TAIANE DA SILVA MARTINS

Diferença esperada na progênie (DEP) para precocidade de tourinhos Nelore e sua relação entre adipogênese, fibrogênese, lipogênese e metabolismo lipídico

Pirassununga
2021

TAIANE DA SILVA MARTINS

Diferença esperada na progênie (DEP) para precocidade de tourinhos Nelore e sua relação entre adipogênese, fibrogênese, lipogênese e metabolismo lipídico

Tese apresentada ao Programa de Pós-Graduação em Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para a obtenção do título de Doutor em Ciências.

Departamento:
Nutrição e Produção Animal

Área de concentração:
Nutrição e Produção Animal

Orientadora:
Profa. Dra. Angélica Simone Cravo Pereira

Pirassununga
2021

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO

(Biblioteca Virginie Buff D'Ápice da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo)

T. 4053
FMVZ

Martins, Taiane da Silva
Diferença esperada na progênie (DEP) para precocidade de tourinhos Nelore e sua relação entre adipogênese, fibrogênese, lipogênese e metabolismo lipídico / Taiane da Silva Martins. – 2021.
200 f. ; il.

Tese (Doutorado) – Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Nutrição e Produção Animal, Pirassununga, 2021.

Programa de Pós-Graduação: Nutrição e Produção Animal.

Área de concentração: Nutrição e Produção Animal.

Orientadora: Profa. Dra. Angélica Simone Cravo Pereira.

1. Bovinos de corte. 2. Espessura de gordura subcutânea. 3. Vias metabólicas. 4. Metabolismo proteico e lipídico. 5. Proteômica. I. Título.

Ficha catalográfica elaborada pela bibliotecária Maria Aparecida Laet, CRB 5673-8, da FMVZ/USP.

CERTIFICADO

Certificamos que a proposta intitulada "Diferença esperada na progênie (DEP) para precocidade de tourinhos Nelore e sua relação entre adipogênese, fibrogênese, lipogênese e metabolismo lipídico.", protocolada sob o CEUA nº 3367170317 (ID 005067), sob a responsabilidade de **Angélica Simone Cravo Pereira** *e equipe; Taiane da Silva Martins; Rafael Aparecido Gomes; Saulo da Luz e Silva; Heidge Fukumasu; Fernando Sebastian Baldi Rey; Adrielle Matias Ferrinho; Lenise Freitas Mueller da Silveira; Tamyres Rodrigues de Amorim; Ingrid Harumi de Souza Fuzikawa; Mariana Zanata* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 20/06/2018.

We certify that the proposal "Expected progeny difference (EPD) for precocity of Nellore bulls and their relationship between adipogenesis, fibrogenesis, lipogenesis and lipid metabolism.", utilizing 80 Bovines (80 males), protocol number CEUA 3367170317 (ID 005067), under the responsibility of **Angélica Simone Cravo Pereira** *and team; Taiane da Silva Martins; Rafael Aparecido Gomes; Saulo da Luz e Silva; Heidge Fukumasu; Fernando Sebastian Baldi Rey; Adrielle Matias Ferrinho; Lenise Freitas Mueller da Silveira; Tamyres Rodrigues de Amorim; Ingrid Harumi de Souza Fuzikawa; Mariana Zanata* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 06/20/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 04/2017 a 11/2017 Área: Nutrição E Produção Animal

Origem:	Prefeitura do Campus da USP de Pirassununga						
Espécie:	Bovinos	sexo:	Machos	idade:	20 a 20 meses	N:	80
Linhagem:	Nelore			Peso:	335 a 365 kg		

Local do experimento: O estudo será realizado no confinamento experimental da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo FZEA/USP, em Pirassununga/SP.

Comentário da CEUA: A pendência foi respondida e apresentado arquivo com n° de identificação de 124 animais disponibilizados pela Prefeitura do campus de Pirassununga.

São Paulo, 23 de março de 2021

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

FOLHA DE AVALIAÇÃO

Autor: Martins, Taiane da Silva

Título: Diferença esperada na progênie (DEP) para precocidade de tourinhos Nelore e sua relação entre adipogênese, fibrogênese, lipogênese e metabolismo lipídico

Tese apresentada ao Programa de Pós-Graduação em Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Doutor em Ciências.

Data: _____/_____/_____

Banca Examinadora

Prof.Dr._____

Instituição:_____Julgamento:_____

Prof.Dr._____

Instituição:_____Julgamento:_____

Prof.Dr._____

Instituição:_____Julgamento:_____

Prof.Dr._____

Instituição:_____Julgamento:_____

Prof.Dr._____

Instituição:_____Julgamento:_____

DEDICATÓRIA

“A persistência é o caminho do êxito”.
Charles Chaplin

Dedico à aqueles que firmes
e fortes me acompanharam, me apoiaram
e torceram por mim durante toda essa jornada.

Aos meus pais, Reiner e Maria de Fátima,
Aos meus irmãos Tamara, Reiner Jr, Tatiane; as minhas sobrinhas Marcella e Alana,
À minha companheira de vida Tainara, e aos meus verdadeiros amigos,
Dedico.

AGRADECIMENTOS

A Deus, nosso pai celestial de infinita bondade. Sabedoria suprema de todas as coisas. Grata pela fé que permitiu que eu chegasse até aqui.

Aos companheiros espirituais, seres de luz que me acompanharam, me ampararam e me aconselharam durante toda essa jornada.

Aos meus amados pais, Reiner e Maria de Fátima, pelo incentivo, paciência e apoio de sempre. Por nunca medirem esforços para me proporcionar o melhor.

A minha companheira de vida, Tainara, que mesmo de longe se fez presente diariamente, dia após dia, durante todo período do doutorado. Obrigada pelo carinho, pela paciência, pela amizade e amor.

Aos meus queridos irmãos, Tamara, Tatiane e Reiner Júnior, pela amizade, companheirismo e compreensão nos momentos difíceis. Pela torcida, força e confiança de sempre.
As minhas sobrinhas Marcella e Alana, por toda alegria, amor e luz.
Aos meus cunhados Marcelo, Gabriel e Claudia pelo apoio durante esse ciclo e por acreditarem em mim.

Aos amigos e todos familiares que apoiaram a realização e concretização desse sonho.

A minha orientadora, Professora Dra. Angélica Simone Cravo Pereira, pela orientação e amizade, pelos ensinamentos profissionais e pessoais, por todas as oportunidades e principalmente pela confiança na execução desse trabalho.

A Dra. Mirele D. Poleti, por abrir as portas do Laboratório de Estudos Avançados em Ciência da Carne (LEACC) e contribuir para que eu ampliasse meus conhecimentos. Muito obrigada pelos ensinamentos laboratoriais, conselhos, correções dos trabalhos, dedicação, acolhimento e por toda amizade durante os últimos anos.

Ao professor Dr. Juliano C. da Silveira, pelos ensinamentos e apoio nas análises realizadas no Laboratório de Morfofisiologia Molecular e Desenvolvimento (LMMD) e por todas as contribuições durante a execução desse projeto.

Ao professor Dr. Júlio César de Carvalho Balieiro, pelo apoio e colaboração na análise proteômica deste estudo, e por todos os ensinamentos e aprendizado durante a disciplina que fui PAE.

Ao professor Fernando Baldi pelo auxílio nas análises estatísticas e orientações durante todo projeto.

Ao professor João Negrão, responsável pelo Laboratório de Fisiologia Animal (LAFA), e a técnica Sandra Oliveira pelo suporte nas realizações das análises hormonais e bioquímicas.

Ao professor Dr. Saulo L Silva, pelo suporte, estrutura e parceria na realização do confinamento experimental.

A toda equipe do Laboratório de Avaliação Animal e Qualidade de Carne (LAAQC/FZEA/USP), em especial a Juliana Silva, Annelise Gomes, Vicente Buarque, Nara Consôlo, Mariane Beline, Juan Gomes e demais.

Aos funcionários da lida diária do confinamento, Ricardinho e Jhonny, e a todos os estagiários que nos acompanharam durante o confinamento experimental.

Ao professor Cesar Garbossa juntamente com a sua digníssima esposa Pollyana, pelas conversas, conselhos, ensinamentos e amizade. Pelos inúmeros momentos de troca de conhecimentos, apoio mútuo e confidências.

A todos os professores dos quais tive oportunidade de ter aula e discutir assuntos pertinentes a nossa área, agradeço pelos valiosos ensinamentos.

A toda equipe do Laboratório de Ciências da Carne (LCC), pela amizade, companheirismo, troca de experiências de vida pessoal e profissional. Agradeço todos os colegas de pós graduação e estagiários do LCC por toda ajuda laboratorial e apoio durante todo doutorado. Obrigada também pelos inúmeros momentos divertidos, de risadas e de choros rsrs. Em especial, Adrielle, Pollyana, Julian, Lenise, Ingrid, Tamyres, Jéssica, Gabriella, HellenCris, Carol, Mariana (Bezerra), Raquel etc.

Agradeço também aos colegas do LEACC, Hugo, Letícia, Ana Giullia e Mirele, pela colaboração na análise proteômica e pelos diversos momentos agradáveis de conversas e cafés.

A todos os colegas do LMMD, em especial a técnica Cibele Zinni e a doutoranda Alessandra Bridi pelo auxilio nas análises de RT-PCR com muita paciência e dedicação.

Aos funcionários e estagiários do LPGC, em especial ao Schmidt por ser amigo, atencioso e sempre estar disposto a nos ensinar.
As secretárias, Alessandra e Fabia, ao secretário da pós graduação João Paulo, e também a técnica Simi e Renata pela paciência, serviços e por todas as dúvidas esclarecidas.

A Maria, colaboradora da Biblioteca Virginie Buff D'Ápice da FMVZ, pela atenção, disponibilidade e elaboração da ficha catalográfica da tese durante essa reta final.

As meninas da limpeza, dona Edna, Cecília, dona Meire e demais, que sempre nos receberam com um sorrisão no rosto alegrando nossos dias.

A amiga Kátia por ter me recebido tão bem em Pirassununga, e ter contribuído para que o ponta pé inicial na USP fosse dado. Obrigada Katinha por ter me acolhido na sua república durante os primeiros meses do doc.
Agradeço também a Connie, Laura e a Sara Mariane, minhas primeiras amigas em Pira.
A Joana, Manuel, Sarah, Lívia, Tainara, Douglas, Tamy, Ingrid, Camila e Karine, pelos diversos momentos compartilhados enquanto dividimos o AP.

A Universidade de São Paulo, Campus Pirassununga pelo conforto e segurança para que as análises fossem desenvolvidas com êxodo. Agradeço também a natureza diversa e linda que a USP nos proporciona. Foi o meu principal refúgio para espairecer a mente cansada.

A Faculdade de Medicina Veterinária e Zootecnia e ao Departamento de Nutrição e Produção Animal pela oportunidade de ingresso ao doutorado.

Agradeço à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pela bolsa de doutorado e pelo auxílio financeiro: processos nº 2017/02349-1; 2016/17433-5 e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (*CNPq*), processo nº 409186 / 2018-0.

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

A todos que contribuíram de alguma forma para que esse projeto fosse concluído, Muito obrigada!

Mas é claro que o sol vai voltar amanhã
Mais uma vez, eu sei
Escuridão já vi pior, de endoidecer gente sã
Espera que o sol já vem

Tem gente que está do mesmo lado que você
Mas deveria estar do lado de lá
Tem gente que machuca os outros
Tem gente que não sabe amar
Tem gente enganando a gente
Veja a nossa vida como está
Mas eu sei que um dia a gente aprende
Se você quiser alguém em quem confiar
Confie em si mesmo
Quem acredita sempre alcança!

Nunca deixe que lhe digam que não vale a pena
Acreditar no sonho que se tem
Ou que seus planos nunca vão dar certo
Ou que você nunca vai ser alguém
Tem gente que machuca os outros
Tem gente que não sabe amar
Mas eu sei que um dia a gente aprende
Se você quiser alguém em quem confiar
Confie em si mesmo
Quem acredita sempre alcança!

Mais uma vez, Renato Russo.

RESUMO

MARTINS, T. S. Diferença esperada na progênie (DEP) para precocidade de tourinhos Nelore e sua relação entre adipogênese, fibrogênese, lipogênese e metabolismo lipídico. 2021. 200 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2021.

Objetivou-se avaliar a expressão de genes relacionados ao metabolismo lipídico, bem como identificar por meio da ferramenta de proteômica diferencial, as principais diferenças no perfil proteico do músculo *longissimus thoracis* (LT) e da espessura de gordura subcutânea (EGS) de progênies Nelore, filhos de touros com DEP contrastante para precocidade sexual e crescimento. Para isso, o presente estudo foi dividido em 3 manuscritos. Em síntese, foram utilizados 105 bovinos, machos não castrados, com idade média de 20 ± 2 meses e 400 ± 24 kg, provenientes de um mesmo rebanho, com informações genéticas para precocidade e crescimento. Os animais foram confinados por 100 dias e realizada a ultrassonografia de carcaça a cada 28 dias. Foram coletadas amostras de sangue para determinar as concentrações metabólicas e hormonais na última pesagem. Os animais foram abatidos após 100 dias de confinamento e durante o abate foram colhidas amostras do músculo LT e da EGS entre a 12ª e 13ª costelas além de amostras da gordura visceral (GV). Todas essas amostras foram imediatamente congeladas em nitrogênio líquido e mantidas em freezer – 80ºC até a realização das análises de expressão gênica por PCR em tempo real (RT-qPCR) e análise e SDS-PAGE, seguida de identificação proteica pela análise de espectrometria de massas acoplada a cromatografia liquida (LC-MS/MS). Durante a desossa foi avaliada a gordura intramuscular (MAR) no músculo LT. Também foram coletados bifes para lipídeos totais, perfil de ácidos graxos e solubilidade do colágeno. Os animais foram selecionados de acordo com a DEP de seus pais (touros). Do total de 105 animais, foram selecionados 6 pais com DEP's simultaneamente contrastantes para precocidade e crescimento, de forma que cada grupo experimental tivessem 3 pais. A partir dos pais, foram formados 2 grupos contrastantes denominados de alta DEP (H_EPD; N=16) e baixa DEP (L_EPD; N=16). Os animais do grupo H_EPD tiveram maior EGS (*P*=0,006); menor LDL (*P*=0,014); maior IGF-1 (*P*=0,064); maior solubilidade do colágeno (*P*=0,098); menor expressão do gene *LPL,* no LT (*P*=0,045). Também este grupo apresentou maior expressão dos genes

envolvidos na lipogênese avaliados na EGS: *ACACA* (*P*=0,060), *LPL* (*P*=0,085), *ACOX1* (*P*= 0,100), *LEP* (*P*=0,030), *SDC* (*P*= 0,009), e *GAPDH* (*P*=0,081), do que os animais do grupo L_DEP. Uma banda eletroforética foi detectada como diferencialmente abundante no músculo LT (banda 16) e três bandas eletroforéticas foram detectadas como diferentemente abundantes na EGS (bandas 24, 30, 32). As vias KEGG do metabolismo de piruvato, glicólise/gluconeogênese, metabolismo de carbono, biossíntese de aminoácidos, dentre outras, foram enriquecidas para as proteínas diferencialmente abundantes identificadas no LT e na EGS. A seleção genética para precocidade e crescimento afeta o proteoma muscular e consequentemente o metabolismo lipídico e proteico de bovinos não castrados. O hormônio IGF-1, o gene LPL e as proteínas PKLR, PKM, ALDOA, DLD, GPI, VIM, ACTC1, OXCT1, GAPDH, LDHA, LDHB, MDH1, MDH2, IDH1, PGK1, SUCLG2 and ACY1 podem ser considerados futuros biomarcadores candidatos para EGS.

Palavras-chave: Bovinos de corte. Espessura de gordura subcutânea. Vias metabólicas. Metabolismo proteico e lipídico. Proteômica.

ABSTRACT

MARTINS, T. S. Expected progeny difference (EPD) for precocity of Nelore bulls and their relationship between adipogenesis, fibrogenesis, lipogenesis and lipid metabolism. 200 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2021.

This study aimed to evaluate the expression of genes related to lipid metabolism, as well as to identify, through the proteomics tool, differences in the protein profile of Longissimus thoracis (LT) and backfat thickness (BFT) proteins of Nelore progenies, offspring of bulls with contrasting factors of expected progeny difference (EPD) for precocity and growth. For this, the present study was divided into 3 manuscripts. In summary, 105 male bulls were used, with a mean age of 20 ± 2 months and 400 ± 24 kg, from the same herd, with the genetic information of precocity and growth. The animals remained confined for 100 days, and the carcass ultrasound was performed every 28 days. Blood samples were collected to determine the metabolic and hormonal profile. The animals were slaughtered after 100 days and during slaughter, muscle LT and BFT were collected between the 12th and 13th ribs, in addition to visceral fat (GV). All of these were immediately frozen in liquid nitrogen and kept in a freezer - 80ºC until the analysis of gene expression by real-time PCR (RT-qPCR) and analysis and SDS-PAGE, followed by the identification of proteins by coupled mass spectrometry liquid chromatography (LC-MS / MS). During boning, the intramuscular fat (MAR) in the LT muscle was evaluated. Steaks for total lipids, fatty acid profile, and collagen solubility were also collected. The animals were selected according to a EPD from their parent bulls. Were selected 6 parents with EPD contrasting simultaneously for precocity and growth, so that each experimental group had 3 parents bulls. Then, were formed, 2 contrasting groups called high EPD (H_EPD; N = 16) and low EPD (L_EPD; N = 16), using 32 progenies. The animals in the H_EPD group had higher BFT (P = 0.006); lower LDL (P = 0.014); higher IGF-1 (P = 0.064); greater collagen solubility (P = 0.098); lower expression of the LPL gene, in LT (P = 0.045). This group also showed greater expression of the genes involved in lipogenesis evaluated in BFT: ACACA (P = 0.060), LPL (P = 0.085), ACOX1 (P = 0.100), LEP (P = 0.030), SDC (P = 0.009), and GAPDH (P = 0.081), than the animals in the L_EPD group. One electrophoretic band was detected as differently abundant in the LT muscle (band 16) and three electrophoretic bands

were detected as differently abundant in the BFT (bands 24, 30, 32). The KEGG pathways of pyruvate metabolism, glycolysis/gluconeogenesis, carbon metabolism, amino acid biosynthesis, among others, were enriched for the differentially abundant proteins identified in the LT and BFT. The genetic selection for precocity and growth affects the muscle proteome and consequently the lipid and protein metabolism of non-castrated cattle. The IGF-1 hormone, the LPL gene, and the PKLR, PKM, ALDOA, DLD, GPI, VIM, ACTC1, OXCT1, GAPDH, LDHA, LDHB, MDH1, MDH2, IDH1, PGK1, SUCLG2, and ACY1 proteins can be considered future biomarkers candidates for BFT.

Keywords: Beef Cattle. Backfat thickness. Metabolic pathways. Protein and lipid metabolism. Proteomics.

.

SUMÁRIO

CAPÍTULO 1

INTRODUÇÃO GERAL

1. INTRODUÇÃO GERAL

Diante do cenário da indústria de carne no Brasil, ao pensar em carne produzida no país, observa-se que as carcaças apresentam composição heterogênea, principalmente em relação ao acabamento ou espessura de gordura subcutânea (EGS). A EGS é essencial para proteger a carcaça durante o resfriamento, evitando problemas como encurtamento pelo frio, perda por gotejamento e escurecimento indesejável da carne (PARRISH et al., 1973, MARSH, 1977). Por esse motivo, alguns frigoríficos brasileiros preconizam o mínimo de três milímetros (mm) de gordura subcutânea depositada para que não haja penalização das carcaças (LUCHIARI FILHO, 2000; LAWRIE, 2006), como tentativa de garantir o padrão mínimo de qualidade da carne, que nem sempre é atendido.

A falta de padronização e acabamento das carcaças provenientes de animais da Raça Nelore, produzidas em maior quantidade no Brasil, podem ser minimizadas por meio de fatores extrínsecos, como por exemplo, o uso de tecnologia intensiva de sistema de produção, com maior aporte energético na dieta, somados aos fatores intrínsecos como ganhos genéticos e com uso de cruzamentos industrial quando se utiliza uma raça taurina, mais precoce, com maior predisposição genética para deposição de gordura.

A prática de se utilizar o cruzamento entre raças ou indivíduos menos aparentados garante maior grau de heterose e consequentemente é utilizada como estratégia de melhorias na qualidade da carcaça e da carne. No Brasil, essa técnica é fundamentada principalmente em relação a precocidade das raças, uma vez que animais taurinos britânicos, por exemplo, apresentam em geral, maior velocidade de crescimento e maior deposição de gordura subcutânea e intramuscular, em relação a animais zebuínos (OWENS et al., 1995, OWENS et al., 1993). Entretanto, diferenças fenotípicas também podem ser encontradas dentro de uma mesma raça para diferentes grupos genéticos, ocasionando diferenças na qualidade do produto final (SMITH et al., 2007).

Dessa forma, a seleção de grupos genéticos mais precoces dentro da raça Nelore pode ser um investimento rentável e replicável. Para isso, uma das ferramentas que pode ser utilizada para identificar o valor genético dos animais para maior maciez da carne e maior deposição de gordura subcutânea e intramuscular, é a Diferença

Esperada da Progênie (DEP). A DEP é uma ferramenta tecnológica que vem sendo explorada e otimizada em diversos programas de melhoramento genético para identificação de touros com maior potencial genético para transmitir as características de interesse, incorporando medidas adequadas de qualidade da carne e assim atender a demanda crescente por carne de alta qualidade (BONIN et al., 2021).

A deposição de gordura é um processo biológico complexo onde microRNAs (miRNAs) podem desempenhar um papel regulador. Existem vários alvos na diferenciação adipogênica regulada por miRNAs e esses desempenham um papel importante na diferenciação dos adipócitos e no metabolismo lipídico (SON et al., 2014; MIAO et al., 2015; ZHANG et al., 2019; YOU et al., 2020). Diferenças encontradas na expressão gênica dos genes relacionados à adipogênese e metabolismo lipídico na gordura subcutânea foram influenciadas pela expressão de miRNAs, sendo moduladas por eles (MUROYA et al., 2020).

Análises proteômicas também podem ser usadas para compreender os mecanismos moleculares envolvidos na deposição de gordura subcutânea e para identificar potenciais biomarcadores relacionados à EGS. No entanto, até onde sabemos, poucos trabalhos foram relatados para compreender ou predizer a capacidade de deposição de gordura subcutânea na carcaça (CECILIANI et al., 2018). A maioria dos estudos proteômicos disponíveis foram realizados utilizando amostras de músculo com foco nas vias de deposição de gordura intramuscular e não na gordura subcutânea (POLETI et al., 2018a; RODRIGUES et al., 2017; MAO et al., 2016; SHEN et al., 2012 ; ZHANG et al., 2010; KIM et al., 2009).

Diante do exposto, O presente estudo teve como objetivo avaliar os efeitos das DEP’s contrastantes para crescimento e precocidade de acabamento, em amostras do músculo *longissimus* e na gordura subcutânea de progênies de bovinos não castrados da raça Nelore, utilizando análise proteômica diferencial, expressão gênica e correlações com características fenotípicas como EGS, MAR, gordura intramuscular (GIM), hormônios e metabolitos.

1.1 Hipóteses:

A seleção genética para precocidade e crescimento afeta o proteoma do músculo e da gordura subcutânea e, consequentemente, o metabolismo lipídico e proteico de bovinos Nelore, não castrados, filhos de touros com DEP's contrastantes simultaneamente para precocidade e crescimento.

Especificamente, hipotetiza-se que animais com alta DEP para precocidade e crescimento:

- Apresentam melhor características da carcaça e qualidade da carne, comparados aos animais do grupo oposto;
- Apresentam diferenças no proteoma do músculo *longissimus thoracis e no proteoma da gordura subcutânea.*
- Possíveis diferenças na abundância de proteínas e genes presentes no tecido muscular esquelético estão associadas às diferenças no teor e solubilidade do colágeno e gordura intramuscular desses animais;
- Possíveis diferenças na abundancia de proteínas presentes na gordura subcutânea estão associadas às diferenças quanto a espessura de gordura subcutânea e metabolismo lipídico desses animais;
- Possíveis diferenças na expressão de genes e fatores transcricionais presentes na gordura subcutânea e visceral influenciam o perfil de ácidos graxos e adiposidade da carcaça.

1.2 Objetivo geral:

Compreender de que forma a seleção de animais Nelore, baseada em DEP's do pai (touro), acuradas para precocidade e crescimento, influencia as diferenças nos fenótipos quanto a características de carcaça, qualidade da carne e deposição de gordura corporal de suas progênies, por meio da caracterização fisiológica e molecular da adipogênese, fibrogênese, lipogênese e metabolismo lipídico.

1.3 Objetivos específicos:

- Caracterizar a carcaça das progênies Nelore avaliando a área de olho de lombo, espessura de gordura subcutânea e espessura de gordura na picanha, utilizando mensurações por ultrassonografia de carcaça, e avaliando o score de gordura intramuscular por meio da utilização de cartão comparativo.

- Avaliar a qualidade da carne das progênies Nelore em relação ao conteúdo e qualidade da gordura intramuscular, utilizando as análises de lipídeo total e perfil de ácidos graxos, e em relação ao conteúdo de colágeno intramuscular total e solúvel.
- Analisar os parâmetros bioquímicos e metabólicos presentes no soro, como: glicose circulante, colesterol, triglicerídeos, LDL, HDL e VLDL e os hormônios insulina, GH, IGF-1 e leptina;
- Analisar a expressão de genes e fatores transcricionais relacionados a adipogênese, lipogênese e fibrogênese presentes no músculo *longissimus thoracis,* na gordura subcutânea e gordura visceral utilizando a técnica de PCR em tempo real (RT-qPCR);
- Identificar as proteínas diferencialmente abundantes no músculo *longissimus thoracis* e na gordura subcutânea, utilizando a técnica de SDS-PAGE, seguida de identificação proteica pela análise de espectrometria de massas acoplada a cromatografia liquida (LC-MS/MS), com base no software MassLynx v.4.1 e Mascot e utilizando o banco de dados Uniprot;
- Explorar as vias metabólicas relacionadas as proteínas identificadas através da análise de bioinformática utilizando a ferramenta web WebGestalt para identificar os processos biológicos e as vias KEGG enriquecidas e o software STRING para análises de Redes de Interação Proteína-Proteína.

2 HISTÓRICO DA PESQUISA

Os procedimentos experimentais foram aprovados pelo Comitê de Ética de Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Pirassununga, São Paulo, Brasil (CEUA / FMVZ / USP), sob protocolo de número 3367170317.

Animais, dieta e delineamento experimental

O experimento foi conduzido nas instalações da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo – FZEA/USP, em Pirassununga/SP em parceria com a Prefeitura do Campus de Pirassununga. Os

animais usados neste estudo foram selecionados a partir de um projeto maior que avaliava os efeitos das DEP's (diferença esperada na progênie) no desempenho animal, carcaça e características de qualidade da carne (Silva et al., 2021). Os animais pertenciam ao rebanho experimental da Universidade de São Paulo, localizado em Pirassununga / SP / Brasil. O rebanho era composto por bovinos Nelore de raça pura, filhos de 16 touros no total, que faziam parte de um programa comercial de avaliação genética, com informações genéticas para precocidade e crescimento. As DEP's foram obtidas usando um modelo animal multitraço (BLUP), sendo considerado informações de precocidade sexual e de acabamento, e ganho de peso ao sobreano respectivamente.

Cento e cinco touros Nelore, não castrados, com peso corporal inicial (PC) de 400 ± 24 kg e 20 ± 2 meses de idade foram alojados em confinamento por 100 dias. As instalações tinham cochos cobertos para ração, piso de concreto e bebedouros automáticos. Todos os animais foram submetidos inicialmente a um período de adaptação de 21 dias. A adaptação da dieta dos animais foi em escada (*step-up*) até chegar à dieta final que consistia em 73% de concentrado e 23% de volumoso. Ao final do período de adaptação todos os animais foram pesados em jejum de 12h antes da alimentação matinal. Durante o período de confinamento, os animais foram alimentados *ad libitum* duas vezes ao dia, às 8h00 e 13h00, com dieta (27:73 forragem: concentrado) contendo silagem de milho, grão de milho, sorgo moído, farelo de soja, polpa cítrica, uréia e núcleo mineral.

A dieta foi formulada pelo programa ração de lucro máximo 3.2 (RLM, ESALQ-USP (tabela 1).

Tabela 1 – Ingredientes e composição química da dieta experimental

Ingrediente	% na MS da dieta
Silagem de milho	27,3
Milho seco moído	20
Sorgo moído	23
Farelo de Soja 45%	8
Polpa cítrica moída	20
Uréia	0,9
Núcleo Mineral [1]	0,8
Composição química	**% na MS da dieta**
Matéria seca	89,1
Nutrientes digestíveis totais	75,7
Proteína Bruta	15,5
Extrato etéreo	3,27
Fibra em detergente neutro	25,3
Fibra em detergente ácido	17,3
Cálcio	0,63
Fósforo	0,40

Nota: Dieta formulada pelo programa ração de lucro máximo 3.2 (RLM) ESALQ-USP.
[1]Núcleo Mineral Minerthal 160MD - Níveis de garantia por quilograma do produto: Cálcio (mín): 208g, Cálcio (máx): 218 g, Cobalto (mín): 148 mg, Cobre (mín): 2.664 mg, Enxofre (mín): 64 g, Fluor (máx): 1.600 mg, Fósforo (mín): 160g, Iodo (mín): 141 mg, Manganês (mín): 2.200 mg, Selênio (mín): 37 mg, Zinco (min): 7.992 mg e Monensina sódica (mín): 4.000mg

O consumo individual foi avaliado diariamente pela pesagem do alimento fornecido e das sobras do dia anterior. O ajuste da oferta de alimento foi realizado diariamente com base na avaliação das sobras do dia anterior, com oferta de matéria seca 5% superior ou inferior dependendo do consumo observado. Todos os animais foram pesados após jejum de 12h para registro do peso corporal final imediatamente antes do abate. O ganho médio diário foi obtido pela diferença do peso corporal final e peso corporal inicial dividido pelos 100 dias de alimentação.

Optou-se pela padronização do uso de três touros (pais) por grupo genético avaliado, uma vez que o objetivo foi avaliar o efeito da DEP nas diferentes características fenotípicas expressas nas progênies e não avaliar de forma individual o touro pai. Os animais foram selecionados, a partir das DEP's do pai, pois esses por terem número significativo de filhos avaliados, possuíam valores de DEP's com maior acurácia. Dessa forma, obteve-se maior representatividade entre os grupos contrastantes.

As características fenotípicas foram avaliadas em 32 progênies (H_EPD, N = 16 e L_EPD, N = 16). Posteriormente, 16 animais foram selecionados aleatoriamente

dentro de cada grupo para análise de expressão gênica e perfil proteômico (H_EPD, N = 8 e L_EPD, N = 8), conforme descrito a seguir:

Seleção dos animais para composição dos grupos experimentais

Os cento e cinco bovinos Nelore, não castrados, pertenciam ao programa de seleção genética da USP (Tabela 2A), e foram ranqueados com base na Diferença Esperada de Progênie (DEP) de seus pais, para precocidade e crescimento e classificadas ambas simultaneamente como DEP alta e baixa. A partir deste ranqueamento, foram selecionados 6 pais (touros) com DEP simultaneamente contrastantes para precocidade e crescimento, de forma que cada grupo experimental tivessem 3 pais cada. A partir dos pais, foram formados 2 grupos contrastantes denominados de alta DEP (H_EPD; N=16) e baixa DEP (L_EPD; N=16). Os três touros Nelore com DEP mais alta eram pais dos animais no grupo H_EPD e três touros Nelore com DEP mais baixa eram pais dos animais do grupo L_EPD (Tabela 2B). Em seguida, foram selecionados do total de animais confinados, 32 animais, sendo 16 animais filhos dos touros de alta DEP e 16 animais filhos dos touros de baixa DEP, para formação dos grupos contrastantes. As características fenotípicas foram avaliadas em 32 progênies (H_EPD, N = 16; L_EPD, N = 16). Posteriormente, 16 animais foram selecionados aleatoriamente dentro do grupos grupos com maior ou menor DEP (H_EPD, N = 8; L_EPD, N = 8) para formar os grupos que foram testados pela análise de expressão gênica e proteômica diferencial Tabela 2C.

Tabela 2A**.** Representação do número de pais selecionados e total de filhos avaliados

Ano	**Progênies avaliadas [2]**	**Pais selecionados (Touros)**	**Progênies selecionadas**
2017[1]	7929[1]	16	105
Confinamento experimental[2]	105	6	32

[1] Fonte: Sumário de Touros Nelore CFM, 2017
[2] Fonte: Auxílio Fapesp (Processo: 2016/17433-5)

Tabela 2B. Distribuição dos pais selecionados pelas DEP's de precocidade e crescimento para formação dos grupos contrastantes

	Precocidade		Crescimento			Progênies
Pai	**DEP**	**Ac**	**DEP**	**Ac**	**Grupo**	**avaliadas**
Touro A	0,63	0,82	10,62	0,81	H_EPD	8
Touro B	0,47	0,72	11,40	0,75	H_EPD	5
Touro C	0,61	0,34	14,94	0,35	H_EPD	3
Touro D	-0,12	0,21	-5,01	0,33	L_EPD	8
Touro E	-0,04	0,18	-4,66	0,29	L_EPD	5
Touro F	-0,01	0,23	-1,34	0,41	L_EPD	3

DEP= Diferença esperada na progênie. H_EPD= grupo de alta DEP e L_EPD= grupo de baixa DEP (contrastantes simultaneamente para precocidade e crescimento); Ac = acurácia.

Tabela 2C: Resumo do número de pais selecionados e filhos avaliados para análise fenotípica e molecular

	Grupos contrastante		
	H_EPD	**L_EPD**	**Animais avaliados**
Pai (Touro)	3	3	6
Progênies avaliadas			
Análises Fenotípicas	16	16	32
Análise molecular	8	8	16

H_EPD= grupo de alta DEP e L_EPD= grupo de baixa DEP (contrastantes simultaneamente para precocidade e crescimento); DEP= Diferença esperada na progênie.

CAPÍTULO 2

REVISÃO DE LITERATURA

1 REVISÃO DE LITERATURA

1.1 Bovinocultura de corte Brasileira

O Brasil possui o maior rebanho comercial de bovinos de corte do mundo, com aproximadamente 238 milhões de cabeças, e ocupa a segunda posição no ranking mundial de produção de carne bovina, consequência da produção de 10,2 milhões de toneladas de carne em 2019 (USDA, 2020). O rebanho brasileiro é composto principalmente por animais zebuínos (*Bos taurus indicus)* da raça Nelore, devido a sua rusticidade e tolerância às condições climáticas tropicais (SIQUEIRA et al., 2012; REGGIORI et al., 2016; RODRIGUES et al., 2017; CARVALHEIRO et al., 2019). Em sua maioria, os animais são não castrados (inteiros), criados em sistema extensivo de produção (a pasto) e comumente abatidos com idade elevada (FERRAZ & FELÍCIO, 2010; MILLEN et al., 2011).

A representatividade da raça Nelore no sistema de produção de carne no país é decorrente de uma série de fatores, tais como: baixo custo de produção, extensão territorial privilegiada, existência de genótipos adaptados ao clima tropical, resistência a parasitas, eficiência na conversão de gramíneas tropicais, longevidade e fertilidade (ALBUQUERQUE et al., 2017; FERNANDES JÚNIOR et al., 2020). Entretanto, do ponto de vista de qualidade de carne, a proporção zebuína presente no rebanho brasileiro contribui para que a raça Nelore seja apontada por alguns pesquisadores, como uma raça que apresenta predisposição genética para baixa deposição de gordura intramuscular e subcutânea, quando comparada a animais de origem europeia (LAGE et al., 2012; PEREIRA et al., 2015; MARTINS et al., 2015; RODRIGUES et al., 2017).

Além disso, em geral, animais zebuínos apresentam taxa reduzida de degradação das proteínas miofibrilares durante a fase *post mortem*, devido a maior atividade da calpastatina que afeta diretamente a maciez da carne (WHIPPLE et al., 1990; KOOHMARAIE, 1994; MARTINS et al., 2017). Ademais, o alto teor de colágeno intramuscular, principal proteína de importância fundamental na constituição da matriz extracelular do tecido conjuntivo, contribui para redução da maciez da carne de animais zebuínos, muito provavelmente, devido a terminação tardia desses animais, quando comparados àqueles de raças de origem europeia (OLIVEIRA et al., 2011).

Por outro lado, as conclusões obtidas nos estudos sobre a deposição de gordura são fundamentadas principalmente na precocidade das raças, uma vez que animais taurinos apresentam maiores taxas de crescimento, maior maciez e maior deposição de gordura subcutânea e intramuscular, em relação a animais zebuínos (OWENS et al., 1995; OWENS et al., 1993).

A taxa de crescimento, a deposição de gordura na carcaça e a solubilidade de colágeno são desafios enfrentados pela indústria da carne brasileira, pois a elevada proporção dos bovinos de corte produzidos no Brasil apresenta terminação tardia, carnes mais duras e menor deposição de gordura intramuscular e subcutânea, quando comparados a animais taurinos (CHARDULO et al., 2013). Tais fatos têm sido associados aos principais entraves para comercialização desses produtos em nichos de mercado específicos, como o de carne *premium* por exemplo e contribui de forma significativa para que a carne *in natura* brasileira tenha baixo valor agregado, junto ao mercado internacional (SCOLLAN et al., 2006; FONT-IFURNOLS, 2014).

1.2 Fatores que afetam a qualidade da carne

A qualidade da carne pode ser afetada por fatores extrínsecos e intrínsecos ao animal, que constituem, por exemplo, a expressão do genótipo individual e das interações desse com o meio ambiente. Dentre inúmeros fatores destacam-se: sistema de produção, efeito do estresse, alimentação, condição sexual, idade de abate, taxa de crescimento, genética e precocidade animal (GUERRERO et al., 2013a; GUERRERO et al., 2013b; ROTTA et al., 2009).

1.2.1Taxa de crescimento

A taxa de crescimento apresenta características alométricas, onde cada tecido animal possui em um determinado momento uma velocidade diferente de crescimento. O primeiro tecido a ser depositado é o nervoso, seguido do ósseo, muscular e tecido adiposo (LAWRIE, 2006). Com o avançar da idade, as carcaças em geral, apresentam maior porcentagem de gordura na carne (subcutânea e intramuscular) e menor solubilidade do colágeno (OLIVEIRA et al., 2011). Os animais abatidos precocemente possuem composição de carcaça e qualidade de carne, com características desejáveis ao mercado consumidor, como quantidade de gordura adequada, peças de tamanho constante, coloração, maciez e sabor desejáveis

(WILLIAMS, 2008; MANCINI & HUNT, 2005; FERRAZ & FELÍCIO, 2010; SCOLLAN et al., 2006). Esses autores enfatizam que os consumidores, mais exigentes, buscam carnes com qualidade comprovada, não se importando em pagar um preço maior por este produto diferenciado.

1.2.2 Genética

A herança genética possui ampla influência na velocidade e intensidade da proteólise durante o processo de conversão do músculo em carne, ocasionando diferenças consideráveis na maciez da carne, sendo 85% da variabilidade na maciez atribuídas às variações no processo enzimático, que levam ao amaciamento da carne bovina, conhecido como maturação. Os 15% restantes seriam devido às diferenças em “marbling” (gordura intramuscular) e colágeno (KOOHMARAIE 1992).

A participação de *Bos indicus* (0,25, 50 e 75% Brahman ou Sahiwal) em cruzamentos resulta em carne mais dura, quando comparada aos mestiços de raças britânicas, com média de força de cisalhamento do músculo *longissimus*, de 4,4 kg, os mestiços 1/4, 1/2 e 3/4 Brahman ou Sahiwal apresentam médias de 5,2, 5,8 e 6,7 kg ou 5,6, 6,6 e 8,4 kg, respectivamente, explicado pela maior atividade bioquímica da calpastatina (enzima inibidora de proteases cálcio-dependentes) em zebuínos (CROUSE et al.,1989).

Abordagens semelhantes foram encontradas em estudos recentes, comparando a raça Angus (taurina) com a raça Nelore (zebuína), em que se verificou maior índice de fragmentação miofibrilar para a raça Angus (69%), em comparação com a Nelore (48%), fato explicado pela maior atividade da enzima calpastatina no músculo esquelético de bovinos Nelore e não na abundância de calpaína e calpastatina (MARTINS et al., 2017).

1.2.3 Precocidade animal

As raças diferem quanto às curvas de crescimento dos tecidos e, consequentemente, à deposição de gordura subcutânea e intramuscular, ou ainda, quanto ao peso e rendimento dos cortes cárneos a um determinado peso de carcaça. Logo, uma raça mais precoce depositará espessura de gordura subcutânea (EGS) adequada mais rápido, alcançará maturidade fisiológica com menor idade e consequentemente poderá ser abatido mais jovem (FELICIO, 1997; SHIOTSUKI, et

al., 2009). O tecido adiposo desempenha função importante como reserva de energia, além de ocasionar modificações na “adiposidade” do animal, de acordo com seu estado fisiológico, como durante a gestação ou fase de terminação (MINER, 2004). Essas características são interligadas aos atributos visuais e sensoriais da carne, sendo apreciadas ou discriminadas dependendo do mercado que se considera (FELICIO, 1997; CHARDULO et al., 2013; BONIN et al., 2021).

No cenário da indústria de carne no Brasil, ao pensar em carne produzida no país, observa-se que as carcaças apresentam qualidade heterogênea, principalmente em deposição de gordura subcutânea (BONIN et al., 2014; BONIM et al., 2021). Uma importante função tecnológica, é que a EGS é essencial para proteger a carcaça durante o resfriamento, evitando problemas na carcaça bovina, como encurtamento pelo frio, perda por gotejamento e escurecimento indesejável da carne (PARRISH et al., 1973; MARSH, 1977; FELICIO, 1998; DEVINE et al., 1999; AALHUS et al., 2001; PINTO NETO et al., 2013). Por esse motivo, alguns frigoríficos brasileiros preconizam o mínimo de 3 mm de gordura subcutânea depositada na carcaça (LUCHIARI FILHO, 2000), como tentativa de garantir o padrão mínimo de qualidade da carne, que nem sempre é atendido.

1.2.4 Metabolismo proteico

Maciez, suculência e sabor permanecem como os três pilares da palatabilidade da carne cozida, todos ligados a satisfação do consumidor (TROY and KERRY, 2010; FELDERHOFF et al., 2020). No momento da compra, os consumidores contam com dicas extrínsecas, como cor, gordura intramuscular (MAR), espessura de gordura subcutanea (EGS), e preço para determinar qual (is) produto (s) de carne comprar. Historicamente, a maciez tem sido o fator mais importante que afeta a palatabilidade da carne bovina, porém o sabor tem se tornado aspecto mais importante de satisfação quando a maciez é aceitável. Os consumidores podem distinguir a MAR e, consequentemente, as diferenças de sabor em alguns músculos e estão dispostas a pagar mais pelo tipo de sabor que preferem (FELDERHOFF et al., 2020).

No entanto, animais *Bos indicus* têm sido relacionados à produção de carne de qualidade inferior em relação a deposição de gordura ou com variabilidade na maciez da carne. Cerca de 85% da variabilidade na maciez da carne bovina estão atribuídas

às variações no processo enzimático, que leva ao amaciamento da carne e está relacionado à proteólise muscular. Os 15% restantes seriam devidos às diferenças em "marbling" (gordura intramuscular) e *turnover* do colágeno, principal proteína de importância fundamental na constituição da matriz extracelular do tecido conjuntivo (KOOHMARAIE, 1992).

1.2.5 Proteólise muscular

Além da influência da genética na maciez, a maturação desempenha um papel fundamental na produção de carnes macias, visto que é um processo que envolve a ativação de sistemas proteolíticos intracelulares, nos quais agem na integridade das proteínas miofibrilares (OUALI et al., 2006; OUALI et al., 2013).

A variabilidade da maciez entre subespécies tem sido atribuída a diferentes níveis de enzimas proteolíticas encontradas nos músculos dos animais (WHIPPLE et al., 1990), especialmente enzimas do sistema calpaína-calpastatina. O sistema proteolítico "calpaína-calpastatina" e o sistema das "caspases" são os principais sistemas que afetam a degradação das fibras musculares durante o período *post mortem* (KOOHMARAIE, 1992; OUALI et al., 2013). Bovinos *Bos indicus* apresentam maior atividade da enzima calpastatina quando comparados aos *Bos taurus*. A atividade elevada de calpastatina diminui a proteólise *post mortem*, afetando negativamente a maciez da carne (KOOHMARAIE, 1994; WHEELER et al, 1990; WHIPPLE et al., 1990; MARTINS et al., 2017; WRIGHT et al.,2018).

Desta forma, o uso de ferramentas biotecnológicas podem ser úteis para identificar potenciais biomarcadores para programas de bovinos de corte e selecionar animais que produzem carnes macias (OUALI et al., 2013). Além disso, os genes pertencentes ao complexo calpaína-calpastatina (*CAPN1*, *CAPN2* e *CAST*) têm se destacado por apresentarem funções importantes, em relação à maciez da carne, entre diferentes grupos genéticos (WRIGHT et al., 2018).

1.2.6 Fibrogênese e turnover do colágeno

Juntamente com a adipogênese, a fibrogênese está ativa durante a fase fetal e gera o tecido conjuntivo que forma o endomísio primordial, perimísio, e epimísio no músculo esquelético durante o final da gestação. Os fibroblastos também produzem enzimas para catalisar as ligações de cross-link do colágeno que contribui para a

dureza da carne. Assim, reduzindo a fibrogênese durante o desenvolvimento muscular contribui para a redução da dureza da carne (Du et al., 2013).

A matriz extracelular (ECM) dos músculos esqueléticos é uma rede complexa que consiste em colágenos, glicoproteínas, proteoglicanos e elastina (HALPER & KJAER, 2014). Como mencionado anteriormente, os colágenos formam uma rede de tecido conjuntivo intramuscular (IMCT), ou seja, os componentes fibrosos centrais da ECM. O IMCT é tipicamente representado e organizado em três camadas: (i) o endomísio, representando a camada mais interna que envolve as fibras musculares individuais, (ii) os grupos de fibras musculares de perimísio e (iii) o epimísio envolvendo todo o músculo (CSAPO, et al., 2020).

De acordo com a complexidade estrutural da rede IMCT evidenciada por microscopia eletrônica de varredura, sugere-se que esta classificação tradicional pode ser simplista e que uma organização de ordem superior da ECM muscular ainda precisa ser definida (GILLIES & LIEBER, 2011; GILLIES et al., 2017). A pesquisa em tecidos fasciais considera ainda as camadas de IMCT como parte de um sistema complexo de tecidos conjuntivos interconectados e entrelaçados que "envolvem, se entrelaçam e interpenetram todos os órgãos, músculos, ossos e fibras nervosas, dotando o corpo de uma estrutura funcional, a fim de fornecer um ambiente que permite que todos os sistemas corporais operem de forma integrada" (ADSTRUM et al., 2017; STECCO et al., 2018).

O colágeno é uma das proteínas mais abundantes no corpo animal, localiza-se dentro da matriz extracelular e tem função estrutural no músculo esquelético formando o endomísio e perimísio (CSAPO, et al., 2020). O endomísio e perimísio compreendem o sistema conjuntivo intramuscular, componente tecidual do músculo esquelético, que contribui principalmente para a maciez da carne. À medida que o colágeno amadurece, a força da fibrila aumenta por meio da formação e do aumento das ligações cruzadas intermoleculares, tornando-se termoestáveis, fato que diminui a maciez da carne com a idade (ROBINS et al., 1973; DUARTE et al., 2011).

As ligações cruzadas estão correlacionadas com a diminuição da maciez da carne (MCCORMICK, 1999). Logo, o conteúdo de colágeno intramuscular tem sido um dos motivos para o aumento dos valores da força de cisalhamento (CROSS et al., 1973; EBARB et al., 2016), resultando em carnes mais duras. Por outro lado, o rápido crescimento muscular que ocorre em animais mais precoces resulta em uma

proporção maior de colágeno menos reticulado (mais solúvel) e associado a maior maciez (BAILEY & LIGHT, 1989) quando comparados a animais com maturidade fisiológica mais mais tardia.

GONZALEZ et al. (2014) demonstraram a contribuição da genética zebuína, sobre a maciez da carne, utilizando como modelo experimental a raça Brahman (zebuíno), em comparação com animais da raça Angus (taurinos) e seus cruzados. Esses autores observaram que as expressões das enzimas *BMP1* e *LOX* foram correlacionadas com características de palatabilidade, sinalizando o efeito dessas enzimas na formação de ligações cruzadas de colágeno intramuscular, afetando a maciez da carne de animais de grupos genéticos diferentes. Desta forma, estratégias a fim de limitar a atividade dessas enzimas são interessantes para melhorar a qualidade da carne.

Durante os estágios de desenvolvimento e crescimento muscular, as colagenases (enzimas que quebram as ligações peptídicas do colágeno), como as metaloproteinases, relacionadas à síntese e degração (*turnover*) do colágeno podem implicar em diferenças na solubilidade do colágeno e consequentemente na maciez da carne. As metaloproteinases da matriz (*MMPS*) desempenham papéis importantes no desenvolvimento tecidual, renovação da matriz de colágeno, reparo e remodelação (YALCINKAYA et al., 2014). Os inibidores teciduais de metaloproteinases (*TIMPS)* são geralmente secretados juntamente com quantidades variáveis de suas *MMPS* e regulam suas atividades proteolíticas, ligando-se firmemente aos seus locais catalíticos.

A razão *MMP* / *TIMP* fornece informações adicionais sobre degradação e modulação da síntese de colágeno. O *turnover* da matriz extracelular é amplamente modulado pela interação entre *MMPS* e seus *TIMPS*. A correlação e influencias recíprocas entre a *MMP* e a *TIMP* determinam o efeito combinado no *turnover* da matriz extracelular (RODERFELD et al., 2007; COGNI et al., 2013; KIM et al., 2012). Portanto, o tipo e *turnover* do colágeno podem influenciar a maciez da carne bovina entre diferentes grupos genéticos (LAURENT, 1987; PURSLOW, 2005).

1.3 Metabolismo lipídico em ruminantes

Em bovinos, a deposição de gordura é resultado da interação entre nutrição, sexo, idade, bio-hidrogenação ruminal e grupo genético (TEIXEIRA et al., 2017). O metabolismo lipídico é responsável pelo desenvolvimento dos adipócitos e pela regulação hormonal da síntese e deposição da gordura na carcaça e na carne, compreendendo os processos conhecidos como adipogênese e lipogênese.

Em animais de produção, o processo de adipogênese é marcado por modificações celulares assíncronas que promovem a formação de células adiposas. O desenvolvimento do tecido visceral, subcutâneos, intermusculares e o tecido adiposo intramuscular segue uma ordem sequencial no gado (ZHAO, HUANG and DU, 2019). Os tecidos adiposos visceral e subcutâneo se desenvolvem primeiro, com inicio no final do primeiro trimestre da gestação em ruminantes (JIANG et al., 2014). A formação de adipócitos intramusculares ocorrem principalmente durante o final da gestação até cerca de 250 dias de idade em bovinos de corte (DU et al., 2015; Figura 1).

Já, a lipogênese é o processo de síntese de ácidos graxos e triglicerídeos, que serão armazenados no fígado (como fonte de energia e de componente estrutural para a construção da membrana) e no tecido adiposo (como deposição de gordura e armazenamento de energia a longo tempo), sendo regulada por fatores nutricionais, hormonais e genéticos (HARVATINE et al., 2006; BAUMAN et al., 2008).

1.3.1 Adipogênese

A adipogênese é o desenvolvimento complexo de pré-adipócitos ou células-tronco mesenquimais para adipócitos maduros e é essencial para a formação de gordura e metabolismo de tecidos adiposos em mamíferos (ROMÃO et al., 2011), sendo acompanhada de alterações morfológicas e bioquímicas. A adipogênese tem sido um dos modelos de diferenciação celular mais intensamente estudados. O tecido adiposo é um componente importante do sistema energético do corpo, além de atuar como um sistema modelo para auxílio das respostas às questões gerais na biologia do desenvolvimento (ROSEN & SPIEGELMAN, 2000; ROSEN et al., 2000).

O tecido adiposo é importante, pois está envolvido na regulação periférica da homeostase do corpo, especificamente, consumo e armazenamento de energia (ROH et al., 2006). Os adipócitos desempenham papel central na regulação do metabolismo

lipídico, atuando tanto no armazenamento de ácidos graxos, quanto como célula endócrina para regular o gasto energético e o comportamento alimentar (ROH et al., 2006). O desenvolvimento do tecido adiposo é controlado pelo equilíbrio entre a proliferação celular (hiperplasia) e o aumento do tamanho (hipertrofia), que se deve à assimilação de ácidos graxos em gotículas lipídicas ricas em triacilglicerol. No adipócito, a hipertrofia envolve principalmente o acúmulo de lipídeos intracelulares.

Os depósitos de gordura individuais apresentam taxas e períodos diferentes de crescimento hiperplástico e hipertrófico (HAMMOND, 1955; ROBELIN, 1986). Os depósitos de gordura visceral, subcutânea, intermuscular e intramuscular diferem em seus momentos de formação e papel metabólico. A gordura visceral é desenvolvida em um primeiro momento, para fornecer isolamento e proteção de órgãos, enquanto a gordura intermuscular e subcutânea são muitas vezes desenvolvidas simultaneamente. A gordura intermuscular é o maior depósito em carcaça de bovino, seguido de gordura subcutânea. A gordura intramuscular é o último depósito a se desenvolver, razão pela qual a maturidade regula fortemente o desenvolvimento deste depósito de gordura (HAMMOND, 1955).

Dessa forma, o insucesso de estudos que visa aumentar o teor de gordura intramuscular, via suplementação alimentar de bovinos de corte na fase de terminação, ocorre possivelmente, pelo fato de que, maior parte da energia suplementar é utilizada para a hiperplasia de adipócitos do tecido adiposo visceral e apenas uma pequena parte da energia consumida é utilizada para deposição de tecido adiposo intramuscular (HAUSMAN et al., 2009; SMITH et al., 2009; DU & DODSON, 2012).

Portanto, uma vez que o desenvolvimento da gordura intramuscular não ocorre de forma independente dos demais tipos de tecidos adiposos (ALBRECHT et al., 2011), a busca pelo aumento da marmorização na carne bovina, sem causar aumento excessivo da deposição de gordura subcutânea e visceral no animal tem se tornado objetivo de algumas pesquisas (DU et al., 2010; ALBRECHT et al., 2011) e há um número crescente de estudos visando compreender os mecanismos moleculares relacionados às diferenças na qualidade da carne bovina entre genótipos zebuínos e taurinos (RODRIGUES et al., 2017; MARTINS et al., 2015; GIUSTI et al., 2013; WEI et al., 2013a, WEI et al., 2013b; DUARTE et al., 2013).

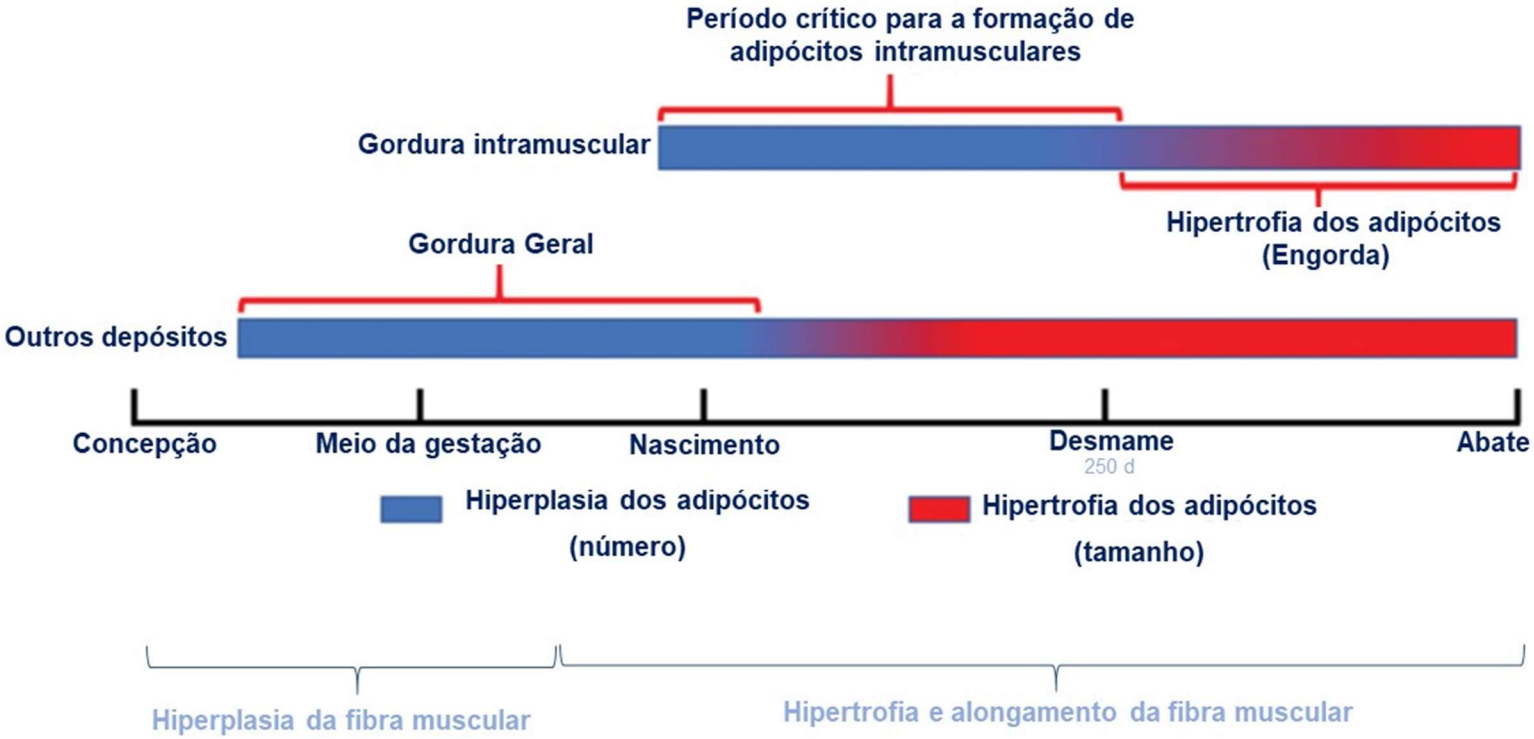

Figura 1: Diferença cronológica da adipogênese em diferentes depósitos de gordura em bovinos de corte. Adaptado Du et al., (2015).

1.3.2 Lipogênese

A lipogênese é o processo de síntese de ácidos graxos e triglicerídeos, que serão armazenados no fígado (como fonte de energia e de componente estrutural para a construção da membrana) e no tecido adiposo (como deposição de gordura e armazenamento de energia a longo tempo). Em ruminantes, a lipogênese acontece principalmente no tecido adiposo (Zhao et al., 2019).

A lipogênese é rigorosamente controlada por fatores nutricionais, hormonais e genéticos (KERSTEN, 2001; LADEIRA et al., 2016; TEIXEIRA et al., 2017). Além disso, existe uma relação estreita entre a taxa de síntese de ácidos graxos e a atividade da enzima acetil-CoA carboxilase, que é codificada pelo gene da acetil-CoA carboxilase alfa (ACACA) e da ação da ácido graxo sintase, complexo multienzimático, codificado pelo gene da ácido graxo sintase (FASN), com a lipogênese (GRIFFIN, 2004; NELSON, 2004). Estudos realizados em várias espécies de mamíferos e aves têm levado a conclusão de que a FASN é controlada principalmente em nível de transcrição do gene (BERGEN, 2005). Por outro lado, a lipogênese também pode ser ativada por mTOR, uma via conhecida por controlar o crescimento e o metabolismo celular em resposta a nutrientes, fatores de crescimento ou estado de energia (LAMMING & SABATINI, 2013; WANG, et al., 2015).

Na maioria das dietas de ruminantes, a gordura está abaixo de 5% da matéria seca (MS). Os ingredientes básicos da ração, incluindo forragens e grãos, geralmente fornecem cerca de 3 a 3,5% de gordura com base na MS total de bovinos de corte (HASSAN et al., 2020). A suplementação de ácidos graxos não é necessária para a proliferação microbiana no rúmen porque os microbiota ruminal pode sintetizar seus próprios ácidos graxos (BROOKS et al., 1954). Consequentemente, os ácidos graxos exógenos são menos evidentes nos tecidos de ruminantes quando comparados aos não ruminantes (BOGGS et al., 1997).

O principal precursor da síntese de ácidos graxos de novo é o acetato (BERGEN & MERSMANN, 2005). Independentemente da fonte de carbono, quase todos os ácidos graxos são produzidos (exceto por alguns resultantes da digestão intestinal mais baixa de microrganismos) via síntese endógena em glândulas adiposas e mamárias apenas durante a lactação. A regulação molecular da lipogênese de novo, deposição lipídica e oxidação é afetada pelos mesmos fatores

de transcrição e mecanismos moleculares no tecido mamário e nos depósitos de gorduras subcutâneas (BAUMAN et al., 2008; HARVATINE & ALLEN, 2006).

O principal substrato de ácido graxo para a bio-hidrogenação ruminal em animais em condições de pastejo é o ácido linoleico, pois é o ácido graxo mais abundante presente nos glicolipídeos e nos fosfolipídeos da forragem. O metabolismo do ácido linoleico no rúmen envolve a formação transitória em ácido linoleico conjugado (CLA), principalmente C18:2 c9, t11, que posteriormente é convertido em ácido vacênico e finalmente ácido esteárico (LOURENÇO et al., 2010). O C18:2 c9, t11 é geralmente o principal isômero CLA encontrado na carne bovina e no leite (FRITSCHE & FRITSCHE, 1998). No entanto, o C18:2 t10, c12 torna-se o intermediário predominante, particularmente em dietas com alta proporção de concentrados ou óleos (GRIINARI et al., 2000).

Ao avaliar o impacto de dietas com alta proporção de concentrado em comparação a dietas ricas em forragem ou com níveis variados de forragem com suplementação de concentrado, French et al. (2000) observaram que as dietas com maior proporção de forragens apresentaram maiores quantidades de ácidos mirístico, palmitoléico e alfa-linolênico, enquanto que as dietas ricas em concentrados apresentaram maiores concentrações de ácidos palmítico e esteárico. A carne comercial provenientes de animais alimentados com grãos geralmente possuiem uma proporção n-6: n-3 maior que 7 (LEHESKA et al., 2008), enquanto a carne bovina alimentada com pasto geralmente tem uma proporção n-6: n-3 menor que 3,0 (FRENCH et al., 2000; LEHESKA et al., 2008).

As maiores proporções de n6 / n3 de animais terminados em confinamento, se deve ao fato do 18: 2n - 6 diminuir no músculo à medida que aumenta a deposição de gordura (WOOD et al., 2008). A principal razão disso é que o fosfolipídio, onde está localizado o 18: 2n - 6, diminui em proporção aos lipídios musculares e aumenta a proporção de lipídios neutros, com seu maior teor de ácidos graxos saturados e monoinsaturados. Isso acontece, principalmente no tecido adiposo, devido a ação da enzima estearoil Co-A dessaturase (SCD), uma importante enzima lipogênica (SCOLLAN et al., 2014).

Em geral a quantidade e qualidade da gordura intramuscular está associado à suculência, sabor, maciez e gosto geral da carne e pode ser influenciada por fatores genéticos ou nutricionais (JEREMIAH et al., 2003, O'QUINN et al., 2012; O'QUINN et al., 2018; FELDERHOFF et al., 2020). Consequentemente, a síntese e

deposição da gordura sofre regulação enzimática e hormonal, como também pode ser influenciada por fatores transcricionais a nível de gene.

1.3.3 Regulação enzimática da síntese e deposição da gordura

O desenvolvimento do tecido adiposo inicia-se ainda no período pré-natal, por volta do terceiro mês de gestação, quando células oriundas do mesênquima embrionário, semelhante aos fibroblastos, diferenciam-se para dar origem aos adipoblastos ou células primitivas do tecido adiposo (DU et al., 2010). Após a diferenciação, não há como a célula retornar ao estado inicial. Uma vez formados, os adipoblastos comprometem-se a uma fase exponencial de multiplicação, formando os pré-adipócitos (HOSSNER, 2005).

Diferentemente do tecido muscular, a hiperplasia dos adipócitos ocorre principalmente durante o final do desenvolvimento fetal até cerca de 250 dias de idade em bovinos de corte (DU et al., 2015). Por sua vez, a hiperplasia muscular ocorre no ambiente intra-uterino durante o período pré-natal e pode ser separado em estágios embrionários e fetais (DU et al., 2010a). Portanto, os tecidos adiposo e muscular tem seu desenvolvimento em diferentes momentos durante sua formação e crescimento em bovinos. A síntese e deposição da gordura e biossíntese de ácidos graxos depende da atividade de várias enzimas-chave como: acetil-CoA carboxilase (ACACA), ácido graxo sintase (FAS), glicose-6-fosfato desidrogenase (G6PDH), enzima málica (ME), estearoil Co-A dessaturase (SCD), Lipase sensível a hormônios (HSL e a lipotroteína lipase (LPL) como demostrado na tabela 1 (VANCE et al., 2002).

Tabela 1: Função das enzimas lipogênicas e lipolíticas no tecido adiposo

Enzima Lipogênica	**Função**
Acetil-CoA carboxilase	Formação irreversível de malonil-CoA a partir de acetil-CoA.
Ácido graxo sintase	Síntese de palmitato a partir de acetil-CoA e malonil-CoA.
Glicose-6-fosfato DH	Fornecimento de NADPH para biossíntese redutiva de ácidos graxos.
Enzima málica	Fornecimento de NADPH para biossíntese redutiva de ácidos graxos.
Estearoil-CoA dessaturase	Transformação de MUFA em SFA.
Hormônios Lipase sensível	Hidrolisa triglicerídeos em ácidos graxos livres.
Lipoproteína lipase	Catalisa a hidrólise de triglicerídeos dos quilomícrons circulantes e das lipoproteínas de densidade muito baixa.

Adaptado VANCE et al., (2002)
DH = desidrogenase; NADPH = fosfato de dinucleótido de dihidronicotinamida adenina; MUFA = ácidos graxos monoinsaturado; SFA = ácidos graxos saturados.

Algumas enzimas são essenciais para o fornecimento de NADPH para a biossíntese de lipídeos. As enzimas conhecidas envolvidas neste processo incluem a enzima málica (ME), glicose-6-fosfato desidrogenase (G6PDH) e 6-fosfoglucose desidrogenase da via da fosfato pentose ao lado da isocitrato desidrogenase (HAO et al., 2014; e HAO et al., 2016). O ACACA é uma enzima citosólica que catalisa a carboxilação de acetil-CoA em malonil-CoA, a primeira etapa limitante da biossíntese de novo de ácidos graxos. Esta sub-via faz parte da biossíntese da via malonil-CoA, e está envolvida no metabolismo lipídico (UniProt Consortium, 2021).

A ação da enzima Estearoil-CoA dessaturase (SCD) reflete uma maior síntese de ácidos graxos, que podem influenciar na eficiência do crescimento, além de alterar a composição dos ácidos graxos depositados na carne (KIM et al., 2000). A SCD é uma enzima do retículo endoplasmático que catalisa a biossíntese de ácidos graxos monoinsaturados, a partir de ácidos graxos saturados que são novamente sintetizados ou derivados da dieta (SMITH et al., 2009).

A lipoproteína lipase (LPL), enzima responsável pela quebra dos triacilgliceróis advindos da circulação em ácidos graxos e glicerol a nível endotelial e sintetizado pelos adipócitos, atua também como sinalizador celular, uma vez que, quando expressada estimula nova onda hiperplástica do tecido adiposo. Após a ocorrência dessa nova onda de proliferação, novos sinalizadores celulares, como a glicerol-3-fosfato desidrogenase (GAPDH) e a enzima ácido graxo sintetase (FAS)

são detectados. As células, então, recebem o sinal para que iniciem o acúmulo de lipídeos, quando passam a ser denominadas de adipócitos propriamente ditos. O GAPDH e o FAS estão presentes nas células recém formadas, marcando o final da diferenciação e início da deposição de lipídios (HOSSNER, 2005).

A deposição de gordura intramuscular é aparentemente regulada por fatores diferentes daqueles que regulam a deposição de gordura em outros tecidos adiposos, existindo diferenças metabólicas entre eles. Adipócitos intramusculares apresentam atividade mais elevada das enzimas hexoquinase e fosfofrutoquinase. Já, o tecido adiposo subcutâneo apresenta níveis mais elevados de enzimas lipogênicas, como a NADP-malatodesidrogenase, fosfogluconate-6-desidrogenase e glicose-6-fosfato desidrogenase, evidenciando papéis exclusivos no metabolismo de lipídeos (MILLER et al., 1991; MAY et al.,1994).

A massa de tecido adiposo, por conseguinte, é controlada pelo balanço entre proliferação de células (hiperplasia) e aumento no tamanho das células (hipertrofia). A assimilação dos ácidos graxos livres no citosol em gotas de lipídeos ricas em triacilglicerol contribui para a hipertrofia dos adipócitos e aumento do tecido adiposo (HOSSNER, 2005).

1.3.4 Regulação hormonal da síntese e deposição da gordura

Em ruminantes, os adipócitos desempenham função importante como reserva de energia, além de ocasionarem modificações na “adiposidade” do animal, de acordo com seu estado fisiológico, como durante a gestação ou fase de terminação. Além disso, os adipócitos atuam como verdadeiras células endócrinas, secretoras de uma série de hormônios, cujos sinais endócrinos estão diretamente relacionados com a produção animal. Dentre esses hormônios, destacam-se a leptina, IGF-1, interleucinas, resistina, entre outros (MINER, 2004).

A quantidade de gordura presente no citoplasma e o consequente tamanho do adipócito são controlados pela expressão da leptina. Este hormônio é produzido especificamente pelos adipócitos, secretado e transportado pelo sangue, até atingir seus receptores localizados em vários tecidos, inclusive o hipotálamo. A leptina tem sua função mediada por meio do neuropeptídeo Y, sendo responsável pela homeostase energética do organismo, que provoca mudanças no consumo alimentar e no gasto energético corporal, além de estar intimamente relacionada a aspectos

reprodutivos, uma vez que sua presença facilita a secreção de gonadotrofinas (MINER, 2004).

Outra importante função da leptina, é que ela está relacionada com o crescimento animal, pois participa da liberação do hormônio do crescimento (GH), via neuropeptídio Y (NPY), que estimula a secreção do hormônio liberador do hormônio do crescimento (GHRH). Portanto, a leptina estimula a secreção de GH via GHRH, através do NPY, uma vez que o efeito de estimulação da leptina é praticamente anulado na presença de anticorpos para GHRH (McMAHON et al., 2001). Além disso, alguns autores relataram que a leptina está associada com a porcentagem de gordura corporal, sendo que animais com maior deposição de gordura, apresentam maiores quantidades de leptina plasmática (CHILLARD et al., 1998; MINTON et al., 1998).

O GH é um hormônio muito importante na regulação do peso e metabolismo dos lipídeos, uma vez que o aumento em sua concentração estimula a síntese proteica, enquanto seu decréscimo sinalizará para a lipogênese. Existe, assim, um feedback entre leptina, NPY e GH para regulação do metabolismo lipídico. Com o GH elevado, há mobilização de lipídeos e inibição de síntese, reduzindo os níveis de leptina, que, por sua vez, deixa de estimular a liberação de NPY (McMAHON et al., 2001).

A insulina é um outro hormônio envolvido na síntese de gordura. Ela estimula a captura de glicose por tecidos periféricos e aumenta a lipogênese ou reduz a lipólise. Dessa forma a concentração plasmática de insulina está positivamente correlacionada com a adiposidade da carcaça (RHOADES et al., 2007). Entretanto, existe variação em relação à sensitividade à insulina entre os diferentes tecidos corporais, sendo um dos principais fatores determinantes da partição de energia entre esses tecidos e o seu crescimento e desenvolvimento (HOSSNER, 2005).

Lee et al. (2013) utilizaram a técnica RNAseq para investigar os transcriptomas de 3 tecidos adiposos (omental, subcutâneo e gordura intramuscular) em bovinos e observaram que os genes lipogênicos foram expressos diferentemente entre os 3 tecidos adiposos indicando diferenças no metabolismo desses depósitos de gordura. Neste estudo, os adipócitos intramuscular apresentaram baixas atividades metabólicas relacionadas ao metabolismo de lipídios baseado na análise do transcriptoma com mais gama de vias metabólicas e genes.

Por outro lado, genes expressos diferencialmente (DEGs) foram regulados para baixo na gordura subcutânea e significativamente enriquecido na via da melanogênese. A sinalização do WNT na via da melanogênese é um importante regulador da adipogênese ou secreção de insulina. Além disso, a análise comparativa do transcriptoma de 3 tecidos mostraram que a interação entre os componentes da matrix extracelular e receptores transmembrana de células de gordura podem influenciar o depósito de gordura (LEE et al., 2013).

1.3.5 Regulação da biossíntese e deposição da gordura por fatores transcricionais

Durante a diferenciação, os pré-adipócitos comprometem-se a mudanças morfológicas, assim como na expressão seletivas de determinados genes. A expressão sequencial de certos fatores de transcrição, tais como o CCAAT/enhancer binding protein factor, SREBP/sterol regulatory elemento binding proteine a família de fatores de transcrição PPAR/peroxime proliferator-activated receptor possuem um papel chave nos estágios de conversão dos pré- adipócitos em adipócitos maduros (GREGORIE et al., 1998).

A adipogênese é regulada em sua maior parte pelos fatores de transcrição pertencentes à família Enhancer binding protein (C/EBP) e o Peroxissome proliferatoractivated receptor (PPAR) (DODSON et al., 2010a; DU et al., 2010b; YAMADA et al., 2009). Durante a adipogênese, C/EBPα é ativado e se liga diretamente aos promotores de PPARγ, que por sua vez são induzidos a expressão do C/EBPα. A expressão de PPARγ desencadeia a expressão de C/EBPα, promovendo uma ação autorreguladora entre estes fatores, fazendo-se necessária a ação conjunta destes para estimular a diferenciação das células mesenquimais em adipócitos levando a maior deposição de gordura (ROSEN et al., 2002).

Em estudos sobre fatores de transcrição do processo de adipogênese foi detectado o fator Zfp423 como marcador para estágios iniciais do processo de adipogênese (GUPTA et al., 2010; GUPTA et al., 2012). O Zfp423 (Zinc-finger protein 423), é um fator que promove a adipogênese, por meio do aumento da expressão do PPARγ. Resultados apresentados por GUPTA et al. (2010) demonstraram que o fator de transcrição Zfp423 é abundantemente expresso na fração estromal-vascular de tecidos contendo pré-adipócitos, bem como em adipócitos maduros. Além disso, o Zfp423 foi identificado como gene responsável pela expressão de grande parte dos

marcadores para pré-adipócitos (GUPTA et al., 2012) e pode regular a expressão do PPARγ em fibroblastos, induzindo a diferenciação de tais células em adipócitos (GUPTA et al., 2010).

Além do Zfp423, o Fator-2 de crescimento de fibroblasto (FGF-2) tem sido relatado como outro fator de transcrição que pode causar aumento da adipogênese (KAKUDO et al., 2007). Inoue et al. (2005) observaram que células-tronco oriundas de tecido adiposo, ao receberem FGF-2 foram induzidas à adipogênese, com aumento de atividade da enzima GAPDH, a qual facilita a conversão de glicose em triglicerídeos.

Em estudo com células-tronco adiposas, Kakudo et al. (2007) sugeriram que o processo de adipogênese ocorre em função do aumento da expressão do FGF-2, que por sua vez desencadeia o aumento da expressão de PPARγ e consequentemente o aumento da adipogênese. Células musculares, adipócitos e fibroblastos são derivados do mesmo pool de células mesenquimais indiferenciadas abundantemente encontradas no tecido muscular esquelético, durante estágios iniciais do desenvolvimento (DU et al., 2010a). Contudo, a maior parte destas células é direcionada à linhagem miogênica, restando uma porção reduzida de células passíveis de se diferenciarem em adipócitos e/ou fibroblastos.

Segundo UEZUMI et al. (2011), fibroblastos e adipócitos competem por células mesenquimais indiferenciadas, por meio de uma via de sinalização comum. Além disso, Joe et al. (2010), demonstraram que células mesenquimais progenitoras contribuem para formação de tecido adiposo em tecido muscular esquelético e apresentam capacidade de se diferenciarem em fibroblastos. Estas células possuem habilidade de induzir a fibrogênese em músculo esquelético, por meio da ativação da expressão dos receptores α de fatores de crescimento derivados de plaquetas (PDGFRα), sugerindo que o tecido adiposo e tecido conectivo são oriundos da mesma população de células (OLSON & SORIANO, 2009).

1.4 Avanços científicos na ciência da carne:

Contribuição das ciências "ômicas" para qualidade da carne

As diferenças na maciez, gordura intramuscular, propriedades sensoriais e a bioquímica relacionada à qualidade da carne podem ser explicadas pela "foodomics" (MUNEKATA et al., 2021). As tecnologias "Omics" fornecem uma visão dos

mecanismos de resiliência e da qualidade do produto em nível ;ular (Figura 2). A aplicação das ciências “ômicas” no contexto da produção de animais tropicais podem melhorar a produtividade de áreas de poucos recursos disponíveis (RIBEIRO et al., 2020).

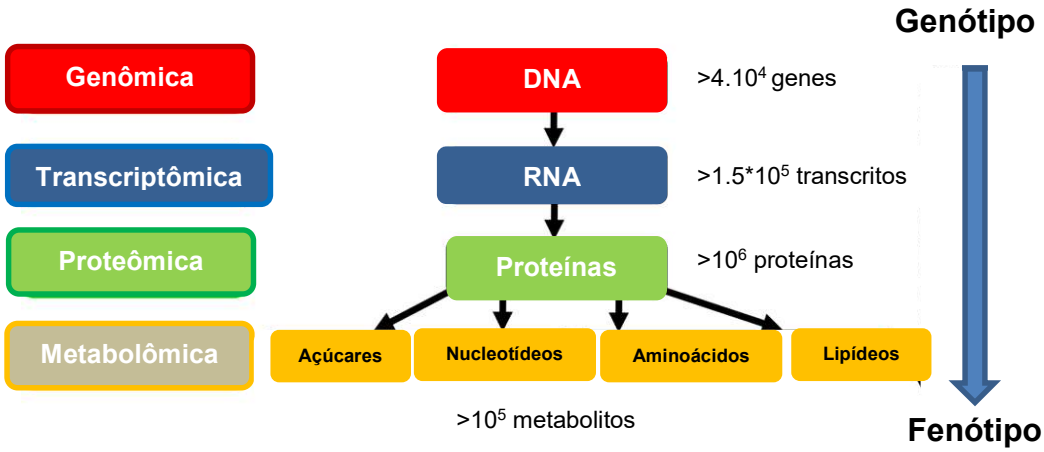

Figura 2. Visão geral da cascata de ômicas em sistemas biológicos (Adaptado Holčapek et al., 2018).

1.4.1 Genômica

Estudos recentes (FERNANDES JÚNIOR et al., 2016; MEDEIROS et al., 2017; SILVA- VIGNATO et al., 2019; CARVALHO et al., 2019) vêm tentando esclarecer os mecanismos envolvidos na deposição de gordura subcutânea de bovinos Nelore (espessura de gordura subcutânea – EGS) e características de interesse econômico como área de olho de lombo (AOL), peso de carcaça quente e marmorização (MAR).

Fernandes Júnior et al. (2016) avaliaram efeitos de SNPs que foram calculados com base nos valores genéticos, obtidos utilizando a abordagem GBLUP de um único passo e um procedimento de incorporação da matriz genômica. Os 10 principais SNPs não sobrepostos explicaram 8,72%, 11,38% e 9,31% da variância genética de AOL, EGS e peso de carcaça quente, respectivamente. Para a AOL, foram identificados, em bovinos da raça Nelore, genes (*CDKN2A* e *CDKN2B*) envolvidos no processo biológico do ciclo celular, que afeta muitos aspectos do crescimento e desenvolvimento dos animais. Os genes *SLC38A1* e *SLC38A2*, ambos da família de transportadores *SLC38*, também foram associados à AOL.

Vários genes identificados para EGS (*SORCS2, AQP3, AQP7, CDC42BPA, ASIP e ACSS2*) foram associados ao metabolismo lipídico em diferentes espécies de mamíferos. Além disso, várias regiões genômicas contem genes candidatos plausíveis que podem estar associados as características de carcaça em bovinos da raça Nelore (FERNANDES JÚNIOR et al., 2016).

Ao utilizar a ferramenta de associação genômica Medeiros et al. (2017) concluíram, que apesar da natureza poligênica das características de AOL e EGS de animais Nelore, alguns genes encontrados nas janelas associadas, têm maior probabilidade de estar relacionados a AOL do músculo Longissimus, como ALKBH3 e HSD17B12 e outros possivelmente, podem estar relacionados com a deposição de gordura em bovinos da raça Nelore, como os genes *PLAG1, CAPN5, MYO7A e XKR4.*

Novas vias de deposição de gordura na carcaça, foram reveladas utilizando ferramentas que exploraram interações gene-gene e correlações gene-fenótipo. Silva- Vignato et al. (2019), destacaram que os genes *RSAD2, EIF2AK2, ACAT1* e *ACSL1*, podem estar relacionados a metabolismo de ácidos graxos e podem ser associados as vias centrais da deposição de EGS em bovinos.

Posteriormente, Carvalho et al. (2019) identificaram, por meio de estudo da associação do genoma geral de bovinos de corte de origem Bos indicus, em particular animais Nelore, regiões e genes associados à qualidade de carcaça, examinando dados fenotípicos e genotípicos. Estes pesquisadores encontraram relação entre peso de carcaça quente e os genes: *LRGUK, TRIM24, SVOPL, TEX37, CA10, OXSR1*; para AOL, os genes: *TWIST2, SFXN1, CMYA5, CPQ e MRS2*; para EGS, os genes: *OR2S2, 5S_rRNA, LOC100299372, LOC523083, LOC532403, LOC613441, SNORA69 e ITGA9* e para MAR, os genes: *EMCN, LNX1, EIF5, SNORA28* e *DSC3*. As várias regiões genômicas associadas as carcterísticas de qualidade de carcaça mostram a complexidade desses fenótipos e não dependem apenas dos efeitos de alguns genes para determinar suas variações (CARVALHO et al., 2019).

Muitos estudos exploraram a variabilidade para selecionar bovinos com alto potencial genético para características de interesse econômico (KOOTS et al., 1994; REVERTER et al., 2003; FERNANDES JÚNIOR et al., 2016; MEDEIROS et al., 2017; SILVA-VIGNATO et al., 2019; CARVALHO et al., 2019). Esses estudos concluíram que a variabilidade genética é uma ferramenta interessante para melhorar os índices

de produção em bovinos, pois também está associada às variações nas características de qualidade de carne e carcaça. Portanto, genes identificados também podem ser úteis para estudos genômicos funcionais adicionais, além de contribuir para uma melhor compreensão do controle genético das características de carcaça.

1.4.2 Transcriptômica

O perfil do transcritoma pode variar dependendo do estado fisiológico, estímulos físicos, químicos, biológicos ou doenças. O transcritoma abrange o conjunto de RNAs mensageiros que codificam as proteínas. Entretanto, pesquisadores incluíram os microRNAs no conceito de transcritoma por representarem uma classe muito importante de pequenos RNAs (contendo aproximadamente 20 a 22 nucleotídeos) que controlam a expressão gênica ao nível pós-transcricional de gene, impedindo a tradução dos RNAm em proteínas (Passos, 2014).

Oliveira et al. (2018) identificaram vários genes e miRNAs como candidatos a reguladores da gordura intramuscular, por meio da análise de expressão diferencial de miRNAs, usando dois métodos diferentes de rede de coexpressão de miRNA-mRNA. Este estudo contribui para o entendimento de potenciais mecanismos regulatórios de redes de sinalização de genes envolvidos em processos de deposição de gordura medidos em músculo. O metabolismo da glicose e os processos de inflamação foram as principais vias encontradas in silico para influenciar deposição de gordura intramuscular em bovinos de corte na análise de coexpressão integrativa de mRNA-miRNA.

Posteriormente, Kappeler et al. (2019) observaram MiRNAs diferencialmente expressos no músculo esquelético de animais com valores genéticos estimados divergentes para maciez da carne bovina. A análise funcional identificou vias importantes relacionadas à sensibilidade, como apoptose e o sistema calpaína-calpastatina. Os resultados obtidos nesse estudo indicam a importância dos miRNAs nos mecanismos regulatórios que influenciam a proteólise muscular e maciez da carne e contribuem para o melhor entendimento do papel dos miRNAs nos processos biológicos associados à maciez da carne.

Também, Cardoso et al. (2021) revelaram, em uma abordagem multimônica, os efeitos do polimorfismo genético no miRNA e destaca os miR-SNPs e genes

candidatos-alvo, que controlam a composição de ácidos graxos da carne de bovinos Nelore. Diante dos resultados observados, mais estudos são necessários para elucidar os mecanismos subjacentes à ligação entre os miRNAs e seus alvos prováveis na determinação de fenótipos de interesse econômico em gado Nelore.

1.4.3 Proteômica

Os mecanismos regulatórios altamente complexos envolvidos nas características fenotípicas de qualidade da carne, tais como maciez, MAR, e EGS, podem ser explicados pelo metabolismo de proteínas e lipídeos no músculo e na gordura intramuscular e subcutânea. Potenciais proteínas biomarcadoras podem ser identificadas usando ferramentas proteômicas (MUNEKATA et al., 2021).

Ao avaliar o proteoma muscular de bovinos, Rodrigues et al. (2017), encontraram diferenças na qualidade da carne entre os bovinos Angus (Bos taurus taurus) e Nelore (Bos taurus indicus) quanto a proteômica e fosfoproteômica. Esses autores relataram que proteínas envolvidas na contração e organização muscular, miofilamentos expressos em fibras de contração rápida ou lenta e proteínas de choque térmico localizadas na mitocôndria ou retículo sarcoplasmático e envolvidas no fluxo celular de cálcio e apoptose podem estar associadas a diferenças na qualidade da carne bovina entre animais Angus e Nelore. Além disso, os pesquisadores relataram que diferenças na fosforilação de miofilamentos e enzimas glicolíticas podem estar envolvidas com diferenças na força de contração muscular, suscetibilidade à calpaína, apoptose e glicólise pós-morte, o que também pode estar relacionado a diferenças na qualidade da carne destes animais.

Poleti et al. (2018a) demonstraram que as proteínas envolvidas no metabolismo da glicólise, sinalização do citoesqueleto, junção de células aderentes e vias para MAPK e insulina são importantes mecanismos biológicos envolvidos na deposição de gordura intramuscular do músculo Longissimus em bovinos. Esses autores sugeriram que o lactato pode ser uma fonte importante de carbono para a deposição de gordura em ruminantes. Além disso, as mudanças gerais do transcriptoma e do proteoma associadas a gordura intramuscular demonstraram que a organização do sarcômero, a transdução de sinal intracelular e a regulação do citoesqueleto de actina são mecanismos alterados para deposição da gordura intramuscular (POLETI et al., 2018a; POLETI et al., 2018b).

Posteriormente, Poleti et al. (2020) revelaram alterações do proteoma muscular bovino, associadas ao conteúdo de ácido oleico e ácido linoléico conjugado cis-9, trans-11. Algumas das proteínas alteradas estão associadas ao metabolismo de ácidos graxos, proteólise, síntese de hormônio da tireóide e estrutura do citoesqueleto. Neste estudo, três potenciais biomarcadores para ambos os ácidos graxos foram identificados (TASP1, UBE4A, HADHA). Esses autores relatam que a integração de dados proteômicos e dados transcriptômicos sugerem um sistema regulatório complexo, em conformidade entre mRNA e níveis de proteína. Esses resultados podem ser aplicados em nutrição e programas de melhoramento genético para a produção de carne bovina de alta qualidade.

Por meio de duas meta-análises recentes de estudos proteômicos sobre cor e maciez da carne bovina, Purslow et al. (2021) destacaram o papel do metabolismo energético post-mortem no estabelecimento das condições para o desenvolvimento da cor e maciez da carne e descreveram a interação complexa entre o metabolismo energético, regulação do cálcio e metabolismo mitocondrial.

1.4.4 Metabolômica

A metabolômica pode ser utilizada como ferramenta para entender o metabolismo muscular. Alguns metabólitos contribuem para várias vias metabólicas, incluindo hipertrofia muscular, deposição de gordura, degradação de proteínas e propriedades sensoriais. Os metabólitos são produtos finais de reações complexas que ocorrem em resposta ao controle genômico e ambiental. Portanto, os metabólitos são indicadores sensíveis da influência genômica sob as carcterísticas fenotípicas (GOLDANSAZ et al., 2017).

Atualmente, a maioria de abordagens “MEATabolomics” combinam técnicas de separação (cromatografia gasosa ou líquida e eletroforese capilar) - abordagens de espectrometria de massa (MS) ou ressonância magnética nuclear (NMR) com as análises multivariadas a jusante, dependendo da polaridade e / ou hidrofobicidade dos metabólitos direcionados (MUROYA et al., 2020).

Ueda et al. (2019) identificaram mais de 80 metabólitos em carne bovina usando metabolômica baseada na análise de espectrometria de massas acoplada a cromatografia gasosa (GC / MS). Esses autores sugerem vários metabólitos (ácido decanóico, ácido úrico, ácido elaídico e ácido 3-fosfoglicérico) como potenciais biomarcadores para gordura intramuscular e para avaliar níveis de marmoreio por

análise GC / MS. Este estudo também indica que o ácido decanóico e a glutamina são potenciais biomarcadores para sabor oleoso, aroma e avaliação geral sensorial da carne de bovinos Wagyu.

Ao analisar animais *Bos indicus* da raça Nelore, selecionados para crescimento e precocidade, Cônsolo et al. (2020) verificaram que animais selecionados para alto crescimento e para alta precocidade, possuem alta concentração de metabólitos de proteínas e mudanças nas vias metabólicas da proteína, enquanto o metabolismo da gordura está mais correlacionada a seleção para precocidade em comparação com a seleção para crescimento.

Jeong et al. (2020) identificaram 28 metabólitos em carne bovina com alto e baixo teor de MAR, usando metabolômica baseada em NMR. Dentre os 28 metabólitos estimados, quatorze metabólitos apresentaram mudanças significativas nos atributos de qualidade da carne. Neste estudo, os principais metabólitos relacionados à pontuação de sabor de palatabilidade, incluindo glutamato e aspartato, não foram alterados entre os grupos de baixo e alto MAR. Porém as notas do painel sensorial, incluindo doçura, acidez e amargor, foram maiores na carne com alta MAR. Portanto, metabólitos podem atuar como biomarcadores potenciais do sabor da carne.

Os resultados destes estudos podem ser úteis para melhorar a avaliação de qualidade da carne por análise metabolômica e futuramente serem usados para melhorar produção e qualidade da carne.

1.4.5 Lipidômica

A lipidômica utiliza os princípios e técnicas da química analítica para estudo do conteúdo lipídico de uma célula, conhecido como lipidoma. As técnicas lipidômicas baseadas em espectrometria de massa (MS), como MS acoplado a cromatografia líquida, lipidômica shotgun, imagem MS e MS de mobilidade iônica têm papéis importantes na lipidômica. O conhecimento das redes e vias metabólicas lipídicas e ferramentas bioinformáticas são essenciais para estudar o metabolismo lipídico usando a lipidômica (HAN, 2016).

Novas tecnologias, metodologias e soluções bioinformáticas estão continuamente sendo desenvolvidos, todos visando melhorar e avançar em várias áreas de conhecimento, inclusive na produção animal e qualidade da carne. Tecnologias e protocolos atualizados permitem para realizar estudos lipidômicos

quantitativos. Os avanços na espectrometria de massa, permitem a observação da bioquímica lipídica localizada nos tecidos, tornando a lipidômica, uma plataforma importante na pesquisa de “foodomics”. Sem dúvidas, isso promoverá um avanço nas ciências básicas e pesquisa clínica e facilitará a indicação de biomarcadores lipídicos em diagnósticos, com o objetivo final de melhorar a saúde humana e a qualidade da carne (HOLČAPEK et al., 2018; BOWMAN et al., 2018; WU et al., 2020).

1.5 Modulação dos genes adipogênicos e lipogênicos por MicroRNAs

Os microRNAs (miRNAs) são uma classe de pequenos RNAs não codificantes que se ligam a sequências nas regiões não traduzidas de múltiplos mRNAs alvo, resultando na regulação pós-transcricional da expressão gênica (FATIMA & MORRIS, 2013). Os miRNAs são reguladores moleculares que participam de vários processos biológicos, incluindo adipogênese e lipogênese, por meio de seus alvos e vias relacionadas (GUO et al., 2017; ROMAO et al., 2012; ROMAO et al., 2011; KIM et al., 2009). A relevância dos miRNAs também é pronunciada quanto ao acúmulo de gordura subcutânea e / ou visceral em resposta ao crescimento e nutrição dietética em camundongos (PARRA et al., 2010; CHARTOUMPEKIS et al., 2012), ovinos (MEALE et al., 20014) e bovinos (ROMAO et al., 2012).

Em bovinos, existe variação dos perfis de miRNA entre diferentes locais de EGS (JIN et al., 2009). Os MiRNAs de tecido adiposo exibiu níveis de expressão diferenciais entre novilhos, de raças distintas, bem como com diferentes níveis de EGS (JIN et al., 2010). MiRNAs também demonstraram ser diferencialmente expressos em tecido adiposo bovino com a expressão de mir-378, variando em relação a EGS (JIN et al., 2010). ROMAO et al. (2012) revelaram que a expressão de miRNAs diferiu entre EGS e gordura visceral, sugerindo que o mecanismo molecular de a adipogênese é dependente do local de deposição de gordura em em bovinos de corte.

Muroya et al. (2016) revelaram que a dieta de bovinos a pasto afetou os miRNAs plasmáticos em comparação com gado terminado em confinamento, com dieta de alto grão. Especialmente, a expressão de miR-10b aumentou na circulação plasmática e músculo em bovinos a pasto. A expressão alterada de miR-10b e miR-2478 no músculo foi ainda associada à expressão dos genes lipogênicos alvo,

ELOVL6 e SCD1, respectivamente. Além disso, a expressão do fator que regula a homeostase da glicose, PTEN, também foi reduzida em animais sob pastejo. Esses resultados indicaram que as mudanças nos miRNAs induzidos por uma dieta rica em grãos estão associados ao miRNA do músculo esquelético e ao potencial de expressão do gene alvo, sugerindo, por sua vez, que miRNAs musculares estão envolvidos na regulação do metabolismo do músculo e de genes envolvidos no metabilismo lipídico, afetando a deposição da gordura e consequentemente a qualidade da carne.

Portanto, deposição de gordura é um processo biológico complexo onde miRNAs podem desempenhar um papel regulador de genes adipogênicos e lipogênicos. No tecido adiposo, o acúmulo de evidências demonstra claramente que os miRNAs desempenham um papel importante na diferenciação dos adipócitos e no metabolismo lipídico (SON et al., 2014; MIAO et al., 2015). Esse autores verificaram que muitos miRNAs tais como let-714, miR-14315–17, miR-17–5p18, miR-1419 e miR-3320–22 regulam a diferenciação de adipócitos e lipogênese por várias vias de sinalização, incluindo Wnt, MAPK, regulação do ciclo celular e via da insulina. Receptores nucleares como proteínas de ligação de CCAAT / potenciador (C / EBPs), receptor ativado por proliferador de peroxissoma (PPARs) e proteínas de ligação do elemento regulador de esterol (SREBPs) também são importantes.

Essas descobertas demonstram que os miRNAs podem participar do acúmulo de lipídeos no tecido adiposo e no músculo de bovinos de corte (SON et al., 2014; MIAO et al., 2015). Vários miRNAs são identificados que podem regular a adipogênese por meio de seus alvos e vias relacionadas. Entre eles, bta-miR-196b regulado para cima e btamiR-874, regulado para baixo podem influenciar a tradução do sinal da via do PPAR por seus alvos envolvidos na via e, consequentemente, regulam a deposição de gordura (GUO et al. 2017). Existem vários alvos na diferenciação adipogênica regulados pelo miR-224 de pré-adipócitos bovinos (KIM et al., 2014). Zhang et al. (2019) sugeriram que miR-224 prejudicaram a diferenciação adipogênica de pré-adipócitos bovinos, por direcionamento de LPL.

De acordo com os resultados relatados por YU et al. (2020), vários genes alvo dos MiRNAs são genes importantes da adipogênese, como FASN, C / EBP, PPAR, ADIPOQ, LPL, SERBP1, DLK1, etc. que podem ser regulados por miR-24-3p, miR22 197, miR-431, miR-409b, miR-11982, miR-10179-5p, miR-326, miR-6715

e miR-370. O PPAR pode ser modulado por miR-10179-5p e o C / EBP pode ser modulado por miR- 10179-5p, miR-11976, miR-370, miR-1343-5p e miR-2887.

Todos esses resultados podem indicar que RNAs diferencialmente expressos estão associados à adipogênese bovina. Recentemente, Muroya et al. (2020) relataram que as diferenças encontradas na expressão gênica dos genes relacionados à adipogênese e metabolismo lipídico na EGS foram influenciadas pela expressão de miRNAs neste tecido adiposo. Dessa forma, mudanças na expressão dos seguintes miRNAs na gordura subcutânea foram relatadas: miR-18a, miR-27b, miR-28, miR-30a-5p, miR-92a, miR-107, miR-126-3p, miR-128, miR-142-5p, miR-185, miR-20a, miR-103, miR-145, miR-148a (MUROYA et al., 2020).

1.6 Estratégias de seleção genética utilizadas para melhorar a qualidade da carne

O uso da informação genômica tem sido um procedimento padrão para avaliação genética em programas de melhoramento genético, a fim de melhorar as características quantitativas dos animais (COUTINHO et al., 2010) e otimizar à utilização de equipamentos de custo oneroso no sistema de produção, como a ultrassonografia de carcaça bovina *in vivo*.

Logo, a possível descoberta de novos marcadores moleculares envolvidos no metabolismo lipídico e a seleção de animais mais precoces para deposição EGS e MAR, seria uma das soluções na busca de qualidade da carne na indústria frigorífica brasileira. Atualmente, muitos frigoríficos já utilizam a EGS como critério de bonificação ao produtor. A MAR também já está inserida em algumas empresas, como critério para atender demandas de um nicho de mercado crescente diferenciado no Brasil

Diferenças fenotípicas com relação à deposição de gordura na carcaça podem ser encontradas dentro de uma mesma raça para diferentes grupos genéticos, ocasionando diferenças na qualidade do produto final (SMITH et al., 2007). Dessa forma, a seleção de animais mais precoces pode ser um investimento rentável e replicável. Para isso, uma das ferramentas mais utilizadas para identificar o valor genético dos animais e aumentar a sua precisão é a diferença esperada da progênie, comumente conhecia pela sigla DEP (HAYES et al., 2009), que vem sendo utilizada em diversos programas de melhoramento genético para identificação de touros mais precoces.

Neste sentido, a DEP possibilita identificar animais mais eficientes geneticamente para as características de interesse e utilizá-los como reprodutores no rebanho, visando transmitir essas características aos seus filhos. Em síntese, a DEP reflete a capacidade de um animal transmitir para sua descendência genes que afetarão o desempenho desta descendência em uma determinada característica (SAPP et al., 2002). As DEP's são usadas para comparar animais, dentro de uma mesma raça, quanto ao desempenho de suas futuras progênies (OKUT et al., 2013).

Contudo, para se alcançar ganhos genéticos consistentes devem ser considerados fatores genéticos e de ambiente inerentes às características que se buscam, como a herdabilidade e a variabilidade, além de suas correlações genéticas com outras características fenotípicas importantes (MOURÃO & FORMIGONI; 2006). Diversos estudos têm mostrado diferenças fenotípicas entre animais com alta e baixa DEP para características de interesse econômico (SAPP et al., 2002; DIKEMAN et al., 2005; BOLIGON et al., 2006), comprovando a eficiência desses métodos para identificar animais superiores em determinadas características consideradas no processo de seleção, tais como rendimento de carcaça quente, espessura de gordura subcutânea, gordura intramuscular e maciez.

Vários estudos foram realizados para avaliação de parâmetros genéticos para características produtivas, reprodutivas e de características de carcaça e qualidade de carne em bovinos de corte (KOOTS et al., 1994; REVERTER et al., 2003; entre outros). Portanto, características de interesse produtivo vêm sendo estudadas de forma a comparar os diferentes fenótipos entre zebuínos e taurinos, como também diferenças dentro de uma mesma raça. Ao avaliarem carcaças de touros Nelore e Brahman, O'Connor et al. (1997) observaram que ambas as raças têm a capacidade depositar gordura intramuscular em sistemas de cruzamento.

Em consonância, King et al. (2006) avaliaram bovinos Nelore e Brahman, em sistemas de cruzamento com Angus e observaram variações na EGS e MAR entre progênies, com a mesma porcentagem de herança de Zebu e essas variações foram maiores àquelas encontradas entre as raças. Da mesma forma, Bonin et al. (2014) investigaram a diferença na qualidade da carne de progênies de três linhagens de touros Nelore e observaram efeito paterno para as características de EGS e MAR do músculo Longissimus. Logo, isso reflete a existência de variabilidade entre os touros Nelore na transferência dos graus de EGS e MAR nas carcaças de suas progênies.

Posteriormente, Pereira et al. (2015) avaliaram as característica da carcaça e da qualidade da carne em progênies de touros Nelore e Cruzados (“Angus x Nelore” e “Brahman x Nelore”) e verificaram que apesar dos cruzados apresentarem qualidade da carcaça e da carne superior aos animais Nelore, algumas progênies de Nelore foram semelhantes às progênies de Angus, com relação a qualidade da carne, com destaque para possível diferença genética dentro da raça Nelore, com indicações de ampla variação genetíca intra - racial para características relacionadas a gordura, em especial MAR, entre as progênies.

Portanto, tais resultados evidenciam a existência de animais pertencentes a uma mesma raça, com potencial genético para aumentar a EGS e MAR em suas progênies. Assim, a variabilidade nestas características para DEP entre animais fornece uma ferramenta para selecionar características de interesse dentro da raça, melhorando atributos de carcaça e carne em bovinos. No entanto, é escasso na literatura o conhecimento sobre o mecanismo genético que envolve a deposição da gordura intramuscular e subcutânea em progênies Nelore de grupos genéticos contrastantes para precocidade e crescimento. Ainda há falta de informações sobre as diferenças transcriptômicas e proteômicas entre os tecidos de deposição de gordura, especialmente sobre as diferenças no perfil de expressão gênica e metabolismo proteico e lipídico em zebuínos, selecionados para precocidade sexual e crescimento, com ênfase na deposição de gordura subcutânea.

Neste sentido, segundo os resultados dos estudos citados anteriormente, sugere-se que é possível que bovinos da raça Nelore, filhos de touros selecionados, a partir de DEP’s altas para precocidade e crescimento, apresentem maior expressão de fatores transcricionais e maior abundancia de proteínas envolvidas no metabolismo lipidico, responsáveis pela deposição de tecido adiposo subcutâneo e maior eficiência de deposição de gordura, em relação aos animais com DEP’s baixas para tais características, o que caracterizaria assim, um fator não somente relacionado à raça, como também relacionado às diferenças entre grupos genéticos dentro de uma mesma raça.

Dessa forma, aspectos fisiológicos e moleculares associados à deposição de gordura subcutânea e metabolismo lipídico e proteico são informações escassas na literatura e devido à complexidade dessas características, é importante esclarecer os mecanismos relacionados a deposição de gordura na carcaça e na carne de

progênies, selecionadas a partir das DEP's de seus pais, contrastantes para características de interesse como precocidade e crescimento.

Diante o exposto, hipotetiza-se que a deposição de gordura na carcaça e na carne de bovinos Nelore é influenciada pela precocidade e crescimento, que garante maior adiposidade nas progênies de touros Nelore, com maior potencial genético para tais características, utilizando as DEP's como ferramenta de avaliação do potencial genético em grupos contrastantes de alta e baixa DEP.

Estudos nesse sentido, são fundamentais não só como contribuição científica, mas para a comunidade em geral, pois fornecerá conhecimentos para que a indústria da carne brasileira aumente sua participação em mercados mais exigentes e competitivos em relação à qualidade da carne, influenciando diretamente a lucratividade de todo o sistema de produção de carne bovina.

Para isso, o presente estudo foi dividido nos 3 próximos capítulos, denominados:

- Nelore progenies from contrasting groups for growth and precocity affect physiological parameters and steak molecular profile

- Subcutaneous fat proteomic analysis reveals pathways related to fat deposition from beef cattle with expected progeny difference for precocity

- Bovines with greater genetic potential for precocity have higher levels of transcription of genes involved in lipogenesis

1.7 REFERÊNCIAS

Aalhus, J. L.; Janz, J. A. M.; Tong, A. K. W.; Jones, S. D. M.; Robertson, W. M. The influence of chilling rate and fat cover on beef quality. **Canadian Journal of Animal Science**, v. 81, p. 315–320, 2001.

Adstrum S.; Hedley G.; Schleip R.; Stecco C.; Yucesoy C. A. Defining the fascial system. **J. Bodyw. Mov**. Ther, v. 21, p. 173–177, 2017. 10.1016/j.jbmt.2016.11.003

Albrecht. E.; Gotoh, T.; Ebara, F.; Xu, J.X.; Viergutz, T.; Nürnberg, G.; Maak, S.; Wegner, J. Cellular conditions for intramuscular fat deposition in japanese black and holstein steers. **Meat Science**, v. 89, p. 13-20, 2011.

Albuquerque, L. G.; Fernandes Júnior, G. A.; Roberto, C. Beef Cattle Genomic Selection in Tropical Environments. in Proc. Assoc. Advmt. Anim. Breed. **Genet**, v. 22, p. 255–263, 2017.

Bailey, A. J., & Light, N. D. (1989). **Connective tissue in meat and meat products**. London: Elsevier Applied Science

Bauman, D. E.; Perfield, J. W.; Harvatine, K. J.; Baumgard, L. H. Regulation of fat synthesis by conjugated linoleic acid: lactation and the ruminant model. **The Journal of Nutrition**, v. 138, p. 403-409, 2008.

Bergen, W.G.; Mersmann, H.J. Comparative aspects of lipid metabolism: impact on contemporary research and use of animal models. **The Journal of Nutrition**, v. 135, p. 2499- 2502, 2005.

Boligon, A. A.; Rorato, P. R. N.; Weber, T.; Everling, D. M. E.; Lopes, J. S. Herdabilidades para ganho de peso da desmama ao sobreano e perímetro escrotal ao sobreano e tendências genética e fenotípica para ganho de peso da desmama ao sobreanoem bovinos Nelore-Angus. **Revista Brasileira de Zootecnia**, v.35, p.1323-1328, 2006.

Boggs, D. L.; Bergen, W.G.; Hawkins D.R. Effects of tallow supplementation and protein withdrawal on ruminal fermentation, microbial synthesis and site of digestion. **Journal of Animal Science**, v. 64, p. 907-914, 1987.

Bonin, M. N.; Ferraz, J. B. S.; Eler, J. P.; Rezende, F. M.; Cucco, D. C.; Carvalho, M. E.; Silva, R. C. G.; Gomes, R. C.; Oliveira, E. C. M. Sire effects on carcass and meat quality traits of young Nellore bulls. **Genetics and molecular research**: GMR, v. 13 (2), p. 3250-3264, 2014.

Bowman, A. P.; Heeren, R. M. A.; Ellis, S. R. Advances in Mass SpectrometryImaging Enabling Observation of Localised Lipid Biochemistry within Tissues. **Trends in Analytical Chemistry**, v. 120, p. 115197, 2018. doi: 10.1016/j.trac.2018.07.012

Brooks, C.; Garner, G.; Gehrke, C.; Muhrer, M.; Pfander, W. The effect of added fat on the digestion of cellulose and protein by ovine rumen microorganisms. **J Anim Sci**, v. 13, p. 758–64, 1954. doi: 10.2527/jas1954.134758x

Cardoso, T.F.; Coutinho, L.L.; Bruscadin, J.J.; da Silva Diniz,W.J.; Petrini, J.; Andrade, B.G.N.; de Oliveira, P.S.N.; Poleti, M.D.; Cesar, A.S.M.; da Silveira, J.C.; et al. Multi-Omics Approach Reveals miR-SNPs Affecting Muscle Fatty Acids Profile in Nelore Cattle. **Genes**, v.12, p. 67, 2021. doi.org/10.3390/genes12010067

Carvalheiro, R.; Costilla, R.; Neves, H. H. R.; Albuquerque, L. G.; Moore, S.; Hayes, B. J . Unraveling genetic sensitivity of beef cattle to environmental variation under tropical conditions. **Genet Sel Evol**, v. 51, p. 29, 2019. doi.org/10.1186/s12711-019-0470-x

Carvalho, M.; Baldi, F.; Alexandre, P.; Santana, M.; Ventura, R.; Bueno, R.; Nadai, B. M.; Rezende, F.; Coutinho, L.; Eler, J.; Ferraz, J. Genomic regions and genes associated with carcass quality in Nellore cattle**. Genetics and molecular research**: GMR v. 18, n. 1, p. 18226, 2019.

Ceciliani, F.; Lecchi, C.; Bazile, J.; Bonnet, M. (2018) Proteomics Research in the Adipose Tissue. In: de Almeida A., Eckersall D., Miller I. (eds) **Proteomics in Domestic Animals: from Farm to Systems Biology**. Springer, Cham. doi.org/10.1007/978-3-319-69682-9_12

Chardulo, L. A. L.; Silveira, A. C.; Vianello, F. Analytical aspects for tropical meat quality. **Food quality, safety and technology**, v. 1, p. 53–62, 2013.

Chartoumpekis, D. V.; Zaravinos, A.; Ziros, P. G.; Iskrenova, R. P.; Psyrogiannis, A. I, et al. Differential expression of microRNAs in adipose tissue after long-termhigh-fat diet-induced obesity in mice. **PLoS One**, v. 7, p. e34872, 2012. doi: 10.1371/journal.pone.0034872

Chillard,Y.; Ferlay, A.; Delavaud, C. E.; Bocquier, F. Plasma leptin in underfed or overfed adult Holstein and Charolais cows and its relationship with adipose tissue cellularity. **International Journal of Obesity**, v. 22, p. 171-179, 1998.

Cross, H. R.; Carpenter, Z. L; Smith, G. C. Effects of intramuscular collagen and elastin on bovine muscle tenderness. **Journal Food Science**, v. 38, p. 998–1003, 1973.

Crouse, J. D.; Cundiff, L. V.; Koch, R. M. Comparisons of Bos indicus and Bos taurus inheritance for carcass beef characteristics and meat palatability. **Journal of Animal Science**, v. 67, n.10, p. 2661-2668, 1989.

Cogni, A. L.; Farah, E.; MInicucci, M. F.; Azevedo, P. S.; Okoshi, K, Matsubara, B. B. Metalloproteinases-2 and -9 predict left ventricular remodeling after myocardial infarction. **Arquivo Brasileiro Cardiologia**, v. 100(4), p. 315–321, 2013.

Cônsolo, N. R. B.; Silva, J.; Buarque, V. L. M.; Higuera-Adilla, A.; Barbosa, L. C. G. S.; Zawadzki, A.; Colnago, L. A.; Saran, Netto, A.; Gerrard, D. E.; Silva, S. L. Selection for growth and precocity alters muscle metabolism in Nellore cattle. **Metabolites**, v. 10, p. 1–12. 2020. doi.org/10.3390/metabo10020058

Coutinho, L. L; Rosário, M. F; Jorge, E. Cristina. **Biotecnologia animal**. Estudos Avançados. vol.24 no.70 São Paulo 2010.

Csapo, R.; Gumpenberger, M.; Wessner, B. Skeletal Muscle Extracellular Matrix - What Do We Know About Its Composition, Regulation, and Physiological Roles? A Narrative Review. **Front Physiol**, v. 11, p. 253, 2020. doi:10.3389/fphys.2020.00253

Devine, C. E.; Wahlgren, N. M.; Tornberg, E. Effect of rigor temperature on muscle shortening and tenderization of restrained and unrestrained beef m. Longissimus thoracicus et lumborum. **Meat Science**, v. 51, p. 61–72, 1999.

DIkeman, M. E.; Pollak, E. J.; ZHang, Z.; Moser, D. W.; Gill, C. A. E Dressler, E. A.; Phenotypic ranges and relationships among carcass and meat palatability traits for fourteen cattle breeds, and heritabilities and expected progeny differences for Warner-Bratzler shear force in three beef cattle breeds. **Journal Animal Science**, v. 83, p. 2461–2467, 2005.

Dodson, M. V.; Hausman, G. J.; Guan, L.; Du, M.; Rasmussen, T. P.; Poulos, S. P.; Mir, P.; Bergen, W. G.; Fernyhough, M. E., Mcfarland, D. C.; Rhoads, R. P.; Soret B.; Reecy, J. M.; Velleman, S. G.; Jiang, Z. A; Lipid metabolism, adipocyte depot physiology and utilization of meat animals as experimental models for metabolic research**. International Journal of Biological Sciences**, v. 6, p. 691-699, 2010a.

Du, M.; and Dodson, M. V. Advanced techniques to enhance marbling in meat. In: S.-T. Joo (ed.) Control of meat quality. **Research signpost**, Kerala, 2012.

Du, M., Y. Huang, A. K. Das, Q. Yang, M. S. Duarte, M. V. Dodson, and M. J. Zhu. Meat science and muscle biology symposium: manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. **J. Anim. Sci**, v. 91, p. 1419–1427, 2013. doi:10.2527/jas.2012-5670

Du, M.; Tong, J.; Zhao, J.; Underwood, K. R.; Zhu, M.; Ford , S. P.; Nathanielsz, P. W. Fetal programming of skeletal muscle development in ruminant animals. **Journal Animal Science**, v. 88, p. E51-60, 2010a.

Du, M.; Yin, J.; Zhu, M. J. Cellular signaling pathways regulating the initial stage of adipogenesis and marbling of skeletal muscle. **Meat Science**, v. 86, p.103-109, 2010b.

Du, M.; Wang, B.; Fu, X.; Yang, Q.; Zhu, M. J. Fetal programming in meat production. **Meat Sci**, v. 109, p. 40–47, 2015. doi:10.1016/j.meatsci.2015.04.010

Duarte, M. S.; Gionbelli, M. P.; Paulino, P. V. R.; Serão, N. V. L.; Martins, T. S.; Tótaro, P. I. S.; Neves, C. A.; Valadares Filho, S. C.; Dodson, M. V.; Zhu, M.; DU, M. Effects of maternal nutrition on development of gastrointestinal tract of bovine fetus at different stages of gestation. **Livestock Science** (Print), v. 153, p. 60-65, 2013.

Duarte, M. S.; Paulino, P. V. R.; Das, A. K.; Wei, S.; Serao, N. V. L.; FU, X. ; Harris, S. M.; Dodson, M. V.; Du, M. Enhancement of adipogenesis and fibrogenesis in

skeletal muscle of Wagyu compared with Angus cattle. **Journal of Animal Science**, v. 91, p. 2938-2946, 2013.

Duarte, M. S.; Paulino, P. V. R.; Fonseca, M. A.; Diniz, L L.; Cavali, J.; Serão, N. V. L, et al. Influence of dental carcass maturity on carcasstraits and meat quality of Nellore bulls. **Meat Science**, v. 88(3), p. 441–6, 2011.

Ebarb, S.; Drouillard, J. S.; MAddock-Carlin, K.; Phelps, K.; Vaughn, M.; Burnett, D.; Bibber-Krueger, V.; Paulk, C.; Grieger, D. M.; Gonzalez, J. M. Effect of growth promoting technologies on Longissimus lumborum muscle fiber morphometrics, collagen solubility, and cooked meat tenderness. **Journal Animal Science**, v. 94, p. 869–881, 2016.

Fatima, A and Morris, D. G. MicroRNAs in domestic livestock. **Physiol Genomics**, v. 45, p. 685– 696, 2013.

Felderhoff, C.; Lyford, C.; Malaga, J.; Polkinghorne, R.; Brooks, C.; Garmyn, A.; Miller, M. Beef Quality Preferences: Factors Driving Consumer Satisfaction. **Foods**, v. 9, p. 289, 2020.

Felicio, P.E. Fatores que Influenciam na Qualidade da Carne Bovina. In: A. M. Peixoto; J. C. Moura; V. P. de Faria. (Org.). Produção de Novilho de Corte. 1.ed.Piracicaba: FEALQ, v. Único, p.79-97, 1997.

Fernandes Júnior, G. A.; Costa, R. B.; Camargo, G. M. F.; Carvalheiro, R.; Rosa, G. J. M.; Baldi, F.; Garcia, D. A.; Gordo, D. G. M.; Espigolan, R.; Takada, L.; Magalhães, A. F. B.; Bresolin, T; Feitosa, F. L. B.; Chardulo, L. A. L; Oliveira, H. N. ; Albuquerque, L. G. Genome scan for postmortem carcass traits in Nellore cattle. **Journal of Animal Science**, v. 94 (10), p. 4087–4095, 2016.

Fernandes Júnior, G. A.; de Oliveira, H. N.; Carvalheiro, R.; Cardoso, F. C.; Fonseca, S. F.; Ventura, R. V.; Albuquerque, L. G. A. Whole-genome sequencing provides new insights into genetic mechanisms of tropical adaptation in Nellore (Bos primigenius indicus). **Scientific Reports**, v. 10, p. 9412, 2020. doi.org/10.1038/s41598-020-66272-7

Ferraz, J. B. S., & Felicio, P. E. D. Production systems – An example from Brazil. Meat Science, v. 84(2), p. 238–243, 2010. doi.org/10.1016/j.meatsci.2009.06.006.

Font-I-Furnols M.; Guerrero L. Consumer preference, behavior and perception about meat and meat products: an overview. **Meat Science**, v. 98, p. 361±71, 2014.

Fritsche, S.; Fritsche, J. Ocurrence of conjugated linoleic acid isomers in beef. **Journal of the American Oil Chemists**' Society, v. 75, p. 1449-1451, 1998.

French, P., C. Stanton, F. Lawless, E. G. O'Riordan, F. J. Monahan, P. J. Caffrey, and A. P. Moloney. 2000. Fatty acid composition including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. **J. Anim. Sci**, v. 78, p. 2849 – 2855. doi:10.2527/2000.78112849x

Gillies, A. R.; Chapman, M. A.; Bushong, E. A.; Deerinck, T. J.; Ellisman, M. H.; Lieber R. L. High resolution three-dimensional reconstruction of fibrotic skeletal muscle extracellular matrix: fibrotic muscle ECM organization. **J. Physiol**, v. 595, p. 1159–1171, 2017. 10.1113/JP273376

Gillies, A. R.; Lieber, R. L. Structure and function of the skeletal muscle extracellular matrix. **Muscle Nerve**, v. 44, p. 318–331, 2011. 10.1002/mus.22094

Giusti, J.; Castan, E.; Dal P. M.; Arrigoni, M. D. B.; Baldin, S. R.; Oliveira, H. N. Expression of genes related to quality of Longissimus dorsi muscle meat in Nellore (Bos indicus) and Canchim (5/8 Bos taurus× 3/8 Bos indicus) cattle. **Meat Science**, v. 94, p. 247-252, 2013.

Goldansaz, S.A.; Guo, A.C.; Sajed, T.; Steele, M.A.; Plastow, G.S.; Wishart, D.S. Livestock metabolomics and the livestock metabolome: A systematic review. **PLoS ONE**, v. 12, p. e0177675, 2017.

Gonzalez, J. M; Johnson, D. D.; Elzo, M. A.; WHite, M. C.; Stelzleni, A. M.; Johnson, S. E. Effect of Brahman Genetic Influence on Collagen Enzymatic Crosslinking Gene Expression and Meat Tenderness. **Animal Biotechnology**, v. 25 (3), p.165-178, 2014.

Gregorie, F. M.; Smas, C. M.; Sul, H. S. Understanding adipocyte differentiation. **Physiological Reviews**, v.78, n.3, p.783–809, 1998.

Griffin, M.J.; Sul, H.S. Insulin regulation of fatty acid synthase gene transcription: Roles of usf and SREBP-1C. **IUBMB Life**, v. 56, p. 595–600. 2004

Griinari, J.M.; Corl, B.A.; Lacy, S.H.; Chouinard, P.Y.; Nurmela, K.V.; Bauman, D.E. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase. **The Journal of Nutrition**, v. 130, p. 2285-2291, 2000.

Guerrero, A.; SAñudo, C.; ALberti, P.; RIpolli, G.; Campo, M. M.; Olleta, J. L.; Panea, B.; Khliji, S.; Santolaria, P. Effect of production system before the finishing period on carcass, meat and fat qualities of beef. **Animal**, v.7, p. 2063-2072, 2013a.

Guerrero, A., ValerO, M. V., CAmpo, M. M.; Sañudo, C. Some factors that affect ruminant meat quality: from the farm to the fork. Review. **Acta Scientiarum Animal Sciences**, v. 35, p. 335-347, 2013b.

Guo, Y.; Zhang, X.; Huang, W.; Miao, X. Identification and characterization of differentially expressed miRNAs in subcutaneous adipose between Wagyu and Holstein cattle. **Scientific Reports**, v. 7, p. 44026, 2017. doi:10.1038/srep44026.

Gupta, R. K. Z.; Arany, P.; Seale, R. J.; Mepani, L.; Ye, H. M.; Conroe, Y. A.; ROBY, H.; KULAGA, R.; REED, R.; Spiegelman, B. M. Transcriptional control of preadipocyte determination by zfp423. **Nature**, v. 464, p.619-623, 2010.

Gupta, R. K.; Mepani, R. J.; Kleiner, S.; Lo, J. C.; Khandekar, M. J.; Cohen, P.; Frontini, A.; Bhowmick, D. C.; Cinti, L. Ye, S.; B. M. Spiegelman. Zfp423 expression

identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. **Cell Metabolism**, v. 15, p.230-239, 2012.

Halper, J.; Kjaer, M. “Basic components of connective tissues and extracellular matrix: elastin, fibrillin, fibulins, fibrinogen, fibronectin, laminin, tenascins and thrombospondins,” **in Progress in Heritable Soft Connective Tissue Diseases ed. Halper J. Dordrecht: Springer**, p. 31– 47. 2014. doi:10.1007/978-94-007-7893-1_3

Hammond, J. Progress in the physiology of farm animals (Vol. 2). London: **Butterworths Scientific Publications**, 1995.

Han, X. Lipidomics for studying metabolism. **Nature Reviews Endocrinology**, v. 12, p. 668–679, 2016. doi.org/10.1038/nrendo.2016.98

Hao, G.; Chen, H.; Du, K.; Huang, X.; Song, Y.; Gu, Z.; et al. Increased fatty acid unsaturation and production of arachidonic acid by homologous over-expression of the mitochondrial malic enzyme in Mortierella alpina. **Biotechnol**. Lett, v. 36, p. 1827–1834, 2014. doi:10.1007/s10529-014-1546-x

Hao, G.; Chen, H.; Zhang, H.; Chen, W.; Chen, Y. Q. Metabolic engineering of Mortierella alpina for enhanced arachidonic acid production through the NADPH supplying strategy. **Appl. Environ. Microbiol**, v. 82, p. 3280–3288, 2016. doi:10.1128/AEM.00572-16

Harvatine, K.J.; Allen, M.S. Fat supplements affect fractional rates of ruminal fatty acid biohydrogenation and passage in dairy cows. **The Journal of Nutrition**, v. 136, p. 677- 685, 2006.

Hassan, F.; Arshad, M. A.; Ebeid, H. M.; Rehman, M. S.; Khan, M. S.; Shahid, S.; Yang, C. Phytogenic Additives Can Modulate Rumen Microbiome to Mediate Fermentation Kinetics and Methanogenesis Through Exploiting Diet–Microbe Interaction. Front. Vet. Sci, v. 7, p. 575801, 2020. doi: 10.3389/fvets.2020.575801

Hausman, G. J.; Dodson, M. V.; AJUwon, K.; Azain, M.; Barnes, K. M., Guan, L.L.; Jiang, Z.; Poulos, S. P.; Sainz, R. D.; Smith, S.; Spurlock, M.; Novakofski, J.; Fernyhough, M. E. Board-invited review: The biology and a regulation of preadipocytes and adipocytes in meat animals. **Journal of Animal Science**, v. 87, p.1218-1246, 2009.

Hayes, B. J.; Bowman, P. J.; Chamberlain, A. C.; VErbyla, K. Accuracy of genomic breeding values in multi-breed dairy cattle populations. **Genetics Selection Evolution**, p. 41-51, 2009.

Holčapek, M.; Liebisch, G.; Ekroos, K. Lipidomic Analysis. **Anal. Chem**, v. 90 (7), p. 4249–4257, 2018. doi.org/10.1021/acs.analchem.7b05395

Hossner, K. L. **Hormonal regulation of farm animal growth** / K.L. Hossner. p. 217. Department of Animal Sciences, Colorado State University, Fort Collins, Colorado,USA. CABI Publishing, 2005.

Inoue, S.; Hori,Y.; Hirano,Y.; Inamoto, T.; Tabata, Y. Effect of culture substrate and fibroblast growth factor addition on the proliferation and differentiation of human adipo-stromal cells. **Journal of Biomaterials Science-Polymer**, v.16, p.57-77, 2005.

Jeong, J. Y.; Kim, M.; Ji, S. Y.; Baek, Y. C.; Lee, S.; Oh, Y. K.; Reddy, K. E.; Seo, H. W.; Cho, S.; Lee, H. J. Metabolomics Analysis of the Beef Samples with Different Meat Qualities and Tastes. **Food science of animal resources**, v. 40 (6), p. 924–937, 2020. doi.org/10.5851/kosfa.2020.e59

Jeremiah, L. E.; Dugan, M. E. R.; Aalhus, J. L.; Gibson, L. L. Assessment of the relationship between chemical components and palatability of major beef muscles and muscle groups. **Meat Science**, v. 65 (3), p. 1013-1019, 2003.

Jin, W.; Dodson, M.; Moore, S.; Basarab, J.; Guan, L. L. Characterization of microRNA expression in bovine adipose tissues: a potential regulatory mechanism of subcutaneous adipose tissue development. **BMC Molecular Biology**, v. 11, p. 29, 2010.

Jiang, Y.; Berry, D. C.; Tang, W.; Graff, J. M. Independent stem cell lineages regulate adipose organogenesis and adipose homeostasis. **Cell Rep**, v. 9, p. 1007–1022, 2014. doi:10.1016/j.celrep.2014.09.049

Jin, W.; Grant, J.; Stothard, P.; Moore, S.; Guan, L. Characterization of bovine miRNAs by sequencing and bioinformatics analysis. **BMC Molecular Biology**, v. 10, p. 90, 2009.

Joe, A. W.; Yi, L.; Natarajan, A.; Grand, L. E. F.; So, L.; Wang, J.; Rudnicki, M.A.; Rossi, F.M. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. **Nature Cell Biology**, v. 12, p. 153-U144, 2010.

Kakudo, N.; SHimotsuma, A.; Kusumoto, K. Fibroblast growth factor-2 stimulates adipogenic differentiation of human adipose-derived stem cells. **Biochemical and Biophysical Research Communications**, v. 359, p. 239-244, 2007.

Kappeler, B. I.G.; Regitano, L. C. A.; Poleti, M. D. et al. MiRNAs differentially expressed in skeletal muscle of animals with divergent estimated breeding values for beef tenderness. **BMC Molecular Biol**, v. 20, 1, 2019. doi.org/10.1186/s12867-018-0118-3

Kersten, S. Mechanisms of nutritional and hormonal regulation of lipogenesis. **Embo Rep**, v. 2, p. 282–286, 2001. doi: 10.1093/embo-reports/kve071.

Kim, V. N.; Han, J.; Siomi, M. C. Biogenesis of small rnas in animals. **Nat Rev Mol Cell Biol**, v. 10, p. 126–139, 2009.

Kim, D.; Kim, J.; Hyun, H.; Kim, K.; Roh, S. A nanoscale ridge/groove pattern arrayed surface enhances adipogenic differentiation of human supernumerary tooth-derived dental pulp stem cells in vitro, **Arch. Oral Biol**, v. 59 (8), p. 765–774, 2014.

Kim, Y. C.; Gomez, F. E.; Fox, B.G; Ntambi, J. M. Differential regulation of thestearoyl-CoA desaturase genes by thiazolidinediones in 3T3-L1 adipo-cytes. **Journal of Lipid Research**, v. 41, p. 1310–1316, 2000.

Kim, Y.; Remacle, A. G.; Chernov, A. V.; Liu H.; Shubayev, I.; Lai, C.; et al. The MMP-9/ TIMP-1 axis controls the status of differentiation and function of myelinforming Schwann cells in nerve regeneration. **PLoS One**, v. 7(3), p. e33664, 2012. doi.org/10.1371/journal.pone.0033664

King, D. A.; Morgan, W. W.; Miller, R. K.; Sanders, J. O.; Lunt, D. K.; Taylor, J. F.; Gill, C. A.; Savell, J. W. Carcass merit between and among family groups of Bos indicus crossbred steers and heifers. **Meat Science**, v. 72, p. 496-502, 2006.

Koohmaraie, M. Muscle proteinases and meat aging. **Meat Science**, v 36, p. 93-104, 1994.

Koohmaraie M. Role of the neutral proteinases in postmortem muscle protein degradation and meat tenderness. In: Proc. Rec. **Meat Conference**, Colorado State University, v. 45, p. 63-71, 1992.

Koots, K.R.; Gibson, J.P.; Wilton, J.W. Analysis of published genetic parameters estimates for beef production traits.1 - Herdability. **Animal Breeding Abstract**, v.62 (5), p.309-338, 1994.

Ladeira, M. M.; Schoonmaker, J. P.; Gionbelli, M. P.; Dias, J.; Gionbelli, T. R. S.; Carvalho, J. R. R.; Teixeira, P. D. Nutrigenomics and beef quality: a review about lipogenesis. **International Journal of Molecular Sciences**, v. 17, p. 918, 2016.

Lage, J. F.; Paulino, P. V. R.; Valadares Filho, S. C.; Souza, E. J. O.; Duarte, M. S., Benedeti, P. D. B.; Souza, N. K.; Cox, R. B. Influence of genetic type and level of concentrate in the finishing diet on carcass and meat quality traits in beef heifers. **Meat Science**, v. 90, p. 770±74, 2012.

Lamming, D. W.; Sabatini, D. M. A Central role for mTOR in lipid homeostasis. **Cell Metab**, v. 18(4), p. 465-9, 2013. doi: 10.1016/j.cmet.2013.08.002.

Laurent, G. J. Dynamic state of collagen: pathways of collagen degradation in vivo and their possible role in regulation of collagen mass. **American Physiological Society**, v. 252, p. C1-9. 1987.

Lawrie, R. A & Ledward, D. A. **Lawrie's meat science**. CRC Seventh English edition, Wood head Publishing Limited and CRC Press LLCp. 442, 2006.

Lourenço, M.; Ramos-Morales, E.; Wallace, R.J. The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. **Animal**, v. 4, p. 1008-1023, 2010.

Lee, H-J.; Jang, M.; Kim, H.; Kwak, W.; Park, W. C.; Hwang, J. Y.; Lee, C-K.; Jang, G. W; Park, M. N.; Kim, H-C.; Jeong, J. Y.; Seo, K. S.; Kim, H.; Cho, S.;

Lee, B-Y. Comparative Transcriptome Analysis of Adipose Tissues Reveals that ECM-Receptor Interaction Is Involved in the Depot-Specific Adipogenesis in Cattle. **PLoS One**, v. 8(6), p. e66267, 2013.

Leheska, J. M.; Thompson, L. D.; Howe, J. C.; Hentges, E.; Boyce, J.; Brooks, J. C.; Shriver, B.; Hoover, L.; Miller, M. F. Effects of conventional and grass-feeding systems on the nutrient composition of beef. **J. Anim. Sci**, v. 86, p. 3575 –3585, 2008. doi:10.2527/jas.2007-0565.

Luchiari Filho, A. **Pecuária da carne bovina**. São Paulo: R Vieira Gráfica e Editora, 2000.

Mancini, R. A., & Hunt, M. C. Current research in meat color. **Meat Science**, v. 71(1), p. 100–121, 2005. doi.org/10.1016/j.meatsci.2005.03.003.

Marsh, B.B. The basis of tenderness in muscle foods. **Journal of Food Science**, v. 42, n. 2, p. 295, 1977.

Martins, T. S.; Sanglard, L. M. P.; Silva, W.; Chizzotti, M. L.; Ladeira, M. M.; Serão, N. V. L.; Paulino, P. V. R.; Duarte, M. S. Differences in skeletal muscle proteolysis in Nellore and Angus cattle might be driven by Calpastatin activity and not the abundance of Calpain/Calpastatin. **Journal of Agricultural Science**, v. 155, p. 1669–1676, 2017.

Martins, T. S.; Sanglard, L. M.; Silva, W.; Chizzotti, M. L.; Rennó, L. N.; Serão, N. V.; Silva, F. F.; Guimarães, S.E.; Ladeira, M. M.; Dodson, M. V.; Du, M.; Duarte, M. S. Molecular factors underlying the deposition of intramuscular fat and collagen in skeletal muscle of Nellore and Angus cattle. **PLoS One**, v. 10(10), p. e0139943, 2015.

Mao,Y.; Hopkins, D. L.; Zhang,Y.; Li,P.; Zhu, L.; Dong, P.; Luo, X. Beef quality with diferente intramuscular fat contente and proteomic analysis using isobaric tag for relative and absolute quantitation of differentially expressed proteins. **Meat Science**, v. 118, p. 96–102, 2016. doi.org/10.1016/j.meatsci.2016.03.028.

May, S. G.; Savell, J. W.; Lunt, D. K. Evidence for preadipocyte proliferation during culture of subcutaneous and intramuscular adipose tissues from Angus and Wagyu crossbred steers. **Journal of Animal Science**, v. 72, n.12, p.3110-3117, 1994.

McCormick R. J. Extracellular modifications to muscle collagen: implications for meat quality. **Poultry Science**, v. 78, p. 785–791, 1999.

McMahon, C.D.; Radcliff, R.P.; Lookingland, K. J.; Tucker, H. A. Neuroregulation of Growth hormone secretion in domestic animals. **Domestic Animal Endocrinology**, v. 20, p.65 – 87, 2001.

Meale, S.J.; Romao, J.M.; He, M.L.; Chaves, A.V.; Mcallister, T.A.; et al. Effect of diet on microRNA expression in ovine subcutaneous and visceral adipose tissues. **J Anim Sci**, v. 92, p. 3328–3337, 2014. doi: 10. 2527/jas.2014-7710

Medeiros, O. S. R.; Bonvino, S. N.; Oliveira, F. B.; Miguel, F. C. G.; Matos, C. T.; Gonçalves, N. S.; Cyrillo, J. Genome-Wide Association Study for Carcass Traits in an Experimental Nelore Cattle Population. **PLoS ONE**, v. 12(1), p. e0169860, 2017.

Miao, X.; Luo, Q.; Qin, X. Genome-wide transcriptome analysis of mRNAs and microRNAs in Dorset and Small Tail Han sheep to explore the regulation of fecundity. Mol. Cell. **Endocrinol**, v. 402, p. 32–42, 2015.

Miao, X.; Luo, Q.; Qin, X. Genome-wide analysis reveals the differential regulations of mRNAs and miRNAs in Dorset and Small Tail Han sheep muscles. **Gene**, v. 562, p. 188–196, 2015.

Miao, X.; Luo, Q.; Qin, X.; Guo, Y. Genome-wide analysis of microRNAs identifies the lipid metabolism pathway to be a defining factor in adipose tissue from different sheep. **Sci. Rep**, v. 5, p. 18470, 2015.

Millen, D-D.; Pacheco, R-D-L.; Meyer, P-M.; Rodrigues, P-H-M. et M. de Beni Arrigoni. Current outlook and future perspectives of beef production in Brazil. **Animal Frontiers**, v. 1, p. 46-52, 2011.

Miller, M. F.; Carr, M. A.; Ramsey, C. B.; Crockett, K. L.; Hoover, L. C. Consumer thresholds for establishing the value of beef tenderness. **Journal Animal Science**, v. 79, v. 3062–3068, 2001.

Miller, M. F.; Cross, H. R.; Lunt, D. K. Lipogenesis in acute and 48h cultures of bovine intramuscular and subcutaneous adipose tissue explants. **Journal of Animal Science**, v.69, n.2, p.162-170, 1991.

Miner, J. L. The adipocyte as an endocrine cell. **Journal of Animal Science**, v.82, p.935-941, 2004.

Minton, J. E.; Bindel, D. J.; Drouillard, J. S.; Titgemeyer, E. C.; Grieger, D. M.; Hill, C. M. Serum leptin is associated with carcass traits in finishing cattle. **Journal of Animal Science**, v. 76, p. 231-239, 1998.

Mourão, G. B.; Formigoni, I. B. Entendendo a seleção: seus conceitos e aplicações. In: TURCO, Cristiane de Paula (Org.) **Melhoramento genético ao alcance do produtor**. Bebedouro: Editora Scot Consultoria, p. 27-48, 2006.

Munekata, P. E. S.; Pateiro, M.; López-Pedrouso, M.; Gagaoua, M.; Lorenzo, J. M. Foodomics in meat quality. Current Opinion in Food Science, v. 38, p. 79-85, 2021. doi.org/10.1016/j.cofs.2020.10.003

Muroya, S.; Ogasawara, H.; Nohara, K.; Oe, M.; Ojima, K.; Hojito, M. Coordinated alteration of mRNA-microRNA transcriptomes associated with exosomes and fatty acid metabolism in adipose tissue and skeletal muscle in grazing cattle. **Asian-Australas Journal Animal Science**, v. 33 (11), p. 1824-1836, 2020. doi.org/10.5713/ajas.19.0682

Muroya, S.; Shibata, M.; Hayashi, M.; Oe, M.; Ojima, K. Differences in circulating microrna s between grazing and grain-fed wagyu cattle are associated with altered expression of intramuscular microrna, the potential target pten, and lipogenic genes. **PLoS ONE**, v. 11(9), p. e0162496, 2016. doi:10.1371/journal.pone.0162496

Muroya, S.; Ueda, S.; Komatsu, T.; Miyakawa, T.; Ertbjerg, P. MEATabolomics: Muscle and Meat Metabolomics in Domestic Animals. **Metabolites**, v. 10 (5), p. 188, 2020. doi.org/10.3390/metabo10050188

Nelson, D. L.; Cox, M. M. **Lehninger Principles of Biochemistry**. New York: W. H. Freeman, p. 1158, 2008

O'connor, S. F.; Tatum, J. D.; Wulf, D. M.; Green, R. D.; Smith, G. C. Genetic effects on beef tenderness in Bos indicus composite and Bos Taurus cattle. **Journal of Animal Science**, v.75, p. 1822-1830, 1997.

O'Quinn, T. G.; Brooks, J. C.; Polkinghorne, R. J.; Garmyn, A. J.; Johnson, B. J.; Starkey, J. D.; Rathmann, R. J.; Miller, M.F. Consumer assessment of beef strip loin steaks of varying fat levels. **Journal of Animal Science**, v. 90 (2), p. 626-634. 2012.

O'Quinn, T. G.; Legako, J.F.; Brooks, J. C.; Miller, M. F. Miller Evaluation of the contribution of tenderness, juiciness, and flavor to the overall consumer beef eating experience. **Trans. Anim. Sci**, v. 2, p. 26-36, 2018.

Okut, H.; Wu, X.; Rosa, G. J. M.; Bauck, S.; Woodward, B. W.; Schnabel, R. D.; Taylor, J. T.; Gianola, D. Predicting expected progeny difference for marbling score in Angus cattle using artificial neural networks and Bayesian regression models. **Genetics Selection Evolution**, v. 45, p. 34, 2013.

Oliveira, I. M.; Paulino, P. V. R., Marcondes, M. I; Valadares, Filho S. D. C.; Cavali, J; Prados, L. F; Duarte, M. S.; Detman, E. Beef quality traits of Nellore, F1 Simmental × Nellore and F1 Angus × Nellore steers fed at the maintenance level or ad libitum with two concentrate levels in the diet. **Revista Brasileira de Zootecnia**, v. 40, p. 2894-2902, 2011.

Olson, L.E.; and Soriano, P. Increased pdgfralpha activation disrupts connective tissue development and drives systemic fibrosis. **Developmental Cell**, v. 16, p. 303-313, 2009.

Oliveira, G. B.; Regitano, L. C. A.; Cesar, A. S. M.; Reecy, J. M.; Degaki, K. Y.; Poleti, M. D.; Felício, A. M.; Koltes, J. K.; Coutinho, L. L. Integrative analysis of microRNAs and mRNAs revealed regulation of composition and metabolism in Nelore cattle. **BMC Genomics**, v. 19, p. 126, 2018. doi.org/10.1186/s12864-018-4514-3

Ouali, A.; Herrera-Mendez, C. H.; Coulis, G.; Becila, S.; Boudjellal, A.; Aubry, L.; Sentandreu, M. A. Revisiting the conversion of muscle into meat and the underlying mechanisms. **Meat Science**, v. 74, p. 44–58, 2006.

Ouali, A.; Gagaoua, M.; Boudida, Y.; Becila, S.; Boudjellal, A.; Herrera-Mendez, C. H.; Sentandreu, M. A. Biomarkers of meat tenderness: present knowledge and perspectives in regards to our current understanding of the mechanisms involved. **Meat Science**, v. 95, p. 854-870, 2013.

Owens, F.N.; Dubeski, P.; Hanson, C.F. Factors that alter the growth and development of ruminants. **Journal of Animal Science**, v.71, p.3138-3150, 1993.

Owens, F.N.; Gill, D.R.; Secrist, D.S.; Coleman, S.W. Review of some aspects of growth and development of feedlot cattle. **Journal of Animal Science**, v.73, p.3152-3172, 1995.

Passos, G. A. (Ed.). (2014**). Transcriptomics in Health and Disease**. 344p. eBook ISBN 978-3-319-11985-4. doi:10.1007/978-3-319-11985-4

Parra, P.; Serra, F.; Palou, A. Expression of adipose microRNAs is sensitive to dietary conjugated linoleic acid treatment in mice. PLoS One, v. 5, p. e13005. 2010. doi: 10.1371/journal.pone.0013005

Parrish, F.C.; Young, R. B.; Miner, B. E.; Andersen, L. D. Effect of postmortem conditions on certain chemical, morphological and organoleptic propertiesof bovine muscle. **Journal of Food Science**, v. 38, p.690-695, 1973. doi.org/10.1111/j.1365-2621.1973.tb02846.x

Pereira, A. S. C.; Baldi, F.; Sainz, R. D.; Utembergue, B.L.; Chiaia, H.L.J.; Magnabosco, C.U.; Manicardi, F. R.; Araujo, F. R. C.; Guedes, C. F.; Margarido, R. C.; Leme, P. R.; Sobral, P. J. A. Growth performance, and carcass and meat quality traits in progeny of Poll Nellore, Angus and Brahman sires under tropical conditions. **Animal Production Science**, v. 55, p. 1295±02, 2015.

Pinto Neto, M.; Beraquet, N. J.; Cardoso, S. Effects of chilling methods and hot-boning on quality parameters of M. Longissimus lumborum from Bos indicus Nelore steer. **Meat Science**, v. p. 93, 201–206. 2013.

Poleti, M. D.; Regitano, L. C. A.; Souza, G. H. M. F.; Cesar, A. S. M.; Simas, R. C.; Silva-Vignato, B.; Oliveira, G. B.; Andrade, S. C. S.; Cameron, L. C.; Coutinho, L. L. Longissimus dorsi muscle label-free quantitative proteomic reveals biological mechanisms associated with intramuscular fat deposition, **J Proteomics**, v. 179, p. 30–41, doi.org/10.1016/j.jprot.2018.02.028, 2018a.

Poleti, M. D.; Regitano, L. C. A.; Souza, G. H. M. F.; Cesar, A. S. M.; Simas, R. C.; Silva-Vignato, B.; Oliveira, G. B.; Andrade, S. C. S.; Cameron, L. C.; Coutinho, L. L. Data from proteomic analysis of bovine Longissimus dorsi muscle associated with intramuscular fat content, **Data Brief**, v. 19, p. 1314–1317, doi.org/10.1016/j.dib.2018.06.004, 2018b.

Poleti, M. D.; Regitano, L. C. A.; Souza, G. H. M. F.; Cesar, A. S. M.; Simas, R. C.; Silva-Vignato, B.; Montenegro, H.; Pértille, F.; Balieiro, J. C. C.; Cameron, L. C.; et al. Proteome alterations associated with the oleic acid and cis-9, trans-11 conjugated

linoleic acid contente in bovine skeletal muscle. **J. Proteomics**, v. 222, p. 103792, 2020. doi.org/10.1016/j.jprot.2020.103792

Purslow, P. P. Intramuscular connective tissue and its role in meat quality. **Meat Science**, v. 70, p. 435–447, 2005.

Purslow, P. P.; Gagaoua, M.; Warner, R. D. Insights on meat quality from combining traditional studies and proteomics. **Meat Science**, v. 174, p. 108423, 2021. doi.org/10.1016/j.meatsci.2020.108423

Reggiori, M. R.; Torres Júnior, R. A. A.; Menezes, G. R. O.; Battistelli, J. V. F.; Silva, L. O. C.; Alencar, M. M.; Oliveira, J. C. K.; Faria, F. J. C. Precocidade sexual, eficiência reprodutiva e desempenho produtivo de matrizes jovens Nelore e cruzadas. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 68, n. 6, p. 1563-1572, 2016. doi.org/10.1590/1678-4162-8933

Reverter, A.; Johnston, D. J.; Ferguson, D. M; Perry, D.; Goddard, M. E.; Burrow, H. M.; Oddy, V. H.; Thompson, J. M.; Bindon, B. M. Geneticand phenotypic characterization of animal, carcass and meat quality traits for temperateand tropically adapted beef breeds. Correlations among animal, carcass and meat quality. **Australian Journal of Agricultural Research**, v. 54, p. 149-158,2003.

Rhoades. R. D.; Sawyer. J. E.; Chung. K. Y.; Schell. M. L.; Lunt. D. K.; Smith. S. B. Effect of dietary energy source on in vitro substrate utilization and insulin sensitivity of muscle and adipose tissues of Angus and Wagyu steers. **Journal of Animal Science**, v. 85. p. 1719–1726. 2007. doi.org/10.2527/jas.2006-498

Ribeiro, D. M.; Salama, A.; Vitor, A. C. M.; Arguello, A.; Moncau, C. T.; Santos, E. M.; Caja, G.; de Oliveira, J. S.; Balieiro, J. C. C.; Hernández-Castellano, L. E. et al.: The application of omics in ruminant production: a review in the tropical and sub-tropical animal production context. **J Proteomics**, v. 227, p. 103905, 2020.

Robelin, J. Growth of adipose tissues in cattle; partitioning between depots, chemical composition and cellularity. **Livestock Production Science**, v. 14, p. 349-364, 1986.

Robins, S. P.; Shimokomaki, M.; Bailey, A. J. The chemistry of the collagen cross-links. Age-related changes in the reducible components of intact bovine collagen fibres. **Biochemstry Journal**, v. 131(4), p. 771−780,1973.

Roderfeld, M.; Graf, J.; Giese, B.; Salguero-Palacios, R.; Tschuschner, A.; Müller-Newen, G. Latent MMP-9 is bound to TIMP-1 before secretion. **Biological Chemistry**, v. 388(11), p. 1227–1234, 2007.

Rodrigues, R. T. S.; Chizzotti, M. L.; Vital, C. E.; Baracat-Pereira, M. C.; Barros, E.; Busato, K. C.; Gomes, R. A.; Ladeira, M. M.; Martins, T. S.; Differences in Beef Quality between Angus (Bos taurus taurus) and Nellore (Bos taurus indicus) Cattle through a Proteomic and Phosphoproteomic Approach. **Plos One**, v. 12, p. e0170294, 2017.

Roh, S-G.; Hishikawa, D.; Hong, Y.-H.; Sasaki, S. Control of adipogenesis in ruminants. **Animal Science Journal**, v. 77, p. 472-477, 2006.

Romao, J. M.; Jin, W.; Dodson, M.V.; Hausman, G. J.; Moore, S. S. et al. MicroRNA regulation in mammalian adipogenesis. **Experimental Biology and Medicine**, v. 236, p. 997–1004. 2011.

Romao, J. M.; Jin, W.; He, M.; McAllister, T.; Guan, L. L. Altered microRNA expression in bovine subcutaneous and visceral adipose tissues from cattle under diferente diet. **PLoS One**, v. 7, p. e40605. 2012. doi: 10. 1371/journal.pone.0040605

Rosen, E. D. Molecular mechanisms of adipocyte differentiation. **Annd'endocrinol,** v. 63, p. 79–82, 2002.

Rosen, E. D; Spiegelman, B. M. Molecular regulation of adipogenesis. **Annual Review of Cell and Developmental Biology**, v. 16, p. 145-171, 2000. doi.org/10.1146/annurev.cellbio.16.1.145

Rosen, E. D; Walkey C. J.; Puigserver, P.; Spiegelman, B. M. Transcriptional regulation of adipogenesis. **Genes & Dev**, v. 14, p. 1293 –1307, 2000. doi: 10.1101/gad.14.11.1293

Rotta, P. P.; Prado, R. M.; PRado, I. N.; Valero, M. V.; Visentainer, J. V.; Silva, R. R. The effects of genetic groups, nutrition, finishing systems and gender of Brazilian cattle on carcass characteristics and beef composition and appearance: a review. **Asian-Australas. Journal of Animal Science**, v. 22, p. 1718-1734, 2009.

Sapp, R. L.; Bertrand, J. K,; Pringle. T. D. E.; Wilson. D. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. **Journal Animal Science**, v. 80, 2017–2022, 2002.

Scollan, N.; Hocquette, J. F.; Nuernberg, K.; Dannenberger, D.; Richardson, I.; Moloney, A. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. **Meat Science**, v. 74 (1), p. 17–33, 2006. doi.org/10.1016/j.meatsci.2006.05.002.

Scollan, N. D.; Dannenberger, D.; Nuernberg, K.; Richardson, I.; MacKintosh, S.; Hocquette, J.-F.; Moloney, A. P. Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. **Meat Sci**, v. 97, p. 384-394, 2014.

Shen, Y. N.; Kim, S. H.; Yoon, D. H.; Lee, H. G.; Kang, H. S.; Seo, K. S. Proteome analysis of bovine Longissimus dorsi muscle associated with the marbling score. Asian-Australasian **Journal of Animal Sciences**, v. 25 (8), p. 1083–1088. 2012 doi.org/10.5713/ajas.2012.12127.

Shiotsuki, L.; Silva, J. A. V.; II, H.; Tonhati, L. G. Albuquerque, Genetic associations of sexual precocity with growth traits and visual scores of conformation, finishing, and muscling in Nelore cattle, **Journal of Animal Science**, v. 87 (5), p. 1591–1597, 2009. doi.org/10.2527/jas.2008-1173

Siqueira, J. B.; Oba, E.; Pinho, R. O.; Guimarães, S. E. F.; Neto, T. M.; Guimarães, J. D. Testicular shape and andrological aspects of young Nellore bulls under extensive farming. **Revista Brasileira Zootecnia**, Viçosa, MG, v. 41 (3), p. 612- 617, 2012.

Silva-Vignato, B.; Coutinho, L.L; Poleti, M. D.; Cesar, A. S. M.; Moncau, C.; Regitano, L. C. A.; Balieiro, J. C. C. Gene co-expression networks associated with carcass traits reveal new pathways for muscle and fat deposition in Nelore cattle. **BMC Genomics,** v. 20, p. 32, 2019. doi.org/10.1186/s12864-018-5345-y.

Smith, S. B.; Kawachi, H.; CHoi, C. B.; Choi, C. W.; Wu, G.; Sawyer, J. E. Cellular regulation of bovine intramuscular adipose tissue development and composition. **Journal of Animal Science**, v. 87, p. E72-82, 2009.

Smith, T.; Domingue, J. D.; Paschal. J. C.; Franke, D. E. Genetic parameters for growth and carcass traits of Brahman steers. **Journal of Animal Science**, v.85, p.1377-1384, 2007.

Son, Y. H.; Ka, S.; Kim, A. Y.; Kim, J. B. Regulation of Adipocyte Differentiation via MicroRNAs. **Endocrinol. Metab**, v. 29, p. 122–135. 2014.

Stecco C.; Adstrum S.; Hedley G.; Schleip R.; Yucesoy C. A. Update on fascial nomenclature. J**. Bodyw. Mov**. Ther, v. 22, p. 354. 2018. 10.1016/j.jbmt.2017.12.015

Teixeira, P. D.; oliveira, D. M.; Chizzotti, M. L.; Chalfun-Junior A.; Coelho, T. C.; Gionbelli, M.; Paiva, L. V.; Carvalho, J. R. R.; Ladeira, M. M. Subspecies and diet affect the expression of genes involved in lipidmetabolism and chemical composition of muscle in beef cattle. **Meat Science**, v. 133, p. 110-118, 2017.

The UniProt Consortium, UniProt: the universal protein knowledgebase in 2021, **Nucleic Acids Research**, v. 49 (D1), p. D480–D489, 2021. doi.org/10.1093/nar/gkaa1100

Troy, D. J and Kerry, J. P. Consumer perception and the role of science in the meat industry. **Meat Sci,** v. 86 (1), p. 214-226, 2010. doi.org/10.1016/j.meatsci.2010.05.009

Ueda, S.; Iwamoto, E.; Kato, Y.; Shinohara, M.; Shirai, Y.; Yamanoue, M. Comparative metabolomics of Japanese Black cattle beef and other meats using gas chromatography–mass spectrometry. **Bioscience, Biotechnology, and Biochemistry**, v. 83, p. 137–147, 2019. doi.org/10.1080/09168451.2018.1528139

Uezumi, A.; Ito, T.; Morikawa, D.; Shimizu, N.; Yoneda, T.; Segawa, M.; Yamaguchi, M.; Ogawa, R.; Matev, M.; Miyagoe-Suzuki, Y.; Takeda, S.; Tsujikawa, K.; Tsuchida, K.; Yamamoto, H.; Fukada, S. Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle. **Journal of Cell Science**, v. 124, p. 3654 - 3664, 2011.

United States Department of Agriculture. Livestock and poultry: world markets and trade [Internet]. USDA; [updated 2021 Jan;]. http://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf

Vance, D.E.; Vance, J.E. **Biochemistry of Lipids, Lipoproteins and Membranes**, 4th ed.; Elsevier Science: Amsterdam, The Netherland, 2002; pp. 1–607.

Wang, Y.; Viscarra, J.; Kim, S-J.; Su, H. S. Transcriptional Regulation of Hepatic Lipogenesis. **Nat Rev Mol Cell Biol**, v. 16(11), p; 678–689, 2015. doi:10.1038/nrm4074.

Wei, S.; Duarte, M. S.; Zan, L.; Du, M.; Jiang, Z.; Guan, L.; Hausman, G. J.; Dodson, M. V. Cellular and Molecular Implications of Mature Adipocyte Dedifferentiation. **Journal of Genomics**, v. 1, p. 5-12, 2013a.

Wei, S.; Du, M.; Jiang, Z.; Duarte, M. S.; Culver, M. F.; Albrecht, E; Will, K.; Zan, L.; Hausman, G. J.; Elabd, E.; Bergen, W. G.; Basu, U.; Dodson, M.V. Bovine dedifferentiated adipose tissue [DFAT] cells: DFAT cell isolation. **Adipocyte**, v. 2, p. 1-12, 2013b.

Whipple, G.; Koohmaraie, M.; Dikeman, M. E. Evaluation of attributes that affect longissimus muscle tenderness in Bos taurus and Bos Indicus cattle. **Journal of Animal Science**, v. 68, n. 9, p.2716-2728, 1990.

Wheeler, T. L; Savell, J. W.; Cross, H. R; Lunt, D. K; Smith, S. B. Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. **Journal of Animal Science**, v. 68, p. 4206-4220, 1990.

Williams, J. L. Genetic control of meat quality traits. In F. Toldra (Ed.). **Meat biotechnology** (pp. 21–61). New York: Springer Science & Business Media. 2008. doi.org/10.1007/978-0-387-79382-5_2.

Wolcott, M. L.; Johnston, D. J.; Barwick, S. A.; Iker, C. L.; Thompson, J. M; Burrow, H. M. Genetics of meat quality and carcass traits and the impact of tenderstretching in two tropical beef genotypes. **Animal Production Science**, v. 49 (6), p. 383–398, 2009. doi.org/10.1071/EA08275.

Wood, J. D.; Fisherg, A.V.; Nute, G. R.; Sheard, P.R.; Richardsons, R. I.; Hughes, S. I.; Whittingtonet, F. M. Fat deposition, fatty acid composition and meat quality: a review. **Meat Science**, v. 78, p. 343-358, 2008.

Wright, S. A.; Ramos, P.; Johnson, D. D.; Scheffler, J. M.; Elzo, M. A.; Mateescu, R. G.; Bass, A. L.; Carr, C. C.; Scheffler, T. L. 2018. Brahman genetics influence muscle fiber properties, protein degradation, and tenderness in an Angus-Brahman multibreed herd. **Meat Science**, v. 135, p. 84–93, 2018.

Wu, B.; Wei, F.; Xu, S.; Xie, Y.; Lv, X.; Chen, H.; Huang, F. Mass spectrometry-based lipidomics as a powerful platform in foodomics research, **Trends in Food Science &Technology**, 2020. doi.org/10.1016/j.tifs.2020.10.045.

Yalcinkaya, E.; Celik, M.; Bugan, B. Extracellular Matrix Turnover: a Balance between MMPs and their Inhibitors. **Arquivo Brasileiro Cardiologia**, v. 102 (5), p. 519–520, 2014.

Yamada, T.; Kawakami, S. I.; N. Nakanishi. Expression of adipogenic transcription factors in adipose tissue of fattening wagyu and holstein steers. **Meat Science**, v. 81, p. 86-92, 2009.

Yu, X.; Fang, X.; Gao, M.; Mi, J.; Zhang, X.; Xia, L.; Zhao, Z.; Albrecht, E.; Maak, Ste_en.; Yang, R. Isolation and Identification of Bovine Preadipocytes and Screening of MicroRNAs Associated with Adipogenesis. **Animals**, v. 10, p. 818, 2020. doi:10.3390/ani10050818

Zhang, Q.; Lee, H. G.; Han, J. A.; Kim, E. B.; Kang, S. K.; Yin, J.; Choi, Y. J. Differentially expressed proteins during fat accumulation in bovine skeletal muscle. **Meat Science**, v. 86 (3), p. 814–820, 2010. doi.org/10.1016/j.meatsci.2010.07.002

Zhang, Y.; Wang, Y.; Wang, H., Ma, X.; Zan, L. MicroRNA-224 impairs adipogenic differentiation of bovine preadipocytes by targeting LPL. **Molecular and Cellular Probes**, v. 44, p. 29–36. 2019. doi.org/10.1016/j.mcp.2019.01.005

Zhao, L.; Huang, Y.; Du, M. Farm animals for studying muscle development and metabolism: dual purposes for animal production and human health. **Animal Frontiers**, v. 9, (3), p. 21–27, 2019. doi.org/10.1093/af/vfz015

CAPÍTULO 3

Nelore progenies from contrasting groups for growth and precocity affect physiological parameters and steak molecular profile
MARTINS et al. 2021

MARTINS, T. S. Nelore progenies from contrasting groups for growth and precocity affect physiological parameters and steak molecular profile. 2021

ABSTRACT

The present study test the hypothesis that genetic selection for precocity and growth can affect physiological parameters and consequently muscle proteomic, lipidomic and transcripts profile of the Zebu cattle. In order to answer this hypothesis we aimed to evaluate the effects of contrasting EPD for precocity and growth on *longissimus thoracis* (LT) muscle by performing proteomics, mRNA analysis, and correlations with phenotypic traits, such as collagen, intramuscular fat, hormones and metabolites. A total of 105 male bulls were used, with a mean age of 20 ± 2 months and 400 ± 24 kg, from the same herd, with the genetic information of precocity and growth. Animals remained confined for 100 days, and the carcass ultrasound was performed every 28 days. Blood samples were collected to determine the metabolic and hormonal profile. The animals were slaughtered after feedlot 100 days and during slaughter, the LT samples were collected between the 12th and 13th ribs and immediately frozen in liquid nitrogen and kept in a freezer - 80ºC until the analysis of gene expression by real-time PCR (RT-qPCR) and analysis and SDS-PAGE, followed by the identification of proteins by coupled mass spectrometry liquid chromatography (LC-MS / MS). After 24 hours of slaughter, during boning, the marbling score (MAR) in the LT muscle was evaluated. Steaks for total lipids, fatty acid profile, and collagen solubility analyses were also collected. The animals were selected according to EPD from their parent bulls. Were selected six parents with EPD contrasting simultaneously for precocity and growth, so that each experimental group had three parents bulls. Then, were formed, two contrasting groups called high EPD (H_EPD; N = 16) and low EPD (L_EPD; N = 16), using 32 progenies. The animals in the H_EPD group had higher backfat thickness (BFT, P = 0.006); lower LDL (P = 0.014); higher IGF-1 (P = 0.064); greater collagen solubility (P = 0.098); lower expression of the LPL gene, in LT (P = 0.045). One electrophoretic band was detected as differently abundant in the LT muscle (band 16). The KEGG pathways of pyruvate metabolism, glycolysis/gluconeogenesis, carbon metabolism, amino acid biosynthesis, among others, were enriched for the differentially abundant proteins within the protein band identified in the LT muscle. The genetic selection for precocity and growth affects the muscle proteome and consequently the lipid and protein metabolism of non-castrated cattle. The IGF-1 hormone, the LPL gene, and the PKLR, PKM, ALDOA, DLD, GPI, VIM, ACTC1, OXCT1, CASQ1, and PCYOX1 proteins can be considered future biomarkers candidates for BFT and may be involved with the muscle protein turnover.

Keywords: Beef Cattle. Backfat thickness. Metabolic pathways. Protein and lipid metabolism. Proteomics.

1 INTRODUCTION

Beef production has a large impact on the global economy (KEADY et al., 2013). Brazil is one of the world's largest beef exporters, with approximately 238 million heads, and occupies the second position in the world ranking of beef production (USDA, 2020). Zebu cattle are extensively used in the tropics, due to their thermo tolerance and resistance to parasites, although *Bos taurus* breeds are more precocious. Most of the Brazilian herd is composed of Zebu cattle (*Bos taurus indicus*), mainly of the Nelore breed (FERRAZ & FELÍCIO, 2010; SARTORI et al., 2010). However, there is wide heterogeneity in carcasses and beef quality, due to variation in deposition of subcutaneous fat, marbling, collagen solubility, and meat tenderness due the total herd of Nelore cattle belongs to different genetic groups.

Thereby, there is an increasing number of studies to understand the phenotypic and genotypic differences between Nelore cattle related to the contrasting characteristics for animal production and beef quality (SILVA et al., 2021; CÔNSOLO et al., 2021; CÔNSOLO et al., 2020; COSTA et al., 2019; SANTANA et al., 2019; BONIN et al., 2014). These studies can provide scientific support to the beef industry by developing strategies to improve the quality of their products.

Growth characteristics and precocity are generally useful selection criteria for beef cattle. Improvements in average daily gains, body weight, muscle mass, and fat deposition in the carcass have been analyzed using genetic merit for growth and precocity as selection criteria (CLARKE et al., 2009). Therefore, the Expected Differences in Progeny (EPD's) are valuable tools for animal production and selection. However, to achieve consistent genetic gains, genetic and environmental factors inherent to the traits intended must be considered, such as heritability and variability, in addition to their genetic correlations with other of interest phenotypic traits (MOURÃO & FORMIGONI, 2006).

Proteomics study comparing muscle from cattle breeds with different beef quality merits were able to identify differentially abundant proteins related to beef sensory attributes (CHAZE et al., 2013; RODRIGUES et al., 2017; POLETI et al., 2020). However, there is a lack of researches about muscle proteomics and the physiological and molecular differences between different genetic groups within the breed of Nelore.

Therefore, the present study hypothesizes that genetic selection for precocity and growth affects the muscle proteome and consequently lipid and protein metabolism of the Zebu cattle. In light of the above, aimed to the present study to evaluate the effects of contrasting EPD's for precocity and growth on *longissimus thoracis* (LT) by differential proteomic and molecular analysis and correlations with phenotypic traits, such as colagen and intramuscular fat, hormones e metabolites.

2 MATERIAL AND METHODS

The experimental procedures were approved by the Ethics Committee on Animal Use (CEUA/FMVZ) from the School of Veterinary Medicine and Animal Science of the University of Sao Paulo (Protocol number 3367170317), Pirassununga, Sao Paulo, Brazil.

2.1 Animals and Experimental design

The animals used in this study were selects from a larger project evaluating the effects of EPD groups on animal performance, carcass, and meat quality traits (SILVA et al., 2021). The animals belonged to USP's genetic selection program, with genetic information for precocity and growth. One hundred and five Nelore bulls, not castrated, with initial body weight (iBW) of 400 ± 24 kg and 20 ± 2 months of age were ranked based on their parent's Expected Progeny Difference (EPD), for precocity and growth and classified as either High and Low EPD. Three bulls Nelore with high EPD were parents of animals in the H_EPD group (average EPD for precocity and growth = 0.57 and 12.32 respectively), and three bulls Nelore with low EPD were parents of animals in the L_EPD group (average EPD for precocity and growth = - 0.05 and - 3.67 respectively). The phenotypic traits were evaluated in 32 progeny (High_EPD, N=16; Low_EPD, N=16). Posteriorly, 14 animals were selected with either highest (H_EPD, N = 7) or lowest (L_EPD, N = 7) and contrasting subcutaneous fat deposition to form to the groups that were tested for differential proteomic analysis.

All the animals were housed in a feedlot facility for 100 days, and individual feed intake was monitored. Facilities had covered feed bunks, concrete floors, and automatic waterers. All animals were initially submitted to adaptation period of

21 days. At the ending of the adaptation period all animals were weighed after a 12 h fasting period prior to the morning feeding. During the feedlot period, the animals were fed ad libitum twice daily, at 08:00h and 16:00h with a diet (27:73 forage: concentrate) containing corn silage, corn grain, ground sorghum, soybean meal, citrus pulp, urea, and mineral core. All animals were weighed after a 12 h fasting to record the final body weight immediately prior to slaughter. The average daily gain was obtained by the difference of final body weight and initial body weigh divided by the 100 days of feeding.

2.2 Carcass ultrasound

Carcass ultrasonography was performed at the begin and at the end of the feedlot to assess rib eye area (REA) and backfat thickness (BFT) in the LT muscle, in the region between the 12th and 13th ribs, and rump fat thickness (RFT), using the Aloka ultrasound equipment. SSD 500 Micrus model (Aloka Co. Ltda.), with 3.5 MHz linear transducers and 17.2 cm in length. The images were stored in the portable microcomputer and records were subsequently interpreted in laboratory using the Lince® software.

2.3 Blood samples collection and processing for biochemical and metabolic parameters

Blood samples were harvested before slaughter, at the end of the finishing period by puncturing the jugular vein or artery before the morning feeding, with fasting the previous night. Blood samples were collected into 10 ml tubes (BD Vacutainer, São Paulo, SP, Brazil), without anticoagulant, for hormones analyses and metabolic parameters. Samples were centrifuged for 15 min at 2000×g at 4 °C; the supernatant serum was transferred into labeled plastic tubes and stored at −80°C until analysis.

Serum concentrations of glucose, triglycerides, total cholesterol, and high-density lipoprotein (HDL) were determined by Immunoenzymatic procedure using Bioclin Diagnostics kits (Belo Horizonte, Brazil) in automated biochemical equipment (Mindray BS-200E), according to manufacturer's instructions. Serum values of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were calculated according to Friedewald et al. (1972), being, VLDL = triglycerides/5; and LDL = total

cholesterol – (HDL + VLDL). Insulin, leptin, GH (growth hormone), and IGF-1 (insulin-like growth factor-1) hormones were determined by the Immunoenzymatic Assay method, according to BioElisa Microplate Reader New Biotechnology and Sigma kits (Bioclin Quibasa, Belo Horizonte, Brazil), following manufacturer's standards.

2.4 Slaughter procedure and longissimus thoracis sampling

At the end of the feeding phase, the animals were slaughtered in the experimental slaughterhouse of the University of São Paulo in Pirassununga, Brazil, according to humanitarian slaughter guidelines, as required by Brazilian law (Brasil 2017). LT samples were harvest from the right half of the carcass at the 12th rib, snap-frozen in liquid nitrogen, and stored at - 80°C until proteins extraction and gene expression analysis. After a 24h chilling period, 2.54cm thick steaks were cut from LT, of the right half of the carcass, between 12th-13th ribs, vacuum packaged, and frozen at −80°C until total lipids analysis and solubility of intramuscular collagen.
Besides that, the marbling score (MAR) was evaluated in the LT, between the 12th and 13th rib, following the methodology described by American Meat Science Association (SMITH et al., 2013).

2.5 Total lipids

The intramuscular fat (IMF) content was determined following the methodology of Bligh and Dyer (1959) in duplicate, for total lipids.

2.6 Total collagen content and heat solubility

Collagen and its heat soluble fraction were measured from the hydroxyproline (OH-Prol) determination (WOESSNER, 1961). LT samples (5.0 g, duplicate) were scissor-cut into small pieces and placed into 50 ml centrifuge tubes with 12 ml saline solution. The centrifuge tubes were placed in water bath at 80 °C for 60 min and stirred regularly. The heat soluble and insoluble collagen fractions were separated through centrifugation at 6000g for 10 min. Each fraction was hydrolyzed at 105 °C for 16 h in 6 N HCl. Then, the samples were filtered (WH1001-150 -WHATMAN). Subsequently, the content obtained was transferred to test tubes,

in duplicate, to perform the color reaction. The hydroxyproline concentrations were measured, by absorbance of 558 nm, by the spectrophotometer (model V-M5 BEL). The soluble and insoluble fractions were multiplied by 7.52 and 7.25, respectively, to calculate the solubility collagen and content total of collagen in each sample (CROSS et al., 1973).

2.7 Total RNA Extraction and cDNA Preparation

Longissimus thoracis samples were macerated and homogenized in liquid nitrogen for total RNA extraction. Total RNA was extracted using Trizol® reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA). Total RNA concentration was quantified using the NanoDrop spectrophotometer (Thermo Scientific, Washington, DE). RNA samples were treated with DNase I (Invitrogen Life Technologies, Carlsbad, CA) before reverse transcription reaction. cDNA was produced using 30 ng of total RNA per analyzed gene using the High Capacity kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. The mRNA levels were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) using gene-specific primers (Table 2). Primer's efficiency was obtained using different cDNA concentrations (5, 15, 30, 60 ng/μL).

2.8 Gene Expression Analyzes

Transcripts abundance were analyzed by QuantStudio 6 Real-Time PCR (Applied Biosystems) using SYBR Green RT-PCR Master Mix (GoTaq® Promega), following the cycle parameters: 95 ° C for 2 min, 45 cycles at 95 ° C for 15 s, and 60 ° C for 1 min. In each reaction were used 5μL of SYBR Green RT-PCR Master Mix, 1.5 μL of each forward and reverse primer at 0.5μM, 1μL of cDNA (30ng of RNA), and MilliQ (ultrapure) water to complete the volume of 10 μL. After amplification, a melting curve (0.01 C / s) was used to confirm the purity of the product. The "threshold cycle" (Ct) values were normalized (ΔCt) based on the geometric mean Cts of the values obtained for GAPDH and ACTB.

Table 1. Sequence of studied primers

Abbreviation	Gene		Sequence 5' - 3'	NCBI	biological process[1]	References
PPARG	Peroxisome Proliferator activated Receptor Gamma	Forward	GTGGAGCCTGTATCCCCACC	NM_181024.2	Caspase activation	Mueller et al., 2021 (not yet published)
		Reverse	TTTATCCCCACAGACCCGGC			
ZNF423	Zinc finger protein 423	Forward	GGATTCCTCCGTGACAGCA	NM_001101893.1	Regulation of adipogenesis	Martins et al., 2015
		Reverse	TCGTCCTCATTCCTCTCCTCT			
TGFB1	transforming growth	Forward	AGCCAGGGGGATGTGCCA	NM_001166068.1	SMAD protein complex	Duarte et al., 2013
	factor beta 1	Reverse	TAGCACGCGGGTGACCTCCT		assembly	
ACACA	Acetyl-CoA carboxylase 1	Forward	TGAAGAAGCAATGGATGAACC	NM_174224.2	Fatty acid biosynthesis	Martins et al., 2015
		Reverse	TTCAGACACGGAGCCAATAA			
LPL	Lipoprotein lipase 1	Forward	CTCAGGACTCCCGAAGACAC	NM_001075120.1	Lipoprotein metabolism	Martins et al., 2015
		Reverse	GTTTTGCTGCTGTGGTTGAA			
ACOX1	Acyl-CoA oxidase 1	Forward	GCTGTCCTAAGGCGTTTGTG	NM_001035289.3	Lipid metabolism	Martins et al., 2015
		Reverse	ATGATGCTCCCCTGAAGAAA			
LEP	Leptin 2	Forward	GGGCACGTCAGCATCTATTA	NM_173928.2	Positive regulation of	Mota et al., 2017
		Reverse	CCTGTCTGCTGTTATGGTCTTA		fatty acid biosynthetic	
FABP4	fatty acid binding	Forward	GGATGATAAGATGGTGCTGGA	NM_174314.2	Fatty acid metabolism	Martins et al., 2015
	protein 4, adipocyte	Reverse	ATCCCTTGGCTTATGCTCTCT			
SCD	Estearoil-CoA	Forward	TTATTCCGTTATGCCCTTGG	NM_173959.4	Unsaturated fatty acid	Teixeira et al., 2017
	dessaturase 3	Reverse	TTGTCATAAGGGCGGTATCC		biosynthetic process	
MSTN	Myostatin	Forward	TTGTGCACCAAGCAAACCCC	NM_001001525.3	Myoblast migration involved	Ferrinho et al., 2021 (not yet published)
		Reverse	CCATGGCTGGAATCTTCCCGTA		in skeletal muscle regeneration	

CAST	Calpastin	Forward	GTCGGATCCAATGAGTTCTACC	NM_174003.2	Calpain inhibitor	Martins et al., 2017
		Reverse	CCTGCGATCCCTTCTTCTTTAT			
CAPN1	μ Calpain 2	Forward	CGCCTCCCTTACCCTCAA	NM_174259.2	Cytoskeletal remodeling	Ferrinho et al., 2021 (not yet published)
		Reverse	CATCCACCCACTCACCAAAC			
CAPN2	m Calpain 3	Forward	CGACTGGAGACACTGTTCAGGA	NM_001103086.1	Cytoskeletal remodeling	Riggs et al., 2015
		Reverse	CTTCAGGCAGATTGGTTATCACTT			
ACTB	actin beta	Forward	CAGCAGATGTGGATCAGCAAGC	NM_173979.3	Normalizing gene	Sangalli et al., 2014
		Reverse	AACGCAGCTAACAGTCCGCC			
GAPDH	Glyceraldehyde-	Forward	AGATAGCCGTAACTTCTGTGC	NM_001034034.2	Normalizing gene	Martins et al., 2015
	3-phosphate5	Reverse	ACGATGTCCACTTTGCCAG			

[1]Biological processes described in NCBI

2.9 Protein Extraction

Approximately 0.5 g of muscle samples were homogenized in 2 mL of RIPA lysis buffer modified containing 150 mm NaCl, 1% Triton X-100, 0,5% sodium deoxycholate, 1% SDS, 50 mM Tris, pH 8.0-plus 1% protease inhibitor cocktail (P8340 Sigma-Aldrich), 1 mM sodium orthovanadate, and 25 mM sodium fluoride, using Ultra Turratec T-10 (IKA) four times for 5 seconds at 30,000 rpm. Afterward, the homogenate was centrifuged at 4 ºC at 10,000 g for 30 minutes. The resulting supernatant was collected and stored at -80 ºC for further analysis. The protein concentration of each sample was measured by the Bradford method, using bovine albumin as standard.

2.10 Unidimensional Electrophoresis - SDS / PAGE

The protein extract was denatured by mixing in a 1: 1 ratio in Laemmli sample buffer (Bio-Rad Laboratories Inc., Hercules, CA, USA) and heated at 100 °C for 45 minutes. The samples (50 µg of protein) were loaded into each lane of the 1.5 mm x 16 cm dual slab gel (SE 600 Ruby Standard Dual Cooled Vertical Unit – GE Healthcare) connected to a Power Supply EPS 1001 (GE Healthcare) and run at 15mA/gel for 30 min. and after adjusted at 50 mA/gel for 4 h at 4ºC, for one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were run through 4% stacking gel, followed by 12% resolving gel. Bio-rad precision plus protein dual color standards (Bio-Rad Laboratories Inc., Hercules, CA, USA) were loaded in the first lane of each gel, to estimate the molecular weight of proteins. After electrophoretic separation, gels were stained by using Coomassie Brilliant Blue G250 solution (0.025% coomassie blue G250, 50% methanol, 7% acetic acid) for 1 hour, on shaker. The gels were then placed in destained solution (40% methanol, 7% acetic acid) for 1 hour and rinsed in deionized water. The gels were digitalized using Image Scanner III (GE Healthcare) and the quantitative analysis of the gel bands was performed with the aid of the Image LabTM software (Bio-Rad). The bands were identified and then analyzed to determine the percentage contribution of each band in relation to the total band volume in the lane to avoid the confounding factor of protein loading (Zapata et al., 2009; Zhao et al., 2014).

2.11 In gel protein digestion

The bands detected as differentially abundant in the statistics analysis were removed from the gel, cut in fragments of approximately 1 mm^3 and placed inside 1.5 ml microtubes. Digestion was performed according to the method of Shevchenko et al. (2006). The gel fragments were washed 3 times of 10 minutes each, using 200 µl of a solution containing 50% acetonitrile (ACN) in 25 mM ammonium bicarbonate pH 8.0 (AMBIC). Then, the gel fragments were dehydrated for 10 minutes after adding 200 µl of 100% ACN. The ACN was removed and the remaining gel residue was kept at room temperature for complete evaporation. The gel fragments were reduced for 40 minutes at 56 ° C with 30 µl of a 20 mM DTT solution in 50 mM AMBIC, after alkylated for 30 min. at room temperature using 40 µl of a 55 mM iodoacetamide solution in 50 mM of AMBIC. The iodoacetamide solution was removed and the gel fragments washed with 200 µl of 25 mM AMBIC. Again, dehydration with 100% ACN was performed twice, as described above. For protein digestion, 15 µL of the trypsin enzyme was added to each sample, at a concentration of 20 ng/µl and incubated for 15 minutes at 4°C. Excess trypsin was removed and 40 µl of 50 mM AMBIC was added. The samples were incubated at 37 ° C for 14 hours after digesting the protein present in the gel, three elution steps were performed. For the first elution, 30 µl of the 5% formic acid was added, and the samples incubated for 10 min. at room temperature. The supernatant was collected and placed in a new tube. For the second elution, 30 µl of the solution made of 5% formic acid in 50% ACN was added, and the samples incubated for 10 minutes at room temperature. The supernatant was recovered and transferred to the same tube used in the previous wash. For the third elution, the second step was repeated. Finally, samples were vacuum concentrated at room temperature, until complete drying.

2.12 Mass spectrometry analysis

For analysis, the samples were resuspended in 30 µl of a 50% Acetonitrile and 0.1% TFA solution, and. an aliquot of 4.5 ul of proteins resulting of peptide digestion was were separated by C18 (100 mm6100 mm) RP-nanoUPLC (nanoAcquity, Waters) coupled with a Q-Tof Premier mass spectrometer (Waters) with nanoelectrospray source at a flow rate of 0.6 ml/min. The gradient

was 2–90% acetonitrile in 0.1% formic acid over 60 min. The nanoelectrospray voltage was set to 3.5 kV, a cone voltage of 30 V and the source temperature was 100uC. The instrument was operated in the ‘top three’ mode, in which one MS spectrum is acquired followed by MS/MS of the top three most-intense peaks detected. After MS/MS fragmentation, the ion was placed on exclusion list for 60 s and for the analysis of endogenous cleavage peptides, a real time exclusion was used.

The spectra were acquired using software MassLynx v.4.1 and the raw data files were converted to a peak list format (mgf) without summing the scans by the software Mascot Distiller v.2.3.2.0, 2009 (Matrix Science Ldt.) and searched against UniProt *Bos Taurus* (release March 23, 2020; 37.513 proteins) and *Bos indicus* database (release March 21, 2020; 42,151 proteins) using Mascot engine v.2.3.01 (Matrix Science Ltd.), with carbamidomethylation as fixed modifications, oxidation of methionine as variable modification, one trypsin missed cleavage and a tolerance of 0.1 Da for both precursor and fragment ions (ARAGAO et al., 2012).

2.13 Data Statistical analysis

Phenotypic data

It was considered a completely randomized design, with 16 repetitions per treatment, each animal was considered an experimental unit. The model includes the parent (bull) as a random effect and considers the groups (H_EPD and L_EPD), the feedlot structure, and slaughter as a fixed effect. Were conducted using SAS software (9.4; SAS Institute Inc., Cary, NC, USA). The significance was considered when $P < 0.1$.

Gene Expression

It was considered a completely randomized design, with 8 repetitions per treatment. The “threshold cycle” (Ct) values were normalized (ΔCt) based on the geometric mean Cts of the values obtained for normalizing genes. The relative expression of mRNAs were performed using Student t-test. All data followed the criteria of normality. The analyses were performed using the JMP software from SAS. The significance was considered when $P < 0.1$.

SDS-PAGE and Correlation between the significant electrophoretic bands

The data were analyzed according to a completely randomized design, with 7 repetitions per treatment, each animal being considered an experimental unit. The model includes the parent (bull) as a random effect and considers the groups (H_EPD and L_EPD), feedlot structure, and slaughter as a fixed effect. For one-dimensional gel analysis, ANOVAs were performed using the SAS software for relative migration (RFs) and molecular weight (MWs). This initial analysis aimed to verify if we were making comparisons of proteins within the same point of the Gel (both for RF and MW). In this case, ANOVAs for RFs and MWs should have always presented non-significant results, indicating that there were no significant changes within each evaluated band. From this preliminary analysis for RFs and MWs, ANOVAs were performed using the SAS software for % Band to detect the differences between the abundance of each band between the H_EPD and L_EPD groups using the test Tukey-Kramer considered significant at *P*-value < 0.1. The correlations between the electrophoretic bands and the phenotypes were estimated by Pearson's correlation coefficient (r), within and between groups, and the data were considered significant when $P < 0.1$.

Protein identification and functional enrichment

The identified proteins were considered candidates when the global Mascot score was greater than 57 with a significance level of $P \leq 0.0001$. Subsequently, the list of proteins identified in each band was submitted to over-representation analysis using WebGestalt web tool (LIAO et al., 2019) to identify the biological processes and KEGG pathways enriched (FDR < 0.05), and also analysis of Protein-Protein Interaction Networks were performed by STRING software.

3. RESULTS

3.1 Phenotypic traits

The average daily gain (ADG) did not differ between the groups (P = 0.459; Table 2). Similarly, the dry matter intake (DMI) did not differ between the groups (P = 0.288; Table 2), such as the final body weight (fBW, P = 0.254; Table 2). On the other hand, its backfat thickness (BFT) and rump fat thickness (RFT), measured by carcass ultrasonography, was higher in H_EPD group animals than L_EPD group

animals (P < 0.1; Table 2), indicating higher precocity of animals from the H_EPD group. Besides this, the marbling score (MAR) was higher in H_EPD group animals compared to L_EPD group animals (P < 0.1; Table 2), despite this, the content of intramuscular fat (IMF) did not differ between animals in the evaluated groups (P = 0.149). The H_EPD group animals showed greater collagen solubility than the L_EPD group animals (P < 0.1; Table 2).

Table 2. Effect of the groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity on the phenotypic traits of Nelore bulls (n=16)

Traits	**GROUP**		***P-value****
	H_EPD	**L_EPD**	
Initial body weight, kg	413.60±9.86	405.37±5.06	0.517
Final body weight, Kg	598.00±11.72	581.62±8.04	0.254
Average daily gain, kg	1.85±0.13	1.76±0.14	0.459
Dry matter intake, kg	12.54±0.77	11.01±0.84	0.288
Rib eye área[1], cm^2	76.62±3.31	77.03±3.58	0.9477
Back fat thickness[1], mm	5.87±0.50	3.78±0.48	0.006
Rump fat thickness[1], mm	8.56±0.58	7.05±0.69	0.086
Intramuscular fat[2]	2.23±0.34	1.34±0.31	0.149
Marbling score [3]	4.57±0.17	4.01±0.15	0.081
Collagen content total (mg/g of beef)	2.88±0.09	2,67±0.09	0.131
Collagen solubility (%)	13.01±0.52	11,76±0.50	0.098

* Significant difference considered when P <0.1
[1] Measured by carcass ultrasonography
[2] Intramuscular fat determined by total lipids analysis (g of lipid / 100 g of meat; Bligh & Dyer, 1959)
[3] Marbling score determined by AMSA, considered select slight: 4.0–4.9; choice small: 5.0–5.9; choice modest: 6.0–6.9; choice moderate: 7.0–7.9; prime: 8.0–9.0 (Smith et al., 2013)

3.2 Biochemical and metabolic parameters

The serum insulin (P = 0.127), GH (P =0.914), and leptin hormone (P = 0.839) did not differ between the groups of high and low EPD (Table 3). On the other, the IGF-1 hormone was greater in H_EPD group than L_EPD group animals (P < 0.1; Table 3). Among the metabolites evaluated, the Cholesterol and the LDL were higher in L_EPD group than H_EPD group animals (P < 0.1; Table 3). There was no difference between groups for glucose serum (P = 0.239; Table 3), triglycerides, HDL (P = 0.462; Table 3), and VLDL (P = 0.886; Table 3).

Table 3. Effect of the groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity on the hormonal and metabolic serum traits from Nelore bulls (n=16)

Variable	GROUP		*P-value**
	H_EPD	L_EPD	
Hormone			
Insulin, ng/mL	4.34±0.98	1.53±1.06	0.127
GH, UI/mL	1.32±0.20	1.27±0.22	0.914
IGF-1, ng/mL	0.29±0.03	0.22±0.02	0.064
Leptin, pg/mL	6.16±3.56	7.54±3.85	0.839
Metabolite			
Glucose, mg/dl	81.75±5.64	94.64±6.09	0.239
Cholesterol, mg/dl	158.45±1.68	222.66±1.82	0.058
Triglycerides, mg/dl	36.77±3.99	37.84±4.31	0.887
HDL, mg/dl	45.40±5.23	38.02±5.64	0.462
LDL, mg/dl	105.69±14.02	177.05±15.16	0.014
VLDL, mg/dl	7.35±0.79	7.57±0.86	0.886

* Significant difference considered when P <0.1
GH = growth hormone; IGF-1 = *insulin-like growth factor-1*; HDL **=** *High Density Lipoproteins*; LDL = *Low Density Lipoproteins;* VLDL = *Very Low Density Lipoprotein*

3.3 Gene Expression

Only gene expression of *LPL* was different between the groups evaluated (*P* = 0.04). L_EPD group showed an increase in *LPL* transcript levels compared to H_EPD group (Figure 1). The other genes analyzed (*PPARG, ZNF423, TGFB1, ACACA, ACOX1, LEP, FABP4, SCD, MSTN, CAST, CAPN1, CAPN2*), related to protein turnover, adipogenesis, lipogenesis, and involvement in general lipid metabolism, did not differ between the groups studied (*P* > 0.1).

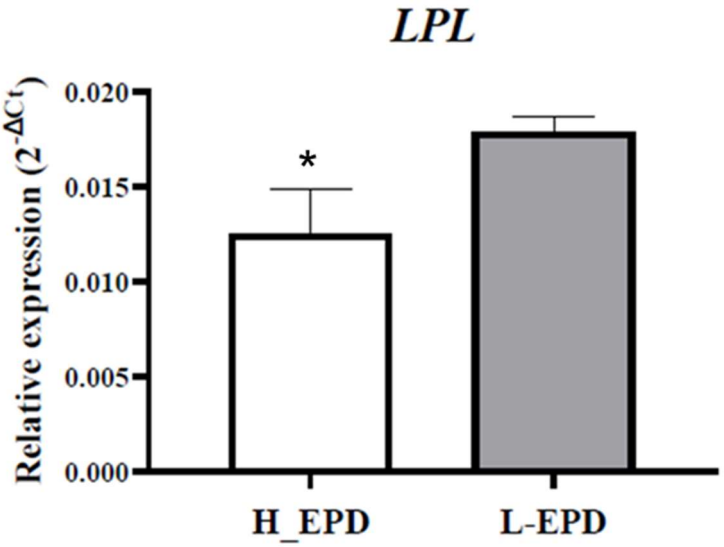

Figure 1: Transcript levels of Lipoprotein lipase (*LPL*) gene in *longissimus thoracis* from groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity. Significant difference: # P < 0.1, * P < 0.05, ** P < 0.01. Transformed value for a graphical representation ($2^{-\Delta Ct}$).

3.4 Unidimensional Electrophoresis - SDS / PAGE

The SDS-PAGE analysis allowed the revelation of 40 bands total from longissimus thoracis that was electrophoretically separated by molecular weight and matched across all samples (Figure 2). One electrophoretic band was differentially abundant between the groups H_EPD and L_EPD by statistical analysis (band 16; Table 4).

The significant electrophoretic band was correlated with productive, biologic, and qualitative phenotypes, such as weight gain, marbling, and subcutaneous fat deposition measured by carcass ultrasound, hormones, and genes evaluated. Also was correlated LPL gene, differently expressed between the groups H_EPD and L_EPD, with all traits mentioned above.

The correlations were investigated independently and within groups (H_EPD and L_EPD). These results were presented in Tables 5, 6 and 7).

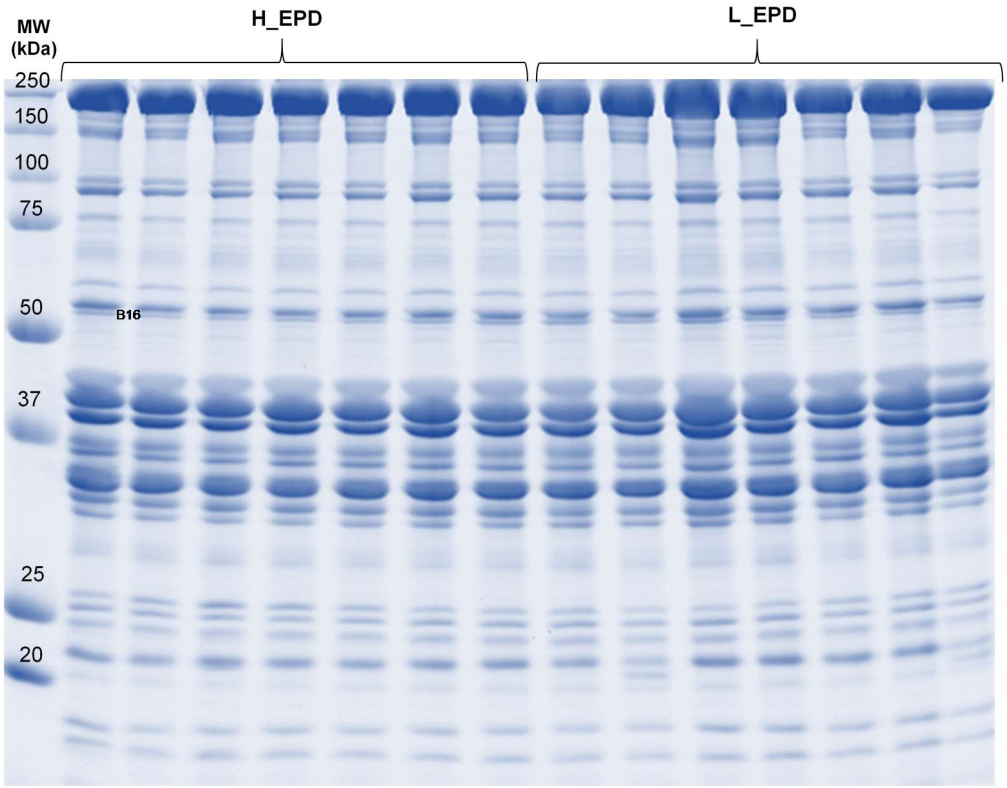

Figure 2. One-dimensional electrophoresis gel (SDS-PAGE 12%) from *longissimus thoracis* of Nelore bulls with high and low expected differences in progeny for growth and precocity.

Table 4. Bands of one-dimensional electrophoresis gel, from *longissimus thoracis* differentially abundant between the groups of Nelore bulls with high (H) and low (L) expected differences in progeny (EPD)

Band	**GROUP**		***P-value***
	H_EPD	**L_EPD**	
16	1.3872 ± 0.07921 B	1.9821 ± 0.07921 A	**0.013**

Means in the same row followed by different letters are significantly different by Tukey-Kramer test considered a significant difference for P < 0.1

3.5 Correlations between differentially abundant band (16) and phenotypes, expression levels, homones and metabolic traits, indepentent and within EPD groups

Independent of the evaluated group it was observed that:

The band 16 presented negativity correlation with final body weight (r = - 0.69; P = 0.05; Table 5), average daily gain (r = - 0.63; P = 0.09; Table 5), dry matter intake (r = - 0.75; P = 0.03; Table 5), Cholesterol (r = - 0.68; P = 0.06; Table 6) and positivity

correlation with transcribed of *ZNF423* (r = 0.68, P = 0.06; Table 7) and *SCD* (r = 0.65, P = 0.07; Table 7).

Within the H_EPD group it was observed that:

The band 16 presented negativity correlation with final body weight (r = - 0.90; P = 0.09; Table 5), average daily gain (r = - 0.94; P = 0.05; Table 5), dry matter intake (r = - 0.99; P = 0.003; Table 5); Intramuscular fat (r = - 0.90; P = 0.09; Table 5) and positivity correlation with transcribed of *TGFB1* (r = 0.91; P = 0.08; Table 7) and *SCD* (r = 0.94; P = 0.05; Table 7).

Within the L_EPD group it was observed that:

The band 16 presented negativity correlation with leptin hormone (r = - 0.93, *P* = 0.06; Table 6).

3.6 Correlations between differentially expressed gene (LPL) and phenotypes, expression levels, homones and metabolic traits, indepentent and within EPD groups

Independent of the evaluated group it was observed that:

The *LPL* gene expression presented negativity correlation with final body weight (r = - 0.63; P = 0.09; Table 5) and positivity correlation with insulin hormone (r = 0.66; P = 0.07; Table 6), *TGFB1* (r = 0.72; P = 0.04; Table 7) and *CAST* (r = 0.76; P = 0.02; Table 7).

Within the H_EPD group it was observed that:

The *LPL* gene expression presented negativity correlation with HDL (r = - 0.95; P = 0.04; Table 6), and positivity correlation with *ZNF423* (r = 0.99; P = 0.003; Table 7), *TGFB1* (r = 0.92; P = 0.07; Table 7) and CAST (r = 0.99, P = 0.007; Table 7).

Within the L_EPD group it was observed that:

The *LPL* gene expression presented negativity correlation with leptin hormone (r = - 0.92; P = 0.07; Table 6).

Table 5. Pearson correlation coefficient between differentilly abundant band (16) or diferentially expressed gene (LPL) in LT of Nelore bulls and phenotypic traits, independent and within of the groups with high and low EPD

	Pearson's correlation coefficient					
Variable	**Independent of group, N = 13**		**H_EPD, N = 7**		**L_EPD, N = 6**	
	Band_P-16	***LPL* gene**	**Band_P-16**	***LPL* gene**	**Band_P-16**	***LPL* gene**
iBW	- 0.69*	- 0.63$^{\#}$	**- 0.90$^{\#}$**			
fBW						
ADG	- 0.63$^{\#}$		**- 0.94***			
DMI	**- 0.75***		**- 0.99****			
BFT						
RFT						
REA						
IMF			**- 0.90$^{\#}$**			
MAR						
COL_S						
COL_T						

H_EPD and L_EPD: group of high (H) and low (L) expected progeny difference (EPD) for growth and precocity
iBW= Initial body weight; fBW= Final body weight; ADG= Average daily gain; DMI= Dry matter intake; BFT= Backfat thickness; RFT= Rump fat thickness; REA= Ribeye area; IMF= Intramuscular fat; MAR= Marbling score; COL_S = Collagen solubility; COL_T= Total collagen contente.
Significance: $^{\#}$ P < 0.10; * P < 0.05; ** P < 0.01. The coefficients higher than 0.75 are in bold.

Table 6. Pearson correlation coefficient between differentilly abundant band (16) or diferentially expressed gene (LPL) in LT of Nelore bulls and hormonal and metabolic traits, independent and within of the groups with high and low EPD

	Pearson's correlation coefficient					
Variable	**Independent of group, N = 13**		**H_EPD, N = 7**		**L_EPD, N = 6**	
	Band_P-16	***LPL* gene**	**Band_P-16**	***LPL* gene**	**Band_P-16**	***LPL* gene**
Hormones						
INS		0.66$^{\#}$				
GH						
IGF-1						
Lep					**- 0.93$^{\#}$**	**- 0.92$^{\#}$**
Metabolites						
Chol	- 0.68$^{\#}$					
Trigl						
HDL				**- 0.95***		
LDL						
VLDL						

H_EPD and L_EPD: group of high (H) and low (L) expected progeny difference (EPD) for growth and precocity
INS= insulin; GH = growth hormone; IGF-1 = *insulin-like growth factor-1*; Lep= lepitin; Chol= cholesterol; Trigl= triglycerides**;** HDL **=** *high density lipoproteins*; LDL = *low density lipoproteins;* VLDL **=** very Low density lipoprotein
Significance: $^{\#}$ P < 0.10; * P < 0.05; ** P < 0.01. The coefficients higher than 0.75 are in bold.

Table 7. Pearson correlation coefficient between differentilly abundant band (16) or diferentially expressed gene (LPL) in LT of Nelore bulls and gen expression levels, independent and within of the groups with high and low EPD

	Pearson's correlation coefficient					
Variable	**Independent of group, N = 13**		**H_EPD, N = 7**		**L_EPD, N = 6**	
	Band_P-16	***LPL* gene**	**Band_P-16**	***LPL* gene**	**Band_P-16**	***LPL* gene**
Genes						
PPARG						
ZNF423	0.68#			**0.99****		
TGFB1		0.72*	**0.91#**	**0.92#**		
ACACA						
LPL						
ACOX1						
LEP						
FABP4						
SCD	0.65#		**0.94***			
MSTN						
CAST		**0.76***		**0.99****		
CAPN1						
CAPN2						

H_EPD and L_EPD: group of high (H) and low (L) expected progeny difference (EPD) for growth and precocity *PPARG*= Peroxisome Proliferator activated Receptor Gamma; *ZNF423*= Zinc finger protein 423; *TGFB1*= *transforming growth fator beta 1; ACACA*= Acetyl-CoA carboxylase 1; *LPL*= Lipoprotein lipase 1; *ACOX1*= Acyl-CoA oxidase 1; *LEP*= Leptin; *FABP4*= fatty acid binding protein 4, adipocyte; *SCD*= Estearoil-CoA dessaturase 3; *MSTN*= Myostatin; *CAST*= Calpastine; *CAPN1*= μ Calpain 2; *CAPN2*= m Calpain 3.

Significance: # P < 0.10; * P < 0.05; ** P < 0.01. The coefficients higher than 0.75 are in bold.

3.7 Mass spectrometry analysis and identification of fat proteins

The differentially abundant band was cut from the gel and submitted to mass spectrometry analysis. A total of twelve proteins were identified in band 16. In Table 8, we reveal the top 10 proteins identified in band 16. It is important to mention that one of the limitations of using one-dimensional electrophoresis is the comigration of proteins within a single band (Zapata et al., 2009; Díaz et al., 2020).

The list of proteins identified in band 16 was submitted to over-representation analysis using the WebGestalt web tool to identify the KEGG pathways that are related to lipid metabolism. The enriched pathways (FDR ≤ 0.05) found are shown in Table 9 and Figure 3. The distribution of proteins identified in each band within GO categories (biological process, cellular component, and molecular function) can be found in Figure 4.

Additionally, a network of predicted protein-protein interactions against the Bos taurus database was performed for the top 10 proteins of the band 16 differentially abundant between the high and low EPD groups, using STRING 10.0 online software (Figure 5).

Table 8. Proteins identified by mass spectrometry in the differentially abundant band of the SDS-PAGE gel between the groups of Nelore bulls with high and low EPD for growth and precocity

Gene	Uniprot	Protein name	Mascot[1]	Mass[2]	Biological processes[3]
GPI	A0A3Q1MP70	Glucose-6-phosphate isomerase	3791	63824	Gluconeogenesis/Glycolysis
PKM	A5D984	Pyruvate kinase	1714	58482	Cellular response to insulin stimulus/glycolytic process
CASQ1	A0A3Q1LN23	Calsequestrin	740	42584	Calcium-binding protein (acts as an internal calcium store in muscle)
DLD	F1N206	Dihydrolipoyl dehydrogenase	425	54723	Mitochondrial electron transport. NADH to ubiquinone
VIM	P48616	Vimentin	167	53752	Positive regulation of collagen biosynthetic process
PKLR	Q1JPG7	Pyruvate kinase	96	57291	Cellular response to insulin stimulus/glycolytic process
PCYOX1	F1N2K1	Prenylcysteine oxidase 1	82	56968	Prenylcysteine catabolic process
ALDOA	A0A3Q1LMG1	Fructose-bisphosphate aldolase	71	40022	Glycolytic process
OXCT1	A0A3Q1LIX4	Succinyl-CoA:3-ketoacid-coenzyme A transferase	68	57256	ketone body catabolic process
ACTC1	A0A3Q1M558	Actin, alpha cardiac muscle 1	58	44111	Actin-myosin filament sliding

[1]The identified proteins were candidates when the global Mascot score was greater than 57 with a significance level of $P \leq 0.0001$.
[2]Molecular weight of the protein, in KDa.
[3]Biological processes described in Uniprot.

Table 9. KEGG pathways enriched for the proteins identified in the differentially abundant band of the SDS-PAGE gel between the groups of Nelore bulls with high and low EPD for growth and precocity

KEGG	Enriched pathways	Protein	*P-value*	FDR[1]
bta00620	Pyruvate metabolism	DLD, PKLR, PKM	0.0000072689	0.00077302
bta00010	Glycolysis / Gluconeogenesis	ALDOA, DLD, GPI PKLR, PKM	$2.8425E^{-05}$	$9.0677E^{-07}$
bta00030	Pentose phosphate pathway	ALDOA, GPI	0.00038835	0.024777
bta01200	Carbon metabolism	ALDOA, DLD, GPI PKLR, PKM	4.9053E-8	0.0000078240
bta01230	Biosynthesis of amino acids	ALDOA, PKLR, PKM	0.000052588	0.0041939

[1]False Discovery Rate - *P value* corrected for multiple tests within each category using the procedure by Benjamini & Hochberg (1995).

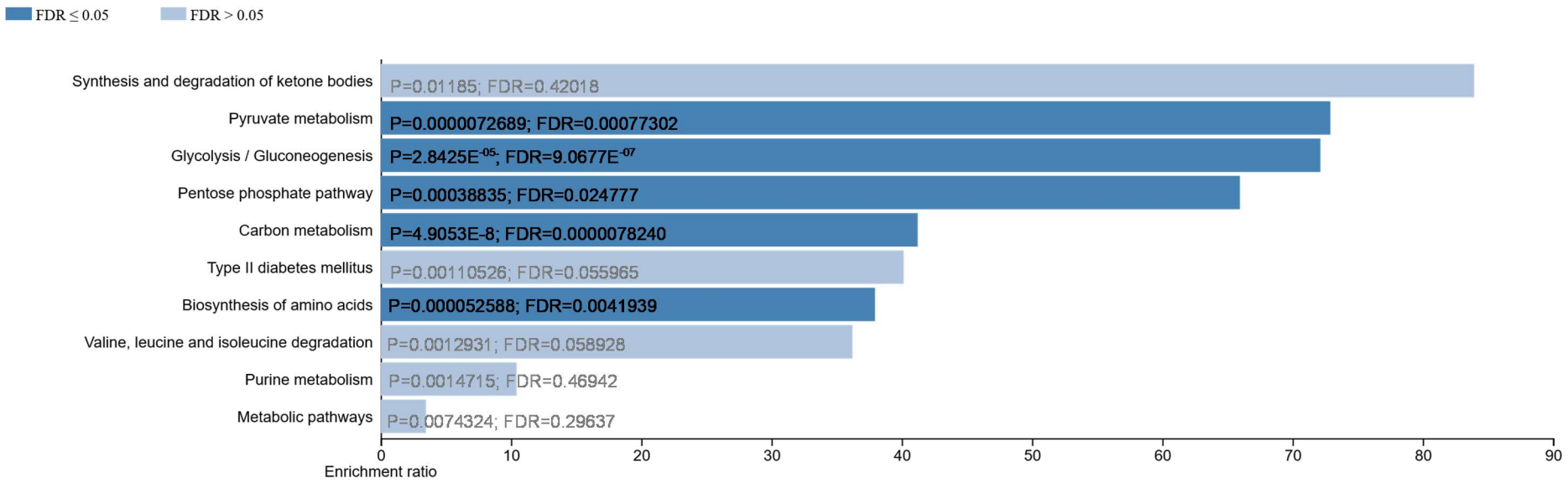

Figure 3: Demonstration of the enrichment rate according to over-representation analysis using WebGestalt web tool, where can observe at an all enriched pathways or no for band 16. FDR =False Discovery Rate – P-value corrected for multiple tests within each category using the procedure by Benjamini & Hochberg (1995).

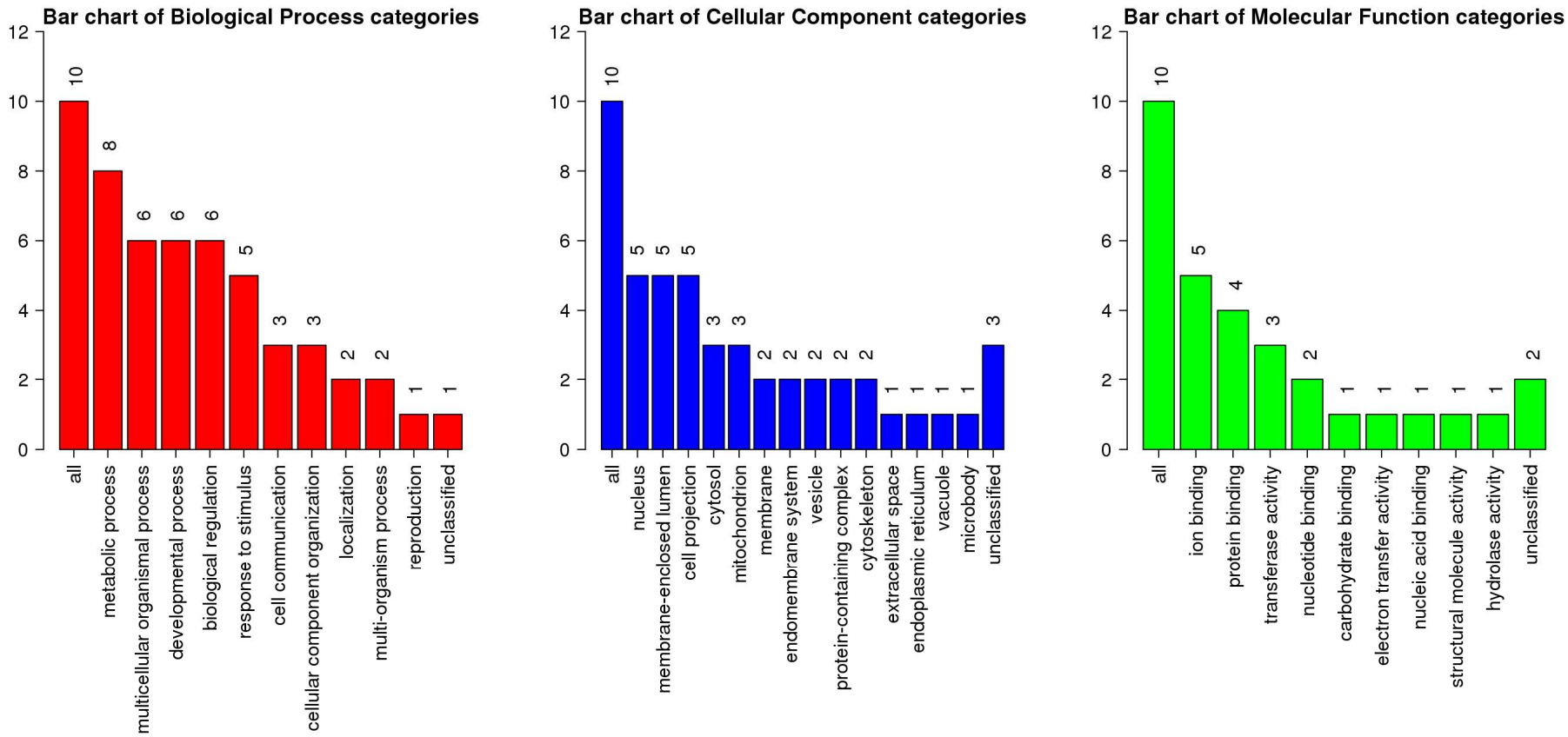

Figure 4. Top 10 Proteins identified in the *longissimus thoracis* organized by category: Biological Process, Cellular Component, and Molecular Function. Each category is represented by a red, blue and green bar, respectively. The height of the bar represents the number of IDs in the user list and also in the category. WebGestalt software.

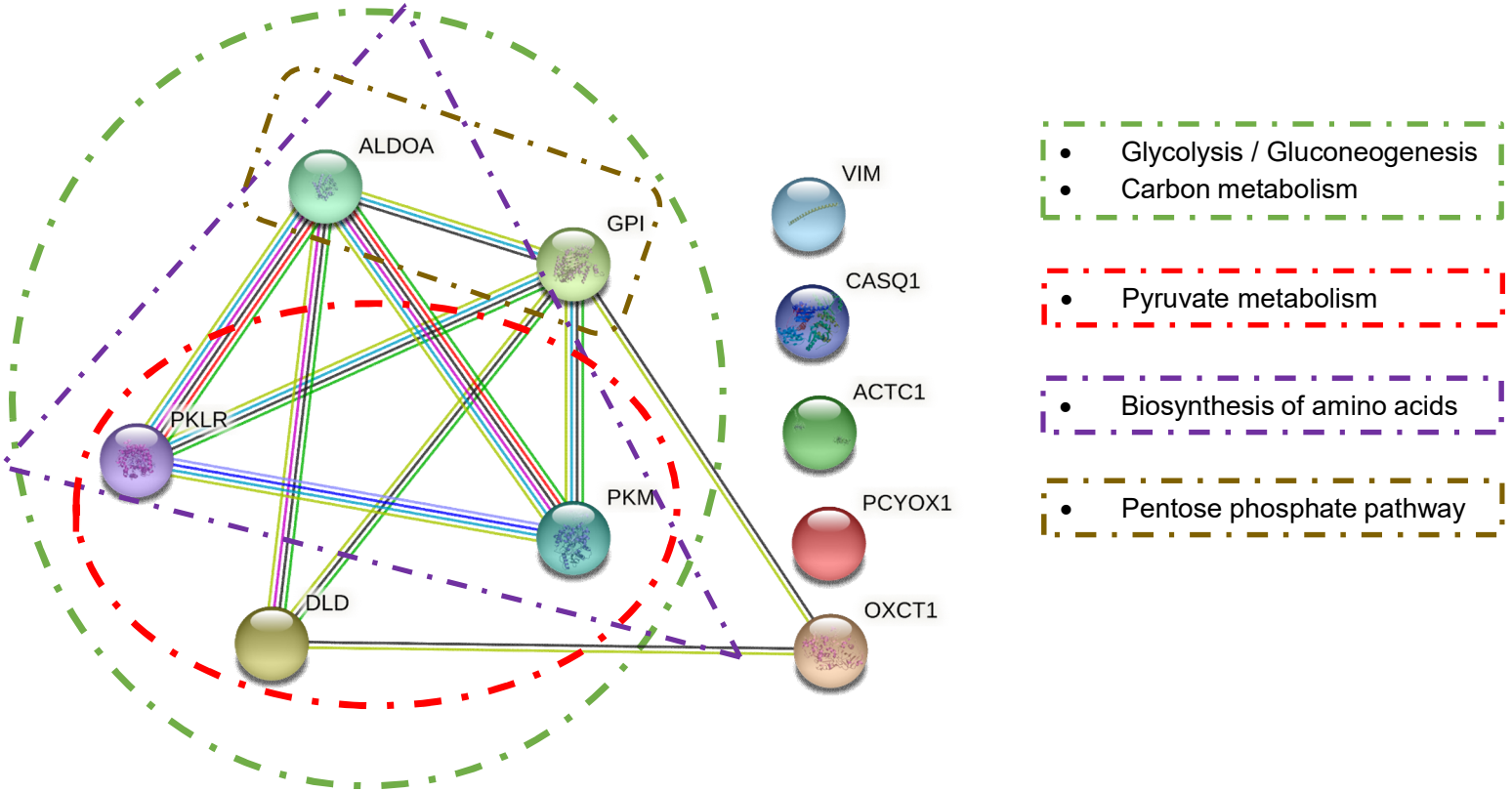

Figure 5. Protein-protein interaction network predicted by the String 11.0 software of the differentially abundant proteins identified between the groups of Nelore bulls with high and low EPD for growth and precocity. The nodes are proteins from the *Bos taurus* database, and the edges represent different evidences of the predicted functional annotations (red line - fusion; green line - neighborhood; blue line - co-occurrence; purple line - experimental; yellow line - text mining; light blue line - database; black line - coexpression). The proteins within the dotted lines represent the metabolic pathways with the color corresponding to the side.

4 DISCUSSION

Several studies have phenotypic differences between animals with high and low EPD for traits of economic interest (FERNANDES et al., 2000; SAPP et al., 2002; DIKEMAN et al., 2005; BOLIGON et al., 2006), proving the methods to identify superior animals in characteristics considered in the selection process are efficient. Furthermore, there is an increasing number of data demonstrating the phenotypic divergences between the contrasting genetic groups within Nelore breed (BONIN et al., 2014; COSTA et al., 2019; SANTANA et al., 2019 CÔNSOLO et al., 2020; CÔNSOLO et al., 2021). Thus, in our study, was evaluated the phenotypic, genotypic, physiological, and proteomic differences of the *longissimus thoracis* between two genetic groups of Nelore progenies, offspring of bulls with contrasting EPD for growth and precocity.

4.1 Phenotypic traits

The H_EPD group showed the highest fat deposition in the rump cap when the RFT was measured by carcass ultrasonography. The "Rump cap" is considered a noble beef cut in Brazil. Besides this, rump fat thickness is an anatomic region deposition fat greater precocious in cattle. Previous studies have shown that ultrasound provides accurate measures of the live animal fat thickness (ROBINSON et al. 1992; HERRING et al. 1994), and rump fat thickness (REALINI et al., 2001).

The evaluated progenies also had a contrasting phenotype for backfat thickness (BFT) between the high and low EPD groups, with the highest subcutaneous fat deposition observed in animals with high EPD. These results can be justified by the theory of preferential deposition of tissues throughout animal growth until reaching their physiological maturation once the animals in the H_EPD group had the higher genetic potential for growth and precocity.

King et al. (2006) evaluated Nelore and Brahman cattle in crossbreeding systems with Angus observed variations in BFT and MAR between progenies, presenting higher variations within Nelore individuals than those found between breeds. Likewise, Bonin et al. (2014) found the paternal effect of BFT and MAR when investigating the difference in meat quality of progenies from 13 strains of Nelore bulls. Therefore, this reflects in variability among Nelore bulls in the transfer of BFT and MAR traits to their progenies.

Pereira et al. (2015) evaluated the carcass traits and meat quality in progenies of Nelore and Crossbreed bulls (“Angus x Nelore” and “Brahman x Nelore”) and found that despite the crossbreeds having carcass and meat quality higher to animals Nelore, some Nelore progenies were similar to Angus progenies to meat quality, highlighting a possible genetic difference within the Nelore breed, with indications of wide intra-racial genetic variation for fat-related characteristics, especially MAR. The marbling score was subtly higher in H_EPD group animals than L_EPD, however, the MAR was still considered " slight" according to Smith et al. (2013).

The H_EPD group animals did not differ from the L_EPD group in the total amount of collagen, yet, the H_EPD group animals showed higher collagen solubility than the L_EPD group animals. The collagen is a factor that contributes to variation in meat tenderness and is related to the increase in cross-links as the animal ages (DUARTE et al., 2011; ETHERINGTON, 1987). However, collagen cross-linking is also affected by the growth rate, nutrition, and genetics (ROMPALA & JONES, 1984; FISHELL et al., 1985; GERRARD et al., 1987; McCORMICK, 1999).

Although the animals are contemporary, the higher collagen solubility in the H_EPD group animals can be due to possible differences related to the collagen turnover due to these animals have higher growth potential. The newly synthesized collagen dilutes the older and less cross-linked collagen than the pre-existing collagen during rapid growth (ETHERINGTON, 1987). Collagen characteristics differ among breeds because of variation in physiological maturity. (BLANCO et al., 2013). These authors relate that interactions observed among total and insoluble collagen contents could be due to differences in precocity between muscles and breeds types. This theory may explain the difference found for collagen solubility between the high and low EPD groups.

4.2 Biochemical and metabolic parameters

The balance between protein synthesis and degradation in muscle fibers is called protein turnover, when positive, results in protein accumulation and muscle growth (SCHIAFFINO et al., 2013). Genetic variation is one of the main factors that influence protein turnover rates, therefore, differences in protein synthesis and deposition may explain part of the changes in growth pathway between different genotypes (ODDY et al., 1995; ANTHONY et al., 2016).

Two main pathways control muscle protein turnover rates in vivo: the IGF-1-protein kinase B pathway (IGF-1-AKT) acting as a positive regulator, and the myostatin pathway, acting as a negative regulator (SCHIAFFINO et al., 2011; SCHIAFFINO et al., 2013). In our results, the IGF-1 hormone serum concentration was higher in H_EPD group animals than L_EPD group animals. This result can be due to the higher growth potential of the H_EPD group animals.

The IGF-1 also acts in the signaling pathway mTOR, which is a protein complex involved in lipolysis and lipid biosynthesis (glycerolipid metabolism). mTORC1 is activated by the presence of growth factors, amino acids, energy status, stress, and oxygen levels, acting in lipid metabolism, autophagy, protein synthesis, and ribosome biogenesis (KANEHISA et al., 2019; KANEHISA et al., 2000).

This study was observed lower LDL content in the H_EPD group than the L_EPD group. The serum HDL content was negatively correlated with the *LPL* gene (r = - 0.95), independent of the group evaluated. Lipoprotein Lipase (LPL) is an extracellular enzyme present in high amounts in skeletal and cardiac muscle and fat tissue. LPL plays a central role in the general metabolism of lipoproteins, generating low-density lipoproteins (LDL). The particles formed from the LPL catalysis contribute to the maturation of high-density lipoprotein (HDL) precursors, involved in the reverse cholesterol transport (PREISS et al., 2002, PILLARISETTI & SAXENA 2003).

Besides this, was observed lower cholesterol content in the H_EPD group than in the L_EPD group. Cholesterol presented a negative correlation with band 16, independent of group evaluated (r = -0.68). The L_EPD group of animals obtained greater abundance for band 16 (P = 0.013) and, consequently, greater expression of the *LPL* gene (P = 0.04), which may have contributed to the higher levels of LDL and cholesterol in the animals of the low EPD group (P = 0.014).

Additionally, the serum insulin content was positively correlated with band 16 independent of the group evaluated (r = 0.66). While, leptin hormone was a high negative correlation with band 16 and LPL gene within the L_EPD group (r = - 0.93, r = - 0.92 respectively). Although no difference was found for the hormone insulin and leptin, the correlations found for these hormones can help us understand the findings BFT and MAR in this study. This because hormonal factors can stimulate food consumption, and the greater dry matter intake leads to an increase in substrates for protein and lipid synthesis. These factors can also promote epigenetic and

nutrigenomic changes sufficient to originate phenotypic differences within the same breed (ZHAO et al., 2019).

4.3 Gene expression

No difference was for the genes involved in protein turnover: *MSTN, CAST, CAPN1, CAPN2*, between the groups, evaluated. On the other hand, differences could have been found for gene expression of collagenases or metalloproteinases involved in collagen turnover and would explain the difference found for collagen solubility in this study. However, this analysis was not carried out.

There was no difference for the following genes involved in adipogenesis and lipogenesis: *PPARG, ZNF423, TGFB1, ACACA, ACOX1, LEP, FABP4, SCD*. This result can be explained by the absence of difference between the H_EPD and L_EPD groups for intramuscular fat.

However, the H_EPD group animals had lower *LPL* gene expression than the L_EPD group animals (P = 0.04). Consequently, the L_EPD group had a greater serum *LDL* concentration than the H_EPD group. Accordingly, *LPL* has negatively correlated with HDL for animals independent of the group evaluated. Our results show us that the higher the *LPL* gene expression, the higher the serum LDL concentration and cholesterol metabolite. Besides this, for this same group, an *LPL* positivity correlation with *ZNF423*, *TGFB1*, and *CAST* transcripts. On the other hand, L_EPD animals correlated negativity with the hormone leptin. Regardless of the groups evaluated, *LPL* showed a positive correlation with the insulin hormone and the *TGFB1* and *CAST* transcripts.

LPL is an essential enzyme in metabolism and lipid deposition and is involved in inflammatory processes that lead to fat deposition, regulates plasma concentrations of triglycerides and HDL, and is a possible gene candidate the biomarker of lipid deposition and energy balance (GUI et al., 2016). Furthermore, the levels of *LPL* transcripts are positively related to *LPL* activity in bovine tissues, including muscles and adipose tissues (HOCQUETTE et al., 1998). Therefore, expected that the animals in the H_EPD group, due to their higher BFT, had higher levels of *LPL* transcription, as reported by Ren et al. (2002) that shows the mRNA expression levels of *LPL* are higher in cattle with higher levels of body fat. But, this fact that did not happen.

Probably, under the conditions in which our study was carried out, due to the difference between the groups concerning physiological maturity, it may be that the transcription had not ended at the time of sample collection and, therefore, lower levels of *LPL* expression were observed in the H_EPD group. In other words, the proteins had already been translated in this group and, therefore, transcript levels were lower. In the L_EPD group, as it is a group of less precocious animals, transcription was still on going, it was delayed compared to the H_EPD group, which may have resulted in higher levels of *LPL* transcripts in the L_EPD group.

However, the *LPL* activity in adipose tissue appears to affect the translation or posttranslational expression of *LPL* (KIRCHGESSNER et al., 1989; DOOLITTLE et al., 1990). Changes seen in enzyme activity may be the result of transcriptional regulation of the *LPL* gene in tissue muscle (LADU et al., 1991).These results may indicate new targets that will assist in research on fat deposition and can contribute to breeding programs to optimize the beef quality.

4.4 Proteomics of longissimus thoracis

In this study, we were able to observe changes in the abundance of proteins in the contrasting groups for EPD's for precocity and growth, such as for, GPI, PKM, CASQ1, DLD, VIM, PKLR, PCYOX1, ALDOA, OXCT1, ACTC1, although one of the limitations of one-dimensional electrophoresis is the co-migration of proteins in a single band (Díaz et al., 2020). The proteins identified in band 16 (more abundant in the L_EPD than H_EPD group; P = 0.013) were enriched for the Pyruvate metabolism pathways (bta00620), Glycolysis / Gluconeogenesis (bta00010), Pentose phosphate pathway (bta00030), Carbon metabolism (bta01200), and Biosynthesis of amino acids (bta01230).

The vimentin (VIM) is a protein that has functioned within the positive regulation of the collagen biosynthetic process, the main component of connective tissue in animals. This protein was identified in band 16. Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. The VIM is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. This protein is involved with LARP6 in the type I collagen mRNAs stabilization for *CO1A1* and *CO1A2* (UniProtKB Consortium, 2021).

As previously reported, was observed in the present study, higher collagen solubility H_EPD group animals and lower collagen solubility L_EPD group animals. The difference in abundance of the VIM can be help explain to difference in collagen solubility between the groups evaluated. Besides this, the VIM protein is related to SMAD protein signal transduction. SMAD protein complex is involved in collagen biosynthesis and has a relation with *TGFB1* (UniProtKB Consortium, 2021). Although no difference was found in *TGFB1* gene expression, was observed a positive correlation between band 16 and *TGFB1* transcript within the H_EPD group (r = 0.72).

Therefore, the animals in the H_EPD group, due to their higher growth rate, may have a higher collagen turnover and consequently higher solubility. This fact may be explained by the lower abundance of VIM in the muscle of H_EPD group animals and higher in the L_EPD group animals. The VIM is physiologically related to SMAD proteins and *TGFB1* transcription factors involved in fibrogenesis. In the fibrotic tissue, type I collagen was the most deposited component, and its alpha1 chain (*Col1a1*) it indicates the level of collagen accumulation (BOLLONG et al., 2017). Myofibroblast expressing a-smooth muscle actin (a-SMA; LI et al., 2013) and VIM is the major cell source accounting for the fibrotic process (LI et al., 2011). This fact may be contributing to lower collagen solubility in the L_EPD group.

Sisto et al. (2018) found a significant increase in the collagen type and VIM protein in the TGF-β1-treated samples, indicating that TGF-β1 induces the epithelial-mesenchymal transition (EMT) via the TGF-β1/SMAD signaling pathway. TGF-β1/SMAD axis could be downregulated by microRNAs. The miR-24 and miR-122 act as fibrogenic inhibitors that reduce the level of SMAD2 and TGFBR2, respectively, whereas SMAD4 suppresses the expression of miRs (SUN et al. 2018).

These findings may explain the difference in collagen solubility between the genetic groups of Nelore cattle with contrasting EPD for growth and precocity.

Calsequestrin (CASQ1) is a muscle protein located sarcoplasmic reticulum (SR) lumen. This protein was identified in band 16 and considered most abundant in the L_EPD group than the H_EPD group. CASQ1 is a high-capacity, moderate affinity, calcium-binding protein and acts as an internal calcium store in muscle. Interestingly the CASQ1 interacts selectively and non-covalently with calcium ions (Ca^{2+}) (UniProtKB Consortium, 2021). CASQ1 is a dynamic Ca^{2+} sensor in the SR that regulates Ca^{2+} release from the SR to the cytosol (WOO et al., 2020). Soon,

extracellular Ca^{2+} released by CASQ1 it's essential for skeletal muscle contraction and relaxation.

Prenylcysteine oxidase 1 (PCYOX1), was identified in band 16 and considered most abundant in the L_EPD group than the H_EPD group. This protein is a pro-oxidant enzyme of low-density lipoproteins (HERRERA-MARCOS et al., 2018). Elevated levels of low-density lipoproteins (LDLs) cause atherosclerotic disease, and proteomic analyses have found that these lipoproteins are full with prenylcysteine lyase (HERRERA-MARCOS et al., 2018). One consequence of its elevated activity could be an increase in hydrogen peroxide, which might help to propagate the oxidative burden of LDLs, thus making PCYOX1 a potential pharmacological target and a new biomarker in cardiovascular disease (CVD) (HERRERA-MARCOS et al., 2018). Once higher intake of processed meat and unprocessed red meat has been associated with a small increased risk of incident CVD (ZHONG et al., 2020). Interestingly, the animals in the L_EPD groups showed higher expression of the *LPL* gene and higher concentrations of LDL and serum cholesterol. There is probably a relationship between the *LPL* gene and the PCYOX1 protein in lipoproteins metabolism in the *longissimus thoracic*.

Succinyl-CoA: 3-ketoacid-coenzyme A transferase (OXCT1) is the key enzyme for ketone body catabolism. This enzyme transfers the CoA moiety from succinate to acetoacetate. Ketone bodies can be used as an energy source as an alternative to glucose (UniProtKB Consortium, 2021). Succinyl-CoA and NAD+ regulate cellular metabolism similarly to acetyl-CoA (NEWMAN & VERDIN, 2014). OXCT1 may function in lipogenesis or lipolysis of adipose tissue. OXCT1 appears to function during the middle period of differentiation, while the LPL expression is an early marker of differentiation, so both can play a role in adipogenesis and provides new insight into adipose deposition (ZENG et al., 2019).

The dihydrolipoyl dehydrogenase (DLD) is a protein identified in band 16 and is present in the enriched pathways: Pyruvate metabolism, Glycolysis / Gluconeogenesis, and Pentose phosphate pathway. One of the functions of the DLD is to transfer electrons from NADH to ubiquinone that occurs during oxidative phosphorylation (UniProtKB Consortium, 2021). The DLD subunit of the ketoglutarate dehydrogenase complex (KGDHC) is known to provide succinyl-CoA to succinyl-CoA ligase, thus supporting mitochondrial substrate-level phosphorylation in the absence of oxygen or inhibition of the mitochondrial respiratory chain

(CHINOPOULOS, 2020). This makes them believe in a possible joint action of the muscle proteins DLD and OXCT1 in the mechanisms involved in post-mortem proteolysis.

The glucose-6-phosphate isomerase (GPI) is a muscle protein located in extracellular space and was identified in the differentially abundant band between the groups of Nelore bulls with high and low EPD in this study. This protein is involved in step 2 of the subpathway that synthesizes D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose. (UniProtKB Consortium, 2021). GPI is present in the enriched pathways: Pyruvate metabolism, Glycolysis / Gluconeogenesis, Pentose phosphate pathway, and Carbon metabolism. Bazile et al. (2019) and Poleti et al. (2018) related the putative contribution of the identified protein GPI to pathways of muscular or carcass adiposity in beef cattle in contrasting groups for intramuscular fat.

The pyruvate kinase enzyme is encoded by two genes: PKLR and PKM, both were identified in band 16 (Table 6). PKLR and PKM act in the glycolytic process and the cellular response to insulin stimulation (UniProtKB Consortium, 2021). The insulin decreases blood glucose concentration and increases cell permeability to monosaccharides, amino acids, and fatty acids, accelerates glycolysis, the pentose phosphate cycle, and glycogen synthesis in the liver. Besides that, insulin has negative regulation of the lipid catabolic process (KANEHISA et al., 2019; KANEHISA et al., 2000). PKLR, PKM, and GPI were energy metabolism-related in cattle (RODRIGUES et al., 2017).

The aldolase protein (ALDOA) also was identified in band 16. Elevated abundance and activity of ALDOA are indicative of increased activity glycolytic (REICHE et al., 2019). Besides that, ALDOA is interconnected with lipid metabolism and the pentose phosphate pathway (OROSZ et al., 2009). ALDOA assists in the creation of cross-links between adjacent actin filaments or in binding troponin to the thin filaments to enhance energy provision needed during contraction, both connections can affect the distance between myofibrils (HUGHES et al., 2019).

ACTC1 is another protein identified in band 16 and is involved with actin-myosin filament sliding. Actin and myosin play a central role in cell biology are responsible not only for muscle contraction (COOPER, 2000). Moreover, the actin cytoskeleton is responsible for the crawling movements of cells across a surface, which appear to be driven directly by actin polymerization as well as actin-myosin

interactions (COOPER, 2000). Given the above, we believe that the ACTC1 protein can help ALDOA submit direct energy during muscle contraction.

PKLR and PKM, as well as ALDOA, are present in almost all enriched pathways for the proteins identified in band 16. These proteins play a central role in these enriched pathways, such as Pyruvate metabolism, Glycolysis / Gluconeogenesis, Pentose phosphate pathway, Carbon metabolism, and Amino acid biosynthesis. It may be that the differences in these enriched pathways influence the groups evaluated, giving rise to the contrasting phenotypes observed in the present study.

In this sense, the *LPL* gene, and the proteins found in *longissimus thoracis* samples in this study, in particular, PKLR, PKM, ALDOA, DLD, GPI, ACTC1, OXCT1, VIM should act on the lipid and protein metabolism in the muscle and may be involved with the muscle protein turnover.

5 CONCLUSION

The genetic selection for precocity and growth affects the muscle proteome and consequently lipid and protein metabolism of the Zebu cattle.

The enriched pathways found in proteomic analysis, such as pyruvate metabolism, glycolysis/gluconeogenesis, pentose phosphate pathway, carbon metabolism, and amino acid biosynthesis, may influence the groups evaluated, giving rise to the contrasting phenotypes for backfat thickness and intramuscular fat. The IGF-1 hormones, the LPL gene, and the proteins PKLR, PKM, ALDOA, DLD, GPI, ACTC1, OXCT1 and, VIM can be considered important factors involved lipid and protein turnover and, therefore, may be able to facilitate the process of selection of beef cattle and the quality of meat from non-castrated, special Zebu animals under global conditions.

These results may suggest new targets that will assist in research on lipid and protein metabolism of the *longissimus thoracis* and can contribute to breeding programs to optimize the quality of beef.

6 PRACTICAL IMPLICATIONS

Cattle of the high EPD group (H_EPD) show carcasses with greater backfat thickness and intramuscular fat than the group of animals with low EPD (L_EPD). Besides, the group with high EPD showed solubility of collagen greater than the group with low EPD. All of these factors are positive for the meat quality. The selection of progenies based on the EPD's of the parents is a viable strategy, within the beef cattle system, as it contributes to a higher degree of finishing on bovine carcasses. That would be a good alternative for producers when complied, allows differentiated payment for the animals, providing added value to the products generated, or not being penalized for the lack of fat deposition on the carcass.

ACKNOWLEDGEMENTS

We are grateful to FAPESP for the scholarship (Grant #2017/02349–1) and financial support (process #2016/17433-5; #2011/21241-0; #2012/50788-0). We also thank CNPq (process #409186/2018-0) and CAPES (Finance Code 001) for financial support. We also thank CNPEM (National Center for Research in Energy and Materials) for the structure to carry out the mass spectrometry analysis.

7 REFERENCES

Anthony, T.G. Mechanisms of protein balance in skeletal muscle**. Domestic Animal Endocrinology**, v. 56, p. S23–S32, 2016. doi:10.1016/j.domaniend.2016.02.012

Bazile, J.; Picard, B.; Chambon, C.; Valais, A.; Bonnet, M. Pathways and biomarkers of marbling and carcass fat deposition in bovine revealed by a combination of gel-based and gel-free proteomic analyses. **Meat Science**, v. 156, p. 146-155, 2019.

Blanco, M.; Jurie, C.; Micol, D.; Agabriel, J.; Picard, B.; Garcia-Launay, F. Impact of animal and management factors on collagen characteristics in beef: A metaanalysis approach. **Animal**, v. 7, p. 1208–1218, 2013.

Bligh E. C.; Dyer W. J. A. Rapid method of total lipid. Extraction and purification. **Canadian Journal of Biochemistry and Physiology**, v.37, p. 911-917, 1959.

Boligon, A. A.; Rorato, P. R. N.; Weber, T.; Everling, D. M. E.; Lopes, J. S. Herdabilidades para ganho de peso da desmama ao sobreano e perímetro escrotal ao sobreano e tendências genética e fenotípica para ganho de peso da desmama ao sobreano em bovinos Nelore-Angus. **Revista Brasileira de Zootecnia**, v.35, p.1323-1328, 2006.

Bonin, M. N.; Ferraz, J. B. S.; Eler, J. P.; Rezende. F. M.; Cucco. D. C.; Carvalho. M. E.; Silva. R. C. G.; Gomes. R. C.; Oliveira. E. C. M. Sire effects on carcass and meat quality traits of young Nelore bulls. **Genetics and molecular research**: GMR, v. 13 (2), p.3250-3264, 2014.

Bollong, M. J.; Yang, B., Vergani, N.; Beyer, B. A.; Chin, E. N.; Zambaldo, C.; Wang, D.; Chatterjee, A. K.; Lairson, L. L.; Schultz, P. G. Small molecule-mediated inhibition of myofibroblast transdifferentiation for the treatment of fibrosis. **Proc. Natl. Acad. Sci**, v. 114, p. 4679–4684, 2017.

Bonin, M.N.; Ferraz, J. B. S.; Eler, J. P.; Silva, S. L; Rezende, F. M.; Cucco, D.C.; Carvalho, M. E.; Silva, R. C. G.; Oliveira, E. C. M. Características de carcaça e qualidade de carne em linhagens da raça Nelore. **Ciência Rural** (UFSM. Impresso), v. 44, p. 1860-1866, 2014.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, p. 248–254, 1976.

Brasil. Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal. **Food of animal origin sanitary and industry inspection**. In. Brasília: Ministério da Agricultura. Pecuária e Abastecimento. 2017.

Chaze, T.; Hocquette, J.F.; Meunier, B.; Renand, G.; Jurie, C.; Chambon, C.; Journaux, L.; Rousset, S.; Denoyelle, C.; Lepetit, J.; Picard, B. Biological Markers for Meat Tenderness of the Three Main French Beef Breeds Using 2-DE and MS Approach. In: Toldrá F., Nollet L. (eds) **Proteomics in Foods**. **Food Microbiology and Food Safety**, v. 2, p 127-146, 2013. doi.org/10.1007/978-1-4614-5626-1_8

Chinopoulos, C. **Acute sources of mitochondrial NAD(+) during respiratory chain dysfunction Exp. Neurol**, v. 327, p. 113218. 2020. 10.1016/j.expneurol.2020.113218

Clarke A. M.; Drennan M .J.; McGee M.; Kenny D.A.; Evans R.D.; Berry D.P. Intake, growth and carcass traits in male progeny of sires differing in genetic merit for beef production. **Animal**, v. 3, p. 791–801, 2009. doi: 10.1017/S1751731109004200.

Cônsolo, N. R. B.; Buarque, V. L. M.; Silva, J.; Poleti, M. D.; Barbosa, L. C. G. S.; Higuera-Padilla, A; Gómez, J.F.M.; Colnago, L. A.; Gerrard, D.E.; Saran Netto, A.; Silva, S. L. Muscle and liver metabolomic signatures associated with residual feed intake in Nelore cattle. **Animal Feed Science and Technology**, v. 271, p. 114757, 2021.

Cônsolo, N.R.B.; Silva, J.; Buarque, V. L. M.; Higuera-Padila, A.; Barbosa, L. C. G. S.; Zawadzki, A.; Colnago, L. A.; Saran Neto, A.; Gerrard, D. E.; Silva, S. L. Selection for growth and precocity alters muscle metabolism in Nelore cattle. **Metabolites**, v. 10, p. 58, 2020.

Cooper, G. M. **The Cell: A Molecular Approach. 2nd edition**. Sunderland (MA): Sinauer Associates; 2000. Actin, Myosin, and Cell Movement. Available from: https://www.ncbi.nlm.nih.gov/books/NBK9961/

Costa, M. B.; Cônsolo, N. R. B.; Silva, J.; Buarque, V. L. M.; Padilla, A. R. H.; Coutinho, I. D.; Barbosa, L. C. G. S.; Colnago, L. A.; Silva, S. L; Saran Netto, A;. Performance, carcass traits and serum metabolomic profile of Nelore males with different genetic potential for post-weaning growth. **Animal**, v. 1, p. 1-8, 2019.

Cross, H. R.; Carpenter, Z. L.; Smith. G. C. Effects of intramuscular collagen and elastin on bovine muscle tenderness. **Journal of Food Science**. v. 38. (6), p. 998-1003. 1973.

D'Alessandro, A.; Zolla, L. Review Meat science: From proteomics to integrated omics towards system biology. **Journal of Proteomics**, v.78, p. 558±77, 2013 doi: 10.1016/j.jprot.2012.10.023

Dikeman, M. E.; Pollak, E. J.; Zhang, Z.; Moser, D. W.; Gill, C. A. E Dressler, E. A.; Phenotypic ranges and relationships among carcass and meat palatability traits for fourteen cattle breeds and heritabilities and expected progeny differences for Warner-Bratzler shear force in three beef cattle breeds. **Journal Animal Science**, v.83, p. 2461–2467. 2005.

Díaz, F.; Díaz-Luis A.; Sierra, V.; Diñeiro, Y.; González, P.; García-Torres, S.; Tejerina, D.; Romero-Fernández, M. P.; Cabeza de Vaca, M.; Coto-Montes, A.; Oliván, M. What functional proteomic and biochemical analysis tell us about animal stress in beef? **Journal of Proteomics**, v. 218, p. 103722. 2020. doi.org/10.1016/j.jprot.2020.103722

Doolittle MH, Ben-Zeev O, Elovson J, Martin D, Kirchgessner TG. The response of lipoprotein lipase to feeding and fasting. **J Biol Chem**, v. 265, p. 4570–7, 1990.

Duarte, M. S.; Paulino, P.V.R.; Das, A. K.; Wei, S.; Serao, N. V. L.; Fu, X.; Harris, S. M.; Dodson, M. V.; Du, M. Enhancement of Adipogenesis and Fibrogenesis in Skeletal Muscle of Wagyu Compared With Angus Cattle. **Journal of Animal Science**, v. 91, p.2938–2946, 2013. doi:10.2527/jas2012-5892

Duarte, M. S.; Paulino, P.V.R.; Fonseca, M. A.; Diniz, L. L.; Cavali, J.; Serao, N. V.; Gomide, L. A; Reis, S. F.; Cox, R. B. Influence of dental carcass maturity on carcass traits and meat quality of Nelore bulls. **Meat Science**,v. 88, p. 441-446. 2011.

Etherington. D. J. Collagen and meat quality: Effects of conditioning and growth rate. In **Advances in Meat Research**. Vol. 4. A. M. Pearson. T. R. Dutsonand A. J. Bailey (Eds.). p 351. AVI. New York. NY. 1987.

Fernandes. H. D.; Ferreira. G. B. Estudo Comparativo de Sete Diferentes Modelos Estatísticos para a Característica Ganho de Peso em Bovinos de Corte. **Revista Brasileira de Zootecnia**. v. 29, p. 1340-1348. 2000.

Ferraz, J. B. S and Felício, P. E. Production systems An example from Brazil. **Meat Science**. v. 84, p. 238-243, 2010.

Fishell, V. K.; Aberle, E. D.; Judge, M. D.; T. W. Perry. Palatability and muscleproperties of beef as influenced by preslaughter growth rate. **Journal of Animal Science**. v. 61. p. 151.15. 1985.

Gerrard, D. E.; Jones, S. J.; Aberle, E. D.; Lemenager, R. P.; Dikeman, M. A.; Judge, M. D. Collagen stability, testosterone secretion and tenderness in growing bulls and steers. **Journal of Animal Science**, v. 65, p.1236–1242. 1987.

Grizard, J.; Dardevet, D.; Papet, I.; Mosoni, L.; Mirand, P.; Attaix, D.; Arnal, M. Nutrient Regulation of Skeletal Muscle Protein Metabolism in Animals. The Involvement of Hormones and Substrates. **Nutrition Research Reviews**, v. 8(1), p. 67-91, 1995. doi:10.1079/NRR19950007

Herrera-Marcos, L. V.; Lou-Bonafonte, J. M.; Martinez-Gracia, M. V.; Arnal, C, Navarro, M. A.; Osada, J. Prenylcysteine oxidase 1, a pro-oxidant enzyme of low density lipoproteins. **Front Biosci**, v. 123, p. 1020-1037, 2018. doi: 10.2741/4631.

Hocquettea, J. F.; Ortigues-Marty, I.; Pethick, D.; Herpin, P.; Fernandez, X. Nutritional and hormonal regulation of energy metabolism in skeletal muscle of meat-producing animals**. Livestock Production Science**, v. 56 (2), p. 115-143. 1998. doi:10.1016/S0301-6226(98)00187-0

Hughes, J.; Clarke, F.; Li, Y.; Purslow, P.; Warner, R. Differences in light scattering between pale and dark beef longissimus thoracis muscles are primarily caused by differences in the myofilament lattice, myofibril and muscle fibre transverse spacings. **Meat Science***,* v. 149, p. 96 –106, 2019.

Kanehisa, M.; Sato, Y.; Furumichi, M.; Morishima, K.;Tanabe, M.; New approach for understanding genome variations in KEGG. **Nucleic Acids Res**, v. 47, p. D590-D595, 2019.

KAnehisa, M.; Goto, S.; KEGG: Kyoto Encyclopedia of Genes and Genomes. **Nucleic Acids Res**, v. 28, p. 27-30, 2000.

King, D. A.; Morgan, W. W.; Miller, R. K.; Sanders, J. O.; Lunt, D. K.; Taylor, J. F.; Gill, C. A.; Savell, J. W. Carcass merit between and among family groups of Bos indicus crossbred steers and heifers. **Meat Science**, v. 72, p. 496-502, 2006.

Kim, D.; Kim, J.; Hyun, H.; Kim, K.;. Roh, S. A nanoscale ridge/groove pattern arrayed surface enhances adipogenic differentiation of human supernumerary tooth-derived dental pulp stem cells in vitro, **Arch. Oral Bio**l, v. 59 (8), p. 765–774, 2014.

Kirchgessner, T. G.; LeBoeuf, R. C.; Langner, C. A.; Zollman, S.; Chang, C. H.; Taylor, B. A.; Schotz, M. C.; Gordon, J. I.; Lusis, A. J. Genetic and developmental regulation of the lipoprotein lipase gene: loci both distal and proximal to the lipoprotein lipase structural gene control enzyme expression. **J Biol Chem,** v. 264, p. 1473–82, 1989.

Koots, K. R.; Gibson, J.P.; Wilton, J.W. Analysis of published genetic parameters estimates for beef production traits.1 - Herdability. **Animal Breeding Abstract**, v. 62 (5), p. 309-338, 1994.

Kuang, J.; Yan, X.; Genders, A. J.; Granata, C.; Bishop, D.J. An overview of technical considerations when using quantitative real-time PCR analysis of gene expression in human exercise research. **PLoS One**, v. 13 (5), p. e0196438, 2018. doi:10.1371/journal.pone.0196438

Ladu, M. J.; Kapsas, H.; Palmer, W. K. Regulation of lipoprotein lipase in adipose and muscle tissues during fasting. **Am J Physiol**, v. 260 (5), p. R953-9. 1991. doi: 10.1152/ajpregu.1991.260.5.R953.

Li, H. Y.; Zhang, Q. G.; Chen, J. W.; Chen, S. Q.; Chen, S. Y. The fibrotic role of phosphatidylinositol-3-kinase/Akt pathway in injured skeletal muscle after acute contusion. **Int. J. Sports Med**, v. 34, p. 789–794. 2013.

Li, H.; Chen, J.; Chen, S.; Zhang, Q.; Chen, S. Antifibrotic effects of Smad4 small interfering RNAs in injured skeletal muscle after acute contusion. **Int. J. Sports Med**, v. 32, p. 735–742. 2011.

Liang, S.; Hu, Y.; Liu, C. *et al.* Low insulin-like growth factor 1 is associated with low high-density lipoprotein cholesterol and metabolic syndrome in Chinese nondiabetic obese children and adolescents: a cross-sectional study. **Lipids Health Dis**, v. 15, p. 112, 2016. doi.org/10.1186/s12944-016-0275-7

Martins T. S.; Sanglard. L. M. P.; Silva. W.; Chizzotti. M. L.; Rennó. L. N.; Serão. N. V. L.; Silva. F. F.; Guimarães. S. E. F.; Ladeira. M. M.; Dodson. M. V.; Du. M.; Duarte. M. S. Molecular Factors Underlying the Deposition of Intramuscular Fat and Collagen

in Skeletal Muscle of Nelore and Angus Cattle. **PLoS ONE**, v. 10. (10), p. e0139943. 2015.

Martins, T. S.; Sanglard, L. M. P.; Silva, W.; Chizzotti, M. L.; Ladeira, M. M.; Serão, N. V. L.; Paulino, P. V. R; Duarte, M. S. Differences in skeletal muscle proteolysis in Nelore and Angus cattle might be driven by Calpastatin activity and not the abundance of Calpain/Calpastatin. **Journal of Agricultural Science**, 155(10), 1669–1676. 2017. doi:10.1017/s0021859617000715

McCormick R. J. Extracellular modifications to muscle collagen: implications for meat quality. **Poultry Science**, v. 78, p. 785–791, 1999.

McCormick R.J. The flexibility of the collagen compartment of muscle. **Meat Science,** v. 36, p. 79. 1994.

Mota, L. F. M.; Bonafé, C. M.; Alexandre, P. A.; Santana, M. H.; Novais, F. J.; Toriyama, E.; Pires, A. V.; Silva, S. L.; Leme, R. P.; Ferraz , J. B.; Fukumasu, H. Circulating leptin and its muscle gene expression in Nelore cattle with divergent feed efficiency. **Journal of Animal Science and Biotechnology**, v. 201, p.78-71, 2017.

Mourão. G. B.; Formigoni. I. B. Entendendo a seleção: seus conceitos e aplicações. In: Turco. Cristiane de Paula (Org.) **Melhoramento genético ao alcance do produtor**. Bebedouro: Editora Scot Consultoria, p. 27- 48. 2006.

Newman, J. C., & Verdin, E. β-hydroxybutyrate: Much more than a metabolite. **Diabetes Research and Clinical Practice**, v. 106(2), p. 173 –181. 2014. doi:10.1016/j.diabres.2014.08.009

Oddy, V.; Speck, P.; Warren, H.; Wynn, P. Protein metabolism in lambs from lines divergently selected for weaning weight. **The Journal of Agricultural Science**, v. 124(1), p. 129-137. 1995. doi:10.1017/S0021859600071331.

Orosz, F.; Olah, J.; Ovadi, J. Triosephosphate isomerase deficiency: New insights into an enigmatic disease. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, v. 1792, p. 1168–1174, 2009.

Preiss-Landi, K.; Zimmermann, R.; Hammerle, G.; Zechner, R. Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. **Curr Opin Lipidol**, v. 13, p. 471–481, 2002.

Pillarisetti, S.; Saxena, U. Lipoprotein lipase as a therapeutic target for dyslipidemia. **Front Biosci**, v. 8, p. d238–241, 2003.

Pereira. A. S. C.; Baldi. F.; Sainz. R. D.; Utembergue. B. L.; Chiaia. H. L. J.; Magnabosco. C. U.; Manicardi. F. R; Araujo. F. R. C.; Guedes. C. F.; Margarido. R. C.; Leme. P. R.; Sobral. P. J. A. Growth performance and carcass and meat quality traits in progeny of Poll Nelore. Angus and Brahman sires under tropical conditions. **Animal Production Science**, v. 55 (10), p. 1295-1302. 2015.

Poleti, M. D.; Regitano, L. C. A.; Souza, G. H. M. F.; Cesar, A. S. M.; Simas, R. C.; Silva-Vignato, B.; Coutinho, L. L. ***Longissimus dorsi*** **muscle label-free**

quantitative proteomic reveals biological mechanisms associated with intramuscular fat deposition. Journal of Proteomics, v. 179, pp. 30-41, 2018. 10.1016/j.jprot.2018.02.028

Poleti, M. D.; Regitano, L. C. A.; Souza, G. H. M. F.; Cesar, A. S. M.; Simas, R. C.; Silva-Vignato, B.; Montenegro, H.; Pértille, F.; Balieiro, J. C. C.; Cameron, L. C.; Eler, J. P.; Coutinho, L. L. Proteome alterations associated with the oleic acid and cis-9, trans-11 conjugated linoleic acid content in bovine skeletal muscle. **Journal of Proteomics**, v. 222, p. 103792, 2020. doi.org/10.1016/j.jprot.2020.103792

Reiche, A. M.; Oberson, J. L.; Silacci, P.; Messadene-Chelali, J.; Hess, H. D.; Dohme-Meier, F.; Terlouw, E. M. C. Pre-slaughter stress and horn status influence physiology and meat quality of young bulls. **Meat Science***,* v. 158, p. 107892. 2019.

M.Q. Ren, J. Wegner, O. Bellmann, *et al.* **Comparing mRNA levels of genes encoding leptin, leptin receptor, and lipoprotein lipase between dairy and beef cattle. Domest. Anim. Endocrinol**, v. 23, p. 371-381, 2002. doi.org/10.1016/S0739-7240(02)00179-0

Reverter, A.; Johnston, D. J.; Ferguson, D. M; Perry, D.; Goddard, M. E.; Burrow, H. M.; Oddy, V. H.; Thompson, J. M.; Bindon, B. M. Genetic and phenotypic characterization of animal carcass and meat quality traits for temperate and tropically adapted beef breeds. Correlations among animal carcass and meat quality. **Australian Journal of Agricultural Research**, v. 54, p. 149-158, 2003.

Riggs, B.P. K.; Vaughn, R. K. DNA markers for beef tenderness in cattle. **Patent Application Publication**. United States. Pub. No.: US 2015/0344952 A1. 2015.

Rodrigues, R.T.S.; Chizzotti, M. L.; Vital, C. E.; Baracat-Pereira, M. C.; Barros, E.; Busato, K. C.; Gomes, R. A.; Ladeira, M. M.; Martins, T. S. Differences in Beef Quality between Angus (*Bos taurus taurus*) and Nelore (*Bos taurus indicus*) Cattle through a Proteomic and Phosphoproteomic Approach. **PLoS ONE**, v. 12 (1), p. e0170294. 2017. doi:10.1371/journal.pone.0170294

Rompala, R. E., & Jones, S. D. Changes in the solubility of bovine intramuscular collagen due to nutritional regime. **Growth**, v. 48 (4), p. 466–472.1984.

Sangalli, J. R.; Chiaratti, M. R.; De Bem, T. H. C.; de Araujo, R. R.; Bressan, F. F.; Sampaio, R.V.; Perecin, F.; Smith, L. C; King, W. A.; Meirelles, F. V. Development to Term of Cloned Cattle Derived from Donor Cells Treated with Valproic Acid. **Plos One**, v. 9(6). pmid:24959750. 2014.

Santana, M. H. A.; Polizel, G. H. G.; Grigoletto, L.; Fernandes, A. C.; Bonin, M. N.; Gomes, R. C.; Carvalho, M. E.; Silva, S. L; Leme, P. R.; Junior, P. R.; Ferraz, J. B. S. The genetic and genomic effects of Nelore lineages on feed efficiency, intake and performance. **Livestock Science**, v. 2019, p. 1, 2019.

Sapp. R. L.; Bertrand. J. K.; Pringle. T. D.; E Wilson. D. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. **Journal Animal Science**, v. 80, p. 2017–2022. 2002.

Sartori, R.; Bastos, M. R.; Baruselli, P. S.; Gimenes, L. U.; Ereno, R. L.; Barros, C. M. Physio-logical differences ans implications to reproductive management of BosTaurus and Bos indicus cattle in a tropical environment. **Soc Reprod Fertil Suppl**, v. 67, p. 357- 375. 2010; doi.org/10.5661/RDR-VII-357.

Schiaffino, S.; Dyar, K. A.; Ciciliot. S.; Blaauw, B.; Sandri, M. Mechanisms regulating skeletal muscle growth and atrophy. **The FEBS Journal**. v. 280 (17), p. 4294-314. 2013. doi: 10.1111/febs.12253.

Schiaffino, S.; Mammucari, C. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: Insights from genetic models. **Skeletal Muscle**, p. 1-4. 2011.

Silva, J.; Cônsolo, N.R.B.; Buarque, V.L.M.; Beline, M.; da Silva Martins, T.; Lobo, A.A.G.; Gómez, J.F.M.; Eler, J.P.; Leme, P.R.; Netto, A.S.; Gerrard, D.E.; Baldi, F.; Silva, S.L. Effect of Different Selection Criteria on Performance, Carcass and Meat Quality of Nellore Young Bulls. *Agriculture*, v. *11*, p. 294, 2021. https://doi.org/10.3390/agriculture11040294

Sisto, M.; Lorusso, L.; Ingravallo, G.; Tamma, R.; Ribatti, D.; Lisi, S. The TGF-β1 Signaling Pathway as an Attractive Target in the Fibrosis Pathogenesis of Sjögren's Syndrome. **Mediators of inflammation**, v. 2018, p. 1965935, 2018. https://doi.org/10.1155/2018/1965935

Smith, G.; Griffin, D.; Johnson, K. Meat Evaluation Handbook. 2. Ed. Champaign, Illinois: **American Meat Science Association**, 2013. 160 p.

Succurro, E.; Arturi, F.; Grembiale, A. et al. Positive association between plasma IGF1 and high-density lipoprotein cholesterol levels in adult nondiabetic subjects. **European Journal of Endocrinology**, v. 163(1), p. 75-80, 2010. DOI: 10.1530/eje-10-0113.

Teixeira, P. D.; Oliveira, D. M.; Chizzotti, M. L.; Chalfun-Junior A.; Coelho, T. C.; Gionbelli, M.; Paiva, L. V.; Carvalho, J. R. R.; Ladeira, M. M. Subspecies and diet affect the expression of genes involved in lipidmetabolism and chemical composition of muscle in beef cattle. **Meat Science**, v. 133, 110-118, 2017 doi.org/10.1016/j.meatsci.2017.06.009.

The UniProt Consortium, UniProt: the universal protein knowledgebase in 2021, Nucleic Acids Research, v. 49 (1), p. D480–D489, 2021. https://doi.org/10.1093/nar/gkaa1100

United States Department of Agriculture. Livestock and poultry: world markets and trade [Internet]. USDA; [updated 2021 Jan;]. http://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf

Wai, Y. A. L.; Yoke, Y. C.; Shih, Y. L.; So, H. T.; Khalid, A. K.; Asharifah, N. A. S. H. Lipoprotein lipase expression, serum lipid and tissue lipid deposition in orally-administered glycyrrhizic acid-treated rats. **Lipids in Health and Disease**, v. 8, p. 31. 2009.

Woessner, J. F. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. **Archives of Biochemistry and Biophysics**, v. 93, p. 440–447, 1961.

Wong, M. L; Medrano, J. F. Real-time PCR for mRNA quantitation. Review. **Bio Techniques**., v. 39. p. 75-85. 2005.

Woo, J. S.; Jeong, S. Y.; Park, J. H. et al. Calsequestrin: a well-known but curious protein in skeletal muscle. **Exp Mol Med**, v. 52, p. 1908–1925, 2020. doi.org/10.1038/s12276-020-00535-1

Zapata, I.; Zerby, H. N.; Wick, M. Functional Proteomic Analysis Predicts Beef Tenderness and the Tenderness Differential. **Journal of Agricultural and Food Chemistry,** v. 57, p. 4956–4963, 2009. doi:10.1021/jf900041j.

Zeng, J.; Zhou, S-W.; Zhao, J. et al. Role of OXCT1 in ovine adipose and preadipocyte differentiation Biochem Biophys Res Commun, v. 512 (4), p. 779-785. 2019. doi.org/10.1016/j.bbrc.2019.03.128

Zhao, L.; Huang, Y.; Du, M. Farm animals for studying muscle development and metabolism: dual purposes for animal production and human health. Animal frontiers: the review magazine of animal, v. 9(3), p. 21–27. 2019. doi.org/10.1093/af/vfz015

Zhang, Y.; Wang, Y.; Wang, H., Ma, X.; Zan, L. MicroRNA-224 impairs adipogenic differentiation of bovine preadipocytes by targeting LPL. **Molecular and Cellular Probes**, v. 44. p. 29–36. 2019. doi.org/10.1016/j.mcp.2019.01.005

Zhong, V. W.; Van, Horn, L.; Greenland, P. et al. Associations of Processed Meat, Unprocessed Red Meat, Poultry, or Fish Intake With Incident Cardiovascular Disease and All-Cause Mortality. **JAMA Intern Med**, v. 180(4), p. 503–512, 2020. doi:10.1001/jamainternmed.2019.6969

CAPÍTULO 4

Subcutaneous fat proteomic analysis reveals pathways related to fat deposition from beef cattle with expected progeny difference for precocity

Martins et al., 2021

MARTINS, T. S. Subcutaneous fat proteomic analysis reveals pathways related to fat deposition from beef cattle with expected progeny difference for precocity.2021

ABSTRACT

The present study hypothesizes that genetic selection for precocity and growth affects the subcutaneous fat proteome and consequently lipid metabolism of the Zebu cattle. Then, the present study aimed to evaluate the effects of contrasting EPD's for precocity and growth on subcutaneous fat by differential proteomic analysis and correlations with phenotypic traits, such as backfat thickness, marbling, total lipid, IGF-1, GH, leptin, and insulin. 105 male bulls were used, with a mean age of 20 ± 2 months and 400 ± 24 kg, from the same herd, with the genetic information of precocity and growth. The animals remained confined for 100 days, and the carcass ultrasound was performed every 28 days. Blood samples was collected to determine the metabolic and hormonal profile. The animals were slaughtered after feedlot 100 days and during slaughter, backfat thickness (BFT) was collected between the 12th and 13th ribs, in addition to visceral fat (GV). All of these were immediately frozen in liquid nitrogen and kept in a freezer - 80ºC until the analysis of gene expression by real-time PCR (RT-qPCR) and analysis and SDS-PAGE, followed by the identification of proteins by coupled mass spectrometry liquid chromatography (LC-MS / MS). After 24 hours slaughter, during boning, the intramuscular fat (MAR) in the LT muscle was evaluated. Steaks for total lipids, fatty acid profile, and collagen solubility were also collected. The animals were selected according to a EPD from their parents (bulls). Were selected 6 parents with EPD contrasting simultaneously for precocity and growth, so that each experimental group had 3 parents bulls. Then, were formed, 2 contrasting groups called high EPD (H_EPD; N = 16) and low EPD (L_EPD; N = 16), using 32 progenies. The animals in the H_EPD group had higher EGS (P = 0.006); lower LDL (P = 0.014); higher IGF-1 (P = 0.064). This group also showed greater expression of the genes involved in lipogenesis evaluated in BFT: ACACA (P = 0.060), LPL (P = 0.085), ACOX1 (P = 0.100), LEP (P = 0.030), SDC (P = 0.009), and GAPDH (P = 0.081), than the animals in the L_EPD group. Three electrophoretic bands were detected as differently abundant in the BFT (bands 24, 30, 32), and 40 proteins identified. The KEGG pathways of pyruvate metabolism, glycolysis/gluconeogenesis, carbon metabolism, amino acid biosynthesis, among others, were enriched. The genetic selection for precocity and growth affects the muscle proteome and consequently the lipid and protein metabolism of non-castrated cattle. The IGF-1 hormone, the GAPDH, LDHA, LDHB, MDH1, MDH2, IDH1, PGK1, SUCLG2, and ACY1 proteins can be considered future biomarkers candidates for BFT.

Keywords: Beef Cattle. Backfat thickness. Metabolic pathways. Protein and lipid metabolism. Proteomics.

1 INTRODUCTION

Fat deposition in beef cattle is affected by the interaction between nutrition, sexual condition, age, ruminal bio-hydrogenation, and genetics (TEIXEIRA et al., 2017). Additionally, several hormones are directly related to fat deposition and composition in the carcass and meat, such as leptin, IGF-1, interleukins and resistin (MINER, 2004). A critical hormone also involved in the synthesis of fat is insulin, which stimulates the capture of glucose by peripheral tissues and increases lipogenesis or reduces lipolysis (RHOADES et al., 2007).

Different metabolic pathways regulate the deposition of subcutaneous fat from those that regulate the intramuscular fat. Intramuscular adipocytes show the greater activity of hexokinase and phosphofructokinase enzymes and use glucose as the main substrate. On the other hand, subcutaneous adipose tissue has higher levels of lipogenic enzymes, such as NADP-malate dehydrogenase, phosphogluconate-6-dehydrogenase, and glucose-6-phosphate dehydrogenase, and preferably uses propionate and acetate as a precursor, evidencing exclusive roles in lipid metabolism (MILLER et al., 1991; MAY et al., 1994). Hereupon, depending on the type and distribution of adipose tissue (visceral, subcutaneous or intramuscular), it has been shown that the metabolism of these types of fat is different, including that visceral fat is much more metabolically active than subcutaneous fat (MELENDEZ et al., 2019).

In tropical conditions, beef production is mainly from Zebu cattle, which are known to produce low-fat content beef when compared to taurine breeds animals. In this context, the heterogeneous quality of Brazilian cattle carcasses, mainly from the Nellore breed, has been identified as a challenge for the beef industry. Precocity and growth are economically relevant traits for the identification of harmonious animals that have been used world with as a basis for the selection and improvement of beef cattle (ELER et al., 2006).

The expected differences in progeny (EPDs) are important tools for animal production and selection. Growth and precocity are generally useful selection criteria for beef cattle. The phenotypes of subcutaneous fat thickness and *Longissimus* muscle area are very easy to measure using real-time ultrasound (YOKOO et al., 2010) and can provide accurate data for estimating genetic values, as reported for young bulls (ROBINSON, 1996). Improvements in

average daily gains, body weight, muscle mass, and fat deposition in the carcass have been reported using genetic merit for growth and precocity as selection criteria (CLARKE et al., 2009). Lopes et al. (2016), states that the *longissimus thoracis* muscle area and the subcutaneous fat thickness measured by real-time ultrasound should be used as a selection criterion. This allowed the estimation of reproduction values before the first mating season, to accelerate the response to individual selection.

However, little is known about the real reason (biological, physiological, or molecular), which stimulates animals with better EPDs for precocity, to present, in general, progenies with greater subcutaneous fat thickness. So, understanding the biological mechanisms involved in the subcutaneous fat deposition in beef can allow great advances in this important economic trait.

Proteomics analyzes can be used to understand the molecular mechanisms involved in subcutaneous fat deposition and to identify potentials biomarkers related to EGS. However, as far as we know, few works have been reported to understand or predict the ability of subcutaneous fat deposition in the carcass (CECILIANI et al., 2018). Most of the available proteomic studies were made using muscle samples with a focus on intramuscular and non-subcutaneous fat deposition pathways (POLETI et al. 2018; RODRIGUES et al., 2017; MAO et al., 2016; SHEN et al., 2012; ZHANG et al., 2010; KIM et al., 2009).

Therefore, the present study hypothesizes that genetic selection for precocity and growth affects the subcutaneous fat proteome and consequently lipid metabolism of the Zebu cattle. Then, the present study aimed to evaluate the effects of contrasting EPD's for precocity and growth on subcutaneous fat by differential proteomic analysis and correlations with phenotypic traits, such as backfat thickness, marbling, total lipid, IGF-1, GH, leptin, and insulin.

2 MATERIAL AND METHODS

The experimental procedures were approved by the Ethics Committee on Animal Use (CEUA/FMVZ) from the School of Veterinary Medicine and Animal Science of the University of Sao Paulo (Protocol number 3367170317), Pirassununga, Sao Paulo, Brazil.

2.1 Animals and experimental design

The animals used in this study were selects from a larger project evaluating the effects of EPD groups on animal performance, carcass, and meat quality traits (Silva et al., 2021). The animals belonged to USP's genetic selection program, with genetic information for precocity and growth. One hundred and five Nelore bulls, not castrated, with initial body weight (BW) of 400 ± 24 kg and 20 ± 2 months of age were ranked based on their parent's Expected Progeny Difference (EPD), for precocity and growth and classified as either High and Low. Three bulls Nelore with high EPD were parents of animals in the H_EDP group (average EDP for precocity and growth = 0.57 and 12.32 respectively), and three bulls Nelore with low EPD were parents of animals in the L_EPD group (average EPD for precocity and growth = - 0.05 and - 3.67 respectively). The phenotypic traits were evaluated in 32 progeny (High_EPD, N=16; Low_EPD, N=16). Posteriorly, 14 animals were selected with either highest (H_EPD, N = 7) or lowest (L_EPD, N = 7) and contrasting deposition of subcutaneous fat to form to the groups that were tested for differential proteomic analysis.

The animals was housed in a feedlot facility for 100 days, and individual feed intake was monitored. Facilities had covered feed bunks, concrete floors, and automatic waterers. All animals were initially submitted to adaptation period of 21 days. At the ending of the adaptation period all animals were weighed after a 12 h fasting period prior to the morning feeding. During the feedlot period, the animals were fed ad libitum twice daily, at 0800 h and 1600 h whit a diet (27:73 forage: concentrate) containing corn silage, corn grain, ground sorghum, soybean meal, citrus pulp, urea, and mineral core. All animals were weighed after a 12 h fasting to record the final body weight immediately prior to slaughter. The average daily gain was obtained by the difference of final body weight and initial body weigh divided by the 100 days of feeding.

2.2 Carcass ultrasound

Carcass ultrasonography was performed every 28 days to assess rib eye area (REA) and backfat thickness (BFT) in the *longissimus thoracis*, in the region between the 12th and 13th ribs, and rump fat thickness (RFT), using the Aloka

ultrasound equipment. SSD 500 Micrus model (Aloka Co. Ltda.), with 3.5 MHz linear transducers and 17.2 cm in length. The images were stored in the portable microcomputer and records were subsequently interpreted in laboratory using the Lince® software.

2.3 Blood samples and biochemical and metabolic parameters

Blood samples were *harveste*d before slaughter, at the end of the finishing period by puncturing the jugular vein or artery before the morning feeding, with fasting the previous night. Blood samples (10 ml) were collected into 10-ml tubes (BD Vacutainer, São Paulo, SP, Brazil), without anticoagulant, for hormones analyses and metabolic parameters. Samples were centrifuged for 15 min at 2000×g at 4 °C; the supernatant serum was transferred into labeled plastic tubes and stored at −80°C until analysis.

Serum concentrations of glucose, triglycerides, total cholesterol, and high-density lipoprotein (HDL) were determined by Immunoenzymatic procedure using Bioclin Diagnostics kits (Belo Horizonte, Brazil) in automated biochemical equipment (Mindray BS-200E), according to manufacturer's instructions. Serum values of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were calculated according to Friedewald et al. (1972), being, VLDL = triglycerides/5; and LDL = total cholesterol – (HDL + VLDL).

Insulin, leptin, GH (growth hormone), and IGF-1 (insulin-like growth factor-1) hormones were determined by the Immunoenzymatic Assay method, according to BioElisa Microplate Reader New Biotechnology and Sigma kits (Bioclin Quibasa, Belo Horizonte, Brazil), following manufacturer's standards.

2.4 Harvesting subcutaneous fat and meat sampling

At the end of the feeding phase, the animals were slaughtered in the experimental slaughterhouse of the University of São Paulo in Pirassununga, Brazil, according to humanitarian slaughter guidelines, as required by Brazilian law (Brasil 2017). Subcutaneous fat samples were collected from the right half of the carcass at the 12th rib, snap-frozen in liquid nitrogen, and stored at -80°C until proteins extraction. After a 24h chilling period, 2.54cm thick steaks were cut

from *longissimus thoracis* (LT), of the right half of the carcass, between 12th-13th ribs, vacuum packaged, and frozen at −80°C until total lipids analysis. Besides that, the marbling score (MAR) was evaluated in the LT, between the 12th and 13th rib, following the methodology described by American Meat Science Association (SMITH et al., 2013).

2.5 Total lipids

The intramuscular fat (IMF) content were determined following the methodology of Bligh and Dyer (1959) in duplicate, for total lipids.

2.6 Protein Extraction

Approximately 1.0 g of subcutaneous fat samples were homogenized in 2 mL of RIPA lysis buffer modified containing 150 mm NaCl, 1% Triton X-100, 0,5% sodium deoxycholate, 1% SDS, 50 mM Tris, pH 8.0-plus 1% protease inhibitor cocktail (P8340 Sigma-Aldrich), 1 mM sodium orthovanadate, and 25 mM sodium fluoride, using Ultra Turratec T-10 (IKA) four times for 5 seconds at 30,000 rpm. Afterward, the homogenate was centrifuged at 4 ºC at 10,000 g for 30 minutes. The resulting supernatant was collected and stored at -80 ºC for further analysis. The protein concentration of each sample was measured by the Bradford method, using bovine albumin as standard.

2.7 Unidimensional Electrophoresis - SDS / PAGE

The protein extract was denatured by mixing in a 1:1 ratio in Laemmly sample buffer (Bio-Rad Laboratories Inc., Hercules, CA, USA) and heated at 100 °C for 45 minutes. The samples (50 µg of protein) were loaded into each lane of the 1.5 mm x 16 cm dual slab gel (SE 600 Ruby Standard Dual Cooled Vertical Unit – GE Healthcare) connected to a Power Supply EPS 1001 (GE Healthcare) and run at 15mA/gel for 30 min. and after adjusted at 50 mA/gel for 4 h at 4ºC, for one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were run throught 4% stacking gel, followed by 12% resolving gel. Bio-rad precision plus protein dual color standards (Bio-Rad Laboratories Inc., Hercules, CA, USA) were loaded in the first lane of each gel, to estimate the molecular weight of proteins. After electrophoretic separation,

gels were stained by using Coomassie Brilliant Blue G250 solution (0.025% coomassie blue G250, 50% methanol, 7% acetic acid) for 1 hour, on shaker. The gels were then placed in destained solution (40% methanol, 7% acetic acid) for 1 hour and rinsed in deionized water. The gels were digitalized using Image Scanner III (GE Healthcare) and the quantitative analysis of the gel bands was performed with the aid of the Image Lab^{TM} software (Bio-Rad). The bands were identified and then analyzed to determine the percentage contribution of each band in relation to the total band volume in the lane to avoid the confounding factor of protein loading (Zapata et al., 2009; Zhao et al., 2014).

2.8 In gel protein digestion

The bands detected as differentially abundant in the statistics analysis were removed from the gel, cut in fragments of approximately 1 mm^3 and placed inside 1.5 ml microtubes. Digestion was performed according to the method of Shevchenko et al. (2006). The gel fragments were washed 3 times of 10 minutes each, using 200 µl of a solution coitaning 50% acetonitrile (ACN) in 25 mM ammonium bicarbonate pH 8.0 (AMBIC). Then, the gel fragments were dehydrated for 10 minutes after adding 200 µl of 100% ACN. The ACN was removed and the remaining gel residue was kept at room temperature for complete evaporation. The gel fragments were reduced for 40 min. at 56 ° C with 30 µl of a 20 mM DTT solution in 50 mM AMBIC, after alkylated for 30 min. at room temperature using 40 µl of a 55 mM iodoacetamide solution in 50 mM of AMBIC. The iodoacetamide solution was removed and the gel fragments washed with 200 µl of 25 mM AMBIC. Again, dehydration with 100% ACN was performed twice, as described above. For protein digestion, 15 µL of the trypsin enzyme was added to each sample, at a concentration of 20 ng/µl and incubated for 15 minutes at 4°C. Excess trypsin was removed and 40 µl of 50 mM AMBIC was added. The samples were incubated at 37 ° C for 14 hoursAfter digesting the protein present in the gel, three elution steps were performed. For the first elution, 30 µl of the 5% formic acid was added, and the samples incubated for 10 min. at room temperature. The supernatant was collected and placed in a new tube.For the second elution, 30 µl of the solution made of 5% formic acid in 50% ACN was added, and the samples incubated for 10 minutes at room temperature.The

supernatant was recovered and transferred to the same tube used in the previous wash. For the third elution, the second step was repeated. Finally, samples were vacuum concentrated at room temperature, until complete drying.

2.9 Mass spectrometry analysis

For analysis, the samples were resuspended in 30 µl of a 50% Acetonitrile and 0.1% TFA solution, and. an aliquot of 4.5 ul of proteins resulting of peptide digestion was were separated by C18 (100 mm6100 mm) RP-nanoUPLC (nanoAcquity, Waters) coupled with a Q-Tof Premier mass spectrometer (Waters) with nanoelectrospray source at a flow rate of 0.6 ml/min. The gradient was 2–90% acetonitrile in 0.1% formic acid over 60 min. The nanoelectrospray voltage was set to 3.5 kV, a cone voltage of 30 V and the source temperature was 100uC. The instrument was operated in the 'top three' mode, in which one MS spectrum is acquired followed by MS/MS of the top three most-intense peaks detected. After MS/MS fragmentation, the ion was placed on exclusion list for 60 s and for the analysis of endogenous cleavage peptides, a real time exclusion was used.

The spectra were acquired using software MassLynx v.4.1 and the raw data files were converted to a peak list format (mgf) without summing the scans by the software Mascot Distiller v.2.3.2.0, 2009 (Matrix Science Ldt.) and searched against UniProt *Bos Taurus* (release March 23, 2020; 37.513 proteins) and *Bos indicus* database (release March 21, 2020; 42,151 proteins) using Mascot engine v.2.3.01 (Matrix Science Ltd.), with carbamidomethylation as fixed modifications, oxidation of methionine as variable modification, one trypsin missed cleavage and a tolerance of 0.1 Da for both precursor and fragment ions (ARAGAO et al., 2012).

2.10 Statistical analysis

Phenotypic data

The data of phenotypic traits were analyzed according to a completely randomized design, with 16 repetitions per treatment, each animal being considered an experimental unit. The model includes the parent (bull) as a

random effect and considers the groups (H_EPD and L_EPD), confinement, and slaughter as a fixed effect. The analyses were conducted using SAS software (9.4; SAS Institute Inc., Cary, NC, USA). The significance was considered when $P < 0.10$.

SDS-PAGE and Correlation between the significant electrophoretic bands

The data were analyzed according to a completely randomized design, with 7 repetitions per treatment, each animal being considered an experimental unit. The model includes the parent (bull) as a random effect and considers the groups (H_EPD and L_EPD), confinement, and slaughter as a fixed effect. For one-dimensional gel analysis, ANOVAs were performed using the SAS software for relative migration (RFs) and molecular weight (MWs). This initial analysis aimed to verify if we were making comparisons of proteins within the same point of the Gel (both for RF and MW). In this case, ANOVAs for RFs and MWs should have always presented non-significant results, indicating that there were no significant changes within each evaluated band. From this preliminary analysis for RFs and MWs, ANOVAs were performed using the SAS software for % Band to detect the differences between the abundance of each band between the H_EPD and L_EPD groups using the test Tukey-Kramer considered significant at *P*-value < 0.10. The correlations between the electrophoretic bands and the phenotypes were estimated by Pearson's correlation coefficient (r), within and between groups, and the data were considered significant when $P < 0.10$.

Protein identification and functional enrichment

The identified proteins were considered candidates when the global Mascot score was greater than 80 with a significance level of $P \leq 0.0001$.

Subsequently, the list of proteins identified in each band was submitted to over-representation analysis using WebGestalt web tool (LIAO et al., 2019) to identify the biological processes and KEGG pathways enriched (FDR < 0.05), and also analysis of Protein-Protein Interaction Networks were performed by STRING software.

3 RESULTS

3.1 Phenotypic traits

The average daily gain (ADG) did not differ between the groups (P = 0.459; Table 1). Similarly, the dry matter intake (DMI) did not differ between the groups (P = 0.288; Table 1), such as the final body weight (fBW, P = 0.254; Table 1). On the other hand, its backfat thickness (BFT) and rump fat thickness (RFT), measured by carcass ultrasonography, was higher in H_EPD group animals than L_EPD group animals (P < 0.1; Table 1), indicating higher precocity of animals from the H_EPD group. Besides this, the marbling score (MAR) was higher in H_EPD group animals compared to L_EPD group animals (P < 0.1; Table 1), despite this, the content of intramuscular fat (IMF) did not differ between animals in the evaluated groups (P = 0.149).

Table 1. Effect of the groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity on the phenotypic traits of Nelore bulls (n=16)

Traits	**GROUP**		***P-value****
	H_EPD	**L_EPD**	
Initial body weight, kg	413.60±9.86	405.37±5.06	0.517
Final body weight, Kg	598.00±11.72	581.62±8.04	0.254
Average daily gain, kg	1.85±0.13	1.76±0.14	0.459
Dry matter intake, kg	12.54±0.77	11.01±0.84	0.288
Backfat thickness[1], mm	5.87±0.50	3.78±0.48	0.006
Rump fat thickness[1], mm	8.56±0.58	7.05±0.69	0.086
Intramuscular fat[2]	2.23±0.34	1.34±0.31	0.149
Marbling score [3]	4.57±0.17	4.01±0.15	0.081

* Significant difference considered when P <0.1
[1] Measured by carcass ultrasonography
[2] Intramuscular fat determined by total lipids analysis (g of lipid / 100 g of meat; Bligh & Dyer, 1959)
[3] Marbling score determined by AMSA, considered select slight: 4.0–4.9; choice small: 5.0–5.9; choice modest: 6.0–6.9; choice moderate: 7.0–7.9; prime: 8.0–9.0 (Smith et al., 2013)

3.2 Biochemical and metabolic parameters

The serum insulin (P = 0.127), GH (P =0.914), and leptin hormone (P =0.839) did not differ between the groups of high and low EPD (Table 2). On the other, the IGF-1 hormone was greater in H_EPD group animals than L_EPD group animals (P < 0.1; Table 2). Among the metabolites evaluated, the Cholesterol and the LDL were higher in L_EPD group animals than H_EPD group animals (P < 0.1; Table 2). There was no difference between groups for glucose

serum (P = 0.239; Table 2), triglycerides, HDL (P = 0.462; Table 2), and VLDL (P = 0.886; Table 2).

Table 2. Effect of the groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity on the hormonal and metabolic serum traits from Nelore bulls (n=16)

Variable	**GROUP**		***P-value****
	H_EPD	**L_EPD**	
Hormone			
Insulin, ng/mL	4.34±0.98	1.53±1.06	0.127
GH, UI/mL	1.32±0.20	1.27±0.22	0.914
IGF-1, ng/mL	0.29±0.03	0.22±0.02	0.064
Leptin, pg/mL	6.16±3.56	7.54±3.85	0.839
Metabolite			
Glucose, mg/dl	81.75±5.64	94.64±6.09	0.239
Cholesterol, mg/dl	158.45±1.68	222.66±1.82	0.058
Triglycerides, mg/dl	36.77±3.99	37.84±4.31	0.887
HDL, mg/dl	45.40±5.23	38.02±5.64	0.462
LDL, mg/dl	105.69±14.02	177.05±15.16	0.014
VLDL, mg/dl	7.35±0.79	7.57±0.86	0.886

* Significant difference considered when P <0.1
GH = *growth hormone*; IGF-1 = *insulin-like growth factor-1*; HDL **=** *High Density Lipoproteins*; LDL = *Low Density Lipoproteins;* VLDL = *Very Low Density Lipoprotein*

3.3 SDS-PAGE and correlation between the significant electrophoretic bands

The SDS-PAGE analysis allowed the revelation of 37 bands total from subcutaneous fat that was electrophoretically separated by molecular weight and matched across all samples (Figure 1). Three electrophoretic bands were differentially abundant between the groups H_EPD and L_EPD by statistical analysis (bands 24, 30, and 32; Table 3).

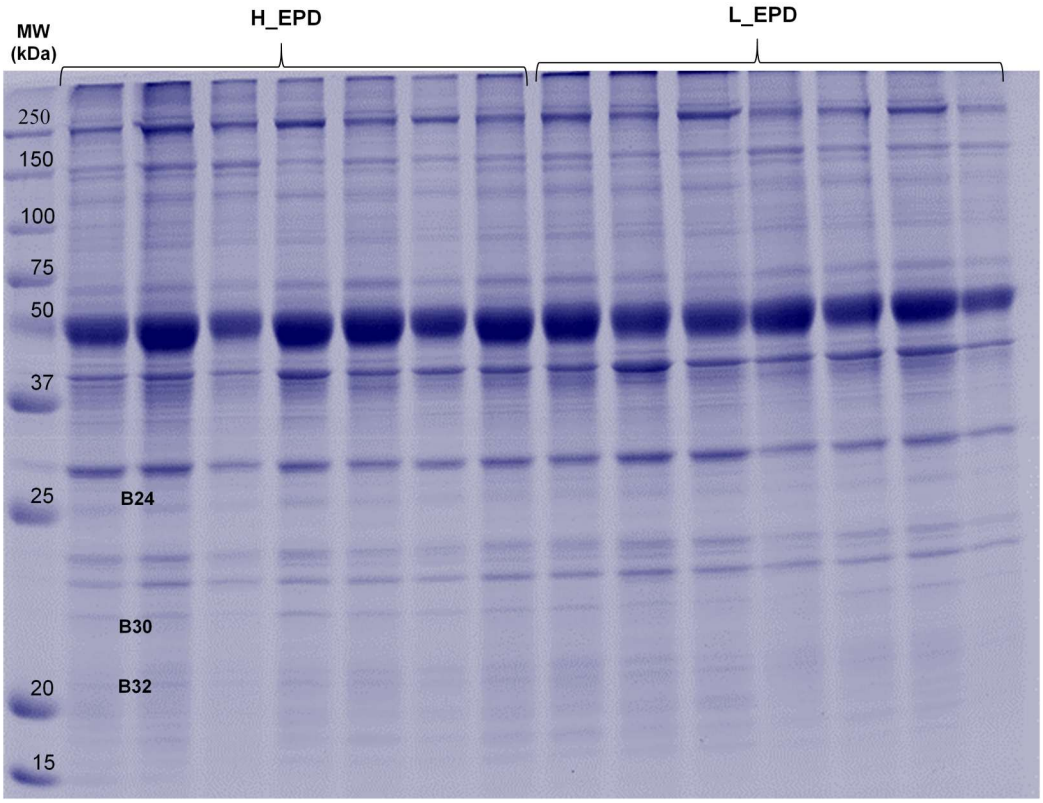

Figure 1. One-dimensional electrophoresis gel (SDS-PAGE 12%) from subcutaneous fat of Nelore bulls with high and low expected differences in progeny for growth and precocity.

Table 3. Bands of one-dimensional electrophoresis gel, from subcutaneous fat differentially abundant between the groups of Nelore bulls with high (H) and low (L) expected differences in progeny (EPD)

Band	GROUP								*P-value*
	H_EPD				L_EPD				
24	5.28	±	1.15	A	0.57	±	1.15	B	0.063
30	1.94	±	0.34	A	0.63	±	0.34	B	0.073
32	0.64	±	0.23	B	1.52	±	0.23	A	0.074

Means in the same row followed by different letters are significantly different by Tukey-Kramer test considered a significant difference for $P < 0.1$

Significant electrophoretic bands were correlated with productive, biologic, and qualitative phenotypes, such as weight gain, hormones, marbling, and subcutaneous fat deposition measured by carcass ultrasound. The correlations were investigated independent and within groups (H_EPD and L_EPD). These results were presented in Table 4A and Table 4B).

Independent of groups, the serum insulin content was positively correlated with band 30 (r = 0.63, P= 0.02; Table 4B). While, beef marbling was positively correlated with band 24 (r = 0.76, P=0.002; Table 4A) and band 30 (r = 0.65, P=0.01; Table 4A). Additionally, positive correlations were observed between beef marbling and the bands 24 (r = 0.80, P=0.03; Table 4A) and 30 (r = 0.86, P=0.01; Table 4A); and between the Intramuscular fat and band 30 (r = 0.77, P=0.04; Table 4A) within H_EPD group. Besides, the results showed a great negative correlation between band 32 and average daily weight gain (r = - 0.85, P=0.01; Table 4A). Furthermore, serum insulin levels were positively correlated with band 30 within the L_EPD group (r = 0.93, P= 0.006; Table 4B).

Table 4A. Pearson correlation coefficient between the differentialy abundant bands from subcutaneous fat of Nelore bulls with the phenotypic traits

	Pearson's correlation coefficient (r)								
	Independent of group, N = 13			H_EPD, N = 7			L_EPD, N = 6		
	Band_P-24	Band_P-30	Band_P-32	Band_P-24	Band_P-30	Band_P-32	Band_P-24	Band_P-30	Band_P-32
iBW									
fBW									
DMI									
ADG						**-0.85****			
BFT									
RFT									
MAR	**0.76****	0.65**		**0.80***	**0.86****	-0.67*			
IMF				0.70*	**0.77***				

iBW = Initial body weight; fBW = Final body weight; DMI = Dry matter intake; ADG = Average daily gain; BFT = Backfat thickness; RFT= Rump fat thickness; MAR = Marbling score ; IMF = Intramuscular fat. Significance: * P < 0.05; ** P < 0.01. The coefficients higher than 0.75 are in bold.

Table 4B. Pearson correlation coefficient between the differentialy abundant bands from subcutaneous fat of Nelore bulls with the Hormonal and metabolic serum traits

	Pearson's correlation coefficient (r)								
	Independent of group, N = 13			H_EPD, N = 7			L_EPD, N = 6		
	Band_P-24	Band_P-30	Band_P-32	Band_P-24	Band_P-30	Band_P-32	Band_P-24	Band_P-30	Band_P-32
CHOL									
TRIG									
HDL									
LDL									
VLDL									
INS		0.63*						**0.93****	
GH									
IGF1									
LEP									

CHOL = cholesterol; TRIG = triglycerides; HDL **=** *High Density Lipoproteins;* LDL = *Low Density Lipoproteins;* VLDL **=** *Very Low Density Lipoprotein;* INS = Insulin; GH = *growth hormone*; IGF-1 = *insulin-like growth factor-1*; LEP = Leptin.
Significance: * P < 0.05; ** P < 0.01. The coefficients higher than 0.75 are in bold.

3.4 Mass spectrometry analysis and identification of fat proteins

The differentially abundant bands were cut from the gel and submitted to mass spectrometry analysis. A total of sixteen proteins were identified in band 24, thirteen proteins for band 30, and sixteen proteins for band 32. In Table 5, we reveal the top 10 proteins identified in each band. It is important to mention that one of the limitations of using one-dimensional electrophoresis is the comigration of proteins within a single band (Zapata et al., 2009; Díaz et al., 2020).

The list of proteins identified in each band was submitted to over-representation analysis using WebGestalt web tool to identify the KEGG pathways that are related to lipid metabolism. The enriched pathways (FDR ≤ 0.05) found are shown in Table 6. No enriched pathway was found for band 32 (FDR > 0.05; Figure 2). The distribution of proteins identified in each band within GO categories (biological process, cellular component, and molecular function) can be found in the (Figure 3).

Additionally, a network of predicted protein-protein interactions against the *Bos taurus* database was performed for the top 10 proteins in each band differentially abundant between the high and low EPD groups, using STRING 10.0 online software (Figure 4).

Table 5. Proteins identified by mass spectrometry in the differentially abundant bands of the SDS-PAGE gel between the groups of Nelore bulls with high and low EPD for growth and precocity

Band	Gene name	Accession Uniprot	Protein name	Score[1]	Mass[2]	Biological process[3]
	CKB	Q5EA61	Creatine kinase B-type	2764	42977	Phosphocreatine biosynthetic process
	ACTG1	P63258	Actin, cytoplasmic 2	1587	42108	Positive regulation of gene expression
	IDH1	A0A140T8A5	Isocitrate dehydrogenase [NADP]	1401	47072	Phospholipid biosynthetic process
	ACTC1	Q3ZC07	Actin, alpha cardiac muscle 1	555	42334	Mesenchyme migration
24	PGK1	Q3T0P6	Phosphoglycerate kinase 1	467	44908	Glycolysis/gluconeogenesis
	ACAA2	A0A3Q1MDH5	3-ketoacyl-CoA thiolase, mitochondrial	377	42428	Lipid metabolism
	ACY1	Q3T0V2	N-acyl-L-amino-acid amidohydrolase	246	41955	Cellular amino acid metabolic process
	OGN	A5D9E8	Mimecan	211	34517	Growth factor
	SUCLG2	Q3MHX5	Succinate--CoA ligase [GDP-forming] mitochondrial	125	47004	Tricarboxylic acid cycle
	RPSA	A0A3Q1MFG3	40S ribosomal protein AS	122	35196	Translation
	ANXA2	P04272	Annexin A2	1940	38873	Calcium ion transmembrane transport
	MDH2	Q32LG3	Malate dehydrogenase, mitochondrial	264	36102	Tricarboxylic acid cycle
	GPD1	Q5EA88	Glycerol-3-phosphate dehydrogenase [NAD(+)]	210	38194	Gluconeogenesis
	LDHB	Q5E9B1	L-lactate dehydrogenase B chain	197	36985	Carbohydrate metabolic process
30	GAPDH	P10096	Glyceraldehyde-3-phosphate dehydrogenase	185	36073	Apoptosis/Glycolysis
	MTARC2	Q1LZH1	Mitochondrial amidoxime reducing component 2	132	37834	Oxidation-reduction process
	MDH1	A0A452DIW4	Malate dehydrogenase	124	36118	Tricarboxylic acid cycle
	LDHA	P19858	L-lactate dehydrogenase A chain	114	36916	Carbohydrate metabolic process
	ANXA1	F1N650	Annexin	95	39240	Insulin secretion

	ANXA5	P81287	Annexin A5	90	36124	Regulation of sequestering of calcium ion
	YWHAE	P62261	14-3-3 protein epsilon	522	29326	Regulation of protein export from nucleus
	PNP	P55859	Purine nucleoside phosphorylase	330	32244	Nucleoside metabolic process
	GAPDH	P10096	Glyceraldehyde-3-phosphate dehydrogenase	179	36073	Apoptosis/Glycolysis
	HSD17B12	A6H7H3	LOC789567 protein	175	35134	Extracellular matrix organization
32	ECHDC1	Q2HJD5	Ethylmalonyl-CoA decarboxylase	122	33806	Fatty acid beta-oxidation
	PSME1	A0A3Q1MK57	Proteasome activator complex subunit 1	98	28812	Proteasome activator complex
	STOM	A8E4P3	STOM protein	94	31497	Regulation of protein targeting to membrane
	CYB5R3	A0A3Q1LMX0	NADH-cytochrome b5 reductase	93	31815	Catalytic activity
	FHL1	G3MZ95	Four and a half LIM domais 1	89	37897	Muscle organ development
	ANXA2	PO4272	Annexin A2	87	38873	Biomineral tissue development

[1]Numeric descriptor of the probability that the protein identification is correct at a *P-value* <0.0001.
[2]Molecular weight of the protein. in KDa.
[3]Biological process described in Uniprot

Table 6. KEGG pathways enriched for the proteins identified in the differentially abundant bands of the SDS-PAGE gel between the groups of Nelore bulls with high and low EPD for growth and precocity

Band	KEGG	Enriched pathways	Protein	*P-value*	FDR[1]
	bta01210	2-oxocarboxylic acid metabolism	ACY1, IDH1	0.00019	0.03112
24	bta00020	Citrate cycle	IDH1, SUCLG2	0.00055	0.04496
	bta01230	Biosynthesis of amino acids	ACY1, IDH1, PGK1	0.00007	0.02550
	bta01200	Carbon metabolism	IDH1, PGK1, SUCLG2	0.00028	0.03112
	bta00620	Pyruvate metabolism	LDHA, LDHB, MDH1, MDH2	9.4510E-05	0.0000030810
	bta00270	Cysteine and methionine metabolism	LDHA, LDHB, MDH1, MDH2	$2.0460E^{-8}$	0.0000033349
	bta00020	Citrate cycle (TCA cycle)	MDH1, MDH2	0.00023163	0.012291
30	bta00630	Glyoxylate and dicarboxylate metabolism	MDH1, MDH2	0.00023163	0.012291
	bta00640	Propanoate metabolism	LDHA, LDHB	0.00026392	0.0122991
	bta00010	Glycolysis / Gluconeogenesis	GAPDH, LDHA, LDHB	0.000014158	0.0015385
	bta01200	Carbon metabolism	GAPDH, MDH1, MDH2	0.000070569	0.0057514
	bta01100	Metabolic pathways	GAPDH, LDHA, LDHB, MDH1, MDH2	0.00082992	0.0.33819

[1]False Discovery Rate – *P-value* corrected for multiple tests within each category using the procedure by Benjamini & Hochberg (1995).

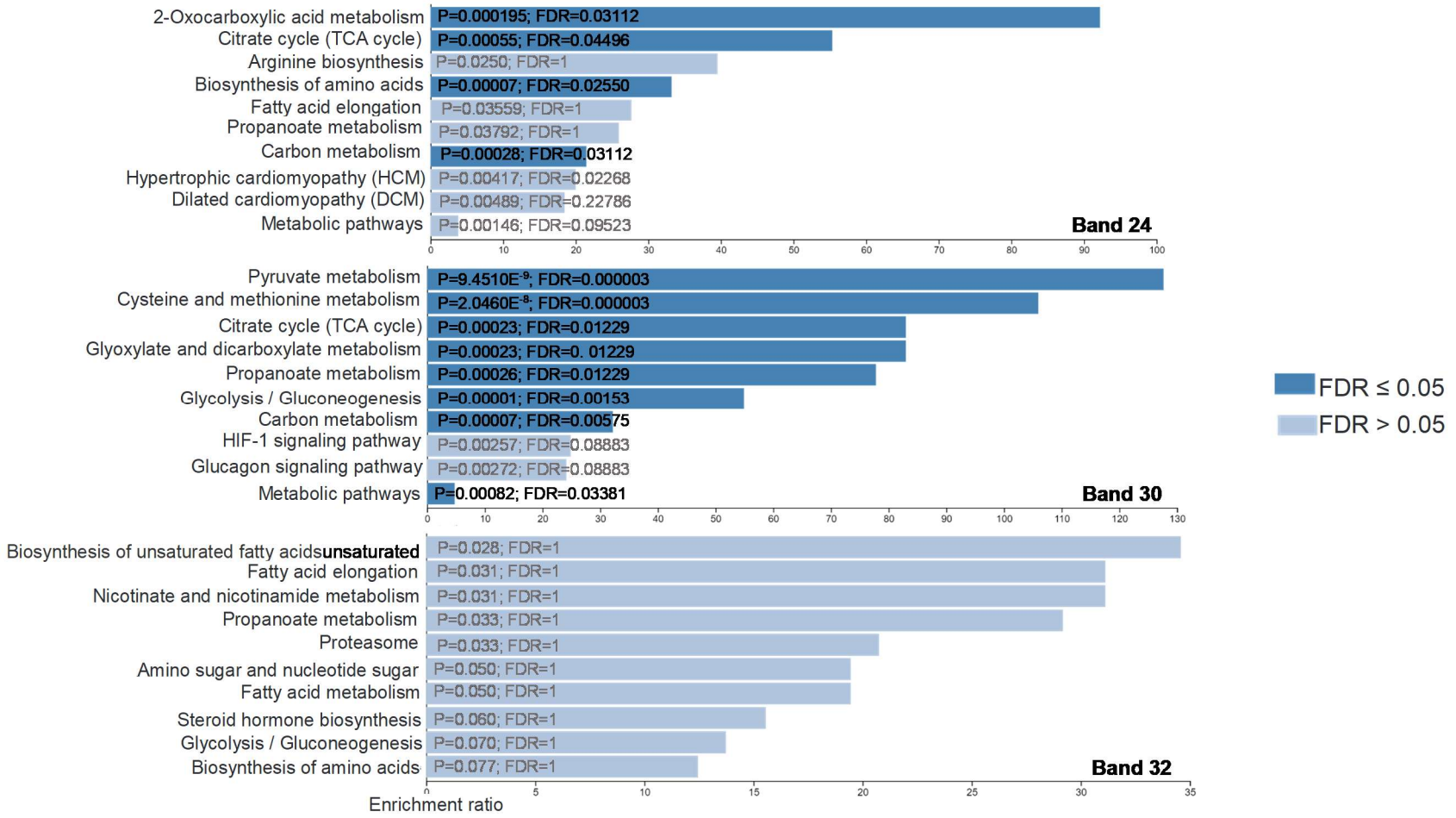

Figure 2: Demonstration of the enrichment rate according to over-representation analysis using WebGestalt web tool, where can to observat is an absence of enriched pathways for band 32. FDR =False Discovery Rate – P-value corrected for multiple tests within each category using the procedure by Benjamini & Hochberg (1995).

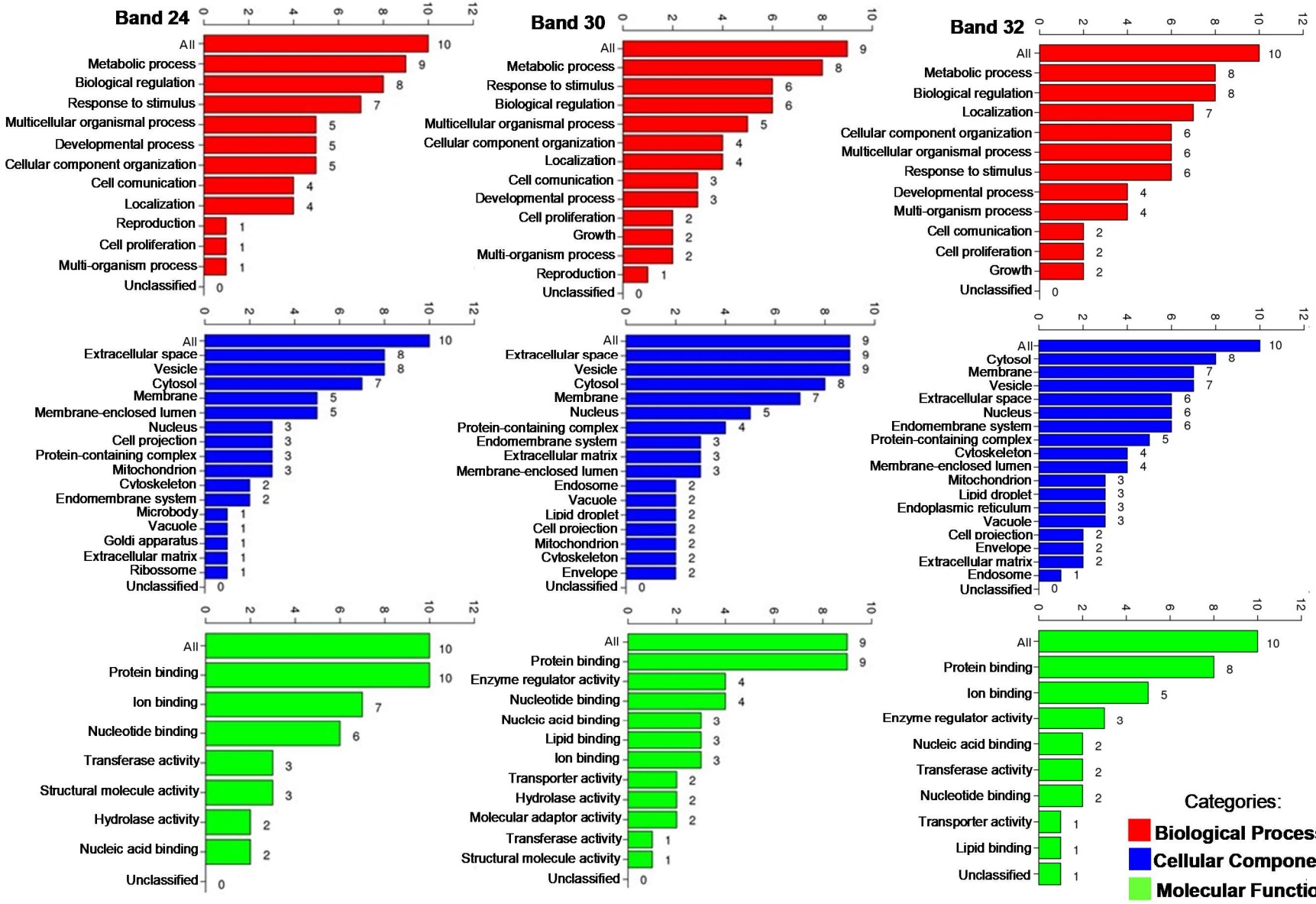

Figure 3: Distribution of proteins identified in each band within GO categories (biological process, cellular component, and molecular function), according over-representation analysis using WebGestalt web tool.

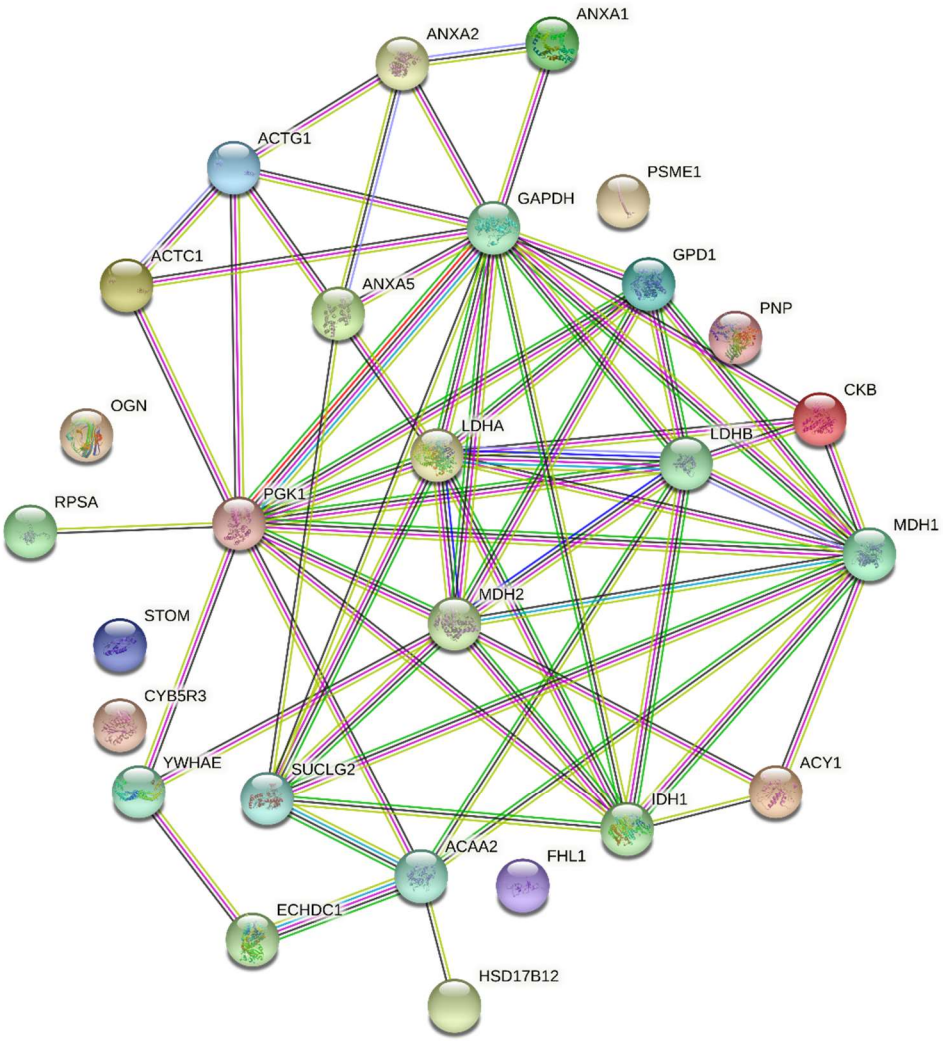

Figure 4. Protein-protein interaction network predicted by the String 11.0 software of the differentially abundant proteins identified between between the groups of Nelore bulls with high and low EPD for growth and precocity. The nodes are proteins from *Bos taurus* database, and the edges represent different evidences of the predicted functional annotations (red line - fusion; green line - neighborhood; blue line - co-occurrence; purple line - experimental; yellow line - text mining; light blue line - database; black line - coexpression).

4 DISCUSSION

The deposition of subcutaneous fat is regulated by different metabolic pathways from those that regulate the deposition of fat in other adipose tissues, such as intramuscular fat. In this context, the major goal of the present study was to understand the effect of genetic selection for growth and precocity on subcutaneous fat metabolism of Zebu cattle by proteomic analysis.

4.1 Phenotypic traits

The H_EPD group showed the highest fat deposition in the rump cap when the RFT was measured by carcass ultrasonography. The "Rump cap" is considered a noble court in Brazil. Besides this, rump fat thickness is an anatomic region deposition fat greater precocious in cattle. Previous studies have shown that ultrasound provides accurate measures of the live animal fat thickness (ROBINSON et al. 1992; HERRING et al. 1994), and rump fat thickness (REALINI et al., 2001).

The evaluated progenies also had a contrasting phenotype for backfat thickness (BFT) between the high and low EPD groups, with the highest subcutaneous fat deposition observed in animals with high EPD. These results can be justified by the theory of preferential deposition of tissues throughout animal growth until reaching their physiological maturation once the animals in the H_EPD group had the higher genetic potential for growth and precocity. This evidence is accordingly some authors that observed greater BFT and MAR for progenies more precocity, presenting higher variations within Nelore individuals than those found between breeds (KING et al., 2006; BONIN et al., 2014a; BONIN et al., 2014b; PEREIRA et al., 2015). The marbling score was subtly higher in H_EPD group animals than L_EPD, however, the MAR was still considered "slight" according to Smith et al. (2013).

4.2 Biochemical and metabolic parameters

The H_EPD group revealed a higher serum concentration of the hormone IGF-1, lower LDL and cholesterol metabolite than the L_EPD group.

Therefore, considering that low levels of IGF-1 were associated with low levels of HDL (SUCCURRO et al., 2010; LIANG et al., 2016), it is possible to infer that a lower concentration of HDL, and consequently, IGF-1, would lead to a higher LDL content, observed in the animals of the L_EPD groups.

A study conducted by Hayden et al. (1993), it was suggested that plasma levels of IGF-1, GH, INS, and thyroid hormones, but not of IGF-2, are affected by alteration of energy intake and are highly correlated with empty body gain and protein deposition in compensatory gain steers.

In addition to its hormone activity and direct contribution to animal production (food consumption and carcass adiposity), insulin has molecular functions such as IGF-1 receiver binding, making it necessary a joint action between INS and IGF-1 to signal the mTOR pathway (UniProtKB Consortium, 2021). In this sense, among numerous functions, IGF-1 acts on the mTOR signaling pathway, a protein complex, involved in lipolysis and lipid biosynthesis.

The mTORC1 is activated by the presence of growth factors, amino acids, energy status, stress, and oxygen levels. This pathway regulates various biological processes, including lipid metabolism, autophagy, protein synthesis, and ribosome biogenesis (KANEHISA et al., 2019; KANEHISA et al., 2000; CHAKRABARTI et al., 2013; KOPCHICK et al., 2020) (Figure 5). Therefore, it is believed that possible differences found for IGF-1 may have an indirect relationship with the contrasting phenotype for BFT.

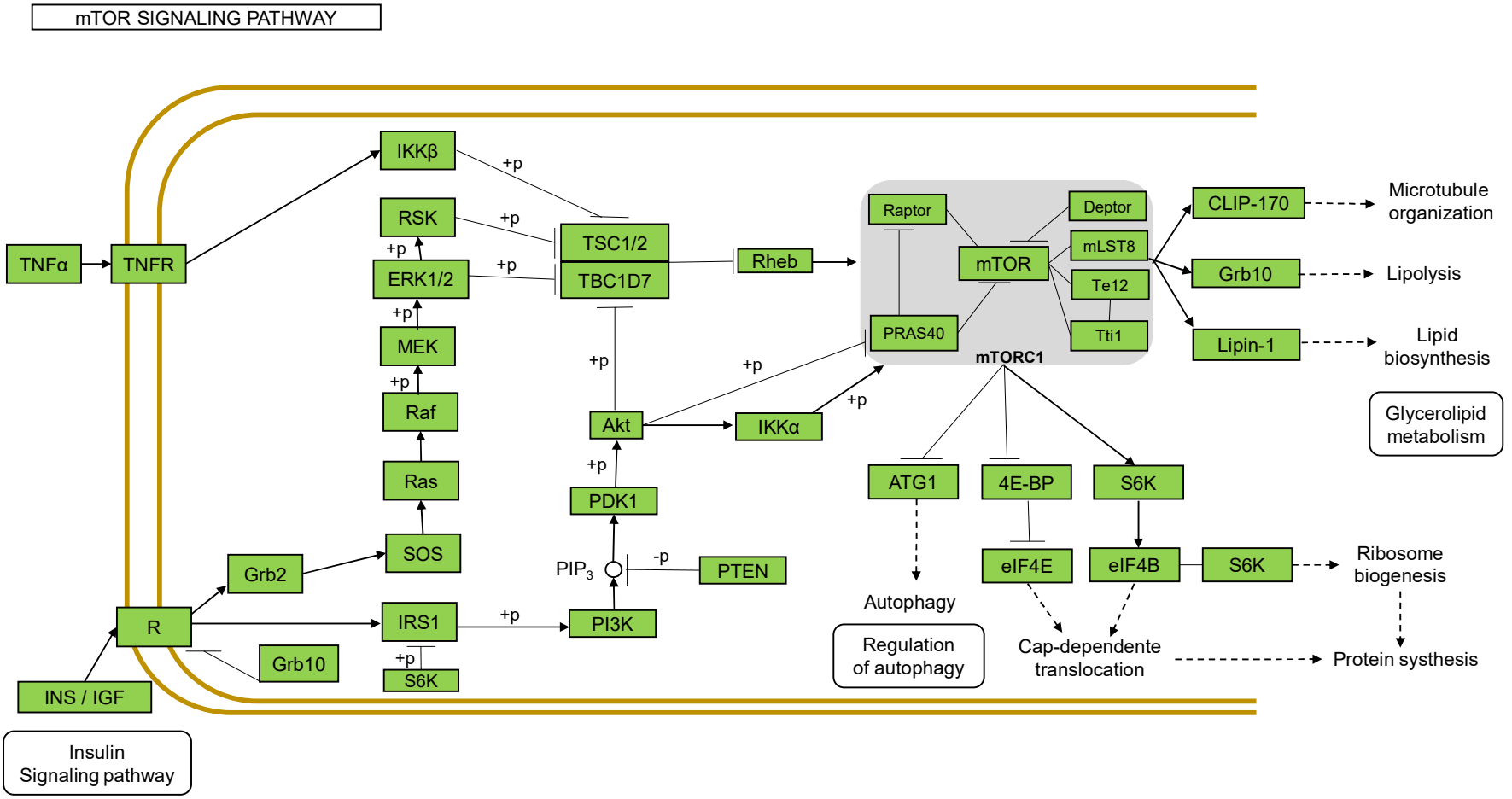

Figure 5. Contribution of the hormone insulin and IGF-1 to mTOR signaling pathway and consequent action on lipolysis and lipid biosynthesis. Adapted Map Kegg bta04150.

4.3 Proteomics of subcutaneous fat

In this study we were able to observe changes in the abundance of proteins in the contrasting groups for EPD's for precocity and growth, such as for, CKB, ACTG1, IDH1, PGK1, ACAA2, ACY1, ONG, SUCLG2, RPSA, ANXA2, MDH2, GPD1, LDHB, GAPDH, MTARC2, MDH1, LDHA, ANXA1, ANXA5, YWHAE, PNP, HSD17B12 ECHDC1, PSME1, STOM, CYB5R3, FHL1, although one of the limitations of one-dimensional electrophoresis is the co-migration of proteins in a single band (Díaz et al., 2020). The proteins identified in bands 24 and 30 (most abundant in the H_EPD group) were enriched for the citrate cycle pathways (bta00020), which is responsible for originating ATP and cAMP necessary for pancreatic cells to synthesize the hormone insulin (Figure 6).

In this study, we observed a high positive correlation between band 30 and insulin. According to Rhoades et al. (2007), bovine carcass adiposity is positively correlated with plasma insulin, corroborating our results. The hormone insulin is considered the most potent physiological inhibitor of lipolysis in adipose tissue, contributes to increasing the deposition of fat in the carcass (ZECHNER et al., 2009; CHAKRABARTI et al., 2013; QAID and ABDELRAHMAN, 2016; KOPCHICK et al., 2020) and is strongly involved in the biosynthesis of fatty acids (RHOADES et al., 2007; SONG et al., 2018).

Besides thatthe protein annexin was identified in band 30. This protein has the function of insulin secretion (Accession Uniprot F1N650_Table 5). Insulin, in turn, stimulates glucose and lipid homeostasis via signaling of genes such as SREBP-c, ACC or ACACA, and FAS, which are directly involved with lipogenesis and fatty acid biosynthesis. Insulin also signals the G6PC, FBP, and PEPCK genes involved in glycolysis (Figure 7) and acts in activation lipogenic and glycolytic enzymes (KERSTEN, 2001). This mechanism indicates a strong relationship between insulin and the TCA cycle pathway in the deposition of subcutaneous fat.

One of the key steps in the TCA cycle is the oxidative decarboxylation of isocitrate to generate α-ketoglutarate (αKG), which is catalyzed by isocitrate dehydrogenase (IDH) in the mitochondrial matrix. The IDH1 protein appears in all enriched pathways in band 24 in the present study (Table 6). The IDH1 along with the upregulation of G6PD and the basal glucose transporter SLC2A1

(GLUT1) is important for NADPH synthesis during lipogenesis (LQBAL et al., 2016; HAUSMAN et al., 2018). These data indicated a pro-lipogenic response in the subcutaneous fat samples. Further evidence for enhanced insulin sensitivity is the greater expression of G6PD, IDH1, and SLC2A1 (GLUT1) also reported in subcutaneous adipose tissue of cows pre-calving, fed high-energy diets (JI et al., 2012).

The protein GAPDH was identified in both bands 30 and 32 (Table 5) and has been enriched for the following pathways: Glycolysis / Gluconeogenesis (bta00010), Carbon metabolism (bta01200), and Metabolic pathways (bta01100) described in Table 6. Besides the lipogenic protein IDH1, the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a central protein in the carbon metabolism pathway and is an essential protein for lipid biosynthesis.

The GAPDH catalyzes the conversion of glyceraldehyde 3-phosphate to 1.3-bisphosphoglycerate in glycolysis, which provides a substrate for acetyl-CoA for the synthesis of various cellular compounds including lipids (OHLROGGE et al., 1995). Within this context, some enzymes are essential for supplying NADPH for lipid biosynthesis. Known enzymes involved in this process include malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphoglucose dehydrogenase from the phosphate pentose pathway beside the isocitrate dehydrogenase previously mentioned (HAO et al., 2014; HAO et al., 2016).

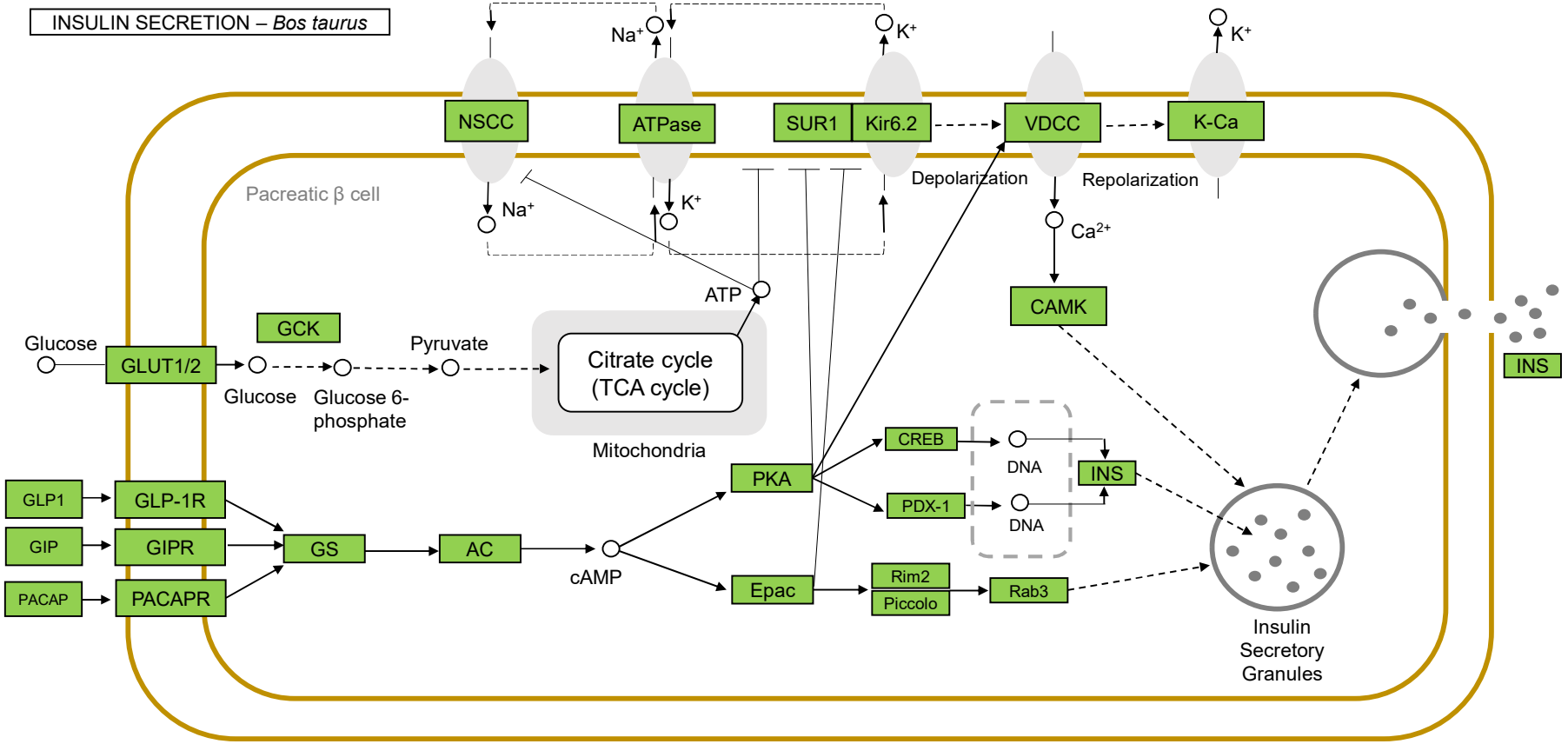

Figure 6. Use of the citrate cycle pathway by pancreatic cells to synthesize the hormone insulin. Adapted Map Kegg bta04911.

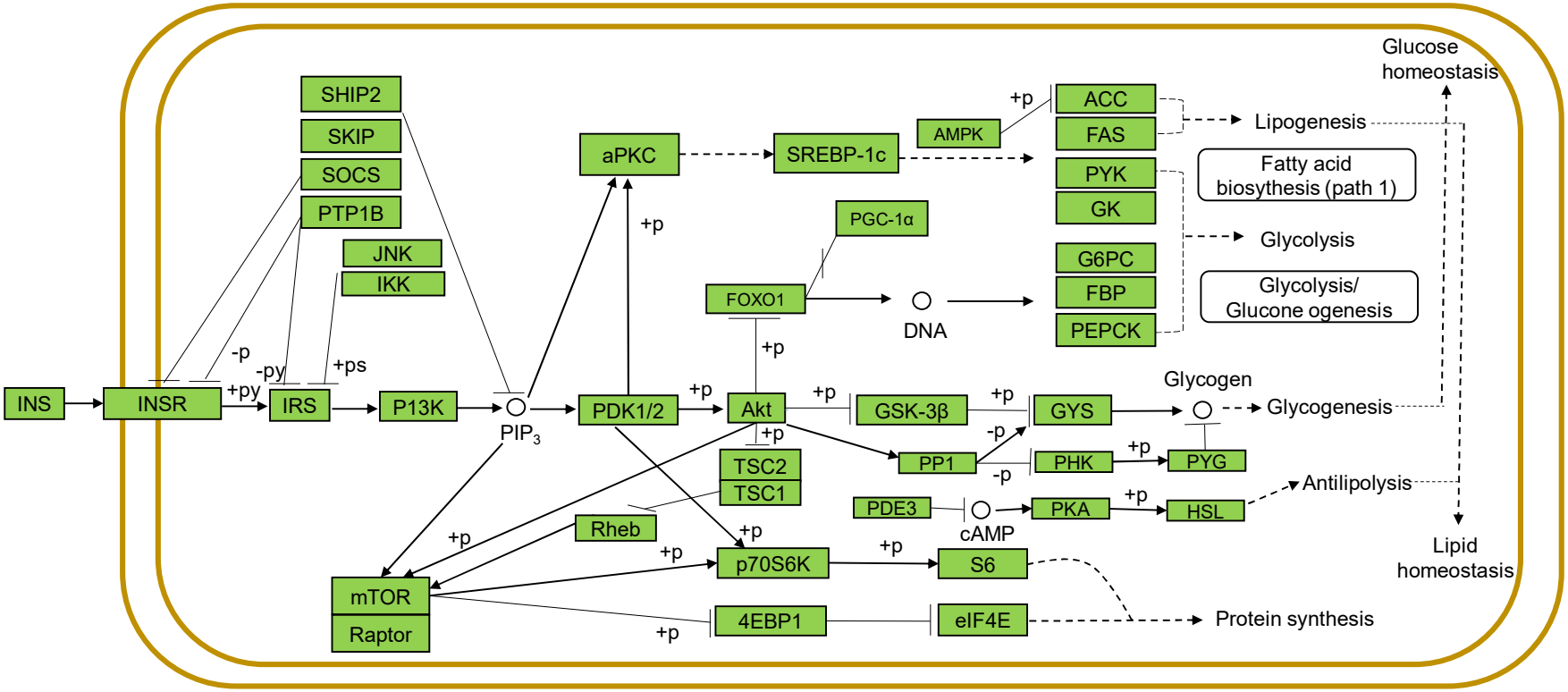

Figure 7. Insulin signaling pathways influence lipogenesis and lipid homeostasis. Adapted Map Kegg bta04910

The proteins Malate dehydrogenase (MDH1), Malate dehydrogenase, mitochondrial (MDH2), L-lactate dehydrogenase A chain (LDHA), and L-lactate dehydrogenase B chain (LDHB) was identified in band 30 (Table 5) and are present in all enriched pathways of band 30, such as pyruvate metabolism (bta00620), cysteine and methionine metabolism (bta00270), citrate cycle (bta00020), glycolysis/ gluconeogenesis (bta00010), carbon metabolism (bta01200) and metabolic pathways (bta01100), described in Table 6. These energy metabolism-related proteins was similar to the one described by Rodrigues et al. (2017) in muscle tissue.

The greater NADP-malate dehydrogenase (MDH1 e MDH2) activity usually indicates a greater capacity to incorporate glucose into fatty acids (KIM et al., 2012; LIANG et al., 2015). This fact may be related to the positive correlation of band 30 with marbling and total lipid, since intramuscular fat preferentially uses glucose for a greater proportion of the carbon source for de novo fatty acid biosynthesis *in vitro* than subcutaneous fat (SONG et al., 2018; LEE et al., 2013; RHOADES et al., 2007). Besides this, the MDH1 qualified as biomarkers of meat marbling in bovine (BONNET et al., 2020; POLETTI et al., 2018).

The cytosolic malic enzyme catalyzes the reversible oxidative decarboxylation of malate to pyruvate, carbon dioxide, and NADPH, the latter contributing to de novo fatty acid synthesis by *FASN* (WISE et al., 1964). Soon, the malic enzyme can catalyze malic acid to generate pyruvic acid and CO_2, accompanied by the reduction of nicotinamide adenine dinucleotide phosphate (NADP+). It provides materials and energy for acetyl-CoA transportation and fatty acid synthesis (DETARSIO et al., 2004). The malic enzyme has also been reported associated with intramuscular fat deposition (HEYER and LEBRET, 2007). Although traits of MAR and LT were not different between groups, the results shown in this study indicate a strong relationship between these band 30 proteins and different fat deposits, which may be an indication of sharing these proteins for the intramuscular and subcutaneous fat deposition pathways.

On the other hand, the bigger subcutaneous fat deposition in the H_EDP group than the L_EPD group in this study leads us to believe that this fat deposit depends on a functional ATP-citrate lyase: NADP-malate dehydrogenase pathway in bovine subcutaneous adipose tissue and that this pathway may be

involved in lipogenesis from acetate as well as lactate (SMITH and CROUSE, 1984; SMITH and PRIOR, 1981). Besides this, feeding high-grain diets increases the activity of lipogenic enzymes such as acetyl-CoA carboxylase (*ACACA*), ATP-citrate lyase (ATP-CL), NADP malate dehydrogenase (NADP-MDH), *G6PDH,* and *6-PGDH* in subcutaneous adipose tissue, which is associated with greater carcass adiposity (SMITH and CROUSE, 1984; SMITH and PRIOR, 1981; SMITH and PRIOR, 1986; LADEIRA et al., 2018). The subcutaneous adipose tissue exhibits higher levels of lipogenic enzymes such as NADP-malate dehydrogenase, fosfogluconate-6-dehydrogenase, and glucose-6-phosphate dehydrogenase, showing unique roles in lipid metabolism (MILLER et al.,1991; MAY et al., 1994).

The protein L-lactate dehydrogenase (LDH) is an enzyme involved in the subpathway that synthesizes (S)-lactate from pyruvate, the pathway pyruvate fermentation to lactate, a process for fatty acid biosynthesis (UniProtKB - P19858 and UniProtKB - Q5E9B1). Lactate is a substrate for lipogenesis in the liver and adipose tissue (O'HEA et al., 1969). Possibly, the most probable fate for most of the lactate produced by adipocytes will be its incorporation into the hepatic lipogenic pathway and their final release as lipoprotein triacylglycerols (ARRIARÁN et al., 2015). Therefore, the LDHA and LDHB enzymes found in band 30 of subcutaneous fat samples may be influencing lipid metabolism, as well as the synthesis of lipoproteins involved in bovine carcass adiposity.

In this sense, the proteins found in subcutaneous fat samples in this study, in particular, GAPDH, LDHA, LDHB, MDH1, MDH2, IDH1, PGK1, SUCLG2 and ACY1, and their phenotypic correlations reveal that they should act on biosynthesis and deposition of subcutaneous and intramuscular fat.

5 CONCLUSION

This study revealed a strong relationship between the subcutaneous fat protein profile and the insulin serum hormonal profile, independent and within the groups of contrasting EPD's for precocity and growth. The proteins involved in the citrate cycle pathway may influence subcutaneous fat deposition. Besides that, possible differences found for IGF-1 may have an indirect relationship with the contrasting phenotypes for backfat thickness.

Our study suggests strong evidence that GAPDH, ACACA, INS, and IGF-1 influence subcutaneous fat deposition. Thus, the exploration of proteins present in subcutaneous fat will allow a better understanding of the metabolic pathways involved in fat deposition cattle. Our results also allow for a better understanding of subcutaneous fat deposition in Nelore progenies, with contrasting EPD's for growth, precocity.

ACKNOWLEDGEMENTS

We are grateful to FAPESP for the scholarship (Grant #2017/02349–1) and financial support (process #2016/17433-5; #2011/21241-0; #2012/50788-0). We also thank CNPq (process #409186/2018-0) and CAPES (Finance Code 001) for financial support. We also thank CNPEM (National Center for Research in Energy and Materials) for the structure to carry out the mass spectrometry analysis.

6 REFERENCES

Aragao. A. Z. B.; Belloni. M.; Simabuco. F. M.; Zanetti. M. R.; Yokoo. S. et al. (2012) Novel Processed Form of Syndecan-1 Shed from SCC-9 Cells Plays a Role in Cell Migration. **PLoS ONE** 7(8): e43521. doi:10.1371/journal.pone.0043521

Arriarán. S.; Agnelli. S.; Sabater. D.; Remesar. X.; Fernández-López. J.Á.; Alemany. M .(2015). Evidences of Basal Lactate Production in the MainWhite Adipose Tissue Sites of Rats. Effects of Sexand a Cafeteria Diet. **PLoS ONE** 10(3): e0119572. doi:10.1371/journal.pone.0119572

Bonin, M.N.; Ferraz, J. B. S.; Eler, J. P.; Rezende. F. M.; Cucco. D. C.; Carvalho. M. E.; Silva. R. C. G.; Gomes. R. C.; Oliveira. E. C. M;. Sire effects on carcass and meat quality traits of young Nelore bulls. **Genetics and molecular research**: GMR. v. 13 n.2. p.3250-3264. 2014a.

Bonin, M.N.; Ferraz, J. B. S.; Eler, J. P.; Silva, S. L; Rezende, F. M.; Cucco, D.C.; Carvalho, M. E.; Silva, R. C. G.; Oliveira, E. C. M. Características de carcaça e qualidade de carne em linhagens da raça Nelore. **Ciência Rural** (UFSM. Impresso), v. 44, p. 1860-1866, 2014b.

Bonnet. M.; Soulat. J.; Bons. J.; Léger. S.; De Koning. L.; Carapito. C.; Picard. B. Quantification of biomarkers for beef meat qualities using a combination of Parallel Reaction Monitoring- and antibody-based proteomics. **Food Chemistry**. v. 1. p. 126317-126376. 2020. doi: 10.1016/j.foodchem.2020.126376.

Brasil. **Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal** (Food of animal origin sanitary and industry inspection). In. Brasília: Ministério da Agricultura. Pecuária e Abastecimento. 2017.

Ceciliani. F.; Lecchi. C.; Bazile. J.; Bonnet. M. (2018) Proteomics Research in the Adipose Tissue. In: de Almeida A.. Eckersall D.. Miller I. (eds) **Proteomics in Domestic Animals**: from Farm to Systems Biology. Springer. Cham. doi.org/10.1007/978-3-319-69682-9_12

Chakrabarti. P.; Kim. J. Y.; Singh. M.; Shin. Y. K.; Kim. J.; Kumbrink. J.; Wu. Y.; Lee. M. J.; Kirsch. K. H.; Fried. S. K.; Kandror. K. V. Insulin inhibits lipolysis in adipocytes via the evolutionarily conserved mTORC1-Egr1-ATGL-mediated pathway. **Molecular and cellular biology**. v. *33*(18). p. 3659–3666. 2013. doi.org/10.1128/MCB.01584-12

Clarke. A.M.; Drennan. M.J.; Mcgee. M.; Kenny. D.A.; Evans. R.D.; Berry. D.P. Intake. growth and carcass traits in male progeny of sires differing in genetic merit for beef production. **Animal**. v. *3*. p. 791–801. 2009.

Cori. C. F. The glucose-lactic acid cycle and gluconeogenesis. **Curr Top Cell Regul**. v. 18. p. 377–387. 1981. PMID: 7273846

Detarsio. E.; Andreo. C. S.; Drincovich. M. F. Basic residues play key roles in catalysis and NADP (+)-specificity in maize (Zea mays L.) photosynthetic NADP (+)-dependent malic enzyme. **Biochem. J. v.** 382. p. 1025–1030. 2004.

Díaz. F.; Díaz-Luis. A.; Sierra. V.; Diñeiro. Y.; González. P.; García-Torres. S.; Tejerina. D.; Romero-Fernández. M. P.; Cabeza de Vaca. M.; Coto-Montes. A.; Oliván. M. What functional proteomic and biochemical analysis tell us about animal stress in beef? **Journal of Proteomics. v.** 218. p. 103722. 2020.

Eler. J. P.; Ferraz. J. B.; Balieiro. J. C.; Mattos. E.C.; Mourão. G. B. Genetic correlation between heifer pregnancy and scrotal circumference measured at 15 and 18 months of age in Nellore cattle. **Genetic Molecular Research**. v. 5(4). p. 569-80. 2006.

Gondret. F.. Vincent. A.. Houée-Bigot. M. *et al.* A transcriptome multi-tissue analysis identifies biological pathways and genes associated with variations in feed efficiency of growing pigs. **BMC Genomics**. v. 18. p**.** 244. 2017.doi.org/10.1186/s12864-017-3639-0

Hao. G.; Chen. H.; Du. K.; Huang. X.; Song. Y.; Gu. Z.; et al. Increased fatty acid unsaturation and production of arachidonic acid by homologous over-expression of the mitochondrial malic enzyme in *Mortierella alpina**. Biotechnol. Lett. v.*** 36. p. 1827–1834. 2014. doi.org/10.1007/s10529-014-1546-x

Hao. G.; Chen. H.; Zhang. H.; Chen. W.; Chen. Y. Q. Metabolic engineering of *Mortierella alpina* for enhanced arachidonic acid production through the NADPH supplying strategy. ***Appl. Environ. Microbiol****. v.* 82. p. 3280–3288. 2016. doi.org/10.1128/AEM.00572-16

Hausman. G. J; Bergen. W. G; Etherton. T. D; Smith. S. B. The history of adipocyte and adipose tissue research in meat animals. **Journal of Animal Science**. v. 96(2). p. 473–486. 2018.

Hausman. G. J.; Dodson. M. V.; AJuwon. K.; Azain. M.; Barnes. K. M.. Guan. L. L.; Jiang. Z.; Poulos. S. P.; SAinz. R. D.; Smith. S.; Spurlock. M.; Novakofski. J.; Fernyhough. M. E. Board-invited review:The biology and aregulation of preadipocytes and adipocytes in meat animals. **Journal of Animal Science**. v. 87. p.1218-1246. 2009.

Hayden. J. M; Williams J.E; Collier. R. J. Plasma growth hormone. insulin-like growth factor. insulin. and thyroid hormone association with body protein and fat accretion in steers undergoing compensatory gain after dietary energy restriction. **Journal of Animal Science***.* v. 71. p. 3327–3338. 1993. doi.org/10.2527/1993.71123327x

Herring, W. O., D. C. Miller, J. K. Bertrand, and L. L. Benyshek. Evaluation of machine, technician, and interpreter effects on ultrasonic measures of backfat and longissimus muscle área in beef cattle. **J. Anim. Sci**, v. 72, p. 2216–2226, 1994.

Heyer. A.; Lebret. B. Compensatory growth response in pigs: Effects on growth performance. composition of weight gain at carcass and muscle levels. and meat quality **J. Anim. Sci**. v. 85. p. 769-778. 2007.

Ji. P; Osorio J; Drackley J; Loor J. Overfeeding a moderate energy diet prepartum does not impairbovine subcutaneous adipose tissue insulin signal transduction and induces marked changes in peri-partal gene network expression. **Journal of Dairy Science**. v. 95. p. 4333–4351. 2012. doi:10.3168/jds.2011-5079

Kanehisa. M. and Goto. S.; KEGG: Kyoto Encyclopedia of Genes and Genomes. **Nucleic Acids Res**. v. 28. p. 27-30. 2000.

Kanehisa. M. and Sato. Y. (2020) KEGG Mapper for inferring cellular functions from protein sequences. **Protein Sci. v.** 29. p. 28-35. 2020.

Kanehisa. M.; Sato. Y.; Furumichi. M.; Morishima. K.;Tanabe. M.; New approach for understanding genome variations in KEGG. **Nucleic Acids Res**. v. 47, p. D590-D595. 2019.

Kersten, S. 2001. Mechanisms of nutritional and hormonal regulation of lipogenesis. **EMBO reports**, v. 2(4), p. 282-286, 2001.

Kim. N. K.; Lee. S. H.; Cho. Y. M.; Son. E. S.; Kim. K. Y.; Lee. C. S.; Yoon D.; Im. S. K.; Oh. S. J.; Park. E.W. Proteome analysis of the m. Longissimus dorsi between fattening stages in Hanwoo steer. **BMB Rep. v.**42 (7). p. 4333–4338. 2009.

Kim. E. Y.; Kim. W. K.; Kang. H. J.; Kim. J. H.; Chung. S. J.; Seo. Y. S.; Park. S. G.; Lee. S. C.; Bae. K. H. Acetylation of malate dehydrogenase 1 promotes adipogenic differentiation via activating its enzymatic activity. Journal of lipid research. v. 53(9). p. 1864–1876. 2012. doi.org/10.1194/jlr.M026567

King, D. A.; Morgan, W. W.; Miller, R. K.; Sanders, J. O.; Lunt, D. K.; Taylor, J. F.; Gill, C. A.; Savell, J. W. Carcass merit between and among family groups of Bos indicus crossbred steers and heifers. **Meat Science**, v. 72: 496-502, 2006.

Kopchick. J. J.; Berryman. D. E.; Puri. V. et al. The effects of growth hormone on adipose tissue: old observations. new mechanisms. **Nature Reviews Endocrinology**. v. 16. p. 135–146. 2020. doi.org/10.1038/s41574-019-0280-9

Ladeira. M. M.; Schoonmaker. J. P.; Swanson. K. C.; Duckett. S. K.; Gionbelli. M. P.; Rodrigues. L. M. et al.. Review: nutrigenomics of marbling and fatty acid profile in ruminant meat. **Animal**. v. 12. p. s282–s294. 2018.

Lee. J-H.; Go. Y.; Kim. D. Y. *et al.* Isocitrate dehydrogenase 2 protects mice from high-fat diet-induced metabolic stress by limiting oxidative damage to the mitochondria from brown adipose tissue. **Exp Mol Med.** v. 52. p. 238–252. 2020. doi.org/10.1038/s12276-020-0379-z

Lee. J-H.; Jang. M.; Kim. H.; Kwak. W.; Park.W. C.; Hwang. J. Y.; Lee. C-K.; Jang. G. W; Park. M. N.; Kim. H-C.; Jeong. J. Y.; Seo. K. S.; Kim. H.; Cho. S.; Lee. B-Y. Comparative Transcriptome Analysis of Adipose Tissues Reveals that ECM-Receptor Interaction Is Involved in the Depot-Specific Adipogenesis in Cattle. **PLoS One** : 8(6): e66267. 2013.

Liao. Y.; Wang. J.; Jaehnig. E. J.; Shi. Z.; Zhang. B.; WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. **Nucleic Acids Research**. v. 47. p. W199–W205. 2019. doi.org/10.1093/nar/gkz401

Lopes. F. B.; da Silva. M. C.; Magnabosco. C. U.; Narciso. M. G; Sainz. R. D. (2016) Selection Indices and Multivariate Analysis Show Similar Results in the Evaluation of Growth and Carcass Traits in Beef Cattle. **PLoS ONE** 11(1): e0147180. doi:10.1371/journal.pone.0147180.

Lorenzen, C.L.; Golden, J.W.; Martz, F.A.; Grün, I.U.; Ellersieck, M.R.; Gerrish, J.R.; Moore, K.C. Conjugated linoleic acid content of beef diers by feeding regime and muscle. **Meat Sci**, v. 75, p. 159–167. 2007.

Lqbal. Z. M.; Akbar. H.; Hosseini. A.; Forteguerri. E. B. R; Osorio. J. S.; Loor. J. J. Digital Cushion Fatty Acid Composition and Lipid Metabolism Gene Network Expression in Holstein Dairy Cows Fed a High-Energy Diet. **PLoS ONE**. v. 11 (7): e0159536. 2016. doi:10.1371/journal.pone.0159536

Liang. Y.-J.; Jiang. J.-G. Characterization of malic enzyme and the regulation of its activity and metabolic engineering on lipid production. **RSC Adv**. v. 5. p. 45558–45570. 2015.

Mao.Y.; Hopkins. D. L.; Zhang.Y.; Li.P.; Zhu. L.; Dong. P.; Luo. X. Beef quality with diferente intramuscular fat contente and proteomic analysis using isobaric tag for relative and absolute quantitation of differentially expressed proteins. **Meat Science**. v. 118. p. 96–102. 2016. doi.org/10.1016/j.meatsci.2016.03.028.

May. S. G.; Savell. J. W.; Lunt. D. K.; Wilson J. J.; Laurenz J. C.; Smith S. B. Evidence for preadipocyte proliferation during culture of subcutaneous and intramuscular adipose tissues from Angus and Wagyu crossbred steers. **Journal of Animal Science**. v. 72. n.12. p. 3110-3117. 1994.

Melendez. P.; Poock. S. E.; Pithua. P.; Pinedo. P.; Manriquez. D.; Moore. S.G.; Neal. J. D.; Taylor. J. F. Genome-wide study to detect single nucleotide polymorphisms associated with visceral and subcutaneous fat deposition in Holstein dairy cows. **Animal**. v. 13. p. 487-494. 2019. doi.org/10.1017/S1751731118001519

Miller. M. F.; Cross. H. R.; Lunt. D. K.; Smith. S. B. Lipogenesis in acute and 48h cultures of bovine intramuscular and subcutaneous adipose tissue explants. **Journal of Animal Science**. v.69. n.2. p.162-170. 1991

.

Miner. J. L. The adipocyte as an endocrine cell. **Journal of Animal Science**. v.82. p.935-941. 2004.

Mohan. C.; Geiger. P. J.; Bessman. S. P. The Intracellular Site of Action of Insulin: The Mitochondrial Krebs Cycle. Current Topics in Cellular Regulation. v. 30. p. 105-142. 1989.

Ohlrogge J. and Browse J. Lipid biosynthesis. **Plant Cell**. v. 7. p. 957–970. 1995.

O'hea. E.K.; Leveille. G. A. Significance of adipose tissue and liver as sites of fatty acid synthesis in the pigand the efficiency of utilization of various substrates for lipogenesis. **J Nutr**. v. 99. p. 338–344. 1969.

Pereira. A. S. C.; Baldi. F.; Sainz. R. D.; Utembergue. B. L.; Chiaia. H. L. J.; Magnabosco. C. U.; Manicardi. F. R;. Araujo. F. R. C.; Guedes. C. F.; Margarido. R. C.; Leme. P. R.; Sobral. P. J. A. Growth performance. and carcass and meat quality traits in progeny of Poll Nelore. Angus and Brahman sires under tropical conditions. **Animal Production Science**. v. 55 (10) :1295-1302 . 2015.

Poleti. M. D.; Regitano. L. C. A.; Souza. G. H. M. F.; Cesar. A. S. M.; Simas. R. C.; Silva-Vignato. B.; … & Coutinho. L. L. *Longissimus dorsi* muscle label-free quantitative proteomic reveals biological mechanisms associated with intramuscular fat deposition. **Journal of Proteomics.** v. 179, p. 30-41, 2018.

Qaid, M. M., and M. M. Abdelrahman. 2016. Role of insulin and other related hormones in energy metabolism-A review. **Cogent Food Agric**, v. 2(1), p. 1267691, 2016.

Rapoport. T. A.; Heinrich. R.; RapoporT. S. M. The regulatory principles of glycolysis in erythrocytes in vivo and in vitro. A minimal comprehensive model describing steady states. quasi-steady states and timedependent processes. **Biochem J**. v. 154. p. 449–469. 1976.

Realini, C. E.; Williams, R. E.; Pringle, T.D.; Bertrand, J.K. Gluteus medius and rump fat depths as additional live animal ultrasound measurements for predicting retail product and trimmable fat in beef carcasses. **J Anim Sci**, v. 79(6), p. 1378-85, 2001. doi: 10.2527/2001.7961378x.

Robinson, D. L., C. A. McDonald, K. Hammond, and J. W. Turner. Live animal measurement of carcass traits by ultrasound: Assessment and accuracy of sonographers. **J. Anim. Sci**, v. 70, p. 1667–1676, 1992.

Rhoades. R. D.; Sawyer. J. E.; Chung. K. Y.; Schell. M. L.; Lunt. D. K.; Smith. S. B. Effect of dietary energy source on in vitro substrate utilization and insulin sensitivity of muscle and adipose tissues of Angus and Wagyu steers. **Journal of Animal Science**. v. 85. p. 1719–1726. 2007. doi.org/10.2527/jas.2006-498

Robinson. D. L. “Models which might explain negative correlations between direct and maternal genetic effects.” **Livest Prod Sci.** v. 47. p. 111-122. 1996. doi.org/10.1016/0301-6226(96)00002-4

Rodrigues. R.T.S.; Chizzotti. M. L.; Vital. C. E.; Baracat-Pereira. M. C.; Barros. E.; Busato. K. C.; Gomes. R. A.; Ladeira. M. M.; Martins. T. S. Differences in Beef Quality between Angus (*Bos taurus taurus*) and Nelore (*Bos taurus indicus*) Cattle through a Proteomic and Phosphoproteomic Approach. **PLoS ONE**. v. 12 (1): e0170294. 2017. doi:10.1371/journal.pone.0170294

Ross. B. D.; Hems. R.; Krebs. H. A. The rate of gluconeogenesis from various precursors in the perfused rat liver. **Biochem J**. v. 102. p. 942–951.1967.

Sahlin K. Lactate formation and tissue hypoxia. **J Appl Physiol**. v. 67. p. 2640. 1989.

Shen. Y. N.; Kim. S. H.; Yoon. D. H.; Lee. H. G.; Kang. H. S.; Seo. K. S. Proteome analysis of bovine Longissimus dorsi muscle associated with the marbling score. **Asian-Australasian Journal of Animal Sciences**. v. *25*(8). p. 1083–1088. 2012 doi.org/10.5713/ajas.2012.12127.

Silva, J.; Cônsolo, N.R.B.; Buarque, V.L.M.; Beline, M.; da Silva Martins, T.; Lobo, A.A.G.; Gómez, J.F.M.; Eler, J.P.; Leme, P.R.; Netto, A.S.; Gerrard, D.E.; Baldi, F.; Silva, S.L. Effect of Different Selection Criteria on Performance, Carcass and Meat Quality of Nellore Young Bulls. **Agriculture**. v. *11*, p. 294. 2121. https://doi.org/10.3390/agriculture11040294

Smith, G.; Griffin, D.; Johnson, K. Meat Evaluation Handbook. 2. Ed. Champaign, Illinois: **American Meat Science Association**, 2013. 160 p.

Smith. S. B. and R. L. Prior. Comparisons of lipogenesis and glucose metabolism between ovine and bovine adipose tissues. **J. Nutr**. v. 116. p. 1279–1286. 1986.

Smith .S. B and Crouse. J. D. Relative contributions of acetate. lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. **J Nut**. v. 114. p. 792–800. 1984.

Smith. S. B.. and R. L. Prior. Evidence for a functional ATP citrate lyase: NADP-malate dehydrogenase pathway in bovine adipose tissue: Enzyme and metabolite levels. **Arch. Biochem. Biophys**. v. 211. p. 192–201. 1981. doi:10.1016/0003-9861(81)90444-6

Song. Z.; Xiaoli. A. M.; Yang. F. Regulation and Metabolic Significance of *De Novo* Lipogenesis in Adipose Tissues. ***Nutrients*** 2018. v. 10. p. 1383. doi.org/10.3390/nu10101383

Steen, R.W.J.; Lavery, N.P.; Kilpatrick, D.J.; Porter, M.G. E_ects of pasture and high-concentrate diets on the performance of beef cattle, carcass composition at equal growth rates, and the fatty acid composition of beef. N. Z. J. Agric. Res., v. 46, 69–81, **2003**.

Teixeira. P. D.; Oliveira. D. M.; Chizzottl. M. L.; Chalfun-Junior A.; Coelho. T. C.; Gionbelli. M.; Paiva. L. V.; Carvalho. J. R. R.; Ladeira. M. M. Subspecies and diet affect the expression of genes involved in lipidmetabolism and chemical composition of muscle in beef cattle. **Meat Science**. v. 133. p. 110-118. 2017

The UniProt Consortium. UniProt: the universal protein knowledgebase in 2021. ***Nucleic Acids Research.*** Volume 49. Issue D1. 8 January 2021. Pages D480–D489. doi.org/10.1093/nar/gkaa1100

USDA. **Official United States standards for grades of carcass beef**. Agric. Marketting Serv., USDA, Washington, D.C., 1999.

Wise. E. M. Jr. and Ball. E. G. Malic enzyme and lipogenesis. **Proc Natl Acad Sci U S**. v. 52. p. 1255–1263. 1964. doi: 10.1073/pnas.52.5.1255.

Yokoo. M. J.; Lobo. R. B.; Araujo. F. R.C.; Bezerra. L. F.; Sainz. R. D.; Albuquerque. L. G. Genetic associations between carcass traits measured by real-time ultrasound and scrotal circumference and growth traits in Nelore cattle. **Journal of Animal Science.** v. 88. p. 52–58. 2010. doi: 10.2527/jas.2008-1028

Zapata. I.; Zerby. N.H.; Wick. M. Functional proteomic analysis predicts beef tenderness and the tenderness differential. **J. Agric. Food Chem**. v. 57. 4956–4963. 2009. dx.doi.org/10.1016/j.livsci.2013.11.030

Zechner R.; Kienesberger P. C.; Haemmerle G.; Zimmermann R.; Lass A. Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. J. Lipid Res. v. 50. p. 3–21. 2009.

Zhang. Q.; Lee. H. G.; Han. J. A.; Kim. E. B.; Kang. S. K.; Yin. J.; ... Choi. Y. J. Differentially expressed proteins during fat accumulation in bovine skeletal muscle. **Meat Science**. v. 86(3). p. 814–820. 2010. doi.org/10.1016/j.meatsci.2010.07.002.

CAPÍTULO 5

Bovines with greater genetic potential for precocity have higher levels of transcription of genes involved in lipogenesis

Martins et al., 2021

MARTINS, T. S. Bovines with greater genetic potential for precocity have higher levels of transcription of genes involved in lipogenesis.2021.

ABSTRACT

The present study hypothesizes that genetic selection for precocity and growth affects the subcutaneous fat transcription of genes and consequently lipid metabolism of the Zebu cattle. Consequently, this study aims to characterize the fatty acid profile intramuscularly and to evaluate the expression of genes adipogenic and lipogenic present in subcutaneous and visceral fat from offspring of bulls with high and low EPD for precocity. 105 male bulls were used, with a mean age of 20 ± 2 months and 400 ± 24 kg, from the same herd, with the genetic information of precocity and growth. The animals remained confined for 100 days, and the carcass ultrasound was performed every 28 days. Blood samples were collected to determine the metabolic and hormonal rules at the last weighing. The animals were slaughtered after 100 days feedlot and during slaughter, backfat thickness (BFT) was collected between the 12th and 13th ribs and immediately frozen in liquid nitrogen and kept in a freezer - 80ºC until the analysis of gene expression by real-time PCR (RT-qPCR). This same procedure was performed to collect visceral fat samples immediately after evisceration. After 24 hours slaughter, during boning, the intramuscular fat (MAR) in the LT muscle was evaluated and steaks for total lipids and fatty acid profile analyses were collected. The animals were selected according to the EPD from their parents (bulls). Were selected 6 parents bulls with EPD contrasting simultaneously for precocity and growth so that each experimental group had 3 parents bulls. Then, were formed 2 contrasting groups called high EPD (H_EPD; N = 16) and low EPD (L_EPD; N = 16), using 32 progenies. The animals in the H_EPD group had a higher BFT (P = 0.006). This group also showed greater expression of the genes involved in lipogenesis evaluated in BFT: ACACA (P = 0.060), LPL (P = 0.085), ACOX1 (P = 0.100), LEP (P = 0.030), SDC (P = 0.009), and GAPDH (P = 0.081), than the animals in the L_EPD group. Although the ACACA, LPL, ACOX1, LEP, SCD, and GAPDH genes expression have been different, the composition of beef fatty acid has not been changed. Therefore, the subcutaneous fat genes do not interfere with the lipid metabolism of intramuscular fat. Bovines with higher genetic potential for precocity have higher levels of transcription of lipogenic genes. This finding can explain the higher fat deposition in the carcass of Nelore progenies with higher EPD for precocity. Therefore so we conclude that the BFT is more related to lipogenic than adipogenic factors.

Keywords: Beef Cattle. Backfat thickness. Metabolic pathways. Protein and lipid metabolism. Proteomics.

1 INTRODUCTION

Lipid metabolism is responsible for adipocyte development, hormonal regulation and, fat deposition in carcass and meat, comprising the processes known as adipogenesis and lipogenesis. Adipogenesis occurs across several depots that develop at different rates and can have unique properties (HAUSMAN et al., 2009). Lipogenesis is the synthesis of fatty acids and triglycerides stored in the liver and adipose tissue. In this sense, the fat deposition in beef cattle is affected by interaction among nutrition, sex, age, ruminal bio-hydrogenation, hormonal and genetic factors (KERSTEN, 2001; HOSSNER, 2005; TEIXEIRA et al., 2017).

The amount and distribution of fat can influence the carcass and meat quality in beef cattle (RITCHIE et al., 1993; WHEELER et al., 1994; LOZEMAN et al., 2001). However, depending on the type, distribution, and anatomical origin of adipose tissue (visceral, subcutaneous, or intramuscular), adipose cells differ in their ability to capture lipids and in their functional attributes, thus presenting a different metabolism (MILLER et al., 1991; JO et al., 2012; MELENDEZ et al., 2019).

Hishikawa et al. (2005) reported differences in gene expression in subcutaneous and visceral fat tissues, using the differential display RT-PCR method, among cattle, mice, and pigs and demonstrated that expression profiles between tissue types were different. Soon, the technology of Real-Time PCR (qPCR) has been used as a technique of molecular genetic for investigations on gene expression of transcripts belonging to the pathways of lipid metabolism in cattle (WONG and MEDRANO, 2005; TEIXEIRA et al., 2017; KUANG et al., 2018).

In this sense, Nelore cattle is the most produced breed raised in Brazil. However, the heterogeneous quality of Brazilian cattle carcasses has been identified as a challenge for the beef industry, especially to the deposition of fatckiness in carcass. Backfat thickness (BFT) is essential to protect the carcass during cooling and to avoid problems such as *cold shortening*, *drip loss*, and *dark meat* (PARRISH et al., 1973; MARSH, 1977; DEVINE et al., 1999; AALHUS et al., 2001; PINTO NETO et al., 2013).

Accordingly, we hypothesized that gene expression of the key adipogenesis regulators and some of the main lipogenic enzymes could be differentially affected

by genetic factors, like animal precocity. Precocity and growth are economically relevant traits for the identification of uniform animals that have been used worldwide as a basis for the selection and improvement of beef cattle (ELER et al., 2006).

The expected differences in progeny (EPD's) are tools for animal production and selection. Precocity and growth traits are generally useful selection criteria for beef cattle. Indeed, the phenotypes of subcutaneous fat thickness are easily to measure using real-time ultrasound (YOKOO et al., 2010) and provide accurate data for estimating genetic values, as reported for young bulls (ROBINSON, 1996). Improvements in average daily gains, body weight, muscle mass, and fat deposition in carcass, were achieved using genetic merit for growth and precocity as selection criteria to improve beef cattle (CLARKE et al., 2009).

However, there is little known about the role molecular functions of adipocytes in bovine species. Further studies are still needed to bridge the gap between the phenotype, backfat thickness, and function of genes expressed in subcutaneous adipose tissue of cattle. Thus, we hypothesizes that genetic selection for precocity and growth affects the subcutaneous fat transcription of genes and consequently lipid metabolism of the *Bos indicus* catlle. Consequently, this study aims to characterize the intramuscular fatty acid composition and, to evaluate the gene expression of key adipogenic and lipogenic enzymes expressed in subcutaneous and visceral fat from offspring of bulls with high and low EPD for precocity.

2 MATERIAL AND METHODS

The experimental procedures were approved by the Ethics Committee on Animal Use (CEUA/FMVZ) from the School of Veterinary Medicine and Animal Science of the University of Sao Paulo (Protocol number 3367170317), Pirassununga, Sao Paulo, Brazil.

2.1 Experimental design

The animals used in this study were selects from a larger project evaluating the effects of EPD groups on animal performance, carcass, and meat quality traits (Silva et al., 2021). The animals belonged to USP's genetic selection program, with genetic information for precocity and growth. One hundred and five Nelore bulls, not

castrated, with initial body weight (BW) of 400 ± 24 kg and 20 ± 2 months of age were ranked based on their parent's Expected Progeny Difference (EPD), for precocity and growth and classified as either High and Low. Three bulls Nelore with high EPD were parents of animals in the H_EDP group (average EDP for precocity and growth = 0.57 and 12.32 respectively), and three bulls Nelore with low EPD were parents of animals in the L_EPD group (average EPD for precocity and growth = - 0.05 and - 3.67 respectively). The phenotypic traits were evaluated in 32 progeny (High_EPD, N=16; Low_EPD, N=16). Posteriorly, 16 animals were selected with either highest (H_EPD, N = 8) or lowest (L_EPD, N = 8) and contrasting deposition of backfat thickness (BFT) to form to the groups that were tested for differential proteomic analysis.

The animals were housed in a feedlot facility for 100 days, and individual feed intake was monitored. Facilities had covered feed bunks, concrete floors, and automatic waterers. All animals were initially submitted to adaptation period of 21 days. At the ending of the adaptation period all animals were weighed after a 12 h fasting period prior to the morning feeding. During the feedlot period, the animals were fed ad libitum twice daily, at 0800 h and 1600 h whit a diet (27:73 forage: concentrate) containing corn silage, corn grain, ground sorghum, soybean meal, citrus pulp, urea, and mineral core. All animals were weighed after a 12 h fasting to record the final body weight immediately prior to slaughter. The average daily gain was obtained by the difference of final body weight and initial body weigh divided by the 100 days of feeding.

2.2 Carcass ultrasound

Carcass ultrasonography was performed in beging and feedlot final to assess rib eye area (REA) and backfat thickness (BFT) in the *longissimus thoracis*, in the region between the 12th and 13th ribs, and rump fat thickness (RFT), using the Aloka ultrasound equipment. SSD 500 Micrus model (Aloka Co. Ltda.), with 3.5 MHz linear transducers and 17.2 cm in length. The images were stored in the portable microcomputer and records were subsequently interpreted in laboratory using the Lince® software.

2.3 Harvesting subcutaneous fat and meat sampling

At the end of the feeding phase, the animals were harvested in the experimental slaughterhouse of the University of São Paulo in Pirassununga, Brazil, according to humanitarian slaughter guidelines, as required by Brazilian law (Brasil 2017). Subcutaneous fat samples were collected from the right side of the carcass at the 12th rib, snap-frozen in liquid nitrogen, and stored at -80°C until gene expression analysis. This same procedure was performed to collect visceral fat samples immediately after evisceration.

After a 24h chilling period, 2.54cm thick steaks were cut from *longissimus thoracis* (LT), of the right half of the carcass, between 12th-13th ribs, vacuum packaged, and frozen at −80°C until total lipids analysis and fatty acid profile. Besides that, the marbling score (MAR) was evaluated in the LT, between the 12th and 13th rib, following the methodology described by American Meat Science Association (SMITH et al., 2013).

2.4 Total lipids and fatty acid composition

The total lipids were determined in fresh samples following the methodology of Bligh & Dyer (1959) in duplicate. Meat fatty acids were extracted from the intramuscular fat of the *longissimus thoracis* as described by Folch et al. (1957). Aliquots of muscle lipids were methylated separately using base (0.5 N sodium methoxide) and acid (5% methanolic HCl) reagents, according to Kramer et al. (1997). The FAs were quantified by gas chromatography (GC-2010 Plus - Shimadzu AOC 20i auto-injector) with an SP-2560 capillary column (100 m × 0.25 mm diameter, 0.02 mm thick, Supelco, Bellefonte, PA). The initial temperature was 45 °C with an increase (13 °C/min) to 175 °C, which was held for 27 min before a further increase to 215 °C (4 °C/min); the final temperature was maintained for 35 min. Hydrogen (H2) was used as the carrier gas at 40 cm3/s. The temperature of the flame ionization detector (FID) was 250 °C, the H2 flow rate was 40 mL/min, the air flow was 400 mL/min (synthetic air), the make-up gas flow was 30 mL/min kPa (N2), and the sampling rate was 40 msec. The FAs were identified by comparing the retention times of methyl esters in the samples with those of the standards, FA C4-C24 (F.A.M.E. mix, Sigma®), GLC 463 Reference Mixture Nu Check, vaccenic acid (V038-1G, Sigma®), linoleic acid (UC-61 M 100 mg), conjugated linoleic acid (CLA) (UC-60M 100 mg, Sigma®), and tricosanoic acid (Sigma®). The FA were quantified by

normalizing the area under the curve of methyl esters using GS Software Solutions (version 2.42). The FA contents were expressed as a percentage of total FA methyl ester quantified.

Δ9 desaturase activity was calculated according to Aldai et al. (2006):

Δ9 desaturase 14 = (100*(14:1 cis9/(14:1 cis9+14:0));

Δ9 desaturase 16 = (100*(16:1 cis9/(16:1 cis9+16:0));

Δ9 desaturase 18 = (100*(18:1 cis9/(18:1 cis9+18:0));

Index related to human health and nutritional quality were calculated according to Zhang et al. (2008), Ulbricht and Southgate (1991), and Santos-Silva, Bessa and Santos-Silva (2002).

Health Index = (MUFA+PUFA)/(4*14:0)+16:0);

Atherogenicity Index = (12:0+(4*14:0)+16:0)/(Σn6+Σn3+ΣMUFA); Thrombogenicity index = (14:0+16:0+18:0)/((0,5*ΣMUFA)+(0,5*Σn6)+(3*Σn3)+n3/n6);

Hypocholesterolemic/Hypercholesterolemic = (18:1 n9cis+18:2 n6cis+20:4 n6+18:3 n3+20:5 n3+22:5 n3+22:6 n3)/(14:0+16:0).

2.5 Total RNA Extraction and cDNA Preparation

Subcutaneous and visceral fat samples were macerated and homogenized in liquid nitrogen for total RNA extraction. Total RNA was extracted using Trizol® reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA). Total RNA concentration was quantified using the NanoDrop spectrophotometer (Thermo Scientific, Washington, DE). RNA samples were treated with DNase I (Invitrogen Life Technologies, Carlsbad, CA) before reverse transcription reaction. cDNA was produced using 30 ng of total RNA per analyzed gene using the High Capacity kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. The mRNA levels were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) using gene-specific primers (Table 2). Primer's efficiency was obtained using different cDNA concentrations (5, 15, 30, 60 ng/μL).

2.6 Gene Expression Analyzes

Transcripts abundance were analyzed by QuantStudio 6 Real-Time PCR (Applied Biosystems) using SYBR Green RT-PCR Master Mix (GoTaq® Promega), following the cycle parameters: 95 ° C for 2 min, 45 cycles at 95 ° C for 15 s, and 60 ° C for 1 min. In each reaction were used 5μL of SYBR Green RT-PCR Master Mix, 1.5 μL of each forward and reverse primer, 1μL of cDNA (30ng of RNA), and MilliQ (ultrapure) water to complete the volume of 10 μL. After amplification, a melting curve (0.01 C / s) was used to confirm the purity of the product. The “threshold cycle” (Ct) values were normalized (ΔCt) based on the geometric mean Cts of the values obtained for *18S* and *ACTB*.

Table 1. Sequence of studied primer

Gene	Gene name		Sequence 5' - 3'	NCBI	biological process[1]	Reference
PPARG	Peroxisome proliferator activated receptor gamma	F	GTGGAGCCTGTATCCCCACC	NM_181024.2	Caspase activation	Mueller et al., 2021 (not yet published)
		R	TTTATCCCCACAGACCCGGC			
ZNF423	Zinc finger protein 423	F	GGATTCCTCCGTGACAGCA	NM_001101893.1	Regulation of adipogenesis	Martins et al., 2015
		R	TCGTCCTCATTCCTCTCCTCT			
CEBPA	CCAAT enhancer binding 1	F	TGCGCAAGAGCCGGGACAAG	NM_176784.2	Fat cell differentiation	Duarte et al., 2013
		R	ACCAGGGAGCTCTCGGGCAG			
ACACA	Acetyl-CoA carboxylase 1	F	TGAAGAAGCAATGGATGAACC	NM_174224.2	Fatty acid biosynthesis	Martins et al., 2015
		R	TTCAGACACGGAGCCAATAA			
LPL	Lipoprotein lipase 1	F	CTCAGGACTCCCGAAGACAC	NM_001075120.1	Lipoprotein metabolism	Martins et al., 2015
		R	GTTTTGCTGCTGTGGTTGAA			
ACOX1	Acyl-CoA oxidase 1	F	GCTGTCCTAAGGCGTTTGTG	NM_001035289.3	Lipid metabolism	Martins et al., 2015
		R	ATGATGCTCCCCTGAAGAAA			
LEP	Leptin	F	GGGCACGTCAGCATCTATTA	NM_173928.2	Positive regulation of fatty acid biosynthetic	Mota et al., 2017
		R	CCTGTCTGCTGTTATGGTCTTA			
FABP4	Fatty acid binding protein 4, adipocyte	F	GGATGATAAGATGGTGCTGGA	NM_174314.2	Fatty acid metabolism	Martins et al., 2015
		R	ATCCCTTGGCTTATGCTCTCT			
SCD	Estearoil-CoA dessaturase 3	F	TTATTCCGTTATGCCCTTGG	NM_173959.4	Unsaturated fatty acid biosynthetic process	Teixeira et al., 2017
		R	TTGTCATAAGGGCGGTATCC			
GAPDH	Glyceraldehyde- 3-phosphate	F	AGATAGCCGTAACTTCTGTGC	NM_001034034.2	Glycolytic process	Martins et al., 2015
		R	ACGATGTCCACTTTGCCAG			
ACTB	Actin beta	F	CAGCAGATGTGGATCAGCAAGC	NM_173979.3	Normalizing gene	Sangalli et al., 2014
		R	AACGCAGCTAACAGTCCGCC			
18 S	18S ribosomal	F	CCTGCGGCTTAATTTGACTC	NM_036642.1	Normalizing gene	Bressan et al., 2013
		R	CTGTCAATCCTGTCCGTGTC			

[1]Biological processes described in NCBI

2.7 Data Statistical analysis

Phenotypic data

The data of phenotypic traits were analyzed according to a completely randomized design, with 16 repetitions per treatment, each animal being considered an experimental unit. The model includes the parent (bull) as a random effect and considers the groups (H_EPD and L_EPD), feedlot, and slaughter as a fixed effect. The analyses were conducted using SAS software (9.4; SAS Institute Inc., Cary, NC, USA). The significance was considered when $P < 0.10$.

Gene Expression

It was considered a completely randomized design, with 8 repetitions per treatment. The “threshold cycle” (Ct) values were normalized (ΔCt) based on the geometric mean Cts of the values obtained for normalizing genes. The relative expression of mRNAs were performed using Student t-test. All data followed the criteria of normality. The analyses were performed using the JMP software from SAS. The significance was considered when $P < 0.1$.

3 RESULTS

3.1 Phenotypic traits

The backfat thickness (BFT) and rump fat thickness (RFT), measured by carcass ultrasonography, was higher in H_EPD group animals than L_EPD group animals ($P < 0.1$; Table 2), indicating higher precocity or greater efficiency into BFT deposition of animals from the H_EPD group. Besides this, the marbling score (MAR) was higher in H_EPD group animals compared to L_EPD group animals ($P < 0.1$; Table 1), despite this, the content of intramuscular fat (IMF) did not differ between animals in the evaluated groups ($P = 0.149$).

Table 2. Effect of the groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity on the phenotypic traits of Nelore bulls (n=16)

Trait	GROUP		*P-value**
	H_EPD	L_EPD	
Initial body weight, kg	413.60±9.86	405.37±5.06	0.517
Final body weight, kg	598.00±11.72	581.62±8.04	0.254
Average daily gain, kg	1.85±0.13	1.76±0.14	0.459
Dry matter intake, kg	12.54±0.77	11.01±0.84	0.288
Back fat thickness[1], mm	5.87±0.50	3.78±0.48	0.006
Rump fat thickness[1], mm	8.56±0.58	7.05±0.69	0.086
Intramuscular fat[2]	2.23±0.34	1.34±0.31	0.149
Marbling score [3]	4.57±0.17	4.01±0.15	0.081

* Significant difference considered when P <0.1
[1] Measured by carcass ultrasonography
[2] Intramuscular fat determined by total lipids analysis (g of lipid / 100 g of meat; Bligh & Dyer, 1959)
[3] Marbling score determined by AMSA, considered select slight: 4.0–4.9; choice small: 5.0–5.9; choice modest: 6.0–6.9; choice moderate: 7.0–7.9; prime: 8.0–9.0 (Smith et al., 2013)

3.2 Total lipids and fatty acid composition

The profile of fatty acids in the meat, as well as the concentration of total lipids, do not differ between the groups (P > 0.10; Table 3). The H_EPD and L_EPD groups did not dissipate for total saturated fatty acids (ΣSFA), total branched-chain fatty acids (ΣBCFA), total monounsaturated fatty acids (ΣMUFA), and total polyunsaturated fatty acids (ΣPUFA). However, there was an individual difference for a single monounsaturated fatty acid (19:1 cis13) and, a single polyunsaturated fatty acid (22: 2 n6). The meat of animals in the H_EPD group had a lower concentration of 19:1 cis13 and a greater concentration of 22: 2 n6, compared to L_EPD group (P < 0.10; Table 3).

Table 3. Effect of the groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity on the fatty acid composition (% of total fatty acids) and total lipids of *longissimus thoracis* from Nelore bulls (n =16)

Variable	Group		*P-value*
	H_EPD	L_EPD	
Total lipids	1.440 ± 0.170	1.78 ± 0.160	0.1660
ΣSFA	37.376±20.0390	40.891±22.1290	0.3531
10:00	0.054±0.0098	0.058±0.0107	0.8512
12:00	0.064±0.0642	0.063±0.0124	0.9755
14:00	2.455±0.3069	2.608±0.3359	0.7801
16:00	21.804±1.0868	24.774±1.1938	0.1638
17:00	0.635±0.0965	0.689±0.1058	0.7675
18:00	11.300±0.8219	12.391±0.9000	0.4917
19:00	0.025±0.0095	0.019±0.0104	0.7431
20:00	0.032±0.0128	0.013±0.0140	0.4416
21:00	0.002±0.0012	0.004±0.0013	0.3213
22:00	0.291±0.0574	0.203±0.0628	0.4341
24:00:00	0.185±0.0461	0.196±0.0505	0.8969
ΣBCFA	0.402±0.1602	0.312±0.1758	0.7573
14:0 ISO	0.02877±0.010	0.021±0.0109	0.675
15:0 ISO	0.093±0.0219	0.071±0.0239	0.5694
15:0 ANTEISO	0.128±0.0258	0.125±0.0284	0.9466
16:0 ISO	0.104±0.0175	0.074±0.0193	0.3419
17:0 ISO	0.04020±0.0997	0.025±0.1101	0.9383
ΣMUFA	51.258±15.9220	47.451±17.5340	0.2014
14:1 cis9	0.861±0.1106	0.791±0.1210	0.7221
16:1 cis9	3.300±0.5373	1.816±0.5924	0.1564
16:1 cis10	0.009±0.0203	0.005±0.0220	0.9075
16:1 cis11	0.227±0.0448	0.135±0.0491	0.258
16:1 trans9	0.233±0.0575	0.236±0.0634	0.9773
15:1 cis10	0.101±0.0263	0.112±0.0288	0.8322
16:1 cis12 trans14	0.035±0.0537	0.019±0.0588	0.8765
16:1 trans10	0.036±0.0353	0.0005±0.0388	0.5647
16:1 trans11/12	0.075±0.0272	0.127±0.0298	0.3246
17:1 cis9	0.626±0.0560	0.576±0.0613	0.6405
17:1 cis10	0.239±0.0589	0.223±0.0645	0.8918
18:1 cis12	0.235±0.0423	0.212±0.0463	0.7782
18:1 cis/trans13	0.413±0.072	0.406±0.0791	0.9601
18:1 cis11	2.252±0.4103	2.224±0.4493	0.9719
18:1 cis14/trans16	0.070±0.0239	0.058±0.0256	0.7672
18:1 n9cis	40.052±1.3230	36.602±1.4607	0.177
18:1 trans6/8	0.067±0.0288	0.0846±0.0316	0.7594
18:1 trans9	0.115±0.1610	0.313±0.1763	0.5253
18:1 trans10	0.060±0.0543	0.222±0.0595	0.1332

18:1 trans11	0.669±0.3372	1.129±0.3693	0.4802
18:1 trans12	0.140±0.1609	0.226±0.1762	0.7796
19:1 cis11	0.182±0.0575	0.140±0.0630	0.7059
19:1 cis13	0.061±0.0254	0.142±0.0280	0.096
19:1 cis15	0.036±0.0115	0.015±0.0126	0.3403
20:1 cis5	0.009±0.0089	0.013±0.0098	0.8193
20:1 cis8	0.051±0.0391	0.037±0.0428	0.853
20:1 cis11	0.192±0.0460	0.127±0.0504	0.4389
22:1 n9	0.961±0.2847	1.062±0.3127	0.8528
ΣPUFA	5.243±0.9448	5.193±1.0346	0.9778
18:2 n6cis	3.865±0.7438	3.972±0.8145	0.9406
18:2 n6trans	0.110±0.0201	0.075±0.0220	0.3754
18:2 trans8 cis13	0.027±0.0093	0.039±0.0102	0.5299
18:2 trans9 cis12	0.020±0.0058	0.005±0.0063	0.1649
18:3 n3	0.215±0.0861	0.338±0.0950	0.4491
18:3 n6*γ*	0.006±0.0083	0.024±0.0091	0.2584
20:2 n6	0.042±0.0149	0.048±0.0163	0.8278
20:4 n3	0.023±0.0099	0.028±0.0109	0.7932
20:4 n6	0.028±0.0159	0.0080±0.0176	0.4927
22:2 n6	0.004±0.0013	0.002±0.0014	0.0074
22:3 n3	0.006±0.0054	0.004±0.0059	0.5348
22:4 n6	0.120±0.0345	0.086±0.0378	0.6034
22:5 n3	0.460±0.1330	0.390±0.1462	0.7822
ΣCLA	0.054±0.0041	0.040±0.0044	0.8571
CLA cis9 trans11	0.015±0.0061	0.0113±0.0061	0.7023
CLA trans10 cis12	0.016±0.0072	0.016±0.0168	0.9947
CLA trans8 cis10/CLA trans9 cis11	0.022±0.0369	0.012±0.0404	0.8907

Significant difference: $P < 0.10$

H_EPD and L_EPD: group of high (H) and low (L) expected progeny difference (EPD) for growth and precocity.Total lipids: Grams of lipid / 100 g of meat; ΣSFA: total saturated fatty acids; ΣBCFA: total branched-chain fatty acids; ΣMUFA: total monounsaturated fatty acids; ΣPUFA: total polyunsaturated fatty acids; ΣCLA: total conjugated linoleic acids.

Regarding Σn3, the proportion n6 / n3 and PUFA/SFA, no difference was observed between the genetic groups (P > 0.10; Table 4).

Table 4. Effect of the groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity on the means of Σn6, Σn3, n6/n3 ratio, PUFA/SFA ratio, Δ9 desaturase 14, 16 and 18 activity, health index (HI), atherogenicity index (AI), thrombogenicity index (TI) and Hypocholesterolemic/Hypercholesterolemic (h/H) of *longissimus thoracis* from Nelore bulls (n =16)

Variable	Group		*P-value*
	H_EPD	L_EPD	
Σn3	0.795±0.1927	0.815±0.2128	0.9562
n6/n3	8.567±8.0787	9.128±8.8468	0.9711
PUFA/SFA	0.147±0.0316	0.127±0.3468	0.7397
Δ9 DESSATURASE 14	26.358±1.5270	22.512±1.6731	0.1995
Δ9 DESSATURASE 16	12.355±2.2072	70.618±2.4372	0.2078
Δ9 DESSATURASE 18	78.121±1.6080	74.649±1.7612	0.2684
HI	1.784±0.1790	1.570±0.1958	0.5076
AI	0.576±0.0652	0.664±0.0716	0.4600
TI	1.211±0.1370	1.372±0.1506	0.5190
h/H	1.474±0.0578	1.571±0.0578	0.2387

Significant difference: $P < 0.10$
H_EPD and L_EPD: groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity
Σn6 (18:2 n6 trans; 18:2 cis9 trans12; 18:2 trans9 cis12; 18:2 n6 cis; 18:3 n6y; 20:2 n6; 20:4 n6; 22:2 n6; 22:4 n6); Σn3 (18:3 n3; 20:3 n3; 20:4 n3; 20:5 n3; 22:3 n3; 22:5 n3; 22:6 n3);
Δ9 desaturase 14 (100*(14:1 cis9/(14:1 cis9+14:0));
Δ9 desaturase 16 (100*(16:1 cis9/(16:1 cis9+16:0));
Δ9 desaturase 18 (100*(18:1 cis9/(18:1 cis9+18:0));
HI: Health Index (MUFA+PUFA)/(4*14:0)+16:0);
AI: Atherogenicity Index (12:0+(4*14:0)+16:0)/(Σn6+Σn3+ΣMUFA);
TI: Thrombogenicity Index (14:0+16:0+18:0)/((0,5*ΣMUFA)+(0,5*Σn6)+(3*Σn3)+n3/n6);
h/H: Hypocholesterolemic/Hypercholesterolemic (18:1 n9cis+18:2 n6cis+20:4 n6+18:3 n3+20:5 n3+22:5 n3+22:6 n3)/(14:0+16:0).

3.3 Gene Expression

The ΔCt value describes the difference between the CT value of the target gene and the CT value of the corresponding endogenous reference gene, such as a housekeeping gene, and is used to normalize for the template amount used:
ΔCt = CT (target gene) – CT (endogenous reference gene). It is worth mentioning that the statistic is performed with ΔCt data. The Ct is proportional to the logarithm of the initial amount of expression of the target gene in a given sample, the lower the initial number of Ct obtained from the target gene in the sample, it is because there was greater amplification of the target gene and, consequently, it presents greater expression. Therefore, the lower the Ct, the greater the gene expression, for this reason, after analysis, the data are transformed for the graphical representation $2^{-\Delta Ct}$.

The *PPARG, CEBPA,* and *ZNF423* gene expressions were not different when measured in backfat thickness (P > 0.1; Figure 1A, 1B, and 1C). Besides that, these genes were not detected in the visceral fat samples in the present study. From the total number of genes ten analyzed in backfat thickness, we identified six genes with a significantly different expression between the H_EPD and L_EPD. We can observe that the genes *LPL*, *ACOX1*, *SCD*, *LEP*, *GAPDH*, and *ACACA*, were more expressed in the BFT from the H_EPD group than the L_EPD group (P < 0.1; Figures: 2A, 2B, 2C, 2D, 3A, and 3B).

For visceral, fat we identified five genes with a different expression between the groups of Nelore cattle with high and low EPD. We can observe that the involved genes in lipid metabolism *ACOX1, FABP4, GAPDH, ACACA,* and *SCD* were more expressed in the visceral fat from the H_EPD group than L_EPD group (P < 0.1, Figures 4C, 5A, 5B, 5C and 5D).

Transcript levels of the genes measures in SUBCUTANEOUS fat

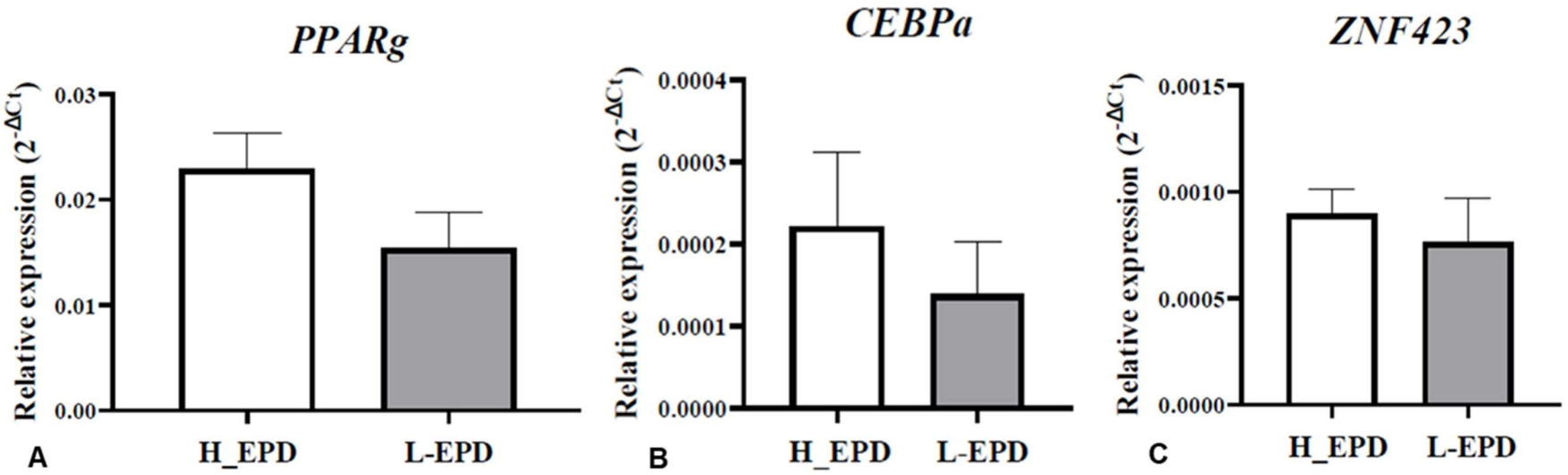

Figure 1: Transcript levels of the genes (A) Peroxisome proliferator-activated receptor gamma (*PPARg*); (B) CCAAT enhancer-binding 1 (*CEBPA);* (C) Zinc finger protein 423 (*ZNF423*) measures in subcutaneous fat from groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity. Non-significant difference: $P > 0.10$. Transformed value for a graphical representation ($2^{-\Delta Ct}$).

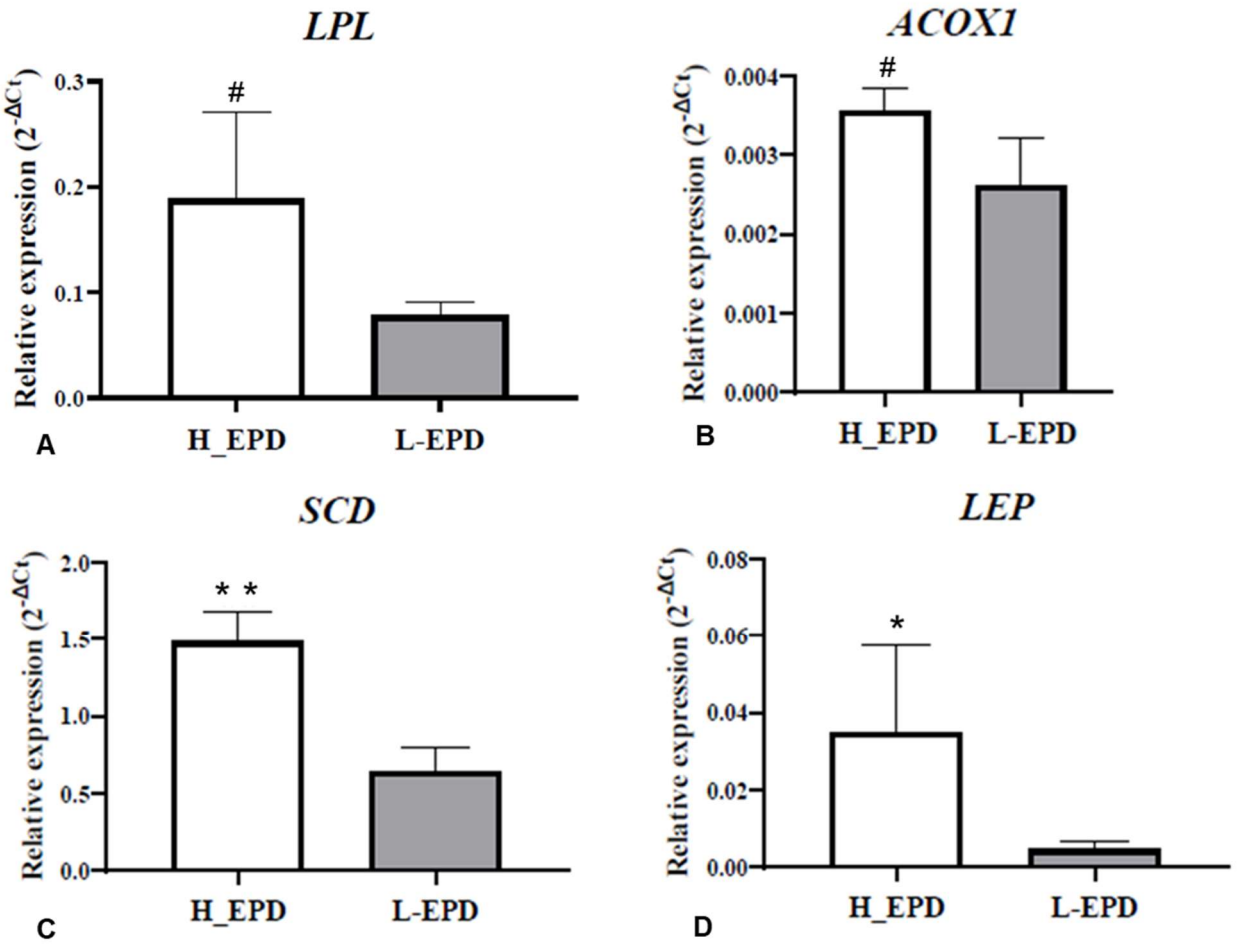

Figure 2: Transcript levels of the genes (A) Lipoprotein Lipase - *LPL*; (B) Acyl-CoA Oxidase - *ACOX1*; (C) Stearoyl-CoA Desaturase -*SCD*; (D) Leptin – *LEP*; measures in subcutaneous fat from groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity. Significant difference: $^{\#}$ P < 0.10, * P < 0.05 and * *P < 0.01. Transformed value for a graphical representation ($2^{-\Delta Ct}$).

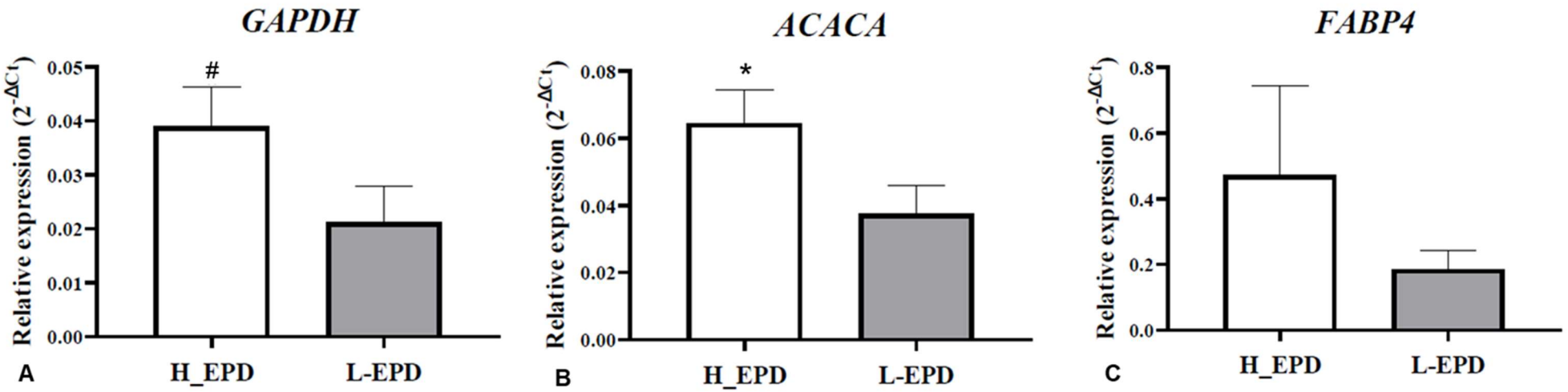

Figure 3: Transcript levels of the genes (A) Glyceraldehyde-3-Phosphate Dehydrogenase- *GAPDH*; (B) Acetyl-CoA Carboxylase 1 - *ACACA*; (C) Fatty Acid Binding Protein 4 - *FABP4*, measures in subcutaneous fat from groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity. Significant difference: $^{\#}$ P < 0.10, * P < 0.05 and * *P < 0.01. Transformed value for a graphical representation ($2^{-\Delta Ct}$).

Transcript levels of the genes measures in VISCERAL fat

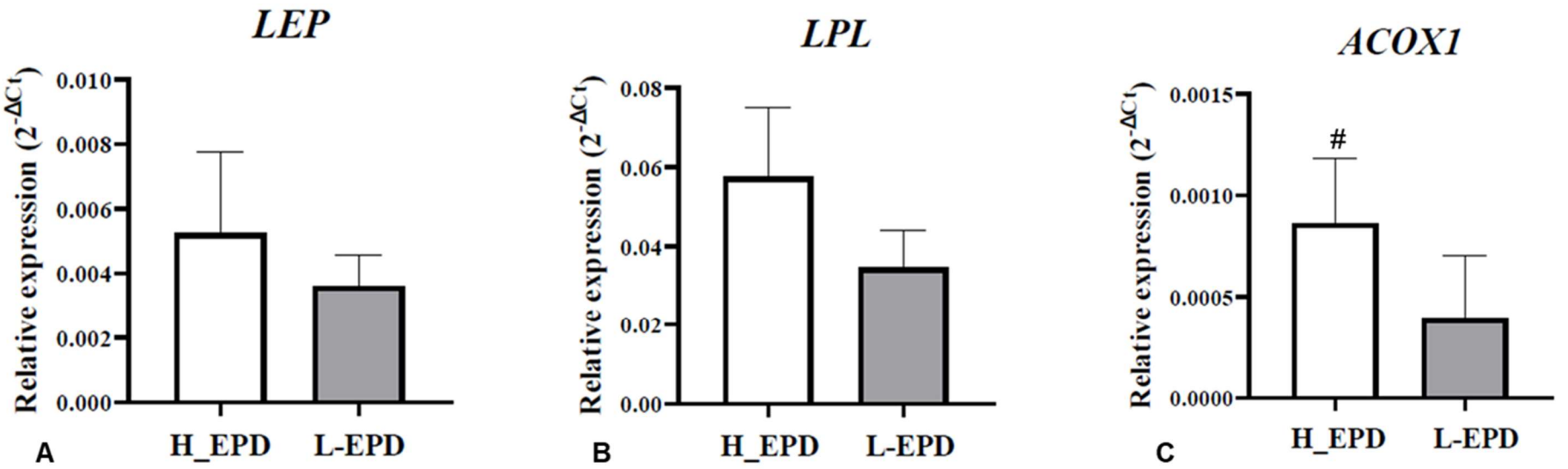

Figure 4: Transcript levels of the genes (A) Leptin – *LEP*; (B) Lipoprotein Lipase - *LPL*; (C) Acyl-CoA Oxidase - *ACOX1* measures in visceral fat from groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity. Significant difference: $^{\#}$ P < 0.10, * P < 0.05 and * *P < 0.01. Transformed value for a graphical representation ($2^{-\Delta Ct}$).

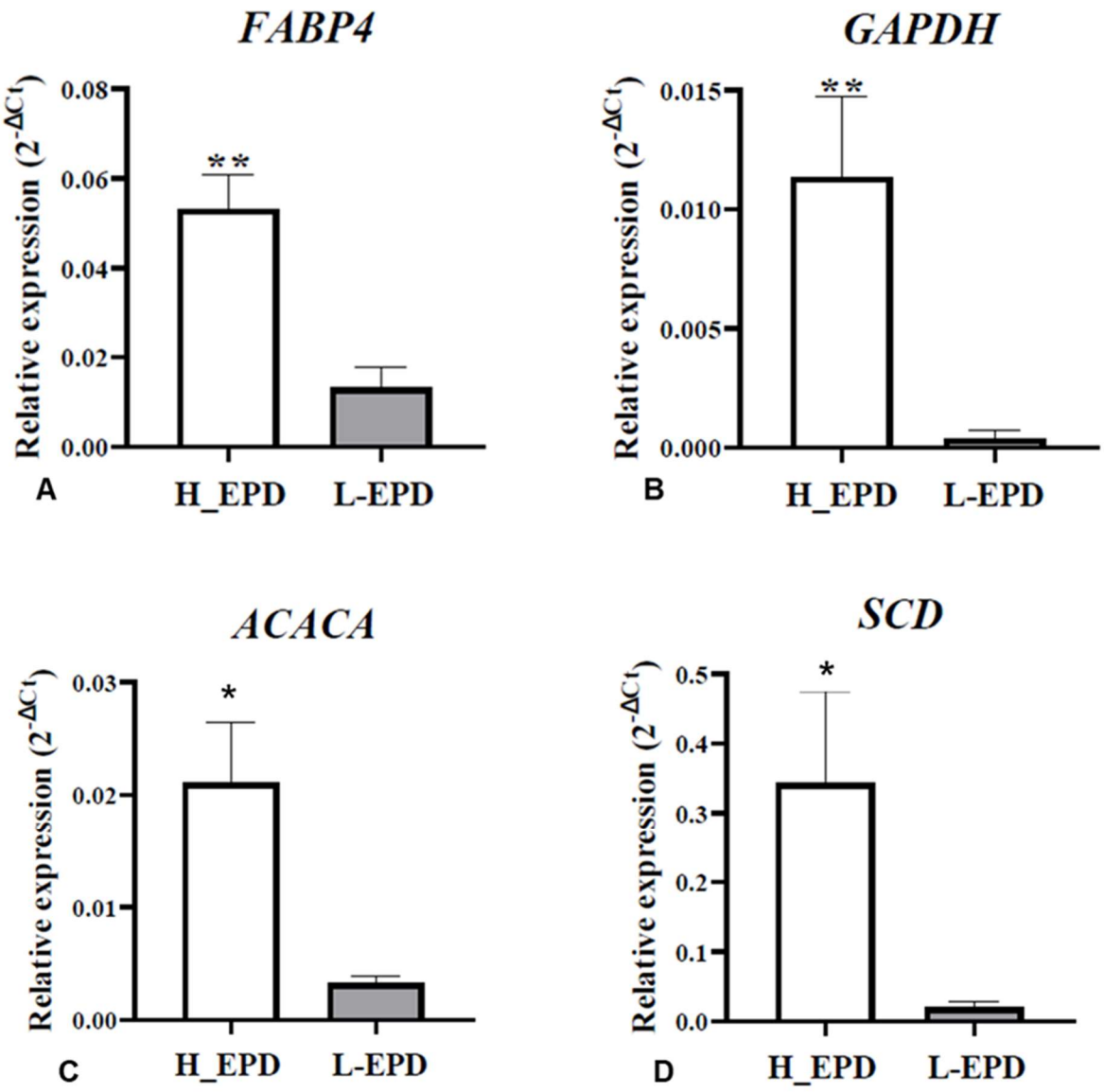

Figure 5: Transcript levels of the genes (A) Fatty Acid Binding Protein 4 - *FABP4*; (B) Glyceraldehyde-3-Phosphate Dehydrogenase -*GAPDH*; (C) Acetyl-CoA Carboxylase 1 - *ACACA*; (D) Stearoyl-CoA Desaturase – *SCD*; measures in visceral fat from groups of high (H) and low (L) expected progeny difference (EPD) for growth and precocity. Significant difference: $^{\#}$ P < 0.10, * P < 0.05 and * *P < 0.01. Transformed value for a graphical representation ($2^{-\Delta Ct}$).

4 DISCUSSION

4.1 Phenotypic traits

Several studies have phenotypic differences between animals with high and low EPD for traits of economic interest (FERNANDES et al., 2000; SAPP et al., 2002; DIKEMAN et al., 2005; BOLIGON et al., 2006), proving the methods of efficiency to identify superior animals in characteristics considered in the selection process. Besides this, the productivity of beef cattle is highly influenced by the rate of muscle growth and precocity that are used to select for improved performance and efficiency. Therefore, precocity is also a measurement of great importance to beef cattle producers.

In the present study, we show that animals presented the same initial body weight. Besides this, no difference was found between the high and low DEP groups for performance characteristics, as such ADG, DMI, and final WB, on the other hand, the H_EPD group animals revealed greater BFT and MAR than L_EPD group animals, indicating higher precocity of animals from the H_EPD group.

In accordance with the foregoing, several studies have been carried out to evaluate genetic parameters and phenotypic, for productive, reproductive, and carcass traits and beef quality between zebu and taurine breeds (KOOTS et al., 1994; REVERTER et al., 2003; among others). On the other hand, an increasing number of researches on phenotypic divergences between the contrasting genetic groups within the Nelore breed (CÔNSOLO et al., 2021; CÔNSOLO et al., 2020; COSTA et al., 2019; SANTANA et al., 2019; BONIN et al., 2014). However, these studies give more emphasis to muscle tissue and intramuscular fat, and rarely subcutaneous fat that is important to the industry, since generally base their charge scales on hot carcass weight (HCW), and the backfat thickness, required by slaughterhouses ranges between 3-6mm (González et al., 2012) levels that facilitate carcass handling, thereby reducing costs to the slaughterhouses.

4.2 Total lipids and fatty acid composition

The concentration of total lipids and the summation of the fatty acids composition in the meat (ΣSFA, ΣBCFA, ΣMUFA, ΣPUFA) not differ between the groups ($P > 0.1$). However, the meat of animals in the H_EPD group had a lower

concentration for a single monounsaturated fatty acid (19:1 cis13) and a greater concentration for a single polyunsaturated fatty acid (22: 2 n6), compared to the beef of animals in the L_EPD group ($P < 0.1$). Although the diet composition was the same for the two groups (27:73 forage/concentrate). Furthermore, the feedlot period may have been limiting for intramuscular fat deposition.

Fat from ruminant animals is a natural source of long-chain polyunsaturated fatty acids, which participate in various metabolic processes beneficial to human health and have been linked to the immune response (FRENCH et al., 2000; HOWE et al., 2006; WOOD et al., 2008). However, there was no difference in the total CLA (ΣCLA) nor for individual CLA (Table 3). Such a result was already expected since the animals were the same age, had the same feedlot time, and the same diet. Furthermore, Nelore animals have a low genetic predisposition for intramuscular fat deposition (PEREIRA et al., 2015; LAGE et al., 2012).

Although the groups did not present any difference for proportion n6 / n3, Table 4 shows higher values than recommended by The British Department of Health recommends an n6 / n3 ratio of 4: 1 for human health (BRITISH DEPARTMENT OF HEALTH, 1994). Animals finished in the feedlot generally present higher proportions of n6/n3 (WOOD et al., 2008), a fact associated with the results verified in our study for this relationship due to the high proportion of concentrate in the diet.

The proportion n6 / n3 has been increasing in human nutrition during the last years (SIMOPOULOS, 2008), a worrying fact because this proportion is related to risk for diseases such as prostate cancer (WILLIANS et al., 2011) and as a factor of risk without increasing obesity (SIMOPOULOS, 2008). Most of the meat produced in Brazil has nutritional advantages because the Nelore cattle are produced mainly in an extensive system whose food is the basis of a high proportion of forage, which brings benefits to the nutritional quality of the meat and human health (BARCELLOS et al., 2017). Thus, grass-fed beef contains higher ratios of n-3 fatty acids, which are beneficial for human health (DANNENBERGER, et al., 2006; MANN, et al., 2003).

The PUFA/SFA ratio was not influenced by the valuated groups (Table 4). The low intramuscular fat content observed in the Nellore cattle of the present study may be a fact intrinsic to the breed or to the feedlot time. These factors, in addition to the use of the same diet with a high content of concentrate, may have contributed to a similar proportion of the PUFA / SFA ratio between the groups evaluated. This

relationship represents the index used to assess the nutritional value of fats (WHO, 2003).

The activity of the delta 9 desaturase enzymes was no different (P > 0.1). Pitchford et al. (2002) related the highest enzyme activity to the highest intramuscular fat content, following the results observed in our study, in whom the intramuscular fat content did not differ. Likewise, there was no difference between the genetic groups for atherogenicity index, thrombogenicity index, and hypocholesterolemic/hypercholesterolemic ratio (P > 0.1). These indices allow for better nutrition, indicating whether food is harmful or not to human health, inferring the risks of coronary and cardiovascular diseases, such as atherosclerosis and stroke (SANTOS-SILVA et al., 2002).

4.3 Gene Expression

The backfat thickness is modulated by different metabolic pathways from those that regulate the deposition of fat in other adipose tissues, such as intramuscular or visceral. Most studies that identified proteins involved in intramuscular fat deposition used muscle instead of subcutaneous fat samples (KIM et al., 2009; MAO et al., 2016; SHEN et al., 2012; ZHANG et al., 2011). On the other hand, Pickworth et al. (2011) observed higher levels of *PPARG*, *FABP4*, *FASN* and *LPL*, messenger RNA (mRNA) in subcutaneous fat than in intramuscular fat in feedlot cattle (Simmental-Angus crossbred).

Accordingly, this study hypothesized that the expression of adipogenic genes (e.g. *PPARG*, *CEBPA*, *ZNF423*) and lipogenic (e.g. *FABP4*, *ACACA*, *LEP ACOX1*, *SDC*, *GAPDH*, and *LPL*) influence the deposition of subcutaneous fat from offspring of bulls Nelore with contrasting precocity EPD.

There was no difference for *CEBPA*, *PPARG*, and *ZNF423* gene expression in subcutaneous fat between the groups evaluated in the present study. These adipogenic genes were also not expressed in our visceral fat samples. We believe that this gene may be more involved with the deposition metabolism of intramuscular fat than with the deposition pathways of subcutaneous or visceral fat.

The transcription factors belonging to the *CEBPA* family actively participate in the regulation of adipogenesis together with *PPARG*. During adipogenesis, *CEBPA* is activated and binds directly to *PPARG* promoters, which in turn are signalings to

express *CEBPA* (DODSON et al., 2010a; DU et al., 2010b; YAMADA et al., 2009). The *PPARG* and *CEBPA* have a self-regulating action which makes necessary it a joint action to signal the differentiation of mesenchymal cells into adipocytes (ROSEN et al., 2002). Similar to *CEBPA*, *ZNF423* is a factor that promotes adipogenesis by increasing the expression of *PPARG*. The *ZNF423* was identified as a marker for the early stages of the adipogenesis is abundantly expressed in the stromal vascular fraction of tissues containing pre-adipocytes and mature adipocytes (GUPTA et al., 2010; GUPTA et al., 2012).

The date revealed that the lipogenic genes (*LPL, ACOX1, SCD, LEP, GAPDH and ACACA*) were more expressed in the subcutaneous fat of the H_EPD group than L_EPD group. As for visceral fat samples, the most expressed lipogenic genes of the H_EPD group than the L_EPD group were: *FABP4*, *ACACA*, *ACOX1*, *SCD*, and *GAPDH*.

It was observed that lipid metabolism differs between subcutaneous and visceral fat, concerning the *LPL* and *LEP* genes, differently expressed between the groups evaluated in subcutaneous fat and similar in visceral fat. These results bring us strong evidence that the biosynthesis of subcutaneous is strongly influenced by lipogenic genes. Animals H_EPD group showed higher expression of *LPL* in subcutaneous fat compared to animals in the L_EPD group. This result shows the relationship of *LPL* with higher carcass adiposity from animals H_EPD group.

The extracellular enzyme, *LPL,* is present in high amounts in skeletal and cardiac muscle and fat tissue. *LPL* is the main enzyme responsible for the hydrolysis of circulating triacylglycerol, generating free fatty acids, which are oxidized in the muscles or re-esterified in the adipose tissues in glycerol, which is returned to the liver (WAI et al., 2009). Therefore, *LPL* plays a central role in the general metabolism of lipoproteins, generating low-density lipoproteins (LDL). The particles formed, from the *LPL* catalysis, contribute to the maturation of high-density lipoprotein (HDL) precursors, involved in the reverse cholesterol transport (PREISS et al., 2002, PILLARISETTI & SAXENA 2003). *LPL* is an essential enzyme in lipid deposition, and tissue metabolism is involved in inflammatory processes that lead to fat deposition, regulates plasma concentrations of triglycerides and HDL, and is a possible gene candidate the biomarker of lipid deposition and energy balance (GUI et al., 2016).

The data presented here also indicated that H_EPD group had a higher gene expression of *ACOX1* and a higher BFT than the L_EPD group. Several studies point

to the critical role of *ACOX1* in the metabolism of fatty acids and its possible functions in the deposition of fat in several animal species, including cattle (WU et al., 2018). *ACOX1* is one of the first enzymes involved in the peroxisomal β-oxidation pathway. This enzyme is regulated by the peroxisome alpha proliferator (*PPARα*) and is essential for the oxidation and deposition of fatty acids, especially in very-long-chain fatty acid lipid metabolism (WU et al., 2018). The *ACOX1* gene can be a potential meat quality enhancer in pigs and cattle (WU et al., 2018). In this sense, ZUO et al. (2007) found that one A / C polymorphism in intron "9" was associated with fat deposition in pigs, and the other (A1865C) in exon "13" was related to BFT and marbling in beef (JIAO et al., 2011). Soon, our data suggest that AOCX1 exerts a direction act on the increase BFT.

The animals belonging to the group with the highest genetic potential for precocity (H_EPD group) showed greater *SCD* gene expression, which may have contributed to the higher subcutaneous fat deposition in these animals. The H_EPD group showed higher SCD gene expression measured in subcutaneous and visceral fat than the L_EPD group. The SCD enzyme can be a negative correlation with weight gain when measured in longissimus thoracis (LADEIRA et al., 2014). These results reinforce that lipid metabolism differs among differents tissues and additionally can influence the phenotypic traits differently.

Variations in *SCD* enzyme activity in mammals are likely to affect various physiological variables, including cell differentiation, insulin sensitivity, metabolic rates, adiposity, atherosclerosis, cancer, and obesity (PATON and NTAMBI, 2009). The action of *SCD* reflects a higher synthesis of fatty acids, which can influence growth efficiency, besides altering the composition of fatty acids deposited in meat (KIM et al., 2000). *SCD* is an endoplasmic reticulum enzyme that catalyzes the biosynthesis of MUFAs, from saturated fatty acids that are synthesized again or derived from the diet (SMITH et al., 2006). The fatty acid composition in the groups evaluated was not different, although a difference was found in the *SCD* gene expression measured in subcutaneous fat. We believe that happened due to the same diet and little feedlot time for groups with contrasting precocity.

As expected, the H_EPD group showed greater expression of the LEP gene in subcutaneous fat, which can explain the contrasting phenotype for BFT between the groups evaluated, in agreement with some authors who report that the leptin is associated with the percentage of the body fat (JI et al., 1998; MINTON et al., 1998).

In the present study, the animals H_EPD group (higher precocity and greater BTF) also had higher *GAPDH* and *ACACA* gene expression than animals from the L_EPD group. The *GAPDH* has been shown as a central protein in the carbon metabolism pathway, acting mainly in glycolytic processes, and is an essential protein for lipid biosynthesis (UniProt Consortium, 2021). The *GAPDH* catalyzes the conversion of glyceraldehyde 3-phosphate to 1, 3 bisphosphoglycerate in glycolysis, which provides a substrate for acetyl-CoA for the synthesis of various cellular compounds including lipids (OHLROGGE et al., 1995).

Within this context, some enzymes are essential for supplying NADPH for lipid biosynthesis. Known enzymes involved in this process include malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphoglucose dehydrogenase from the phosphate pentose pathway beside the isocitrate dehydrogenase (HAO et al., 2014; and HAO et al., 2016). The *ACACA* is a cytosolic enzyme that catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, the first limiting step of de novo fatty acid biosynthesis. This subpathway does part of the pathway malonyl-CoA biosynthesis, which is itself part of lipid metabolism (UniProt Consortium, 2021).

We did not find a difference in *FABP4* expression in the samples of subcutaneous fat. On the other hand, the H_EPD group animals revealed higher *FABP4* gene expression in visceral fat, than L_EPD group animals under the conditions in which the present study was executed. The *FABP4* is a lipid transport protein in adipocytes. This protein binds both long-chain fatty acids and retinoic acid and delivers long-chain fatty acids and retinoic acid to their receptors in the nucleus. The FABP4 acts in the fatty acid metabolic process and cholesterol homeostasis, besides brown and white fat cell differentiation (UniProt Consortium, 2021).

As noted, bovines with higher genetic potential for precocity have higher levels of transcription of lipogenic genes. This can explain the higher fat deposition in the carcass of Nelore progenies with higher EPD for precocity. Therefore, we conclude that the BFT is more related to lipogenic than adipogenic factors. However, further studies are needed to investigate the action of microRNAs in genetic groups of Zebu cattle with different potentials for precocity and deposition of divergent subcutaneous fat. These studies present evidence for new targets that will assist in research on fat deposition and can contribute to breeding programs to optimize beef quality.

5 CONCLUSION

Adipogenesis and lipogenesis are complex processes and involves the interplay of diverse transcription factors, and several other mechanisms will also be considered.

Although the *ACACA*, *LPL*, *ACOX1*, *LEP*, *SCD*, and *GAPDH* genes expression had been different, the composition of beef fatty acid has not been changed. Therefore, the subcutaneous fat genes do not interfere with the lipid metabolism of intramuscular fat.

Bovines with higher genetic potential for precocity have higher levels of transcription of lipogenic genes. This can explain the higher fat deposition in the carcass of Nelore progenies with higher EPD for precocity. Therefore, we conclude that the BFT is more related to lipogenic than adipogenic factors. Our study suggests that the genes *ACACA, LPL, ACOX1, LEP, SCD*, and *GAPDH* can be are important factors involved in subcutaneous fat deposition.

Nevertheless, there is a doubt, and we encourage the continuation of this investigation to verify possible post-transcriptional regulation by microRNAs of the genes mentioned above. Finally, the exploration of genes and microRNAs will allow a better understanding of the metabolic pathways involved in fat deposition in the carcass cattle and help instill industry confidence in the contribution of this process to improvements in carcass and meat quality in beef cattle.

ACKNOWLEDGEMENTS

We are grateful to FAPESP for the scholarship (Grant #2017/02349–1) and financial support (process #2016/17433-5; #2011/21241-0; #2012/50788-0). We also thank CNPq (process #409186/2018-0) and CAPES (Finance Code 001) for financial support.

6 REFERENCES

Aalhus, J. L.; Janz, J. A. M.; Tong, A. K. W.; Jones, S. D. M.; Robertson, W. M. The influence of chilling rate and fat cover on beef quality. **Canadian Journal of Animal Science**, v. 81, p. 315–320, 2001.

Barcellos, V. C.; Mottin, C.; Passetti, R. A. C.; Guerrero, A.; Eiras, C. E.; Prohmann, P. E. F.; Vital, A. C. P.; Prado, I. N. do. Carcass Characteristics and Sensorial Evaluation of Meat from Nellore Steers and Crossbred Angus vs Nellore Bulls. **Acta Sci. Anim. Sci**, v. 39, p. 437 - 448, 2017. doi.org/10.4025/actascianimsci.v39i4.36692

Berton, M. P., Fonseca, L. F.S., Gimenez, D. F.J. et al. Gene expression profile of intramuscular muscle in Nellore cattle with extreme values of fatty acid. **BMC Genomics**, v. 17, p. 972, 2016. doi.org/10.1186/s12864-016-3232-y

Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology**, v. 37, p. 911-917, 1959.

Boligon, A. A.; Rorato, P. R. N.; Weber, T.; Everling, D. M. E.; Lopes, J. S. Herdabilidades para ganho de peso da desmama ao sobreano e perímetro escrotal ao sobreano e tendências genética e fenotípica para ganho de peso da desmama ao sobreano em bovinos Nelore-Angus. **Revista Brasileira de Zootecnia**, v.35, p.1323-1328, 2006.

Bonin, M. N.; Ferraz, J. B. S.; Eler, J. P.; Rezende, F. M.; Cucco, D. C.; Carvalho, M. E.; Silva, R. C. G.; Gomes, R. C.; Oliveira, E. C. M. Sire effects on carcass and meat quality traits of young Nellore bulls. **Genetics and molecular research: GMR**, v. 13, (2), p.3250-3264, 2014b.

Bonin, M.N. ; Ferraz, J. B. S. ; Eler, J. P. ; Silva, S. L ; Rezende, F. M. ; Cucco, D.C. ; Carvalho, Minos Esperândio ; Silva, R. C. G. ; Oliveira, E. C. M. . Características de carcaça e qualidade de carne em linhagens da raça Nelore. **Ciência Rural** (UFSM. Impresso), v. 44, p. 1860-1866, 2014a.

Brasil. Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal (**Food of animal origin sanitary and industry inspection**). In. Brasília: Ministério da Agricultura, Pecuária e Abastecimento. 2017.

British Department of Health. Nutritional aspects of cardiovascular diseases. **Report on health and social subjects n°46**. London: H.M. Stationary Office, 1994.

Clarke, A.M.; Drennan, M.J.; Mcgee, M.; Kenny, D.A.; Evans, R.D.; Berry, D.P. Intake, growth and carcass traits in male progeny of sires differing in genetic merit for beef production. **Animal**, v. 3, p. 791–801, 2009.

Cônsolo, N.R.B.; Silva, J.; Buarque, V.L.M.; Higuera-Adilla, A.; Barbosa, L.C.G.S.; Zawadzki, A.; Colnago, L.A.; Saran Netto, A.; Gerrard, D.E.; Silva, S.L. Selection for Growth and Precocity Alters Muscle Metabolism in Nellore Cattle. **Metabolites**, v. 10, p. 1–12, 2020. doi.org/10.3390/metabo10020058

Cônsolo, N.R.B.; Buarque, V.L.M.; Silva, J; Poleti, M.D.; Barbosa, L.C.G.S.; Higuera-Padilla, A.; Gomez, J.F.M.; Colnago, L.A.; Gerrard, D.E.; Saran Netto, A.; Silva, S.L. Muscle and liver metabolomic signatures associated with residual feed intake in Nellore cattle, **Animal Feed Science and Technology**. 2021. doi: doi.org/10.1016/j.anifeedsci.2020.114757

Costa, M. B.; Cônsolo, N. R. B.; Silva, J.; Buarque, V. L. M.; Padilla, A. R. H.; Coutinho, I. D.; Barbosa, L. C. G. S.; Colnago, L. A.; Silva, S. L; Saran Netto, A. Performance, carcass traits and serum metabolomic profile of Nellore males with different genetic potential for post-weaning growth. **Animal**, v. 14, p. 873-880, 2020.

Dannenberger, D.; Nuernberg, K.; Nuernberg, G.; Ender, K. Carcass and meat quality of pasture vs concentrate fed German Simmental and German Holstein bulls. **Archiv Tierzucht**, v. 49, p. 315 – 328, 2006.

Devine, C. E.; Wahlgren, N. M.; Tornberg, E. Effect of rigor temperature on muscle shortening and tenderization of restrained and unrestrained beef m. Longissimus thoracicus et lumborum. **Meat Science**, v. 51, p. 61–72, 1999.

Dikeman, M. E.; Pollak, E. J.; Zhang, Z.; Moser, D. W.; Gill, C. A. E.; Dressler, E. A.; Phenotypic ranges and relationships among carcass and meat palatability traits for fourteen cattle breeds, and heritabilities and expected progeny differences for Warner-Bratzler shear force in three beef cattle breeds. **Journal Animal Science**, v.83, p. 2461–2467, 2005.

Dodson, M. V.; Hausman, G. J.; Guan, L.; Du, M.; Rasmussen, T. P.; Poulos, S. P.; Mir , P.;BErgen, W. G.; Fernyhough, M.E., Mcfarland, D. C.; Rhoads, R. P.; Soret B.; Reecy, J. M.; Velleman, S. G.; Andjiang, Z. A. Lipid metabolism, adipocyte depot physiology and utilization of meat animals as experimental models for metabolic research. **International Journal of Biological Sciences**, v. 6, p. 691-699, 2010b.

Dodson, M. V.; Jlang, Z.; chen, J.; Hausman, G.J.; Guan, L.L.; Novakofski, J.; Thompson, D.P.; Lorenzen, C.L.; Fernyhough, M.E.; Mir, P.S.B.; Allied industry approaches to alter intramuscular fat content and composition in beef animals. **Journal of Food Science**, v. 75, p. R1-R8. 2010a.

Du, M.; J. Yin.; M. J. Zhu. Cellular signaling pathways regulating the initial stage of adipogenesis and marbling of skeletal muscle. **Meat Science**, v. 86, p.103-109, 2010.

Duarte, M. S.; Paulino, P.V.R.; Das, A. K.; Wei, S.; Serao, N. V. L.; Fu, X.; Harris, S. M.; Dodson, M. V.; Du, M. 2013. Enhancement of Adipogenesis and Fibrogenesis in Skeletal Muscle of Wagyu Compared With Angus Cattle. **Journal of Animal Science**. v. 91. p. 2938–2946. 2013. doi:10.2527/jas2012-5892

Eler, J. P.; Ferraz, J. B.; Balieiro, J. C.; Mattos, E.C.; Mourão, G. B. Genetic correlation between heifer pregnancy and scrotal circumference measured at 15 and 18 months of age in Nellore cattle. **Genetic Molecular Research**, v. 5(4), p. 569 - 80, 2006.

Fernandes, H. D. and Ferreira, G. B. Estudo Comparativo de Sete Diferentes Modelos Estatísticos para a Característica Ganho de Peso em Bovinos de Corte. **Revista Brasileira de Zootecnia**, v. 29, p1340-1348, 2000.

Ferraz, J. B. S and Felício, P. E. Production systems An example from Brazil. **Meat Science**. v. 84, p. 238-243, 2010.

French, P.; O´riordan, E. G.; Monahan, F. J. et al. Meat quality of steers finished on autumn grass, grass silage or concentrate based diets. **Meat Science**, v. 56, p. 173–180; 2000.

Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipides from animal tissues. **The Journal of biological chemistry**, v. 226, n. 1, p. 497–509, 1957.

González, L. A.; Schwartzkopf-Genswein, K. S.; Bryan, M.; Silasi, R.; Brown, F. Factors affecting body weight loss during commercial long haul transport of cattle in North America. **J Anim Sci**, v. 90(10), p. 3630-3639, 2012. doi: 10.2527/jas.2011-4786.

Gui, L.; JIa, C.; Zhang, Y.; ZHao, C.; Zan, L. Association studies on the bovine lipoprotein lipase gene polymorphism with growth and carcass quality traits in Qinchuan cattle. **Molecular and Cellular Probes**, v. 2, p. 61-5. 2016

Gupta, R. K. Z.; Arany, P.; Seale, R. J.; Mepani, L.; YE, H. M.; Conroe, Y. A.; Roby, H.; Kulaga, R.; Reed, R.; Spiegelman, B. M. Transcriptional control of preadipocyte determination by *Zfp423*. **Nature**, v. 464, p.619-623, 2010.

Gupta, R. K.; Mepani, R. J.; Kleiner, S.; Lo, J. C.; Khandekar, M. J.; Cohen, P.; Frontini, A.; Bhowmick, D. C.; Cinti, L. Ye, S.; B. M. Spiegelman. *Zfp423* expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. **Cell Metabolism**, v. 15, p.230-239, 2012.

Hao, G.; Chen, H.; Du, K.; Huang, X.; Song, Y.; Gu, Z.; et al. Increased fatty acid unsaturation and production of arachidonic acid by homologous over-expression of the mitochondrial malic enzyme in Mortierella alpina. **Biotechnol. Lett**, v. 36, p. 1827–1834, 2014. 10.1007/s10529-014-1546-x

Hao, G.; Chen, H.; Zhang, H.; Chen, W.; Chen, Y. Q. Metabolic engineering of Mortierella alpina for enhanced arachidonic acid production through the NADPH supplying strategy. **Appl. Environ. Microbiol**, v. 82, p. 3280–3288, 2016. 10.1128/AEM.00572-16

Hausman, G. J; Bergen, W. G; Etherton, T. D; Smith, S. B. The history of adipocyte and adipose tissue research in meat animals. **Journal of Animal Science**, v. 96 (2), p. 473–486, 2018.

Hausman, G. J.; Dodson, M. V.; AJuwon, K.; Azain, M.; Barnes, K. M., Guan, L.L.; Jiang, Z.; POulos, S. P.; SAinz, R. D.; Smith, S.; Spurlock, M.; Novakofski, J.; Fernyhough, M. E. Board-invited review: The biology and aregulation of

preadipocytes and adipocytes in meat animals. **Journal of Animal Science**, v. 87, p.1218-1246, 2009.

Hishikawa, D.; Hong, Y.H.; Roh, S.G.; Miyahara, H.; Nishimura, Y.; Tomimatsu, A., Tsuzuki, H.; Gotoh, C.; Kuno, M.; Choi, K.C.; Lee, H. G., Cho, K.K.; Hidari, H., Sasaki, S. Identification of genes expressed differentially in subcutaneous and visceral fat of cattle, pig, and mouse. **Physiol. Genomics**, v. 21, p. 343–350, 2005.

Hossner, K. L. **Hormonal regulation of farm animal growth** / K.L. Hossner.p. 217. Department of Animal Sciences, Colorado State University, Fort Collins, Colorado,USA. CABI Publishing, 2005.

Howe, P.H.; Meyer, B.; Record, S.; Bughurst, K. Dietary, intake of ong-chain x-3 polyunsaturated fatty acid: contribution of meet source. **Nutrition**, v. 22, p. 47-53. 2006.

Ibrahim, M. M. Subcutaneous and visceral adipose tissue: structural and functional differences. **Obes Ver**, v. 11(1), p. 11-8. 2010. doi: 10.1111/j.1467-789X.2009.00623.x.

Jiao, Y.; Zan, L. S.; Liu, Y. F.; Wang, H. B.: Molecular characterization, polymorphism of the ACOX1 gene and association with ultrasound traits in Bos Taurus. **Genetic Molecular Research**, v. 10, p. 1948–1957, 2011.

Ji, S. Q.; Willis, G. M.; Scott, R.; R. E Spurlock, M. E. Partial cloning and expression of the bovine leptin gene. **Animal Biotechnology**, v.9, p.1–14, 1998.

Jo, J.; Shreif, Z.; Periwal, V. Quantitative dynamics of adipose cells. **Adipocyte**, v. 1, p. 80–88, 2012.

Kim, D.; Kim, J.; Hyun, H.; Kim, K.; Roh, S. A nanoscale ridge/groove pattern arrayed surface enhances adipogenic differentiation of human supernumerary tooth-derived dental pulp stem cells in vitro. **Arch. Oral Biol**, v.59 (8), p. 765–774, 2014

Kramer, J. K. G.; Fellner, V.; Dugan, M. E. R. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. **Lipids,** v. 32, p. 1219-1228, 1997.

Kuang J.; Yan X.; Genders A. J.; Granata C.; Bishop D. J. An overview of technical considerations when using quantitative real-time PCR analysis of gene expression in human exercise research. **PLoS One**, v. 13 (5), p. e0196438. 2018. doi:10.1371/journal.pone.0196438

Kwon, E. G.; Park, B. K.; Kim, H. C.; Cho, Y. M.; Kim, T.; Chang, S. S.; Oh, Y. K.; Kim, N. K.; Kim, J. H.; Kim, Y. J.; Kim, F-J.; Im, S. K.; Choi, N-J. Effects of Fattening Period on Growth Performance, Carcass Characteristics and Lipogenic Gene Expression in Hanwoo Steers. **Asian-Aust. J. Anim. Sci**, v. 22, p. 1654 – 1660, 2009.

Ladeira, M.; M.; Machado Neto, O. R.; Santarosa, L. C.; Chizzotti, M. L.; Oliveira, D. M.; Carvalho, J. R. R.; Alves, M. C. L. Performance, carcass traits, and gene expression in young bulls fed lipids and monensin. **Pesquisa Agropecuária Brasileira**. vol.49 no.9 Brasília Sept. 2014.

Lage, J.F.; Paulino, P.V.R.; Valadares Filho, S.C.; Souza, E.J.O.; Duarte, M.S., Benedeti, P.D.B.; Souza, N.K.;COX, R.B. Influence of genetic type and level of concentrate in the finishing diet on carcass and meat quality traits in beef heifers. **Meat Science**, v. 90, p. 770±74, 2012.

Lozeman, F. J.; Middleton, C. K.; Deng, J.; Kazala, E. C.; Verhaege, C.; Mir, P. S.; et al. Characterization of microsomal diacylglycerol acyltransferase activity from bovine adipose and muscle tissue. **Comp Biochem Physiol B Biochem Mol Biol**, v. 130, p. 105–15. 2001.

Mann, N. J; Ponnampalam, E. N.; Yep, Y.; Sinclair, A. J. Feeding regimens effect fatty acid composition in Australian beef cattle. **Asia Pacific Journal of Clinical Nutrition**, S38 (Suppl.), 2003.

Marsh, B.B. The basis of tenderness in muscle foods. **Journal of Food Science**, v. 42, n. 2, p. 295, 1977.

Martins, T. S.; Sanglard, L. M.; Silva, W.; Chizzotti, M. L.; Rennó, L. N.; Serão, N. V.; Silva, F. F.; Guimarães, S. E.; Ladeira, M. M.; Dodson, M. V.; Du, M.; Duarte, M. S. Molecular factors underlying the deposition of intramuscular fat and collagen in skeletal muscle of Nellore and Angus cattle. **PLoS One**, v. 10; p. 10:e0139943, 2015.

Melendez, P.; Poock, S. E.; Pithua, P.; Pinedo, P.; Manriquez, D.; Moore, S.G.; Neal, J. D.; Taylor, J. F. Genome-wide study to detect single nucleotide polymorphisms associated with visceral and subcutaneous fat deposition in Holstein dairy cows. **Animal**, v. 13, p. 487-494. 2019. doi.org/10.1017/S1751731118001519

Miller, M. F.; Cross, H. R.; Lunt, D. K.; Smith, S. B. Lipogenesis in acute and 48h cultures of bovine intramuscular and subcutaneous adipose tissue explants. **Journal of Animal Science**, v.69, n.2, p.162-170, 1991.

Miner, J. L. The adipocyte as an endocrine cell. **Journal of Animal Science**, v.82, p.935-941, 2004.

Minton, J. E., Bindel, D. J., Drouillard, J. S., Titgemeyer, E. C., Grieger, D. M.; Hill, C. M. Serum leptin is associated with carcass traits in finishing cattle. **Journal of Animal Science**, v. 76, p. 231-239, 1998.

Muroya, S.; Ogasawara, H.; Nohara, K.; Oe, M.; Ojima, K.; Hojito, M. Coordinated alteration of mRNA-microRNA transcriptomes associated with exosomes and fatty acid metabolism in adipose tissue and skeletal muscle in grazing cattle. **Anim Biosci**, v, 33(11), p. 1824-1836, 2020. doi.org/10.5713/ajas.19.0682

Mota, L. F. M.; Bonafé, C. M.; Alexandre, P. A.; Santana, M. H.; Novais, F. J.; Toriyama, E.; Pires, A. V.; Silva, S. L.; Leme, R. P.; Ferraz , J. B.; Fukumasu, H.

Circulating leptin and its muscle gene expression in Nelore cattle with divergent feed efficiency. **Journal of Animal Science and Biotechnology**, v. 201, p.78-71, 2017.
NCBI (**National Center for Biotechnology Information**) link: https://www.ncbi.nlm.nih.gov/

Ohlrogge J. and Browse J. Lipid biosynthesis. **Plant Cell**, v. 7, p. 957–970, 1995.
Park, S.; Yan, Z.; Choi, C.; Kim, K.; Lee, H.; Oh, Y.; Jeong, J.; Lee, J.; Smith, S. B.; Choi, S. Carcass and Meat Characteristics and Gene Exp-ression in Intramuscular Adipose Tissue of KoreanNative Cattle Fed Finishing Diets Supplementedwith 5% Palm Oil. **Korean Journal for Food Science of Animal Resources**, v. 37(2), p. 168-174, 2017. Doi: doi.org/10.5851/kosfa.2017.37.2.168

Parrish, F.C. et al. Effect of postmortem conditions oncertain chemical, morphological and organoleptic propertiesof bovine muscle. **Journal of Food Science**, v.38, p. 690-695, 1973

Paton, C. M and Ntambi, J. M. Biochemical and physiological function of stearoyl-CoA desaturase. **American Journal of Physiology-Endocrinology and Metabolism**, v. 297, p. E28–E37, 2009.

Pereira, A. S. C.; Baldi, F.; Sainz, R. D.; Utembergue, B. L.; Chiaia, H. L. J.; Magnabosco, C. U.; Manicardi, F. R.; Araujo, F. R. C.; Guedes, C. F.; Margarido, R. C.; Leme, P. R.; Sobral, P. J. A. Growth performance, and carcass and meat quality traits in progeny of Poll Nellore, Angus and Brahman sires under tropical conditions. **Animal Production Science**, v. 55 (10), p. 1295-1302, 2015.

Pflanzer, S. B. and Felício, P. E. Moisture and fat content, marbling level and color of boneless rib cut from Nellore steers varying in maturity and fatness. **Meat Science**, v. 87, p. 7-11, 2011.

Pflanzer, S. B. and Felício, P. E. Effects of teeth maturity and fatness of Nellore (Bos indicus) steer carcasses on instrumental and sensory tenderness. **Meat Science**, v. 83, p. 697-701, 2009.

Pillarisetti S and Saxena U. Lipoprotein lipase as a therapeutic target for dyslipidemia. **Front Biosci**, v. 8, p. d238–241, 2003.

Pickworth, C. L.; Loerch, S. C.; Velleman, S. G.; Pate, J. L.; Poole, D. H.; Fluharty, F.L. Adipogenic differentiation state-specific gene expression as related to bovine carcass adiposity. **Journal of Animal Science**, v. 89, p. 355–366,2011.

Pinto Neto, M.; Beraquet, N. J.; Cardoso, S. Effects of chilling methods and hot-boning on quality parameters of *M. Longissimus lumborum* from *Bos indicus* Nelore steer. **Meat Science**, v. p. 93, 201–206, 2013.

Pitchford, W. S.; Deland, M. P. B.; Siebert, B. D.; Malau-Aduliand, A. E. O.; Bottema, C. D. K. Genetic variation in fatness and fatty acid composition of crossbred cattle. **Journal of Animal Science**, v.80, p.2825-2832, 2002.

Preiss-Landi, K.; Zimmermann, R.; HAmmerle, G.; Zechner, R. Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. **Curr Opin Lipidol**, v. 13, p. 471–481, 2002.

Reverter, A.; Johnston, D.J.; Ferguson, D.M; Perry, D.; Goddard, M.E.; Burrow, H. M.; Oddy, V. H.; Thompson, J.M.; Bindon, B M. Genetic and phenotypic characterization of animal, carcass and meat quality traits for temperate and tropically adapted beef breeds. Correlations among animal, carcass and meat quality. **Australian Journal of Agricultural Research**, v. 54, p. 149-158, 2003.

Ritchie, H. D.; Rust, S. R.; Merkel, R. A.; Bergen, W. G. Getting rid of excess fat is not an easy task. Feedstuffs, v. 13. 1993.

Robinson, D. L. "Models which might explain negative correlations between direct and maternal genetic effects." **Livest Prod Sci**, v. 47, p. 111-122, 1996. doi: doi.org/10.1016/0301-6226(96)00002-4

Rodrigues, R.T.S.; Chizzotti, M. L.; Vital, C. E.; Baracat-Pereira, M. C.; Barros, E.; Busato, K. C.; Gomes, R. A.; Ladeira, M. M.; Martins, T. S. Differences in Beef Quality between Angus (Bos taurus taurus) and Nelore (Bos taurus indicus) Cattle through a Proteomic and Phosphoproteomic Approach. **PLoS ONE**, v. 12 (1), p. e0170294. 2017. doi:10.1371/journal.pone.0170294

Rosen, E. D.; Hsu, C.-H.; Wang, X.; Saka, S.; Freeman, M. W.; Gonzalez, F. J.; Spiegelman, B. M. C/ebpα induces adipogenesis through pparγ: A unified pathway. **Genes & Development**, v. 16: 22-26, 2002.

Sangalli, J. R.; chiaratti, M. R.; Bem, T. H. C.; Araujo, R. R.; Bressan F. F.; Sampaio, R. V. Development to Term of Cloned Cattle Derived from Donor Cells Treated with Valproic Acid. **Plos One**, v. 9, n. 6, p. mid: 24959750, 2014.

Santana, M. H. A.; Polizel, G. H. G.; Grigoletto, L.; Fernandes, A. C.; Bonin, M. N.; Gomes, R. C.; Carvalho, M. E.; Silva, S. L.; Leme, P. R.; Junior, P. R..; Ferraz, J. B. S. The genetic and genomic effects of Nellore lineages on feed efficiency, intake and performance**. Livestock Science**, v. 2019, p. 104-108, 2019.

Santos-Silva, J.; Bessa, R. J. B.; Santos-Silva, F. Effect of genotype, feeding system and slaughter weigt on the quality of light lambs. II. Fatty acid composition of meat. **Livestock Production Science**, v. 77, p. 187-194, 2002.

Santos-Silva, J.; Mendes, I. A.; Bessa, R. J. B. The effect of genotype, feeding system and slaughter weight on the quality of light lambs 1. Growth, carcass composition and meat quality. **Livestock Production Science**, v. 76, p. 17-25, 2002.
S
app, R. L,; Bertrand, J. K,; Pringle. T. D.; E Wilson. D. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. **Journal Animal Science**, v. 80, 2017–2022, 2002.

Silva, J.; Cônsolo, N.R.B.; Buarque, V.L.M.; Beline, M.; da Silva Martins, T.; Lobo, A.A.G.; Gómez, J.F.M.; Eler, J.P.; Leme, P.R.; Netto, A.S.; Gerrard, D.E.; Baldi, F.;

Silva, S.L. Effect of Different Selection Criteria on Performance, Carcass and Meat Quality of Nellore Young Bulls. ***Agriculture***. v. *11*, p. 294. 2021. https://doi.org/10.3390/agriculture11040294

Simopoulos, A. P. Minireview: The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. **Experimental Biology and Medicine**, v. 233, p. 674–688, 2008.

Simopoulos, A. P. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. **Nutrients**, v.8, p. 128, 2016

Smith, S. B.; Kawachi. H.; Choi. C. B.; Choi. C. W.; Wu. G.; J. E. Sawyer. Cellular regulation of bovine intramuscular adipose tissue development and composition. **Journal of Animal Science**, v. 87, p. E72-82, 2009.

Smith, T.; Domingue, J. D.; Paschal. J.C.; Franke, D.E. Genetic parameters for growth and carcass traits of Brahman steers. **Journal of Animal Science**, v.85, p.1377-1384, 2007.

Teixeira, P. D.; Oliveira, D. M.; CHizzottI, M. L.; Chalfun-Junior A.; Coelho, T. C.; Gionbelli, M.; Paiva, L. V.; Carvalho, J. R. R.; Ladeira, M. M. Subspecies and diet affect the expression of genes involved in lipidmetabolism and chemical composition of muscle in beef cattle. **Meat Science**, v. 133, p. 110-118. 2017

The UniProt Consortium, **UniProt: the universal protein knowledgebase in 2021**, Nucleic Acids Research, v. 49 (D1), p. D480–D489, 2021. doi: doi.org/10.1093/nar/gkaa1100

Ulbricht, T. L. V.; Southgate, D. A. T. Coronary heart disease: seven dietary factors. **The lancet**, v. 338, p. 985-992, 1991.

Wai, Y. A. L.; Yoke, Y. C.; Shih, Y. L.; So, H. T.; Khalid, A. K.; Asharifah, N. A. S.H. Lipoprotein lipase expression, serum lipid and tissue lipid deposition in orally-administered glycyrrhizic acid-treated rats. **Lipids in Health and Diseas,** v. 8, p. 31. 2009.

Wheeler, T. L.; Cundiff, L. V.; Koch, R. M. Effect of marbling degree on beef palatability in Bos taurus and Bos indicus cattle. **J. Anim. Sci**, v. 72, p. 3145–3151. 1994

Williams, C.; Whitley, B.; Hoyo, C.; Grant, D.; Iraggi, J.; Newman, K.; Gerber, L.; Taylor, L. A.; Mckeever, M. G.; Freedland, S. J. A high ratio of dietary n-6/n-3 polyunsaturated fatty acids is associated with increased risk of prostate cancer. **Nutrition Research**, v. 3, p. 1–8, 2011.

Wood, J. D.; Fisherg, A.V.; Nute, G. R.; Sheard, P.R.; Richardsons, R. I.; Hughes, S. I.; Whittingtonet, F. M. Fat deposition, fatty acid composition and meat quality: a review**. Meat Science**, v. 78, p. 343-358, 2008.

Who. Diet, nutrition and the prevention of chronic diseases. **Report of a joint WHO/FAO expert consultation**. WHO technical report series 916, Geneva, 2003.

Wong, M. L and Medrano, J. F. Real-time PCR for mRNA quantitation. Review. **BioTechniques**, v. 39, p. 75-85, 2005. doi:10.2144/05391rv01

Wu, X.-F.; Liu, Y.; Gao, C.-F.; Chen, X.-Z.; Zhang, X.-P.; Li, W.-Y. Novel alternative splicing variants of ACOX1 and their differential expression patterns in goats, **Archives Animal Breeding**, v. 61, p. 59 –70, 2018.

Yamada, T.; S. I. Kawakami, and N. Nakanishi. Expression of adipogenic transcription factors in adipose tissue of fattening wagyu and holstein steers. **Meat Science**, v. 81, p. 86-92, 2009.

Yokoo, M.J.; Lobo, R.B.; Araujo, F.R.C.; Bezerra, L. F.; Sainz, R. D.; Albuquerque, L.G. Genetic associations between carcass traits measured by real-time ultrasound and scrotal circumference and growth traits in Nelore cattle. **Journal of Animal Science**, v. 88, p. 52–58, 2010. doi: 10.2527/jas.2008-1028

Yu, X.; Fang, X.; Gao, M.; Mi, J.; Zhang, X.; Xia, L.; Zhao, Z.; Albrecht. E.; Maak, S.; Yang, R.; Isolation and Identification of Bovine Preadipocytes and Screening of MicroRNAs Associated with Adipogenesis. **Animals,** v. 10, p. 818, 2020. doi:10.3390/ani10050818

Zhang, S.; Knight, T. J.; Reecy, J. M.; Beitz, D. C. DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition. **Animal Genetics**, v. 39, p. 62-70, 2008.

Zhang, Y.; Wang, Y.; Wang, H., Ma, X.; Zan, L. MicroRNA-224 impairs adipogenic differentiation of bovine preadipocytes by targeting LPL. **Molecular and Cellular Probes**, v. 44, p. 29–36, 2019. doi.org/10.1016/j.mcp.2019.01.005

Zuo, B.; Yang, H; Wang, J.; Lei, M. G.; Xiong, Y. Z.: Molecular characterization, sequence variation and association with fat deposition traits of ACOX1 gene in pigs, **J. Anim. Feed. Sci**, v. 16, p. 433–444, 2007.

CONSIDERAÇÕES FINAIS

CONSIDERAÇÕES FINAIS

Ao considerarmos os resultados verificados em nosso estudo, confirmamos nossa hipótese de que a seleção genética para precocidade e crescimento alteram o proteoma do músculo e da gordura subcutânea e, consequentemente, o metabolismo lipídico e proteico de bovinos Bos *indicus*, Nelore, não castrados, filhos de touros com DEP's contrastantes simultaneamente para precocidade e crescimento. Ao utilizarmos a metodologia de SDS-PAGE, seguida de identificação proteica, por meio da análise de espectrometria de massas, acoplada a cromatografia liquida (LC-MS/MS) e a técnica de PCR em tempo real (RT-qPCR), foi possível identificar proteínas e genes no músculo *longissimus thoracis* e na gordura subcutânea diferentemente abundantes. Além disso, as análises de bioinformática permitiram verificar as vias metabólicas enriquecidas em que essas proteínas e genes estavam envolvidos.

Nosso estudo também investigou o perfil hormonal e metabólico dos animais de alta e baixa DEP e revelou que os animais com maior potencial de crescimento e precocidade apresentam maior concentração sérica do hormônio IGF-1 e menor concentração sérica dos metabólitos LDL e colesterol. Foi possível notar diferenças em relação aos genes avaliados na gordura intramuscular, subcutânea e visceral. A expressão do gene LPL avaliado no *longissimus thoracis* foi maior no grupo de baixa DEP, enquanto que a expressão dos genes lipogênicos avaliados na gordura subcutânea foi maior para o grupo de alta DEP. Portanto, confirmou-se diferença no metabolismo desses depósitos de gordura, o que também foi revelado ao avaliar o proteoma do *longissimus thoracis* e da gordura subcutânea, verificando-se diferentes vias metabólicas envolvidas.

No capítulo 3, objetivou-se avaliar os efeitos de DEP's contrastantes para precocidade e crescimento, por meio da proteômica diferencial do *longissimus thoracis* e análises moleculares, bem como por correlações com características fenotípicas, como colágeno e gordura intramuscular, hormônios e metabólitos. As vias enriquecidas encontradas na análise proteômica: metabolismo do piruvato, glicólise / gliconeogênese, via da pentose fosfato, metabolismo do carbono e biossíntese de aminoácidos podem influenciar o metabolismo de lípideos no músculo. Os hormônios IGF-1, o gene LPL e as proteínas PKLR, PKM, ALDOA, DLD, GPI, ACTC1, OXCT1 e VIM podem ser considerados fatores importantes envolvidos

no turnover lipídico e proteico e, portanto, podem ser capazes de facilitar o processo de seleção de bovinos de corte e melhorar a qualidade da carne de animais zebuínos, não castrados, produzidos em condições tropicais.

No capítulo 4, avaliamos os efeitos de DEP's contrastantes para precocidade e crescimento, por meio da análise de proteômica diferencial da gordura subcutânea e correlações com características fenotípicas, como EGS, MAR, lipídios totais, IGF-1, GH, leptina e insulina (INS). Observamos nos resultados uma forte relação entre o perfil proteico da gordura subcutânea e o perfil hormonal da insulina sérica, independente e dentro dos grupos de DEP's contrastantes para precocidade e crescimento. Sugerimos que as proteínas envolvidas na via do ciclo do citrato influenciam a deposição de gordura subcutânea. Nosso estudo sugere ainda, fortes evidências de que GAPDH, ACACA, INS e IGF-1 são fatores importantes envolvidos na biossíntese da gordura subcutânea.

No capítulo 5, objetivamos caracterizar a composição de ácidos graxos intramusculares e avaliar a expressão gênica das principais enzimas adipogênicas e lipogênicas expressas na gordura subcutânea e visceral de progênies Nelore, filhos de touros com alta e baixa DEP para precocidade e crescimento. Verificou-se que bovinos com maior potencial genético para precocidade e crescimento apresentam maiores níveis de transcrição dos genes lipogênicos: ACACA, LPL, ACOX1, LEP, SCD e GAPDH. Desta forma, a gordura subcutânea está mais relacionada aos fatores lipogênicos do que adipogênicos. Embora a expressão dos genes lipogênicos tenham sido diferentes, a composição dos ácidos graxos da carne bovina não foi alterada. Portanto, os genes da gordura subcutânea não influenciaram o metabolismo lipídico da gordura intramuscular dos animais, independente dos grupos de alta e baixa DEP para precocidade.

No entanto, ainda há dúvidas com relação a biossíntese da gordura subcutânea em bovinos Nelore com diferente potencial de precocidade e crescimento. Devido a isso, encorajamos a continuação de estudos futuros a fim de verificar uma possível regulação pós-transcricional por microRNAs dos genes mencionados acima. Os microRNAs são moléculas pequenas de RNA fita simples, não codificadores de proteínas, que agem como potentes reguladores pós-transcricionais da expressão gênica. A exploração dos genes e microRNAs permitirá um melhor entendimento das vias metabólicas envolvidas na deposição de gordura

na carcaça de bovinos e contribuirá com a indústria em relação a melhorias na qualidade da carcaça e da carne em bovinos de corte.

As carcaças relacionadas ao grupo de alta DEP apresentaram maior EGS e MAR, em relação àquelas de baixa DEP. Além disso, o grupo com alta DEP apresentou maior solubilidade do colágeno. Destacamos, portanto, que estes resultados favorecem a qualidade da carne em animais , filhos de touros selecionados para alta DEP's. Portanto, a seleção de progênies com base nas DEP's dos progenitores é uma ferramenta viável, no sistema de pecuária de corte, pois contribui, entre outras, para um maior escore de acabamento das carcaças bovinas. Essa seria uma alternativa interessante para os produtores, que recebem remuneração diferenciada pelos animais ao abate, agregando valor aos produtos gerados, ou evitando a penalização pela falta de padronização de deposição de gordura na carcaça. Para a indústria esta estratégia permite maior padronização e consistência nos cortes, possibilitando à mesma um elevado potencial para atingir diferentes nichos de mercados.

Taiane da Silva Martins e
Profa Dra Angélica Simone Cravo Pereira

Agradecemos à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pela bolsa de doutorado e pelo auxílio financeiro: processos nº 2017/02349-1; 2016/17433-5; 2012/50788-0 e 2011/21241-0.
O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001 e do Conselho Nacional de Desenvolvimento Científico e Tecnológico (*CNPq*), processo nº 409186 / 2018-0.

Agradecemos também aos seguintes laboratórios pelas instalações e apoio no desenvolvimento desta pesquisa:
Laboratório de Ciências Carnes (LCC/FMVZ/ USP);
Laboratório de Estudos Avançados em Ciências da Carne (LEACC / FZEA / USP);
Laboratório de Morfofisiologia e Desenvolvimento Molecular (LMMD/FZEA/USP);
Laboratório de Avaliação Animal e Qualidade de Carne (LAAQC/FZEA/USP);
Laboratório de Fisiologia Animal (LAFA/FZEA/USP);
Laboratório de Ciências Carnes (LCC/DZO/ UFV);
E ao Centro Nacional de Pesquisa em Energia e Materiais (CNPEM).