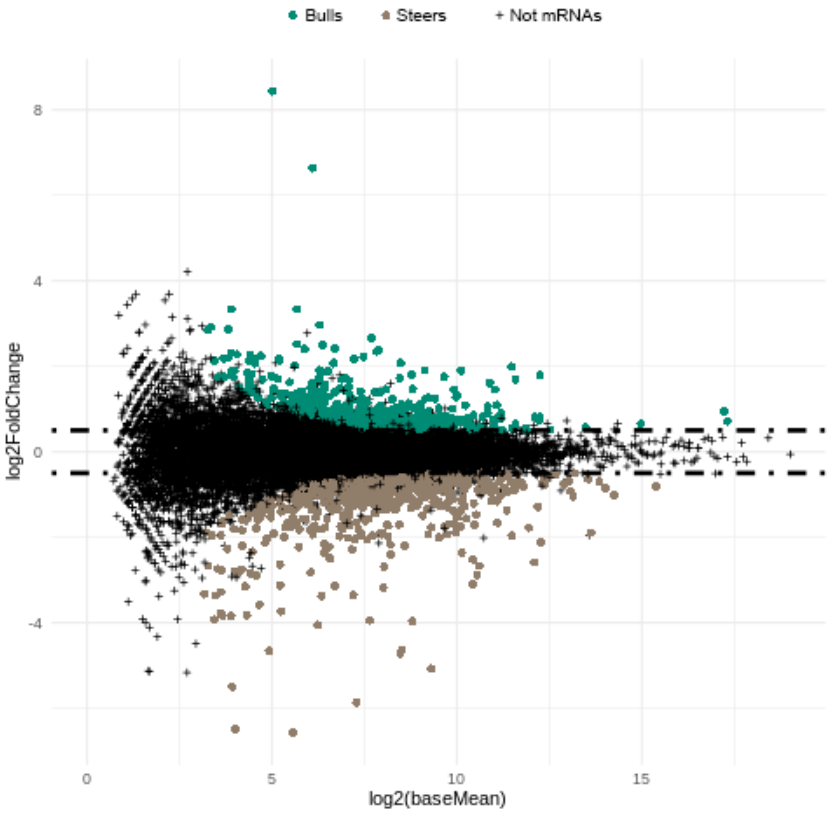


Figure 3. MD plot showing the log2 fold change versus log2 mean of differentially expressed RNAs. Green dots indicate genes that are up-regulated, and grey dots indicate genes that are down-regulated in bovine muscle (Bulls *versus* Steers).

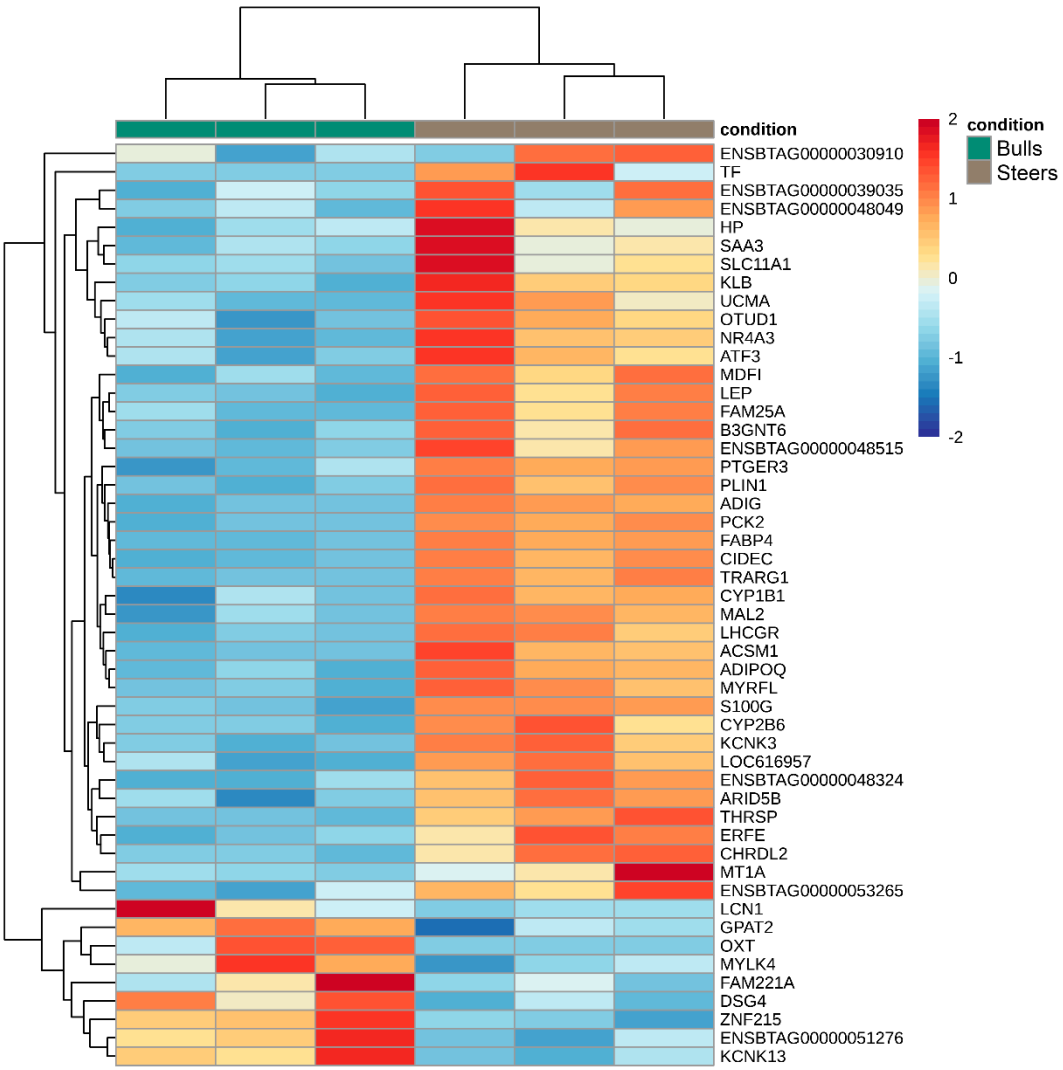


Figure 4. The top 50 genes identified as differentially expressed between F1 Angus-Nellore bulls and steers.

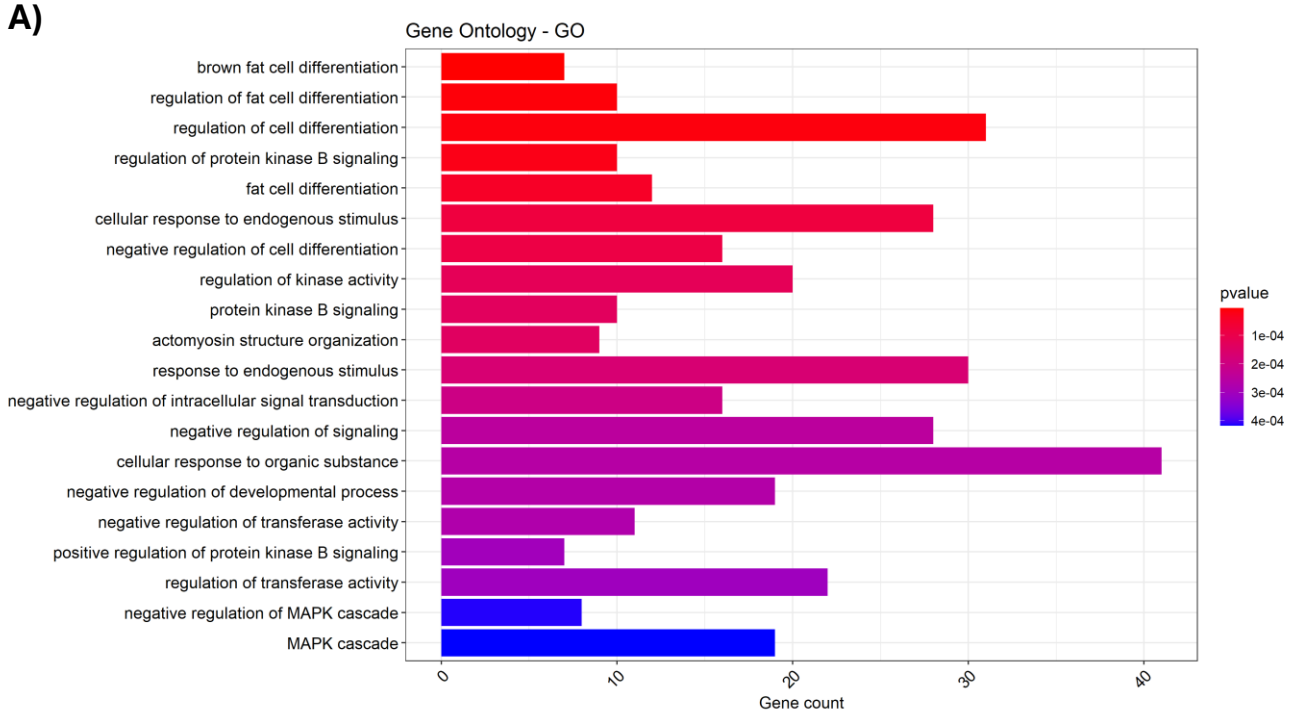
3.2 Gene ontology enrichment and KEGG pathway analysis of related DEGs

Evaluation of the relationship between DEGs and IMF deposition revealed 202 significantly ($P < 0.05$) enriched GO terms, 174 in biological processes, 13 in cellular components, and 15 in molecular functions (Fig. 5 and Table S5). Among the overrepresented pathways for up-regulated genes the following stand out: regulation of cell differentiation (GO:045595; $P = 0.04$), dephosphorylation (GO:016311; $P < 0.01$), muscle structure development (GO:061061; $P = 0.06$), sarcomere (GO:030017; $P = 0.05$; Table S6). In contrast, down-regulated genes had the following pathways highlighted: blood vessel development (GO:01568; $P < 0.01$), inflammatory response

(GO:06954; *P* = 0.04), fat cell differentiation (GO:045444; *P* < 0.01), response to insulin (GO:032868; *P* < 0.01; Table S7).

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis permitted to elucidate the molecular interactions and biological functions of the DEGs. The KEGG database was used to identify the main candidate genes. Ninety-five pathways were enriched (*P* < 0.10; Table S8), including the AMPK signaling pathway (bta04152; *P* < 0.01), PI3K-Akt signaling pathway (bta04151; *P* < 0.01), cAMP signaling pathway (bta04024; *P* < 0.01), insulin resistance signaling (bta04931; *P* < 0.01) and PPAR signaling pathway (bta03320; *P* = 0.02). The main KEGG pathways identified are shown in Fig. 6. The results obtained in this study highlight that fat deposition in steers is regulated by many transcription factors, signaling pathways, and expressed genes (Fig. 7), including AMPK, regulation of lipolysis in adipocytes, and PI3K-Akt. This fact suggests that their interaction may regulate fat metabolism in steers.

A)



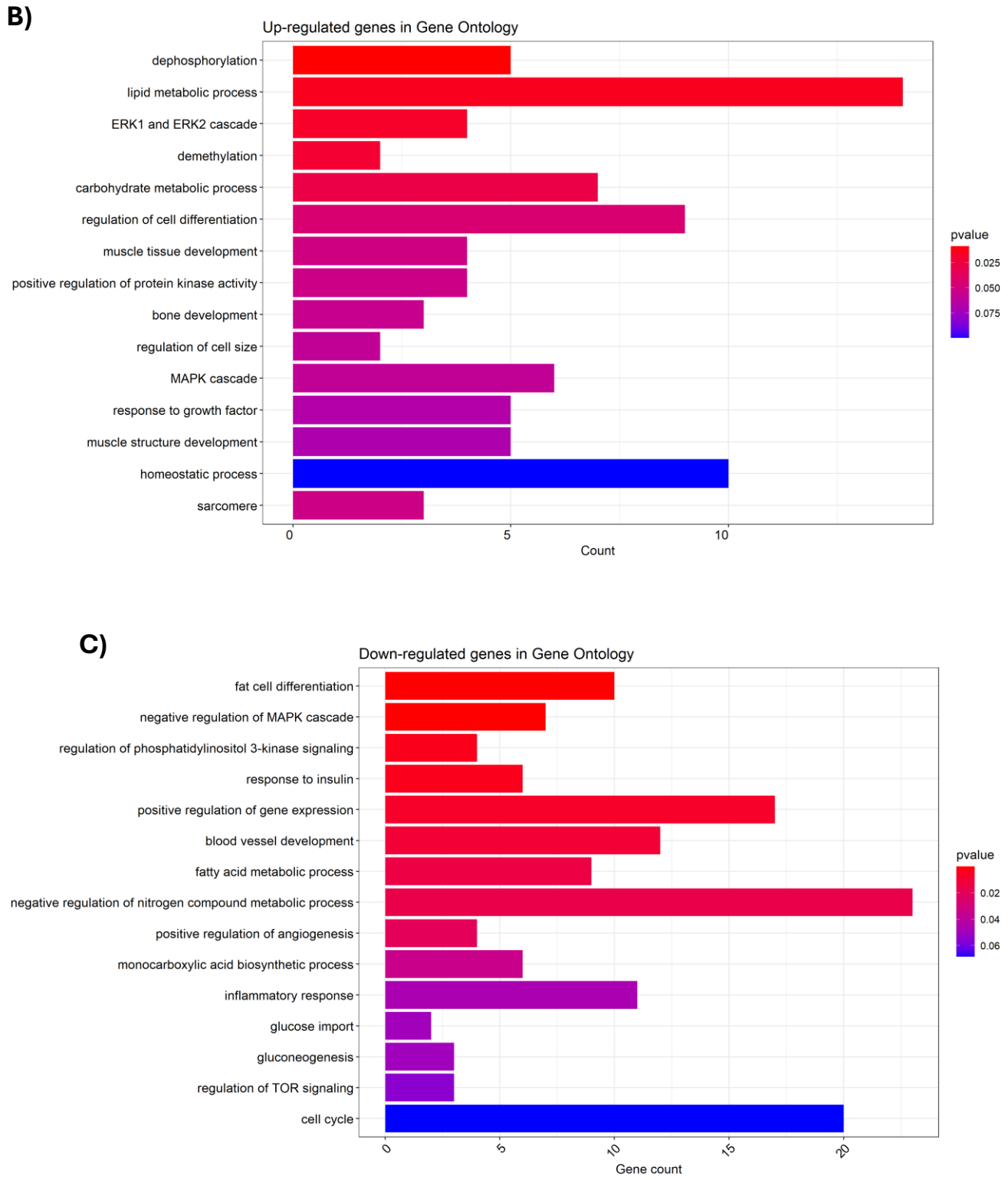


Figure 5. Gene ontology (GO) enrichment analysis of genes that are differentially expressed in F1 Angus-Nellore bulls versus steers. **(A)** GO terms for all genes differentially expressed **(B)** GO terms for up-regulated genes differentially expressed **(C)** GO terms down-regulated genes differentially expressed.

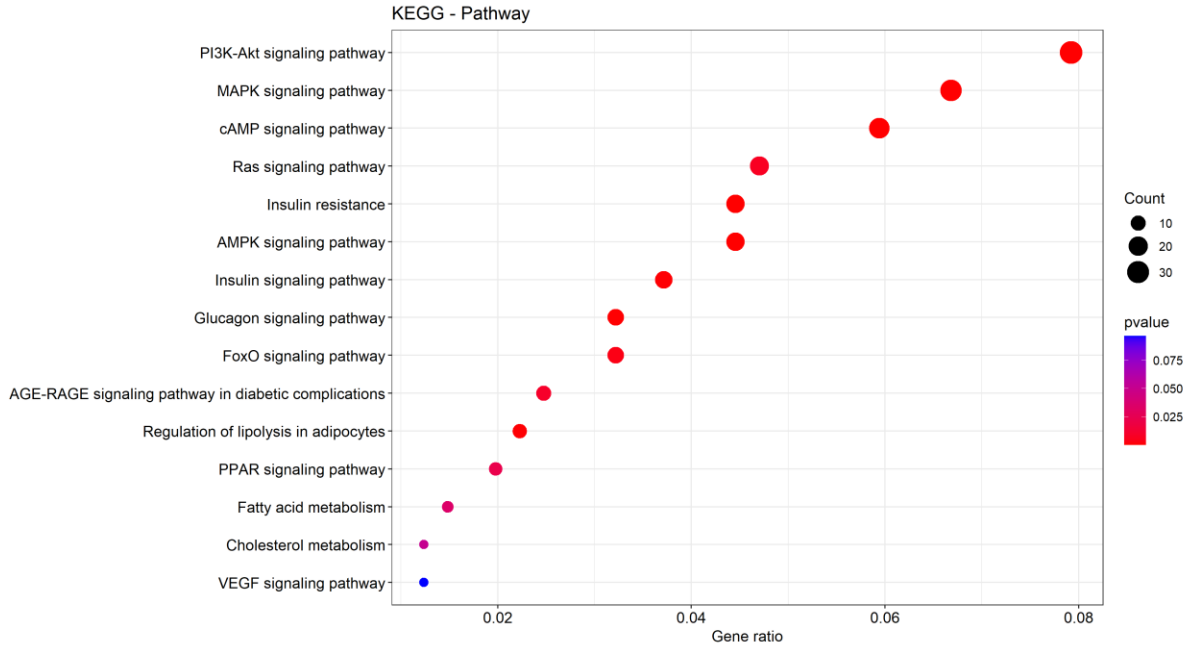


Figure 6. Analysis of related KEGG pathways in F1 Angus-Nellore bulls and steers.

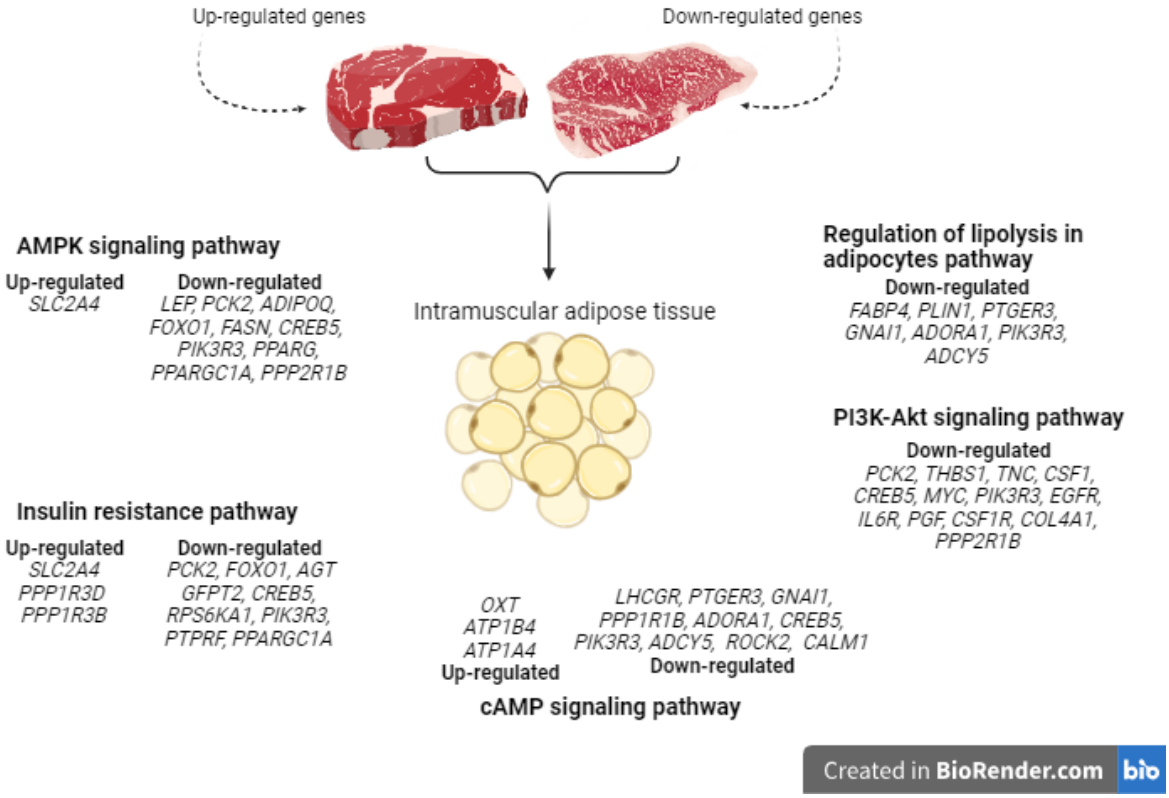


Figure 7. Regulatory mechanisms of signaling pathways and genes associated with fat metabolism and deposition in F1 Angus-Nellore bulls and steers

3.3 Hub genes analysis and correlation

The CeTF analysis results in 14 hub genes for the bulls *versus* steers contrast (Table S4), six up-regulated (*ASB4*, *CTSC*, *HMGA1*, *KPNA3*, *LGALSL*, *PRPF19*) and eight down-regulated (*EPAS1*, *KLHL40*, *LOC533308*, *MYL6B*, *PNRC1*, *TBCD*, *VPS13D*, *YWHAH*). Two correlation analyses were conducted. The first analysis examined the correlation between DEGs and hub genes with phenotypes (REA and IMF), while the second analysis explored the interactions between hub genes and DEGs to identify gene interactions (Fig. 8). Therefore, the hub genes *ASB4* (P = 0.01; r2 = -0.90 – Table S10 and Table S11), *HMGA1* (P = 0.04; r2 = -0.81), *KPNA3* (P = 0.03; r2 = -0.84), *LGALSL* (P = 0.01; r2 = -0.91, and *PRPF19* (P < 0.01; r2 = -0.92) were negatively correlated with IMF content. In contrast, the genes *EPAS1* (P = 0.03; r2 = 0.84), *LOC5300308* (P = 0.01; r2 = 0.88), *VPS13D* (P = 0.03; r2 = 0.83), and *YWHAH* (P < 0.01; r2 = 0.93) were positively correlated. The correlation analysis between hub genes and DEGs is important for revealing only the gene interactions among them. Positive or negative correlations are not discussed due to the imprecision of the analysis in inferring whether the expression of hub genes is the cause or consequence of DEG transcription. Thus, the focus was on hub genes that interacted with lipogenic genes (*ADIPOQ*, *FABP4*, *FASN*, *PPARG*), which actively participate in well-characterized pathways in fat deposition.

Therefore, the hub genes that had lipogenic genes in their interaction networks were *ASB4*, *HMGA1*, *KPNA3*, and *LGALSL*. The interactions with the *ADIPOQ* gene were significant for *HMGA1* (P = 0.02 – Table S12 and Table S13), *KPNA3* (P = 0.04), and *LGALSL* (P = 0.01). For *FABP4*, the significant interactions were with *HMGA1* (P = 0.02), *KPNA3* (P = 0.01), and *LGALSL* (P < 0.01). The interactions with *FASN* were significant for *HMGA1* (P = 0.04), *KPNA3* (P = 0.01), and *LGALSL* (P < 0.01), while for *PPARG*, the significant interactions were with *ASB4* (P = 0.02), *HMGA1* (P = 0.03), *KPNA3* (P = 0.01), and *LGALSL* (P < 0.01). The hub genes with a positive correlation with IMF were *EPAS1* and *VPS13D*. *EPAS1* had interactions with *CREB5* (P = 0.02), *HMGCR* (P = 0.04), *ITGA7* (P = 0.04), *RBM4* (P = 0.03), *RPS6KA1* (P = 0.01), and *THBS2* (P = 0.04), while *VPS13D* had many interactions, notably with *ADIG* (P = 0.04), *CREB5* (P = 0.03), *HMGCR* (P = 0.03), and *PIK3R3* (P = 0.03).

For the correlation with phenotypic traits, the genes correlated negatively with IMF were *CAV3* (P < 0.01; r2 = -0.91), *MSTN* (P = 0.03; r2 = -0.83). While the DEG

correlated positively were *ADIPOQ* (*P* = 0.03; r2 = 0.84), *ADCY5* (*P* = 0.02; r2 = 0.86), *ADIG* (*P* < 0.01; r2 = 0.95), *ADORA1* (*P* = 0.03; r2 = 0.84), *AKT2* (*P* = 0.01; r2 = 0.88), *CALM* (*P* = 0.04; r2 = 0.81), *CLIC1* (*P* = 0.02; r2 = 0.87), *CREB5* (*P* < 0.01; r2 = 0.95), *FABP4* (*P* = 0.02; r2 = 0.87), *FASN* (*P* = 0.01; r2 = 0.89), *FOXO1* (*P* < 0.01; r2 = 0.92), *GDI1* (*P* < 0.01; r2 = 0.95), *GNAI1* (*P* < 0.01; r2 = 0.91), *HMGCR* (*P* < 0.01; r2 = 0.97), *HSP90AB1* (*P* = 0.01; r2 = 0.89), *PCK2* (*P* < 0.01; r2 = 0.92, *PIK3R3* (*P* = 0.02; r2 = 0.88), *PLIN1* (*P* = 0.04; r2 = 0.81), *PPARG* (*P* < 0.01; r2 = 0.97), *PTBP1* (*P* = 0.04; r2 = 0.81), *PTGER3* (*P* = 0.01; r2 = 0.91), *RBM4* (*P* = 0.01; r2 = 0.90), *TFE3* (*P* = 0.03; r2 = 0.84), *YWHAH* (*P* < 0.01; r2 = 0.93). Among the DEGs that had correlation only with REA, stand out *SLC2A4* (*P* = 0.03; r2 = 0.84).

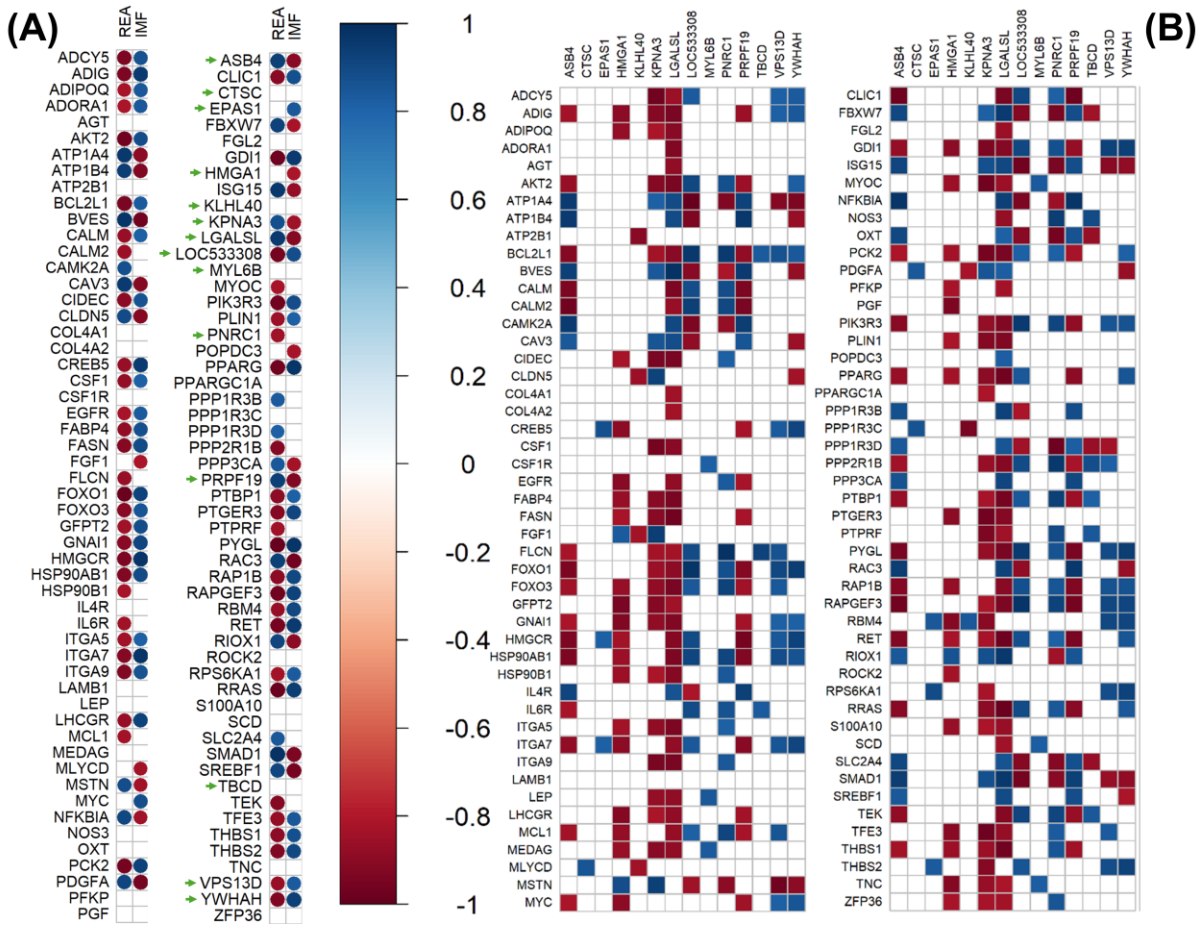


Figure 8. Correlation analysis. **(A)** Heatmap of the correlation between DEG and hub genes with phenotype (Ribeye area – REA and Intramuscular fat – IMF) in Bulls and Steers, previously reported in Santiago et al. [3, 25]. **(B)** Heatmap of the correlation between hub genes with DEG. The plotted points represent significant correlations between variables.

4. Discussion

Castration usually alters the body composition of cattle, slowing down muscle mass gain and rapidly increasing the development of adipose tissue [30]. These effects are caused by a decrease in the production of sex hormones, promoting benefits in fat and carbohydrate metabolism by improving carcass fatness and IMF deposition in meat [8, 9]. Meat quality traits are associated with biological processes during growth, development, and fat deposition, which are regulated by multiple genes, metabolic pathways, and vital activities. However, few studies have compared alterations in the muscle tissue transcriptome between bulls and steers, especially in terms of the mechanisms underlying the differences in meat quality between these animals [31].

The present study compared for the first time the transcriptome of feedlot-finished bulls and steers F1 crossbred Angus-Nellore with divergent IMF deposition. Enriched metabolic pathways were reported in muscle tissue, which helps to explain the differences in IMF deposition. These pathways include the AMPK signaling pathway, cAMP signaling pathway, insulin resistance pathway, and reported for the first time, the PI3K-Akt signaling pathway.

Functional enrichment analysis of the DEGs allowed to identify a set of genes that were considered to be the most important for the regulation of IMF deposition. Five significantly enriched metabolic pathways that are involved in energy metabolism, adipogenesis, and lipid metabolism and that act together in the regulation of IMF were selected. Studies investigating the mechanism of IMF deposition in cattle have identified genes involved in IMF deposition that were also identified in the present study, such as *FASN*, *FABP4*, *PPARG*, and *PLIN* [13, 32, 33].

Moreover, previous studies have also analyzed the expression profiles of candidate genes in order to identify molecular differences between bulls and steers [6, 8, 34]. Several DEGs that control IMF deposition in steers were identified in the present study. It was demonstrated for the first time in F1 Angus-Nellore cattle that the *FOXO1*, *PCK2*, *CALM1*, *LEP*, *ADIPOQ*, *FABP4*, *PLIN1*, *AGT*, *GFPT2*, and *ROCK2* genes are influenced by castration-induced hormonal alterations and are associated with greater IMF deposition observed in steer’s meat.

4.1 Insulin resistance pathway

The insulin pathway is strongly involved in muscle development since glucose utilization for energy production in fat and muscle cells is mediated by an increase in intracellular insulin signaling [35–37]. However, insulin resistance has been associated with lipid accumulation and is an important mechanism involved in IMF deposition in bovine muscle [38, 39]. Similar to the current study, insulin has been associated with adipogenesis and IMF deposition, improving traits such as meat tenderness, reducing drip losses, and stimulating the expression of genes that encode lipogenic enzymes in adipose tissue [40], confirming the results of the present study.

The *FOXO1* gene plays a key role in the regulation of glucose metabolism, and it is important for maintaining energy homeostasis by acting as a switch in the synthesis of carbohydrates and lipids in skeletal muscle [41]. In addition, *FOXO1* can repress the expression of the peroxisome proliferator-activated receptor gamma (*PPARγ*), a transcription factor that promotes adipogenesis through the formation of the *PPARγ–RXRa* heterodimer of target genes [42, 43]. However, depending on the stage of adipose tissue development, the *FOXO1* gene can act as a promoter of adipogenesis, especially during the stage of differentiation or lipogenesis [44]. In the present study, FOXO1 and FOXO3 were more highly expressed in steers and showed a positive correlation with IMF content. This indicates that *FOXO1* and *FOXO3* are key molecules contributing to greater intramuscular fat levels.

Moreover, the members of the FOXO family interact with AMPK and mTOR in response to nutrient availability and control protein synthesis and energy homeostasis. According to Schumacher et al. [45], they are only a few of the transcription factors involved in fat accumulation. Weikard et al. [46] reported that the *PPARGC1A* gene plays a critical role in several aspects of glucose, fat, and energy metabolism, with this gene being more expressed in samples from steers compared to bulls in the current study.

The *SLC2A4* gene that encodes the GLUT4 glucose transporters has been described as a key molecule in the regulation of meat quality and carcass traits because of its role in muscle and adipose tissue glucose metabolism [47]. However, in the present study, this gene was up-regulated in bulls and had positive correlation with the REA. Similar to the current study, Cesar et al. [48] also reported that Nellore steers with less marbling scores had a greater expression of the SLC2A4 gene.

Therefore, it is possible that glucose transport and the regulation of GLUT4 transporters mediated by insulin tend to be greater in animals that experience greater muscle hypertrophy, as is the case of non-castrated cattle [49].

The *PCK2* gene participates in the metabolic pathway of gluconeogenesis [50, 51]. This gene might be involved in the regulation of fat deposition, promoting the differentiation of adipocytes. Studying LT muscle of pigs, Wang et al. [51] reported a significant correlation between this gene and IMF deposition in the animals, which is in great agreement with the present study where *PCK2* was more expressed (top 50 DEGs in steers when compared to bulls) in cattle with greater IMF (steers). In the present study, the *PCK2* gene also has a positive correlation with IMF content and a negative correlation with REA.

4.2 AMPK signaling pathway

The adenosine monophosphate-activated protein kinase (AMPK) pathway acts as a key intracellular energy sensor that maintains the energy balance inside the cell and plays a critical role in the control of glucose and lipid metabolism [52]. According to Underwood et al. [53], the AMPK pathway is involved in IMF deposition in beef cattle, altering IMF and glycogen levels in bovine muscle. Similarly, in the present study, the AMPK pathway was fundamental for the greater IMF content in steers. Considering its role as a cellular fuel gauge and in energy partition, the genes encoding AMPK may be related to meat quality traits in cattle of the present study. These genes include *LEP*, *PCK2*, *ADIPOQ*, *FOXO1*, *FASN*, and *PPARG*.

Similarly, Heras-Saldana et al. [41], who studied DEGs in the *Longissimus* muscle of Hanwoo steers, also reported the *FOXO1*, *LEP*, *ADIPOQ*, *PCK2*, and *PIK3R3* genes to be related to the AMPK pathway. Once activated, the AMPK pathway induces glycolysis in skeletal muscle and directly controls lipid metabolism through the phosphorylation of acetyl-CoA carboxylase [54, 55]. Fatty acid synthase encoded by the *FASN* gene is one of the main lipid synthesis genes of the AMPK pathway. This enzyme is involved in the *de novo* synthesis of saturated fatty acids and is expressed mainly in adipose tissue [56, 57], this explains the result found in the present study, in which steers showed greater activation of the AMPK pathway. Among the DEGs belonging to AMPK pathway, the genes *ADIPOQ*, *AKT2*, *CREB5*, *FASN*, *FOXO1*,

HMGCR, *PCK2*, *PIK3R3* and *PPARG* had a positive correlation with IMF content and negative correlation with REA.

4.3 cAMP signaling pathway

According to Ravnskja et al. [58], cyclic adenosine monophosphate (cAMP) plays a significant role in adipogenesis, particularly in the differentiation and function of adipocytes, therefore affecting IMF deposition in cattle. Preadipocytes in adipose tissue retain the ability to differentiate when stimulated by adipogenesis, increasing intracellular cAMP levels [59].

However, the onset of adipogenesis has been shown to be associated with transcriptional changes in regulators of adipogenesis, for example, the receptor activated by *PPARγ* and CCAAT/enhancer binding protein α (C/ EBPα) as effectors of the cAMP pathway [60]. Studying the adipose tissue transcriptome of pigs, Xu et al. [61] reported that cAMP was involved in the regulation of pigs' feed efficiency by directly affecting lipid metabolism. This finding suggests that cAMP regulates both gene expression and enzymatic activities associated with fat metabolism [62].

Similar results were observed in the present study, in which the cAMP pathway regulated the expression of some genes of interest, such as *CALM1* and *ADCY5*, in the muscle of steers. The *ADCY* genes are regulators of lipolysis in adipocytes through the G protein pathways, which stimulate protein kinase A interposed by cAMP in adipocytes [63]. Li et al. [64] reported the *ADCY5* gene to be involved in growth, development, and lipid metabolism, for this study the *ADCY5* gene was positive correlated with IMF content and negatively correlated with REA.

This gene has been selected as a candidate gene for beef quality traits. Results from the present study suggest that calcium and cAMP act together to control fat metabolism in cattle, which helps to explain the greater IMF content observed in steers compared to bulls. Other genes were also expressed in steers (*LHCGR*, *PTGER3*, *GNAI1*, *PPP1R1B*, *CREB5*, *PIK3R3*, *ROCK2*, and *PDE4B*). Although literature data are scarce, our results suggest the association of these genes within the cAMP pathway and their involvement in the regulation of lipid metabolism in steers.

4.4 Regulation of lipolysis in adipocytes signaling pathway

The regulation of the lipolysis pathway has been suggested to play a critical role in the determination of meat quality [65]. However, little information is available in the literature. Therefore, it is still necessary to investigate which genes of this pathway are involved in the determination of meat quality and, more specifically, in the phenotypic differences in marbling or IMF. Similar to the data observed in steers from the current study, Lim et al. [66] also reported that the expression levels of the *FABP4* and *ADIPOQ* genes were significantly greater in animals with greater marbling. Other studies investigating the effect of castration on the regulation of *FABP4* also reported greater expression of this gene in castrated animals compared to non-castrated animals [67–69]. The *ADIPOQ* and *FABP4* were correlated positively with IMF and negatively with REA.

In agreement with these findings, in the present study, the *PLIN1* gene was regulated in steers that had greater IMF deposition. This gene is reported in the literature as encoding proteins in the structure of lipid droplets that regulate lipid storage. In humans and pigs, *PLIN1* has been described to be expressed exclusively in adipocytes [70]. The levels of *FABP4*, *PLIN1*, and *PPARG* suggest a larger number and a larger size of adipocytes in samples with more IMF after castration [7, 71]. In the present study, these genes were up-regulated in animals with a greater IMF content, suggesting that the castration-induced hormonal alterations may directly affect this pathway, which, in turn, exerts a direct effect on marbling in cattle. Regarding the *PTGER3* and *GNAI1* genes, few studies are available in literature; however, the results of the present study confirm the direct participation of these genes in animals with high marbling as they are positively correlated with IMF.

The adenosine A1 receptor (*ADORA1*) is known to play an important role in processes such as lipid catabolism, cell proliferation, and hormone secretion [72, 73]. When expressed abundantly, *ADORA1* attenuates lipolysis via the G-protein-coupled pathway and increases adipogenesis in peripheral adipose tissue [74, 75]. In pigs, this gene was confirmed to be fundamental for the deposition of lipids, being strongly linked to the regulation of muscle growth and fat deposition [73]. Additionally, in humans, transcription of the *ADORA1* gene predominantly occurs in mature adipocytes [76]. Within this context, the regulation of *ADORA1* in steers observed in the present study may indicate that IMF deposition starts earlier during the feedlot

period in these animals when compared to bulls, as demonstrated by its positive correlation with IMF.

4.5 PI3K-Akt signaling pathway

Adipogenesis involves a cascade of transcription factors that regulate the expression of genes involved in the development of adipocytes. The phosphatidylinositol 3-kinase (PI3K)-Akt pathway plays an important role in the differentiation of adipocytes, promoting adipogenesis through the phosphorylation of some substrates [77]. Recent studies have investigated the association of the FoxO, PI3K-Akt, and cAMP pathways with lipid metabolism [78]. However, the regulatory mechanisms of some genes expressed in the PI3-Akt pathway for fat deposition in cattle are poorly understood. According to the literature, the expression of the *PCK2* and *PIK3R3* genes, which are involved in the signaling pathways, favors IMF deposition [54, 79, 80].

In the present study, a significant number of genes involved in the PI3-Akt pathway were more expressed in steers. In addition to the two genes mentioned above, these genes are *THBS1*, *TNC*, *CSF1*, *CREB5*, *MYC*, *EGFR*, *IL6R*, *PGF*, *CSF1R*, *COL4A1*, and *PPP2R1B*, whose function still needs to be further explored since the regulatory mechanisms remain unclear in studies on beef cattle. These findings suggest for the first time the importance of the PI3K-Akt pathway and its genes in the lipid metabolism of steers, which can be confirmed by the greater fat deposition in these animals. Studies on pigs have observed that the PI3K-Akt pathway was associated with greater IMF deposition [81, 82].

4.6 Regulation of cell differentiation

The significant difference in the transcriptional profile between bulls and steers in the regulation of cell differentiation pathway can be attributed to hormonal difference between the two groups. Bulls had higher testosterone circulation and therefore exhibited higher expression of the *SRD5A1* gene, responsible for testosterone catabolism [83]. Greater testosterone levels in bulls results in a regulation of gene expression patterns directed towards muscle hypertrophy, as most of the genes in this pathway that were upregulated were positively correlated with REA.

The role of testosterone in glucose metabolism and body adiposity has been extensively studied in humans and various animals, but it remains a subject of controversy. According to the theory of Herbst and Bhasin [84], testosterone stimulates the commitment of mesenchymal pluripotent cells to myogenesis and inhibits the differentiation and maturation of adipocytes. Baik et al. [85], on the other hand, propose that testosterone is a crucial factor in regulating and controlling fatty acid uptake and synthesis in all tissues. Its deficiency leads to alterations in lipid metabolism, increasing the circulation of free fatty acids and cholesterol, resulting in greater fat deposition.

Indeed, steroid hormones, not just testosterone, significantly influence the proliferation and differentiation of adipocytes. In a cell culture study, dehydroepiandrosterone (DHEA) and 17 β-estradiol demonstrated effects on the development of 3T3-L1 cells, thus reducing the differentiation and proliferation of pre-adipocytes [86].

4.7 Castration and expression of the leptin gene

The metabolic pathways associated with energy metabolism and fat deposition in steers were influenced by the leptin (*LEP*) gene. This gene is involved in mechanisms that regulate feed intake, energy metabolism, reproduction, and the immune system [87]. The *LEP* gene is therefore considered a candidate gene for quantitative traits of economic interest, particularly fat deposition, meat tenderness, sexual precocity, and milk fat percentage. This gene is considered one of the most effective biomarkers related to body fat [88, 89] and is crucial for biological processes related to the growth performance and carcass and meat quality of cattle [90, 91].

Expression of the *LEP* gene mainly occurs in adipocytes and is activated by *C/EBPα* [92]. In the present study, a difference in *LEP* expression was observed between sex classes, with this gene being in steers compared to bulls. In addition, the gene was among the top 50 DEGs in steers when compared to bulls. This result may be related to the growth of fat deposits, which regulate the expression of the *LEP* gene [93]. Additionally, corroborated with the regulation of *ADORA1*, that may indicate that intramuscular adipocytes are completely mature in the steers studied here. This greater expression of the *LEP* gene in animals with greater IMF content observed in

the present study is due to the influence of adipocyte size on the synthesis and secretion of leptin, with mRNA being more abundant in larger adipocytes [93, 94].

4.8 Correlation of hub genes

In the current study, the hub genes *HMGA1*, *KPNA3*, *LGALSL*, *ASB4*, and *PRPF19* were found to interact with lipogenic genes, showing correlations with genes such as *ADIPOQ*, *FABP4*, *FASN*, and *PPARG*. However, they appear to act as inhibitory factors in fat deposition. Although members of the Asb family are reported as regulators in insulin signaling [95], the expression of *ASB4* is crucial in the regulation of protein turnover due to its ubiquitination/proteasomal system [96], indicating it as an important regulator in muscle growth and development [97]. This makes the *ASB4* gene a candidate genetic marker for animals with muscle growth and hypertrophy, as observed for bulls in this study.

Similarly, the hub gene *HMGA1* likely exerts an inhibitory regulation on fat deposition. The gene *HMGA1* encodes a non-histone protein that regulates chromatin structure and gene expression. In transgenic mice overexpressing *HMGA1*, its regulatory role in adipogenesis has been reported, hindering differentiation in a manner not associated with glucose homeostasis [98]. This supports our current study, where *HMGA1* showed a negative correlation with IMF.

Conversely, the hub genes *KPNA3* and *LGALSL* were negatively correlated with IMF and positively correlated with REA, indicating that their regulation of lipid metabolism genes is likely repressive. In a study with chickens, *KPNA3* was identified as an important gene for muscle development and rapid growth [99]. Regarding the *LGALSL* hub gene, a study with Holstein cows demonstrated that cows with higher milk yields had higher expression of *LGALSL* [100]. Therefore, the genes *KPNA3* and *LGALSL* are candidate genes for enhancing productivity and increasing meat deposition in animals, traits commonly associated with bulls.

In the case of hub genes positively correlated with IMF, *EPAS1* and *VPS13D* stand out. The hub gene *EPAS1* showed interactions with a few genes, all of which were correlated with IMF, making this hub gene a target for studies aimed at understanding adipogenesis and lipogenesis. The gene *EPAS1* has a crucial role in myogenesis development in cattle, sheep, and pigs [101], and also shows potential in adipocyte differentiation, as evidenced in cell culture by Shimba et al. [102]. On the

other hand, the hub gene *VPS13D* is associated with mitochondrial communication with lipid droplets [103] and has shown interactions with genes involved in energy metabolism. Therefore, the hub genes *EPAS1* and *VPS13D* are key candidate genes that require further studies for a better understanding of their roles in lipid metabolism in bovine muscle tissue.

5. Conclusion

The castration significantly affected the expression of genes that modulate IMF deposition. There was greater expression of *FOXO1*, *PPARG*, *PCK2*, *LEP*, *ADIPOQ*, *FASN*, *FABP4*, *PLIN1*, *PIK3R3*, *ADCY5*, *CALM1*, and *ADORA1* in LT muscle of steers compared to bulls, as well as enrichment of the insulin resistance, AMPK, cAMP, regulation of lipolysis in adipocytes, and PI3K-Akt pathways. Furthermore, genes such as *LHCGR*, *PTGER3*, *GNAI1*, *ROCK2*, *PDE4B*, *THBS1*, *TNC*, *CSF1*, *CREB5*, *MYC*, *EGFR*, *IL6R*, *PGF*, *COL4A1*, *AGT*, *GFPT2*, and *RPS6KA1* were more expressed in steers and, together, affected IMF deposition. Furthermore, four hub genes (*ASB4*, *HMGA1*, *KPNA3* and *LGALSL*) important for muscle growth and two (*EPAS1* and *VPS13D*) related to intramuscular fat deposition were highlighted. The results obtained can contribute to expanding the understanding of the effects of castration on the mechanisms that regulate IMF deposition in feedlot-finished F1 Angus-Nellore cattle, providing new insights into the biological model studied.

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CHAPTER 3 - A proteomics characterization about the effects of growth-promoting technologies on muscle in Angus and Brahman steers finished in a feedlot system[1]

[1]Este capítulo corresponde ao artigo científico que será submetido à revista *Journal of Animal Science*

Abstract: The objective of the study was to investigate the skeletal muscle proteome of Angus (*Bos taurus*) and Brahman (*Bos indicus*) steers managed with and without the use of growth-promoting technologies (GPT). Two hundred steers, 100 Brahman and 100 Angus, were used in a 2×2 factorial design to evaluate the effects of GPT on feedlot growth performance and carcass traits. Steers received either GPT (anabolic growth-hormonal implants, and an in-feed ionophore, antibiotic, and β-adrenergic agonist), designated as TRT (treatment) or no GPT, designated as CON (control). All animals underwent a 21-day diet adaptation before being finished on a grain-based diet for 159 days (total of 180 days on feed). At the end of the feeding period, carcasses were harvested and *Longissimus thoracis* muscle samples (five per treatment within breed) were analyzed by mass spectrometry–based proteomics to assess protein expression differences related to breed and GPT use. Angus steers had greater (P < 0.01) average daily gain (ADG) and final body weight (FBW) compared to Brahman steers. Steers that GPT were applied exhibited greater (P < 0.01) ADG, FBW, hot carcass weight (HCW) and *longissimus* muscle area (LMA) than steers managed without GPT. A total of 87 differentially abundant proteins (DAPs) were more abundant in the muscle of steers in the Angus TRT group, while 27 DAPs were more abundant in the Angus CON group; moreover, 23 DAPs were more abundant in the muscle of steers in the Brahman TRT group, while 17 DAPs were more abundant in the Brahman CON group. Regardless of breed, steers on the TRT group had a greater DAPs related to cellular energy metabolism, muscle hypertrophy and cell proliferation (NDUFS, NDUFB, CKMT2, HSPB, HSPA9, PREP, MYOF, MYH6, TPM2), while CON steers had more DAPs associated with catabolic processes and oxidative stress (FKBP4, MAPK1, GALM, LMO7, CALAR, UBE2M, PLIN1). The DAPs expressed in the TRT steers compared to CON cattle were related to more efficient

cellular metabolism, favoring muscle growth. However, CON cattle presented DAPs associated with muscle degradation, and oxidative stress, which help to explain the smallest FBW and ADG compared to TRT cattle. The current study has demonstrated that GPT enhances muscle metabolism by activating anabolic pathways. Therefore, changes in cellular respiration pathway led to improved growth performance in cattle that use GPT compared to CON cattle.

Keywords: beef cattle, cellular respiration, energy metabolism, muscle growth, protein abundance.

1. Introduction

The USA beef industry is the world's largest beef producer, producing approximately 12.29 million metric tons in 2024, representing 20% of global production (USDA, 2025). This significant impact on global beef production is strongly influenced by the use of growth-promoting technologies (GPTs) over the past decades (Drouillard, 2018). These technologies directly impact efficiency, average daily gain, and carcass weight (Johnson et al., 2013; Parr et al., 2016). Growth technologies include hormonal implants, β-agonists, ionophores, and antibiotics, which are agents that modulate metabolic characteristics that result in accelerated lean muscle growth (Johnson et al., 2013).

In addition to the use of GPTs, cattle breed also influences growth performance and herd composition (Drouillard, 2018). In the USA beef production system, the Angus breed (*Bos taurus*) is the predominant breed due to its superior carcass quality, maturity, and rapid growth rate compared to other traditional beef breed (Feuz et al., 2022; Scheffler, 2022). In contrast, the Brahman breed (*Bos indicus*) is characterized by its resistance and ability to adverse climatic conditions, nutritional stress and disease resistance, but these animals tend to have less desirable carcass and meat quality characteristics, particularly marbling and tenderness (Elzo et al., 2015; Scheffler, 2022).

These two breeds (Angus vs Brahman) have distinct physiological, genetic and phenotypic properties, which directly affect muscle development and, consequently, meat production (Okamoto et al., 2025). The differences observed between *Bos taurus* and *Bos indicus* cattle can be explained by molecular variations. Thus, proteomic profile represents an important tool for characterizing and obtaining specific information about proteins present in the muscle tissue of livestock (Gagaoua et al., 2024), which helps to understand the molecular processes involved in muscle growth, particularly when considering the impact of GPTs and differences between breeds. However, there are no reports in the literature that use a proteomic approach to explain the effects of GPT on growth performance and carcass characteristics.

To the authors knowledge, this is the first study to investigate the effects of GPT that influence growth characteristics. We hypothesize that the use of GPT distinctly alters the muscle proteome, and these effects may differ between Angus and Brahman

breeds, potentially helping to understand the molecular changes that explain the observed differences in growth performance and carcass characteristics. Therefore, the objective of this study was to investigate the skeletal muscle proteome of Angus (*Bos taurus*) and Brahman (*Bos indicus*) steers managed with and without the use of GPTs

2. Material and Methods

This study was approved by the Colorado State University Institutional Animal Care and Use Committee (protocol number 3712-13), and the experiment was carried out at the Colorado State University Agricultural Research, Education, and Development Center (ARDEC) experiment station in Fort Collins, Colorado, during the summer season, from April to October 2023.

2.1 Animals and muscle samples

The experiment was conducted with 200 steers, including Brahman (*Bos indicus,* n = 100) (initial body weight (IBW) = 342 ± 31 kg) and Angus (*Bos taurus*, n = 100) (IBW = 341 ± 21 kg). Steers of each breed were randomly assigned to a management treatment (TRT), animals that received GPT or control (CON) animals that did not receive any GPT, in a 2×2 factorial arrangement. Steers in TRT received a hormonal in-ear implant on 0 d (100 mg trenbolone acetate/14 mg estradiol benzoate; Synovex Choice, Zoetis, Parsippany, NJ, USA) and 84 d (200 mg trenbolone acetate/28 mg estradiol benzoate; Synovex Plus, Zoetis). Steers in TRT also received an in-feed ionophore (35 g/ton DM basis; monensin, Rumensin, Elanco, Greenfield, IN, USA), antibiotic (7 g/ton DM basis; tylosin, Tylan, Elanco), and BAA during the last 42 d of the feeding period (27 g/ton DM basis; ractopamine hydrochloride, Actogain, Zoetis), allowing for a 2 d withdrawal period prior to harvest. The CON steers did not receive any of the above-listed GPT.

After randomization, all steers were housed in pens by (breed/GPT and Control). These pens were designed to measure individual animal feed intake daily, as well as an estimate of the daily amount of methane production per animal (C-Locke Inc; Rapid City, SD, USA). All steers were transitioned to the final finishing diet after a 21 d adaptation from a starter diet to a finishing diet, including two intermediate step-up diets. The finish diet consisted of 65% steam-flaked corn, 20% corn silage, 7%

distillers grains w/ solubles. The individual body weight of the animals was collected at intervals of approximately 28 days throughout the study.

On day 180 all steers were weighed and shipped to the JBS facility in Greeley, Colorado, and individual carcass data were collected. Hot carcass weight (HCW) were assessed and the carcasses were chilled for 24h at −4°C, approximately 24 h after slaughter, the carcasses were ribbed between the 12th and 13th ribs, carcass data were collected by cameras, including *longissimus* muscle area (LMA) and marbling score. The Marbling score was evaluated on a scale: 300 = slight, 400 = small, 500 = modest, 600 = moderate (USDA, 1997). Samples of *Longissimus thoracis* were collected (10 per breed, n=20) and stored in an ultrafreezer (-80 °C) for proteomics analysis using Mass Spectrometry Analysis. Based on the random collection of samples, they were divided in 10 samples for Angus (5 samples for CON and 5 for TRT) and 10 samples for Brahman (5 samples for CON and 5 for TRT).

2.2 Muscle Sample Preparation

Protein Extraction

The tissue was weighed and transferred into a 2mL bead beating tube containing 4 stainless steel 1mm balls, 100mg of 0.1mm zirconia silica beads, and 1mL Alkaline-7 SDS buffer (5% SDS, 50 mM Tris-HCl, pH 8.5; 0.15 M NaCl; 0.1 mM EDTA; 1 mM MgCl2; 50 mM Dithiothreitol (DTT). Samples were subjected to bead beating at 6m/s for 5 cycles of 30 seconds, with a 2-minute dwell between cycles. Tubes were then centrifuged at 10,000 ×*G* for 10 minutes, and 400 µL of supernatant transferred to a new tube. 1.6 mL of -20 °C acetone was added to each sample, then frozen overnight at -20 °C for protein precipitation. After precipitation, samples were thawed and centrifuged at 16,000 × *G* for 20 minutes at 4 °C to pellet proteins. The supernatant was discarded, and the pellets were subjected to acetone washing (see Protein Acetone Wash and BCA).

Protein pellets were washed with 300 µL chilled (-20°C acetone) followed by briefly vortexing and centrifugation at 16,000 × *G* for 10 mins at 4°C. Acetone was removed and replaced with a fresh 1mL of chilled acetone and centrifuged. The acetone washing procedure was repeated for 3 total washes. Protein pellets were air-dried until all acetone evaporated, then solubilized in 500 uL of lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA). Pellets were sonicated for 10 minutes, then

subjected to 2 cycles of 10-minute freeze/thaw and vigorous vortexing to resolubilize. Samples were briefly microcentrifuged and the supernatant used for protein quantification. Total protein content of the samples was measured using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) following manufacturer instructions. Absorbance at 550nm was measured on a BioRad 680 microplate reader and total protein concentrations calculated based on a bovine serum albumin standard curve fit to a quadratic (Bio-Rad Laboratories, Hercules, CA, USA).

Protein Digestion

Samples were digested using the EasyPep Mini MS Sample Prep Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. Briefly, 100 µg total protein was aliquoted from each sample and raised to 100 µl with lysis buffer. Reduction and alkylation solutions were sequentially added followed by incubation at 95 ˚C for 10 minutes. After cooling to room temperature, 50 µg of a 0.2 µg/µL Trypsin/LysC mixture was added, and samples were digested with shaking at 37 ˚C for 2 hours. The enzymes were then deactivated with the Digestion Stop Solution and contaminants removed using mixed mode peptide clean up columns. Peptide eluate was dried in a vacuum evaporator and resuspended in 3% acetonitrile/0.1% formic acid (roughly 50 µL per sample to normalize to 1mg/mL). Once resolubilized, absorbance at 205nm was measured on a NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) and total peptide concentration was subsequently calculated using an extinction coefficient of 31 (Scopes, 1974).

Liquid Chromatography-Tandem Mass Spectrometry - LC-MS/MS

Chromatography was performed using water with 0.1% formic acid (A) and 80% acetonitrile with 0.1% formic acid (B). A total of 1 µg of peptides were randomly purified and concentrated using an on-line enrichment column (Thermo Fisher Scientific, Waltham, MA, USA PepMap Neo C18 5 µm, 300 µm ID x 0.5 cm). Peptides were eluted directly into the mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Nanospray Flex ion source (Thermo Fisher Scientific, Waltham, MA, USA) and spectra were collected over a *m*/*z* range of 375–2000 under positive mode ionization. Ions with charge state +2 or higher were accepted for MS/MS using

a dynamic exclusion limit of 1 MS/MS spectra of a given *m*/*z* value with an exclusion duration of 60s.

2.3 Data Analysis and Statistics

Data Processing

Growth performance data were analyzed with R (R Core Team, 2021, v. 4.4.1) software by three-way repeated measures (breed × production system × period and their interactions). The model diagnostics included testing for normal distribution of the error residuals, homogeneity of variance, and independence among observations. Differences were declared significant at $P \leq 0.05$.

Proteome Discoverer (PD) 3.0 was used for data processing (Thermo Fisher Scientific, Waltham, MA, USA). A precursor detector node with S/N=1.5 was used to identify additional precursors within the isolation window of the precursor spectrum when chimeric or mixed spectra is present. Spectra from all samples were searched using the Sequest HT node (Eng et al., 1994), setting methionine oxidation and acetylation of the N-terminus as dynamic and cysteine Carbamidomethylation as fixed modification. Subsequently, the data was submitted to an intensity-based rescoring process using a deep learning algorithm to predict fragment ion intensities (Zolg et al., 2025). Raw data was interrogated in separated analyses based on each breed. All samples were interrogated against the FASTA of the reference proteome for *Bos taurus* (ARS-UCD2.0) from Uniprot taxon ID: 9913. Additionally, for each analysis the cRAP proteome was included (The common Repository of Adventitious Proteins -cRAP- contains commonly found contaminant proteins in proteomics experiments) fasta file “cRAP_100518”. Sequest HT was searched with a fragment ion mass tolerance of 0.60 Da and a parent ion tolerance of 10 PPM. Peptide spectral matches (PSMs) were validated using the Percolator node (Käll et al., 2007). Thresholds were set such that a false discovery rate (FDR) of ≤1% and protein identification was defined with at least one peptide.

Bioinformatics analyses were conducted for the classification of differentially abundant proteins (DAPs) in muscle tissue from animals on MetaboAnalyst 6.0. The data were subsequently normalized by automatic scaling. The Volcano plot was performed to visualize the relationship between the magnitude of change and the statistical significance of differential protein expression between each group. Partial

Least-Squares Discriminant Analysis (oPLS-DA), and Student's *t*-test were then performed. The oPLS-DA was then performed to check whether the proteins profile was able to distinguish between TRT and CON group. A heatmap was constructed with the significant variables obtained from Student's *t*-test. The Gene Ontology (GO) pathway and process enrichment analyses were conducted using the Metascape® (https://metascape.org/) to investigate the main enriched GO terms (Biological Processes) from DAPs influenced by the factors (Breed, CON and TRT).

3. Results

Body weight increased significantly ($P < 0,01$) during the experimental period, with a magnitude greater for Angus than Brahman in final body weight (FBW). The average daily gain (ADG), hot carcass weight (HCW) and *longissimus* muscle area (LMA) were greater ($P < 0.03$), mainly in animals that used GPT, according to previous studies using the same animals (Schilling-Hazlett et al., 2025; Branine et al., 2025). However, the marbling score was reduced ($P < 0.01$) for cattle that were managed with GPT (Table 1).

Table 1. Growth performance and carcass data from Angus and Brahman steers managed with and without the use of growth-promoting technologies.

Item[1]	Angus TRT[2]	Angus CON[2]	Brahman TRT[2]	Brahman CON[2]	SEM[3]	*p*-Value[4]		
						B	T	B x T
ADG, kg	2.63	1.90	2.15	1.55	0.14	-	-	0.01
FBW, kg	652	594	604	564	4.1	<0.01	<0.01	<0.01
HCW, kg	430	390	368	356	11.59	0.04	0.03	0.23
Marbling score	483	600	329	344	15.1	<0.01	<0.01	<0.01

[1] ADG = average daily gain; FBW = final body weight; HCW = hot carcass weight; marbling score scale: 300 = slight, 400 = small, 500 = modest, 600 = moderate according to USDA standards.
[2] Experimental management treatment. CON= cattle managed without growth-promoting technologies, TRT = cattle managed with the use of growth-promoting technology.
[3] Standard error of the mean.
[4] The individual effects of breed (B), management treatment (T), and their interaction (B x T) were tested. Differences were considered significant when $P \leq 0.05$.
For ADG, only the breed × treatment interaction (B × T) was reported.

3.1 Proteome

In the current study, 1,543 proteins were identified in the muscle of cattle, of which 155 were abundant among treatments. Specifically, 23 DAPs were up-regulated in Angus CON, 87 DAPs down-regulated in Angus TRT, 17 DAPs in Brahman CON, and 28 DAPs in Brahman TRT (fold change of 1.5 and $P < 0.05$; Figures 2 and 3). When comparing breeds (Angus x Brahman), 110 proteins were differentially abundant in Angus steers, while 45 proteins were differentially abundant in Brahman steers (fold change of 1.5 and $P < 0.05$).

In Angus CON, differentially abundant proteins (DAPs) such as FKBP4, NPM1, GALM, CDKN1B, GPHN, KYAT1, EIF3L, MAPK1, ABCF1, and CZIB were identified. These groups of proteins are mainly related to muscle degradation, autophagy, and oxidative stress. In Angus TRT, DAPs such as NDUFS3, CKMT2, PSMD2, NDUFA7, NDUFB9, NDUFB8, NDUFA6, NDUFA2, IST1, and COQ3 were identified, which are related to mitochondrial and energy metabolism functions. However, in Brahman CON, DAPs including CALAR, UBE2M, IGSF23, PPIB, PLIN1, JPT2, CLASP1, RBM38, and LZTFL1 were identified, which are related to protein homeostasis, basal cellular stress, and tissue maintenance. However, Brahman TRT had greater DAPs such as PREP, MYOF, ITIH4, SERPINB6, TPM2, APOC3, MYH6, MTAP, ACY1, and LDHB, which are related to muscle hypertrophy, muscle growth and remodeling, changes in lipid metabolism, cellular response to chemical stress, and cell proliferation and tissue regeneration.

The oPLS-DA (Figure 4) demonstrates the clear separation between the groups and the highlighting of key proteins that contribute to variation between treatments. The heat maps report the list of DAPs in Angus (Figure 5) and Brahman (Figure 6) comparing the treatments (CON and TRT).

The current study has identified crucial biological pathways across breeds and treatments (Figure 7). In Angus TRT cattle, enriched Gene Ontology (GO) terms such as cellular respiration (GO:0045333), response to oxygen levels (GO:0070482), reactive oxygen species metabolic process (GO:00724593), response to temperature stimulus (GO:0009266), positive regulation of blood vessel endothelial cell migration (GO:0043536), mitochondrial electron transport (GO:0006122), tricarboxylic acid cycle (GO:0006099), carboxylic acid metabolic process (GO:0019752), and acetyl-CoA biosynthetic process from pyruvate (GO:0006086) were identified. While in Angus

CON cattle, the enriched pathways were microtubule polymerization (GO:0031113), protein-containing complex localization (GO:0031503), response to growth factor (GO:0070848), and autophagy (GO:0006914).

In Brahman TRT cattle, the enrichment GO terms comprises negative regulation of catabolic process (GO:0009895), muscle contraction (GO:006936), regulation of neurotransmitter secretion (GO:0046928), cytoplasmic translation (GO:0002181), carboxylic acid metabolic process (GO:0019752), endomembrane were enriched system organization (GO:0010256), purine-containing compound metabolic process (GO:0072521), and cellular response to chemical stress (GO:0062197). In Brahman CON cattle, the enriched pathways were cell-substrate adhesion (GO:0010810), regulation of cell junction assembly (GO:1901888), regulation of protein localization to cell periphery (GO:1904375), and negative regulation of protein depolymerization (GO:1901880).

For the biological processes identified in the GO (Supplementary table 2), distinct characteristics were observed between the groups. In Angus TRT cattle, 52.94% of DAPs were associated with the cellular respiration process (Log10(P) -75.94), and 17.65% with the carboxylic acid metabolic process (Log10(P) -8.34). For Angus CON, 13.64% of these proteins were associated with autophagy (Log10(P) -2.77) and 13.64% with response to growth factor (Log10(P) -2.12). In Brahman TRT cattle, 18.52% of the DAPs were associated with the carboxylic acid metabolic process (Log10(P) -3.23) and 11.63% with the cytoplasmic translation process (Log10(P) -5.33). However, in Brahman CON cattle, 12.50% of the enriched DAPs were associated with the negative regulation of protein depolymerization (Log10(P) -3.18) and 12.50% with the regulation of protein localization at the cell periphery (Log10(P) -2.51). Among the differentially enriched proteins between Angus and Brahman, only one was shared in both groups, being regulated differently between the breeds (Figure 8).

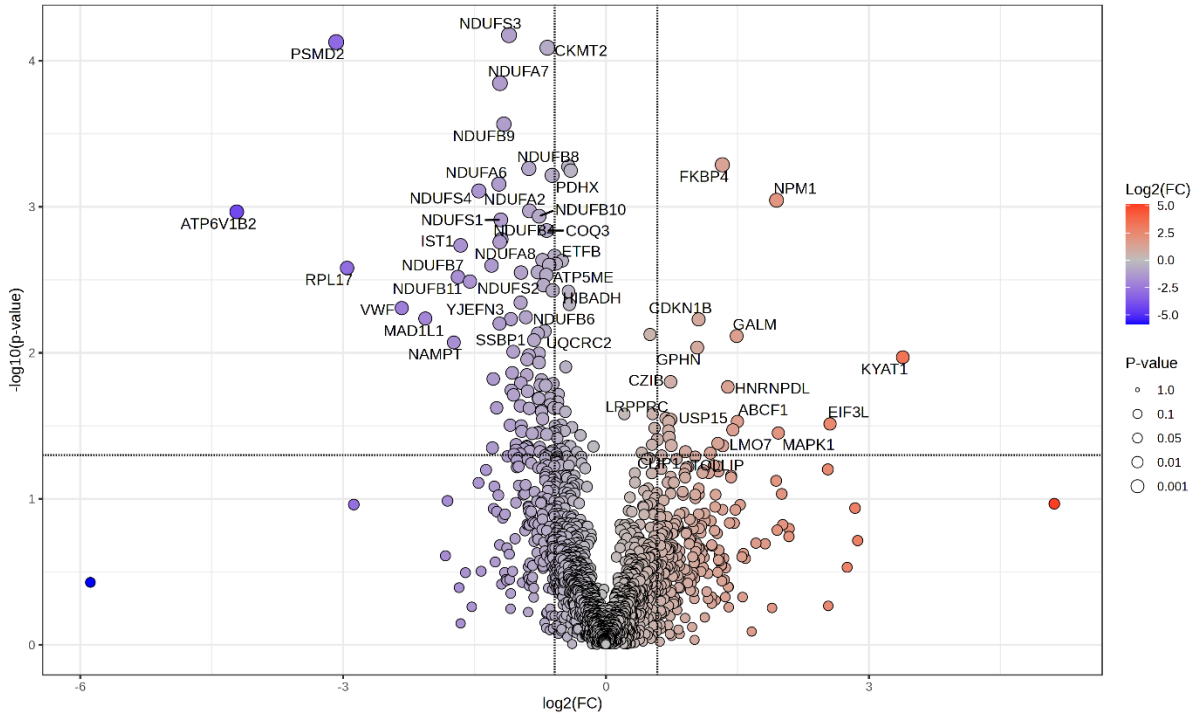

Figure 2. The Volcano plot of differentially expressed proteins in Angus CON and Angus TRT. (CON = red balls/right side, TRT = purple balls/left side). CON= cattle managed without growth-promoting technologies, TRT = cattle managed with the use of growth-promoting technology.

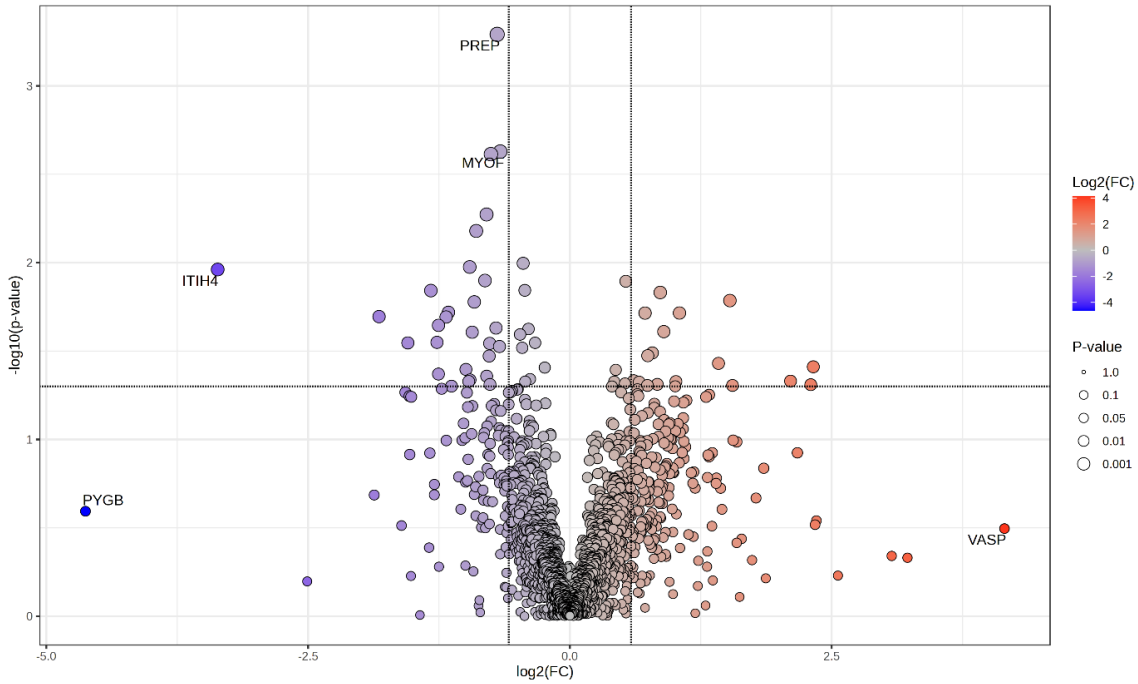

Figure 3. The Volcano plot of differentially expressed proteins in Brahman CON and Brahman TRT. (CON = red balls/right side, TRT = purple balls/left side). Experimental treatment: CON= cattle managed without growth-promoting technologies, TRT = cattle managed with the use of growth-promoting technology.

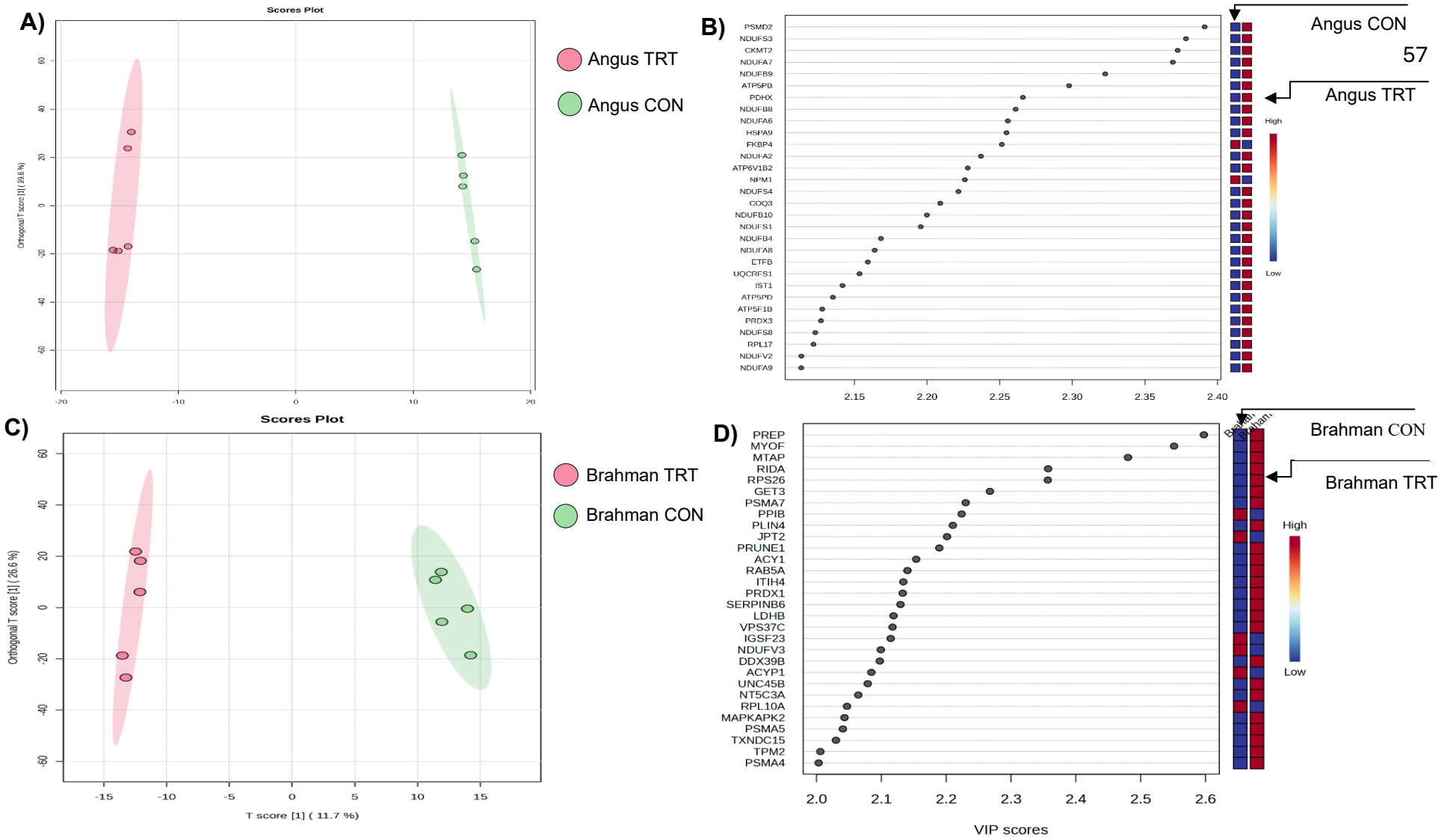

Figure 4. The oPLS-DA reports clear separation between each breed and treatment and the protein **v**ariable importance in projection (VIP) Score, which plays a role in explaining the variation in each group. **A)** oPLS-DA in Angus CON and TRT - (CON = pink balls/ left side, TRT = green balls/right side) **B)** Protein VIP Score in Angus CON and TRT - (CON = blue color/right side, TRT = red color/left side). **C)** oPLS-DA in Brahman CON and TRT. **D)** Protein VIP Score in Brahman CON and TRT. Experimental treatment: CON= cattle managed without growth-promoting technologies, TRT = cattle managed with the use of growth-promoting technology.

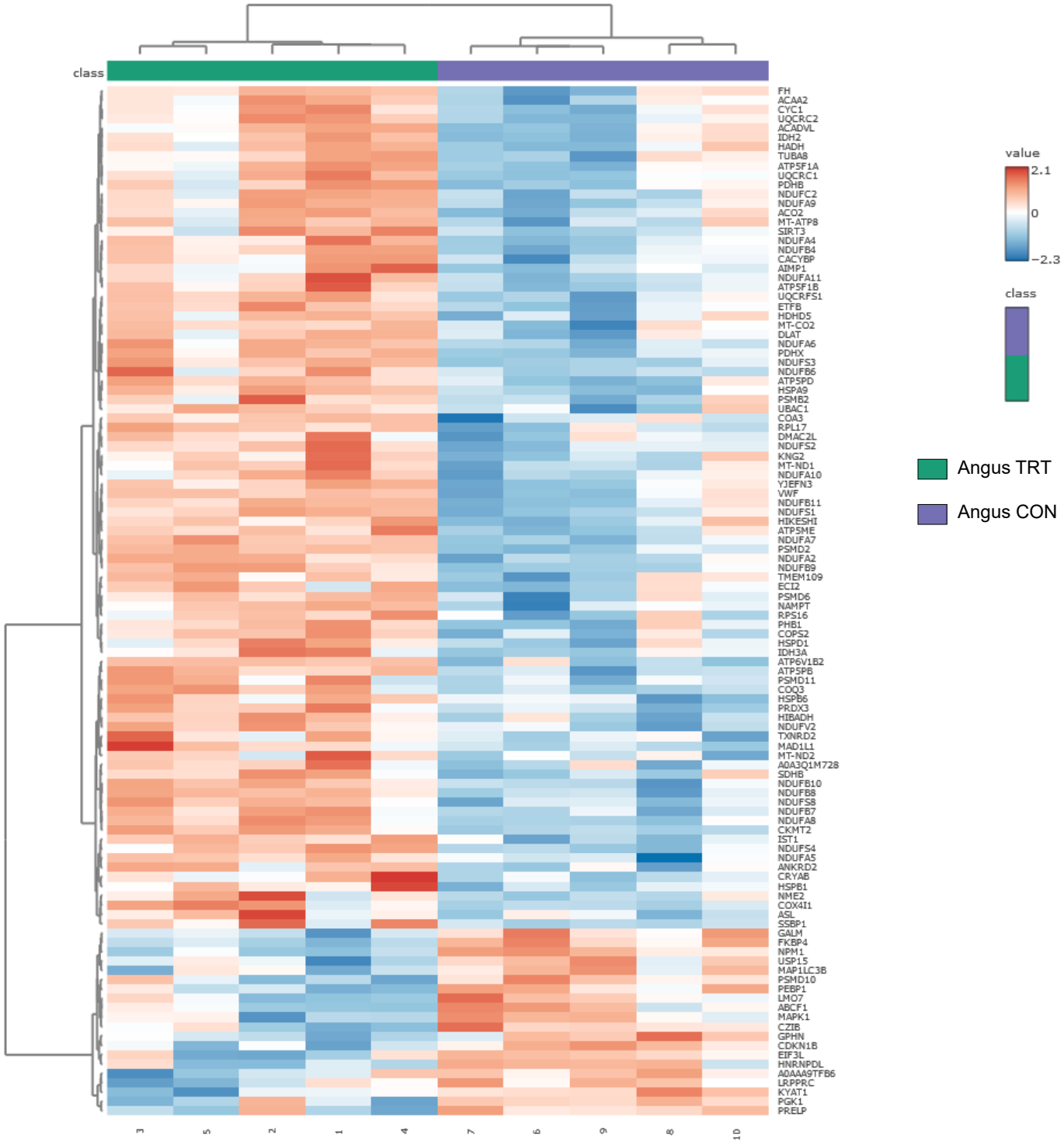

Figure 5. Heatmap with differentially expressed proteins in Angus CON and TRT Experimental treatment; CON= cattle managed without growth-promoting technologies, TRT = cattle managed with the use of growth-promoting technology. (CON = purple color/right side, TRT = green color/left side)

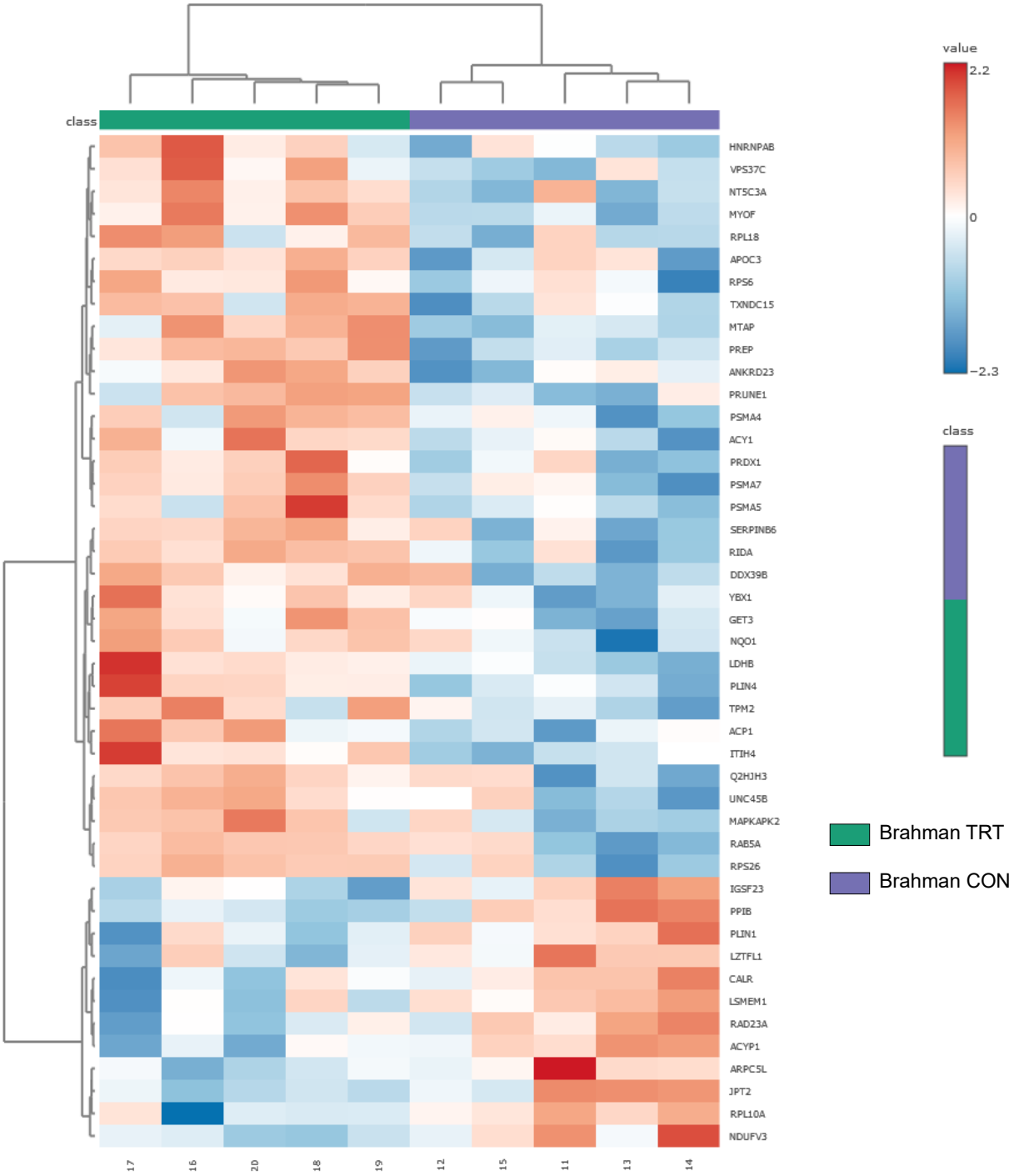

Figure 6. Heatmap with differentially expressed proteins in Brahman CON and TRT. Experimental treatment: CON= cattle managed without growth-promoting technologies, TRT = cattle managed with the use of growth-promoting technology. (CON = purple color/right side, TRT = green color/left side).

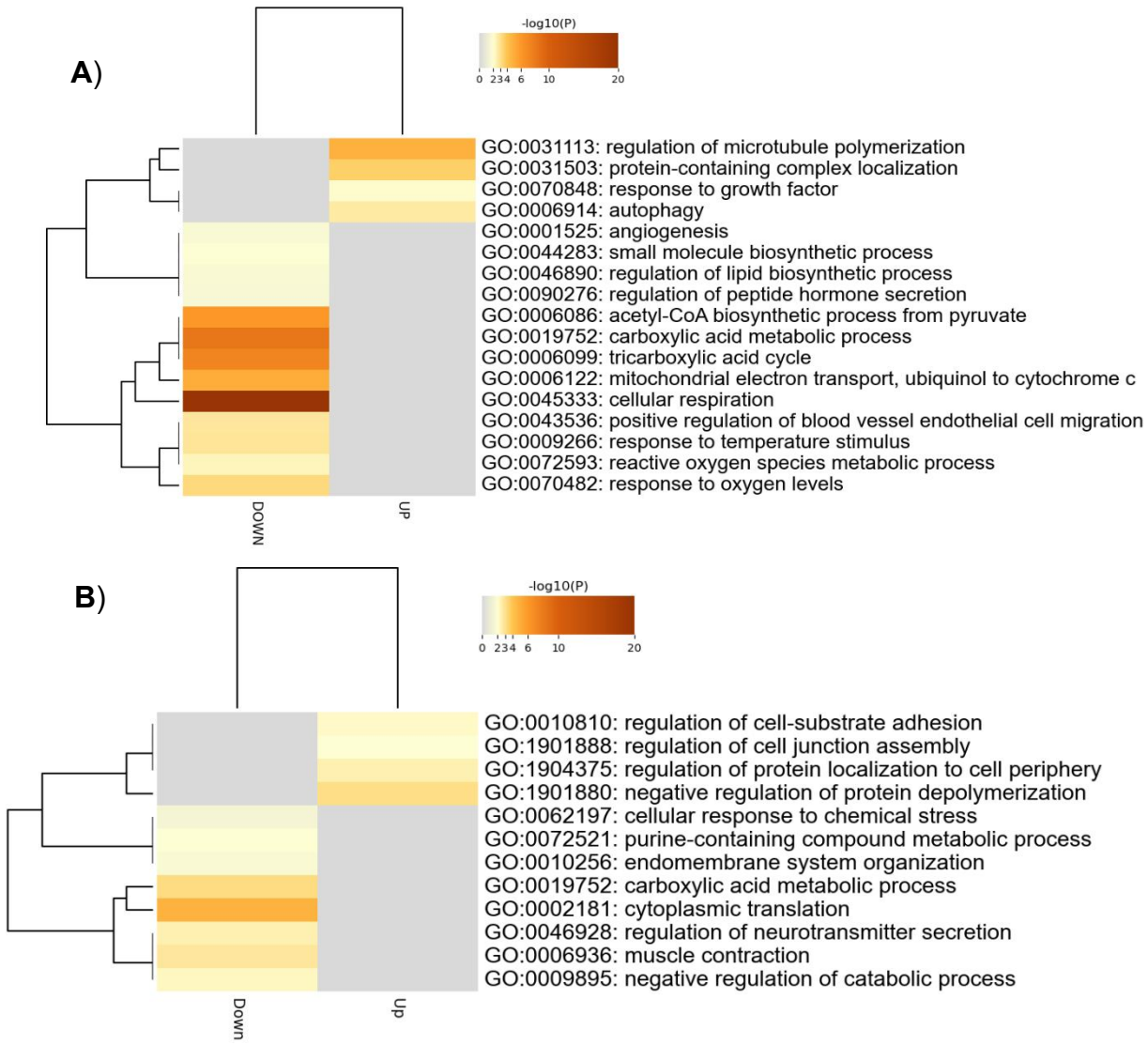

Figure 7. Heatmap clustering compares the enriched gene ontology (GO) terms within Angus and Brahman, as well as the main biological processes in each condition. **A)** GO in Angus TRT (Down regulated) and CON (Up regulated); **B)** GO in Brahman TRT (Down regulated) and CON (Up regulated). The heatmaps colored by P-value are indicated by color; palest brown indicates a low P-value, and darkest brown indicates a high P-value. Experimental treatment: CON= cattle managed without growth-promoting technologies, TRT = cattle managed with the use of growth-promoting technology.

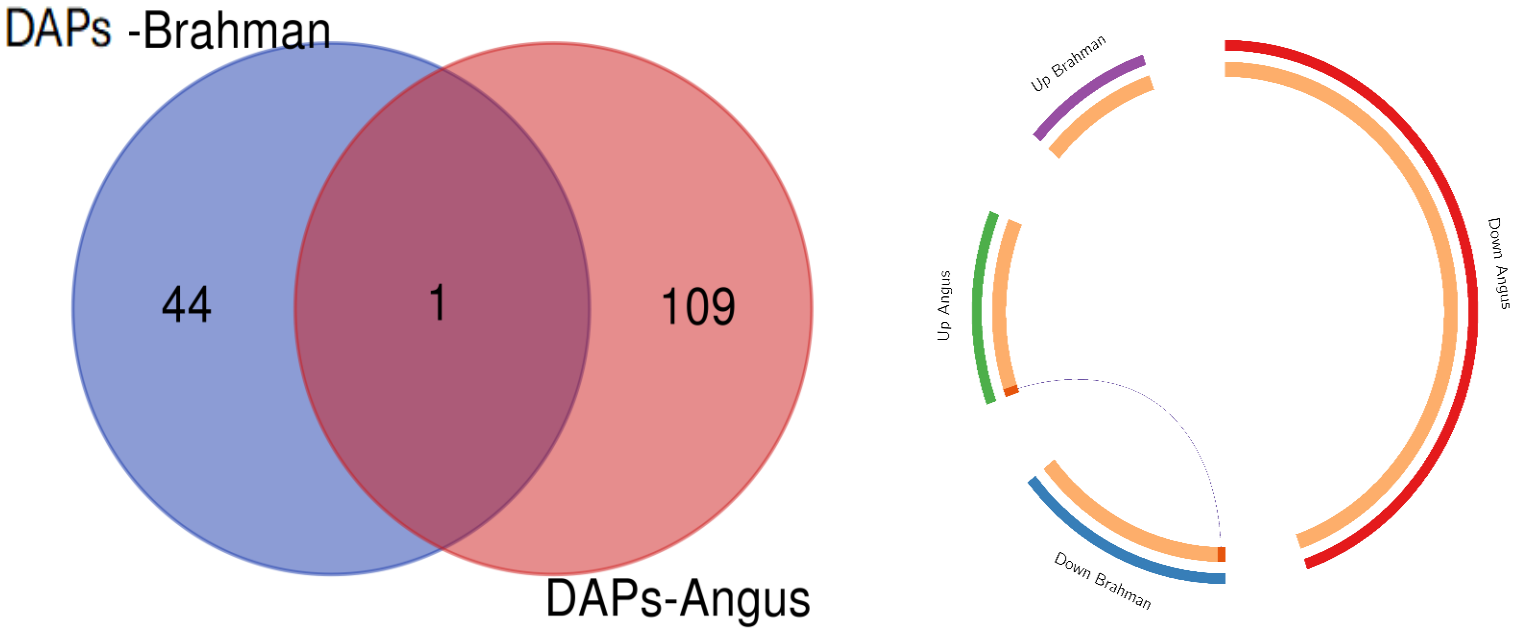

Figure 8. Comparison of differentially abundant proteins (DAPs) in Angus and Brahman. The Venn plot shows the unique DAP overlap between Angus and Brahman. Colors indicate the proteins that are Up (green and purple) or Down (red and blue) abundance, while the dotted line connects the only protein in common between Angus and Brahman, which showed opposite regulation between the groups. UP = CON: cattle managed without growth-promoting technologies; DOWN = TRT = cattle managed with the use of growth-promoting technology.

4. Discussion

Muscle development and growth characteristics, which are economically important traits in beef cattle production, can be affected by genetic and environmental factors. These factors modulate proteins, genes, and pathways that act on growth mechanisms (Zhou et al., 2016). This study focused on evaluating the skeletal muscle proteome of Angus (*Bos taurus*) and Brahman (*Bos indicus*) steers, particularly in the context of managing these animals with and without GPTs.

In the Angus TRT, the identified pathways are directly associated with mitochondrial function. Mitochondria are responsible for approximately 90% of cellular energy and a large proportion of adenosine triphosphate (ATP) synthesis, playing a crucial role in mammalian metabolic efficiency (McKenna et al., 2020; Herd and Arthur, 2009). Thus, variations in its efficiency alter the conversion of nutrient energy into ATP, resulting in differences in metabolic efficiency and productive performance (Hudson et al., 2017). As a result, animals that have more efficient mitochondria produce more ATP per unit of oxidized substrate, resulting in greater energy availability for processes such as muscle growth. Under stress conditions, however, nutrients incorporated into the cells require metabolic adjustments to maintain the balance between energy supply and utilization (Herd and Arthur, 2009). The results reported in this study are consistent with those cited by the authors, as TRT steers exhibited a greater growth rate and better performance than Angus CON steers, possibly due to more efficient energy utilization for muscle development.

Our results suggest that the use of GPTs may be associated with changes in DAPs, such as NADH ubiquinone oxidoreductase subunit (NDUF), which acts in the cellular respiration pathway and improves animal growth performance. Proteins within this subunit are important for cell growth, nutrient supply, and ATP production (Knocker and Yang, 2014). In a study by Karisa et al. (2013), which evaluated genes associated with carcass characteristics in beef cattle, NDUF subunit gene was reported to be related to muscle growth, such as ribeye area. Similarly, a study by Carmelo and Kadarmideen (2020), evaluating the muscle transcriptome and feed efficiency of pigs, observed that the NDUF was associated with animals with better feed efficiency, and that mitochondrial activity is a critical factor for the feed conversion rate in these animals.

The regulation of the protein mitochondrial creatine kinase 2 (CKMT2) is related to muscle energy metabolism, primarily responsible for regulating muscle growth and development (Wang et al., 2025). This protein is known to play a significant role in energy transport in tissues with high energy demands (Bai et al., 2020). This fact suggests that Angus TRT has a higher energy demand, as the use of GPTs directly influences the metabolic changes that may contribute to accelerated muscle mass growth (Parr et al., 2016).

The role of the protein increased sodium tolerance-1 (IST1) is not entirely clear in the muscle growth in livestock. However, there are reports that IST1 is involved in cytokinesis, hypertrophy, cell regeneration, and auxiliary roles in endosomal protein sorting (Bajorek et al., 2009; Clippinger et al., 2024). This set of proteins is involved in cellular respiration, mitochondrial electron transport, response to oxygen levels, and regulation of lipid biosynthesis, processes directly related to enhanced and efficient cellular metabolism. This, in turn, influences the factors that regulate growth and protein synthesis. Our results reinforce the potential of these proteins to enhance cellular metabolism, which could contribute to enhanced HCW and LMA in Angus TRT compared to Angus CON.

In contrast, the DAPs regulated in Angus CON are associated with fundamental biological processes for homeostasis, oxidative stress, and cell proliferation. Therefore, these animals tend to direct energy toward maintenance, defense, and regeneration, rather than rapid and efficient growth. Among them, FKBP4, a progestin receptor (PR) co-chaperone protein, plays a role in muscle regeneration. The FKBP4 has two during the cell proliferation process: in the initial phase, it acts rapidly, while in the late phase, it inhibits the myoblast differentiation process (Ruiz-Estevez et al., 2018). Furthermore, FKBP4 is a binding protein that interacts with the heat shock protein HSP90 to ensure that other proteins in muscle are correctly folded, active, and stable (Zhang et al., 2018). This process is important in conditions that require high metabolic demand or adaptation, especially for cellular maintenance and stress response.

The nucleophosmin protein (NPM1) belongs to the chaperone family and is an abundant and multifunctional phosphoprotein that acts in cell cycle control and cellular stress stimuli (Liu et al., 2023). The protein galactose mutarotase (GALM) is involved in glucose metabolism and plays a crucial role in oxidative pathways in skeletal muscle (Myers et al., 2013). Moreover, the CDKN1B (Cyclin-dependent kinase inhibitor 1B)

acts to inhibit cell proliferation and is essential for the heat stress response (Logan and Somero, 2011; Chen et al., 2023).

The mitogen-activated protein kinase (MAPK) pathway is well-described for its roles in cellular events, including cell proliferation and differentiation. In skeletal muscle, it plays an essential role in muscle fiber specialization and maintenance of muscle mass (Geisler et al., 2013). In cattle, its role is also known to affect lipid metabolism. In a study by Silva-Vignato et al. (2017), which examined the comparative muscle transcriptome associated with carcass traits in Nellore cattle, the researchers observed that the MAPK signaling pathway is involved in both muscle and fat deposition.

In relation to the regulation of the MAPK protein this may be consistent with our results. It is known that there are some concerns regarding the use of GPTs and its impact on meat quality, particularly the decrease in marbling score (Webb et al., 2020; Schilling-Hazlett et a., 2025). The use of GPT may reduce marbling compared to cattle without GPT, as it prioritizes the disposition of lean muscle tissue over adipose tissue (Parr et al., 2016), reinforcing that the expression of MAPK1 in Angus CON may be associated with greater fat deposition.

Other expressed proteins, such as GPHN, KYAT1, EIF3L, ABCF1, and CZIB, are not well described in the literature regarding their functions in bovine skeletal muscle. However, they appear to be associated with the maintenance of cellular homeostasis and oxidative stress. These results suggest that the DAPs expressed in Angus CON help explain the smaller growth performance response compared to Angus TRT, as these DAPs are more related to oxidative stress and control of cell proliferation than to functions that promote more efficient growth.

Conversely, the DAPs in Brahman TRT were related to muscle hypertrophy, muscle growth and remodeling, changes in lipid metabolism, cellular response to chemical stress, cell proliferation, and tissue regeneration. Among them is Myoferlin (MYOF), a muscle-specific transmembrane protein with an essential function in myoblast fusion. Thus, skeletal muscle growth requires several steps accompanied by the fusion of myoblasts, which are precursor cells to form muscle fibers (Han et al., 2016). These myoblasts fuse to increase the myofiber (Posey Jr et al., 2011). This protein becomes essential for muscle growth and development, especially when animals receive some stimulus, as observed in Brahman using GPT. This fact may be

associated with the acceleration of the muscle hypertrophy process and protein deposition in these animals compared to CON Brahman.

The Inter-Alpha-Trypsin Inhibitor Heavy Chain 4 protein (ITIH4) acts in the regulation of inflammation and stabilization of the extracellular matrix (Wu et al., 2023). Although there are no defined reports on its function in cattle, this protein may help in some inflammatory processes that may occur, as TRT animals present a more accelerated growth of muscle tissue, that is, this protein may contribute to cell regeneration. The Serpin Family B Member 6 protein (SERPINB6) is responsible for promoting cell proliferation. According to Zhao et al. (2024), evaluating the transcriptome and genome of chickens, the authors reported that SERPINB6 was associated with carcass characteristics such as carcass weight, dressed weight and breast muscle weight. The expression of this gene may promote the proliferation of fibroblasts and myoblasts, which favors muscle growth and regeneration. This result also helps us understand that the regulation of this protein may have contributed to the improved FBW in Brahman TRT, stimulating muscle hypertrophy, as reported in other species.

Tropomyosin (TPM2) is an action-binding protein that regulates muscle contractile activity and also regulates cell growth and development (Xu et al., 2012). In bovine skeletal muscle, the function of TPM2 is linked to myoblast proliferation and differentiation (Cho et al., 2016; Dube et al., 2021). According to Wang et al. (2017), studying key proteins that affect muscle growth in pigs, they found TPM2 to be one of the proteins involved in regulating muscle growth. This result is consistent with our results, where this protein was down-regulated in Brahman TRT, which may be associated with the use of GPTs and its effect on muscle growth. Moreover, there are no clear reports in the literature on the proteins PREP, APOC3, MTAP, ACY1, and LDHB in bovine muscle, their regulation in Brahman TRT suggests participation in some metabolic processes related to the modulation of responses associated with accelerated muscle growth.

In contrast, the DAPs in Brahman CON are related to protein homeostasis, basal cellular stress, and tissue maintenance. For example, the Ubiquitin-conjugating enzyme (UBE2M) acts as an E2 ubiquitin-conjugating enzyme, mainly involved in the regulation of cellular response and protein degradation processes (Zhou et al., 2024). It is a stress-induced protein, regulated according to cellular responses to environmental stimuli (Zhou et al., 2018). This fact helps to understand why the

UBE2M protein was up-regulated in Brahman CON, suggesting that animals that do not use GPT may have a greater metabolic demand to maintain weight gain, that is, generating a greater response to oxidative stress. In this condition, muscle cells try to adapt using a stress response mechanism and do not prioritize the deposition of muscle mass.

The Perilipin 1 (PLIN1) is a structural protein of lipid droplets that regulates lipolysis (Shijun et al., 2020). In some studies, PLIN1 has been described at the transcriptional level as a gene associated with the upregulation of adipogenesis in cattle and pigs (Gandolfi et al., 2011; Li et al., 2018; Shijun et al., 2020; Shi et al., 2024). This gene is considered a candidate marker for improving fat deposition characteristics in livestock. Our results indicate that this protein may be related to the fact that animals using GPT tend to have less intramuscular fat deposition. Thus, GPT are associated with better animal growth performance and protein accumulation in the carcass through an increase in the area of the longissimus muscle (Smith et al., 2007; Tokach et al., 2010; Johnson et al., 2018), that is, as the accumulation of lean muscle is prioritized, there may be a decrease in the deposition of adipose tissue in the muscle (Parr et al., 2016).

Interestingly, the protein Heterogeneous Nuclear Ribonucleoprotein (HNRNPDL) was identified in both Angus CON and Brahman TRT groups. This is an RNA-binding protein involved in mRNA transcription and regulation (Batlle et al., 2020), and also participates in different biological functions, including controlling the expression of numerous proteins and growth factors, as well as maintaining muscle function (Li et al., 2019).

We suggest that the regulation of this protein may be related to its activity in maintaining muscle function in Angus CON. According to Hu et al. (2017), HNRNPDL plays an important role in normal muscle development, and its inactivation can retard growth. As well, in the metabolic adaptation process in Brahman TRT, this protein may modulate other proteins in response to increased energy demand, potentially influenced by the use of GPT. According to Li et al. (2019), HNRNPDL is responsible for the transcriptional regulation of mechanisms that enable cells to modulate gene expression in response to physiological conditions, as verified in Brahman TRT.

5. Conclusion

The current study has demonstrated that GPT distinctly affect the skeletal muscle proteome of Angus (*Bos taurus*) and Brahman (*Bos indicus*) steers. In Angus, GPT can influence mitochondrial and energy metabolism proteins (NDUF, CKMT2, COQ3), enhancing ATP production and muscle growth efficiency. Conversely, Angus control exhibited proteins linked to cellular maintenance and stress response, suggesting smaller growth potential and greater fat deposition. In Brahman steers, GPT increased the abundance of proteins related to myogenesis and tissue remodeling (MYOF, SERPINB6, TPM2), whereas Brahman control presented greater expression of proteins associated with oxidative stress and lipid metabolism (UBE2M, PLIN1). These breed-specific proteomic responses indicate that GPT modulates distinct biological pathways influencing energy use, muscle hypertrophy, and carcass composition.

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