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RAFAELA MARTINS

ESTUDO DE ASSOCIAÇÃO GENÔMICA AMPLA (GWAS) PARA CARACTERÍSTICAS DE CARCAÇA MENSURADAS POR ULTRASSONOGRAFIA EM BOVINOS DA RAÇA NELORE

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Dissertação apresentada como requisito para obtenção do título de Mestre em Zootecnia, no Programa de Pós-Graduação em Zootecnia, da Universidade Estadual de Ponta Grossa, na área de concentração: Produção Animal, com ênfase em Melhoramento Genético e Reprodução Animal.

Orientador: Prof. Drº Victor Breno Pedrosa
Co-orientador: Prof. Drº Luiz Fernando Brito

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" ESTUDO DE ASSOCIAÇÃO GENÔMICA AMPLA (GWAS) PARA CARACTERÍSTICAS DE CARCAÇA MENSURADAS POR ULTRASSONOGRAFIA EM BOVINOS DA RAÇA NELORE"

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Ponta Grossa, 21 de Agosto
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RESUMO

A raça Nelore (*Bos taurus indicus*) é utilizada em larga escala em rebanhos brasileiros, entretanto, os animais zebuínos apresentam menores teores de gordura na carcaça. Isso ocorre, devido ao impasse de seleção das características de carcaça, pois, muitas vezes essas são medidas *post-mortem*, além dos altos custos e dificuldades na mensuração. Com a inclusão da genômica, o processo de seleção, bem como os estudos de associação genômica ampla (GWAS) para identificação de regiões genômicas envolvidas nas expressões dessas características, tornaram-se mais simples. Com isso, o objetivo foi realizar GWAS para as características de espessura de gordura subcutânea (EGS), espessura de gordura na picanha (EGP8), e acabamento (ACAB), afim de identificar regiões genômicas de maiores efeitos, genes candidatos, e ainda, verificar as funções biológicas dos mesmos. Para isso, foram utilizadas informações de 1440 animais, todos com registros de ultrassom e genotipados com o painel GGP-Indicus 35K. As análises foram realizadas pelo método de *single-step GWAS* (ssGWAS) com os programas da família BLUPF90, que calcularam as variações para janelas genômicas contendo 10 polimorfismos de nucleotídeo único (SNPs) consecutivos, e posteriormente, as regiões que obtiveram ais que 0,5% da variância genética aditiva total foram utilizadas para examinar os genes candidatos. Após a identificação dos genes candidatos pelo banco de dados ENSEMBL, foram realizadas análises de ontologia genética (GO, PANTHER) e de redes de conexões de genes (REVIGO), para avaliar quais vias biológicas os genes atuavam, e também, a ligação desses genes sob cada característica abordada. Os principais genes de EGS (*TBL1XR1, AHCYL2, SLC4A7, AADAT, VPS53, IDH2* e *ETS1*), EGP8 (*GSK3β, LRP1B, EXT1, GRB2, SORCS1*, *e SLMAP*) e ACAB (*GSK3β, LRP1B, EXT1, SORCS1, NR1L2*, *e APAF1*), esses podem estar ligados com a deposição de gordura. A análise de enriquecimento gênico revelou processos que podem influenciar diretamente nas características estudadas, alguns desses genes, pertencem a vias relacionadas ao metabolismo de lipídios, polissacarídeos, carboidratos, homeostase lipídica, e do colesterol, além de outros que possam influenciar na composição de EGS, EGP8 e ACAB. Os resultados aqui relatados, ajudarão na compreensão dos mecanismos que formam as características supracitadas, além disso, várias das regiões genômicas identificadas apontaram processos relacionados à deposição de gordura, tais processos podem ser úteis para futuros estudos genômicos em bovinos da raça Nelore.

Palavras chaves: bovinos zebuínos, características produtivas, genes candidatos, marcadores moleculares, ssGWAS.

ABSTRAT

The Nelore breed (Bos taurus indicus) is used on a large scale in Brazilian herds, however, Zebu animals have lower levels of fat in the carcass. This is due to the impasse in the selection of carcass characteristics, as these are often post-mortem measures, in addition to the high costs and difficulties in measuring. With the inclusion of genomics, the selection process, as well as studies of broad genomic association (GWAS) for the identification of genomic regions involved in the expression of these characteristics, became simpler. With this, the objective was to perform GWAS for the characteristics of subcutaneous fat thickness (EGS), fat thickness in the sirloin (EGP8), and finishing (ACAB), in order to identify genomic regions of greater effects, candidate genes, and yet, check their biological functions. For this, information from 1440 animals was used, all with ultrasound records and genotyped with the GGP-Indicus 35K panel. The analyzes were performed using the single-step GWAS (ssGWAS) method with the BLUPF90 family programs, which calculated the variations for genomic windows containing 10 consecutive single nucleotide polymorphisms (SNPs), and later, the regions that obtained more than 0, 5% of the total additive genetic variance was used to examine the candidate genes. After the identification of candidate genes by the ENSEMBL database, analyzes of genetic ontology (GO, PANTHER) and gene connection networks (REVIGO) were carried out, to assess which biological pathways the genes acted on, and also, the connection of these genes under each characteristic addressed. The main genes of EGS (TBL1XR1, AHCYL2, SLC4A7, AADAT, VPS53, IDH2, and ETS1), EGP8 (GSK3β, LRP1B, EXT1, GRB2, SORCS1, and SLMAP) and ACAB (GSK3β, LRP1B, EXT1, S1, EXT1, S1, and APAF1), these may be linked to fat deposition. The gene enrichment analysis revealed processes that can directly influence the characteristics studied, some of these genes belong to pathways related to the metabolism of lipids, polysaccharides, carbohydrates, lipid homeostasis, and cholesterol, in addition to others that may influence the composition of EGS, EGP8, and ACAB. The results reported here, will help in understanding the mechanisms that form the aforementioned characteristics, in addition, several of the identified genomic regions pointed out processes related to fat deposition, such processes may be useful for future genomic studies in Nellore cattle.

Keywords: candidate genes, molecular markers, single-step genome-wide association, ssGWAS, productive traits, zebu cattle.

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1.1 INTRODUÇÃO

O Brasil é classificado como o terceiro maior consumidor da carne bovina no mundo e o principal exportador deste produto, atendendo mais de 130 países (ABIEC, 2018). A exigência do mercado consumidor com relação a qualidade da proteína animal tornou-se crescente, o que influenciou, a transição da cadeia de produção da carcaça bovina. Com isso, o principal objetivo de produção estava voltado a quantidade de carcaça e não a qualidade, como observado nos dias atuais. Neste contexto, uma das maiores dificuldades do país, é produzir carcaças que possibilite atender aos padrões de qualidade exigidos pelos mercados que melhor remuneram. Assim, o conhecimento das características de interesse econômico, que estão intimamente ligadas com a qualidade da carcaça, são essenciais para atingir a taxa de expansão exigida mundialmente (SIMIANER, 2016).

O rebanho bovino brasileiro é composto por cerca de 70% de raças zebuínas (*Bos taurus indicus*), em que destes, estima-se, que 90% sejam da raça Nelore, devido, principalmente, a sua adaptação ao clima tropical e subtropical predominante no país (CARVALHO *et al*., 2018). Apesar da raça Nelore ser responsável por uma expressiva parte da produção cárnea brasileira, os animais zebuínos apresentam, no geral, menores características de acabamento, cada vez que, comparados com animais taurinos (*Bos taurus taurus*, BRESSAN *et al*., 2016). Isso pode decorrer, em consequência aos longos períodos de seleção genética dos programas de reprodutores taurinos, em comparação aos programas mais recentes de seleção de reprodutores zebuínos, os quais concentravam-se em melhorar as características de crescimento, com menor atenção para características qualitativas do produto (Decker *et al.,* 2012). Para a bovinocultura de corte, algumas características apresentam bons indicativos da composição quantitativa e qualitativa da carcaça como: espessura de gordura subcutânea (EGS), espessura e gordura na picanha (EGP8) e de modo geral o acabamento da mesma (ACAB) (TONUSSI *et al*., 2015).

Embora alguns programas de melhoramento incluam as características de qualidade da carcaça em seus programas de avaliação genética clássica, o conhecimento de marcadores moleculares ligados a essas características ainda é limitado, especialmente em animais zebuínos. Por isso, faz-se necessário que estudos

moleculares sejam intensificados, com o intuito de possibilitar o aumento do progresso genético, por meio do possível aumento da acurácia de seleção e a intensidade da mesma (SANTANA *et al.,* 2015).

Os avanços nas tecnologias genômicas, permitem a genotipagem de centenas de milhares de marcadores de DNA, espalhados por todo o genoma (KHATKAR *et al.,* 2014). Antes do advento da genômica, para identificar animais superiores, eram utilizados somente informações de pedigree e de desempenho do próprio animal ou de parentes. Assim a acurácia do valor genético do animal avaliado, dependia exclusivamente do número de indivíduos que estavam relacionados a matriz de parentesco do mesmo.

A inclusão da avaliação genômica, tornou as informações eficientes para predição e tomadas de decisões de seleção, incluindo o mapeamento de QTLs (*Quantitative Trait Loci*), SNPs (*Single Nucleotide Polymorphisms*) e mais recentemente, a utilização do GWAS (Estudos de Associação Genômica Ampla). Comparando-a com a seleção tradicional, a inclusão de informações genômicas resultam na seleção de animais mais jovens, os quais ainda não possuem dados fenotípicos coletados, de maneira que à redução dos intervalos de gerações, o que torna mais eficiente os ganhos genéticos e reduz os custos em até 90%, em relação ao uso de programas de seleção tradicionais (PRYCE *et al.*, 2012).

Os estudos de locais específicos do genoma, podem ser utilizados para mapear genes que estão envolvidos no controle fisiológico das características fenotípicas (SNELLING *et al.*, 2010). Dessa forma, ao agregar informações de estudos de genômica, juntamente ao conhecimento de pedigree e fenótipos já obtidas, aumenta-se a confiabilidade das estimativas dos valores genômicos, o que reduz a probabilidade de uma seleção errônea de reprodutores. Além da identificação de genes, que possam desempenhar papéis importantes nas atividades em vias metabólicas, os quais são essenciais para estudar os processos biológicos das características fenotípicas de interesses (WANG *et al.,* 2010). Com isso, objetivou-se identificar possíveis regiões cromossômicas com maior efeito sobre as características de carcaça em bovinos da raça Nelore, aplicando-se GWAS, pelo método single-step GBLUP (MISZTAL *et al.*, 2018).

1.2 REVISÃO DE LITERATURA

De acordo com os dados da ABIEC (2018), o Brasil em 2017, possuía o maior rebanho comercial mundial, sendo de 221,81 milhões de cabeças, com um volume total de 9,71 milhões de toneladas de carcaça, ocupando a segunda posição mundial em produção, atrás apenas dos Estados Unidos (12,1 milhões de toneladas). Do total da produção brasileira, 20% abasteceu o mercado interno e 80% foi destinada para exportação, posicionando o Brasil como o principal exportador de carne bovina mundial.

A produção de carne bovina exerce grande importância econômica no agronegócio brasileiro, como demonstrado no primeiro semestre de 2018, em que a exportação rendeu um total de US$ 3,5 bilhões (ABPA, 2018). Apesar da produção de carne gerar grandes rendimentos na economia, a produtividade e a qualidade da carcaça estão muito aquém do seu verdadeiro potencial. Como comparação, em termos de produtividade, o rendimento da carcaça bovina no Brasil é de 52% (SEAB 2013), nos Estados Unidos o rendimento médio é 63% (GONZALEZ; PHELPS, 2018). O menor rendimento da carcaça bovina brasileira, colabora para que a produção em toneladas, seja inferior ao total produzido nos Estados Unidos, que por sua vez, possuem um maior rendimento do seu produto. Essa variação no rendimento de carcaça, pode ser provocada, pelas características do sistema de produção do Brasil, visto que, a produção brasileira é voltada ao sistema extensivo de criação, por possuir grandes áreas territoriais.

Grande parte do rebanho brasileiro de gado de corte é caracterizado pela subespécie Zebuína, também denominada como *Bos taurus indicus*, da qual a raça Nelore predomina, seja pura ou produtos advindos de suas cruzas, devido à grande adaptação ao clima brasileiro com temperaturas altas, ao se comparar ao gado taurino (*Bos taurus taurus*). Medeiros *et al*. (2015), ao analisarem bovinos das raças Nelore e Angus, conclui-se que animais predominantes *Bos taurus indicus* possuem uma menor deposição de gordura, quando comparados com bovinos de origem *Bos taurus taurus.* Ressalta-se que a baixa taxa de deposição de gordura, é um dos principais problemas do mercado cárneo brasileiro, deixando-o menos competitivo mundialmente, devido aos baixos indicadores de deposição de gordura na carcaça, como gordura intramuscular e gordura subcutânea.

Conforme Hadlich *et al.* (2013), a padronização da carcaça com relação a sua qualidade, é um grande desafio na pecuária de corte brasileira, em termos práticos, essas diferenças dificultam os mecanismos de classificação e tipificação de carcaças, para prever as características de qualidade da mesma.

1.3 CARACTERÍSTICAS PRODUTIVAS

As características de qualidade da carcaça, estão diretamente relacionadas à decisão de compra do produto pelos consumidores. Os autores Ferraz, Felício (2010), expõem uma das características mais relacionadas com a aquisição do produto, como o teor de gordura, que exerce grande influência sobre a qualidade da carcaça. Essa característica, está diretamente ligada às características sensoriais da carne como palatabilidade, suculência, e aspectos de coloração (FEITOSA *et al*., 2017). Segundo Gordo *et al.* (2018), características como EGS e EGP8 são importantes para a comercialização da cadeia produtiva bovina, devido essas serem indicadores da composição quantitativa e qualitativa da carcaça bovina.

Os bovinos zebuínos, tem baixa propensão a deposição de gordura subcutânea e menor acabamento, com impactos nas características visuais e sensoriais da carcaça, ao comparar-se a porcentagem das mesmas em raças taurinas (HOCQUETTE *et al*., 2012). Um dos fatores ambientais que podem estar relacionados com a baixa deposição de gordura, é a forma de criação desses animais, onde cerca de 93% dos bovinos são criados de forma extensiva (TERRA *et al.,* 2019). As principais alterações na porcentagem de gordura na carcaça, ocorrem devido ao tipo de terminação adotado, animais com dietas ricas em grãos, com maiores densidades energéticas, tendem a ter uma carcaça melhor acabada (ALVES *et al.,* 2019). Entretanto, essas características não são influenciadas apenas pelo ambiente de produção, assim, está intimamente ligada com os aspectos do genoma do bovino Nelore (DE CASTRO *et al.*, 2014). Desta forma, a principal fonte de variação genética entre indivíduos da mesma espécie, pode ser atribuída às diferenças da expressão gênica, devido à alguma alteração sofrida ao longo da evolução (STAMATOYANNOPOULOS, 2004).

De acordo com Igarasi *et al.* (2008), 54% das características de carcaça é de responsabilidade ambiental e 46% deve-se aos genes que formam as características correlacionadas com a qualidade da carcaça. Assim sendo, a genética possui uma

contribuição significante nas características visuais e sensoriais da carcaça, ou seja, é possível mudar o padrão de qualidade da mesma, através de seleção genômica, selecionando animais que possuam marcadores significativos para tais características, como EGS, EGP8 e ACAB.

1.3.1 Espessura de gordura subcutânea – EGS

A cobertura de gordura subcutânea, é uma medida tomada diretamente sobre o músculo *longissimus dorsi,* entre a 12ª e 13ª costelas. Com escalas que variam de 1 a 5, sendo, 1) ausente de EGS , 2) de 1 a 3mm , 3) de 3 a 6mm, 4) de 6 a 10mm e 5) acima de 10mm de EGS (BRASIL, 2004).

A EGS é de extrema importância, devido a ação de conservação, essa reduz possíveis perdas de peso evaporativo da carcaça ao refrigera-lá, além de ser um bom indicativo do acabamento da carcaça (DIBIASI *et al*., 2010). Além disso, a EGS está relacionada com a qualidade da carcaça bovina, através da proteção proporcionada ao músculo assim que exposto ao frio, essa proteção evita que ocorra o encurtamento do sarcômero após o abate no processo de resfriamento, o qual resultaria, em uma carne mais dura. Assim, uma carcaça de boa qualidade, deve ter uma espessura de gordura suficiente para garantir a sua preservação (MALHEIROS *et al.,* 2015).

Para a característica EGS as herdabilidades encontradas nos estudos de Gordo *et al.* (2018) e de Tizioto *et al.* (2013) foram coincidentes em 0,21, o que exemplifica que, a seleção de animais com genes que influenciam a característica, possam transmitir os mesmos genes para a progênie, o que aumenta o progresso genético e concomitantemente a qualidade da carcaça.

1.3.2 Espessura de gordura na Picanha - EGP8

A EGP8, situa-se na intersecção dos músculos *Glúteos medius* e *Biceps femoris,* localizados entre o ílio e o ísquio do animal. A deposição de gordura na EGP8 inicia-se antes que a da costela, além de ser uma característica indicadora do grau de acabamento da carcaça (YOKOO *et al.,* 2008). De acordo com o mesmo autor, a EGP8 juntamente com a EGS, são fundamentais no processo de industrialização da carcaça, devido a proteção da mesma quando exposta às baixas temperaturas. Essas

características fornecem ao processo um resfriamento lento e gradual da carcaça, evitando o encurtamento das fibras, evitando assim, o endurecimento do produto final.

Para a característica EGP8 as herdabilidades encontradas em estudos variavam de 0,28 a 0,43 (GORDO *et al.*, 2012, YOKOO *et al.*, 2009).

1.3.3 Acabamento de carcaça - ACAB

De acordo com a Associação Nacional de Criadores e Pesquisadores (ANCP), a característica ACAB está relacionada com a precocidade no acabamento da carcaça. A ACAB é resultado da análise em conjunto das características EGP8 e EGS. De acordo com Yokoo *et al.* (2009) as correlações entre EGS e EGP8 com a característica precocidade (P), são de 0,40 e 0,42 respectivamente. Essas correlações ressaltam a informação que ao aumentar as características EGP8 e EGS, as mesmas aumentarão de forma positiva e moderada, a característica ACAB.

A característica precocidade relaciona a profundidade da costela com a altura dos membros, e quando visualizada é um indicativo de indivíduos que irão depositar gordura mais precocemente (KOURY FILHO *et al*., 2006). A precocidade, fornece uma terminação homogênea com relação a cobertura de gordura da carcaça, e esse quesito, é de extrema importância, para que o produto mantenha sua qualidade.

1.4 ESTUDO DE ASSOCIAÇÃO GENÔMICA AMPLA – GWAS

O genoma bovino é composto por 60 cromossomos (2n=30), organizados em pares, com função de armazenar todas as informações genotípicas e garantir que a herança genética seja repassada a progênie (GARNER, 2008). Os genes são sequências de nucleotídeos, formados por pares de bases, ou seja, frações de DNA, que são transcritos em moléculas de RNA, essas são traduzidas em proteínas que poderão influenciar as características de interesse. O genoma bovino, possui mais de 2670 milhões de pares de base, desses aproximadamente 94% nos cromossomos autossômicos, e o restante nos cromossomos sexuais e DNA mitocondrial (GEER *et al*., 2009).

Algumas características são controladas por diversos genes, que exercem pequenos efeitos sobre as mesmas, a localização destes ao longo do genoma é denominada de locus, os genes nesses contidos, podem afetar determinadas

características qualitativas (QTLs). De acordo com Meuwissen, Hayes, Goddard, (2013), a utilização da seleção genômica, é uma forma de seleção assistida por SNPs, quais estão contidos em todo o genoma. As características de importância econômica são quantitativas, ou seja, são influenciadas por vários genes, que se situam, em diferentes locus no genoma.

De acordo com Silva *et al.* (2019), as características de qualidade da carcaça, são regidas por diversas variantes, e em sua grande maioria de efeitos pequenos, ou seja, cada marcador encontrado explica uma percentagem do fenótipo da característica, o que ressalta o efeito poligênico destas. Isso aumenta a importância da realização da seleção através da genômica, vez que, essas características possuem um valor elevado para mensuração, sendo muitas vezes complexas ou com resultados tardios na vida do animal (TIZIOTO *et al*., 2013).

Grande parte dos programas de melhoramento genético animal, ainda utilizam registros de desempenho e dados de pedigree. A incorporação de informações genômicas em avaliações genéticas, e a possibilidade dessa implementação na indústria de bovinos de corte, é relativamente nova (*ROLF et al.*, 2014). A análise por GWAS, tem como objetivo principal, associar regiões do genoma com fenótipos de interesse, com a finalidade de identificar essas regiões e buscar suas funções biológicas. Esta identificação, tem como intuito, aumentar a compreensão da influência genética, sobre o desempenho do animal, em relação a sua produtividade e qualidade do produto final (MEUWISSEN; HAYES; GODDARD, 2001). Atualmente, através da genotipagem em larga escala, é possível avaliar os efeitos dessas regiões, que contém inúmeros marcadores de DNA, os quais são estimados, simultaneamente, ao combinar informações fenotípicas. O arranjo dessas informações, auxiliam na estimação de valores genômicos, que podem, explicar parte da variação genética de uma característica quantitativa (RESENDE *et al*., 2008).

A genotipagem de marcadores baseados em SNPs, concedeu novas perspectivas para a identificação de QTLs, que possuem baixas taxas de mutações e contemplam todo o genoma. Os marcadores indicam os QTLs que estão em desequilíbrio de ligação, bem como, genes candidatos e mutações causais (GARCIA *et al*., 2010, BERG *et al*., 2013). A genotipagem de animais candidatos à seleção com painéis de marcadores de alta densidade é muitas vezes inviável pelo alto valor investido. Nos estudos de GWAS faz-se necessário uma amostra representativa de

animais genotipados para encontrar possíveis variações causais (MISZTAL *et al.*, 2011).

Entretanto, o principal desafio que envolvem estudos de GWAS, estariam na adequação de modelos e metodologias estatísticas, o tamanho amostral utilizado, a colinearidade proveniente do desequilíbrio de ligação entre os marcadores e principalmente a interpretação dos resultados (CANTOR *et al.*, 2010). Nessa perspectiva, o tamanho amostral é de grande importância ao visar a acurácia das informações, pois para que o SNP possa ser considerado um marcador molecular é necessário que ele esteja presente no mínimo em 1% da população avaliada (BROOKES, 1999). Assim, quanto maior o número de indivíduos genotipados, maior será a confiabilidade do efeito dos SNPs relacionados a característica alvo.

A utilização da genômica, para identificação de genes e/ou regiões cromossômicas com efeitos importantes, que melhorem as características da qualidade da carcaça, são alternativas eficientes e levam a grandes avanços genéticos, vez que parte da variação das características é resultado dos genes de efeitos aditivos (ZUIN *et al*., 2012). Neste contexto, estudos de GWAS, tem sido uma ferramenta importante para a detecção de variantes genéticas associadas a características complexas (UTSUNOMIYA *et al.*, 2013). No momento, qual se encontra marcadores associados a essas características, mesmo que esses genes exerçam cerca de 1% da variação fenotípica encontrada, já são de extrema importância para o entendimento do processo biológico para formação da característica (CANTOR *et al.*, 2010).

A seleção genômica, pode ser implementada de duas formas ao aplicar-se de multi-step e single-step. Ao utilizar-se o multi-step, requer uma avaliação baseada em pedigree e após a avaliação genômica é realizada. Uma alternativa para o multi-step, é o single-step, que consiste, em integrar todas as informações genotípicas e fenotípicas disponíveís (de animais genotipados e não-genotipados), em um único procedimento, que cria um pedigree genômico combinado com uma matriz de relacionamento (MISZTAL *et al*., 2009). Com predição subsequente, dos valores genômicos estimados de todos os animais (MISZTAL *et al.*, 2014, WANG *et al.*, 2012).

Por meio do método single-step são incorporadas, conjuntamente, informações fenótipicas, de pedigree e genômicas. A matriz de relacionamento do single-step baseia-se no pedigree (**A**), que é combinada com a matriz de relação genômica (**G**) e são associadas formando uma única matriz de relação (**H**). Aguilar *et*

al. (2010), ao analisar a precisão do melhor preditor linear não viesado single-step (ssGBLUP), observou-se que, as avaliações que utilizam o ssGBLUP foram tão precisas quanto as realizadas pelo multi-step. De acordo com Lourenço *et al.* (2014), o ssGBLUP tem vantagem sobre o multi-step, por ser um método simples, em que a computação de pseudo-fenótipos ou um índice não é necessária, utiliza-se o mesmo modelo das avaliações tradicionais. Sendo que todos os animais genotipados podem ser considerados e possivéis viés da avaliação genômica pode ser sanada por pequenas modificações como a imputação de dados adicionais (VITEZICA *et al.*, 2011).

1.4.1 Uso de GWAS em características produtivas

As características de qualidade de carcaça, ao realizar o melhoramento genético tradicional, podem trazer uma boa representatividade no ganho genético, entretanto, em um longo período de tempo, visto que, as características de qualidade são medidas tardiamente na vida do animal, geralmente apenas no post-mortem (GORDO *et al*., 2018). Em consequência disto, o melhoramento genético tradicional tem sido lento, devido a seleção de touros para essas medidas requererem que os animais sejam submetidos ao teste de progênie. Sendo esse, relativamente demorado, assim reduz o progresso genético, devido ao aumento do intervalo de gerações (MAGALHÃES *et al.,* 2019). Por sua vez, quando se usa a seleção genômica, não há necessidade de realizar o teste de progênie, o que reduz o intervalo de gerações e aumenta o progresso genético ao longo dos anos, vez que, sabemos quais animais são realmente melhoradores.

Algumas características como EGS e EGP8 são características mensuradas por ultrassonografia ou *post-mortem,* que estão relacionadas com o ganho de peso diário, rendimento de carcaça, precocidade de acabamento, sabor e suculência da carne (CARTAXO *et al*., 2011).

Com isso, a identificação de genes responsáveis pelas variações fenótipicas das características EGS e grau de acabamento são passos importantes para o desenvolvimento de métodos de seleção de indivíduos de genótipos superiores (MAGALHÃES *et al.*, 2016). A identificações de genes e/ou regiões genômicas, que possam estar envolvidos com o desenvolvimento ou a fisiologia de uma determinada característica são denominados de genes candidatos. Esses genes são sequênciados

e seus polimorfimos detectados e associados com a característica que o mesmo exerce influência, afim de elucidar a base genética de diferentes espécies (BROWN *et al.,* 2013). Diversos estudos realizados encontraram SNPs que possam afetar o rendimento e a qualidade da carcaça de bovinos.

Os genes *ACAT1* e *ACSL1* estão relacionados com o metabolismo lipídico, contribuem para a biossíntese, transporte, armazenamento, e degradação de ácidos graxos (HUANG *et al*., 2017). Outro estudo, revelou o *ACSL1* relacionado com a gordura localizada no músculo *longissimus dorsi* em bovinos Nelore (POLETI *et al.,* 2018).

O gene *MYOD1* (Diferenciação da Miosina do tipo I) descrito por Rexroad *et al.* (2001), está envolvido no desenvolvimento muscular esquelético, regeneração de tecidos, diferenciação dos mioblastos, adaptação da fibra muscular, entre outros processos (BHUIYAN *et al.*, 2009, BLUM, DYNLACHT, 2013). Em estudo de Bhuiyan *et al.* (2009), ao comparar animais *Bos taurus indicus* com *Bos taurus taurus,* observaram maior presença do gene *MYOD1* em animais *Bos taurus indicus,* o que indica um menor desenvolvimento muscular da raça Nelore.

Casas *et al.* (2003) em estudo para a detecção de QTLs para crescimento e composição da carcaça, notou-se um gene de grande importância econômica sobre a deposição de gordura na carcaça, o gene conhecido como *ASAP1* localizado no cromossomo 14 bovino, onde haviam diversos QTLs relatados para espessura de gordura e marmoreio. Veneroni *et al.* (2010), encontraram QTLs para espessura de gordura em bovinos da raça Canchim, constatou-se um polimorfismo localizado no íntron 13 (sequências não codificantes do DNA), do gene *ASAP1*.

Silva *et al.* (2017) analisaram os resultados de GWAS para características de carcaça em bovinos da raça Nelore, identificaram o gene *HTR2B*, localizado no cromossomo 2, sendo este associado a EGS. O gene *XKR4* localizado no cromossomo 14 de bovinos da raça Nelore foi associado com EGS, como observado por Porto Neto *et al.* (2012) nas raças Belmont Red e Santa Gertrudis, em que foram encontrados 3 SNPs ligados ao referido gene, em que um destes SNPs explicou 5,9% da variância genética aditiva para EGS. Ainda, o gene *XKR4* foi relatado por Lindholm-Perry *et al.* (2012) como um gene candidato para o aumento do consumo médio diário de ração em bovinos mestiços, que por sua vez, estarão indiretamente relacionado com a taxa de deposição de gordura na carcaça do animal.

Utsunomiya *et al.* (2013) destacaram a presença do gene denominado como *PLAG1* (Pleomorphic adenoma gene1), qual está intimamente associado com espessura de gordura na carcaça. Ao avaliar uma mutação no gene *PLAG1* verificou a possibilidade do mesmo estar ligado com a estatura de bovinos, pois, por meio de haplótipos foi analisado que este gene exerce efeitos sobre o tamanho corporal, peso e reprodução (UTSUNOMIYA *et al.,* 2017). A influência do gene *PLAG1* sobre essas características pode ser explicado devido a algumas evidências funcionais já relatadas em alguns estudos, como por exemplo, ser um dos reguladores do fator de crescimento semelhante a insulina do tipo II (IGFII) (VOZ *et al.,* 2000).

1.5 ANÁLISES FUNCIONAIS

Os genes encontrados em diferentes estudos podem ser: 1º) genes candidatos posicionais, ou 2º) genes candidatos funcionais, em que o primeiro está relacionado a localização em uma região cromossômica que está associada com uma característica de interesse econômico, o segundo pode ser tanto um gene posicional como funcional, quando estão envolvidos em vias metabólicas, codificam proteínas que podem estar relacionadas com a expressão de uma característica (TIZIOTO *et al.,* 2016).

Estudos de GWAS permitem a identificação de regiões genômicas envolvidas em processos biológicos relacionados as características de interesse (MACLEOD *et al.,* 2016). A análise funcional, gera o entendimento da maneira que os genes desempenham suas atividades em uma via metabólica, essencial para o estudos dos processos fisiológicos que influenciam o fenótipo de interesse (WANG *et al.,* 2010). estudo de ontologia gênica, é capaz de esclarecer de maneira mais simplificada a função fisiológica de genes, diante da necessidade de padronizar as anotações das funções gênicas, surgiram diversos bancos de dados públicos, como exemplos, Visualization and Integrated Discovery (DAVID) (HU *et al.,* 2009), Networks and Pathways (VisANT) (HUANG; SHERMAN; LEMPICKI, 2009), Kyoto Encyclopedia of Genes and Genomes (KEGG) (OGATA *et al.,* 1999).

O conhecimento das rotas biológicas que essas regiões genômicas podem estar influenciar é importantíssimo para explicar as variações fenotípicas. Magalhães *et al.* (2016) observaram janelas de SNPs que continham fatores olfativos que participavam da transformação de guanosina difosfato em guanosina trifosfato, os

quais são reguladores da proteína G, e podem ser utilizados como fonte de energia para a célula. Esses receptores olfativos, também, são conhecidos por atuarem no tecido adiposo e na diferenciação dos adipócitos, o que pode acarretar no aumento do acúmulo de gordura no tecido muscular (VON DER HEYDE *et al.,* 2014).

A investigação das associações significativas de SNPs, tem o intuito, de encontrar possíveis genes candidatos funcionais, que possam estar conectados à processos fisiológicos. A partir de estudos, Santana *et al.* (2015) detectaram marcadores significativos em genes candidatos funcionais, observou-se os genes *OSBPL3* e *SUDS3* para EGS e *RARRES1* e *VEPH1* para EGP8. Sendo alguns desses relacionados ao metabolismo lípidico (*CLSTN2*, *OSBPL3*, *RARRES1*, e *VEPH1*). Braz *et al*. (2019) observou que os genes *SUCLG1*, *THOC5*, *NIPSNAP1*, *IQCK*, *TM7SF2*, *VPS51*, *PTPRK*, *ECHDC2*, e *SCP2*, relacionados com diversas vias reguladoras da miogênese, metabolismo dos lipídios e de ácidos graxos, quais as vias podem estar associadas com a deposição de gordura na carcaça.

Leal-Gutiérrez *et al.* (2019) identificaram genes como *EVC2*, *ANXA10*, e *PKHD1* relacionados com a qualidade da carne. Os genes *EVC2* e *PKHD1* codificam proteínas transmembranas que estão associadas à organização do citoesqueleto de miócitos e na proteólise no *post-mortem*. O gene *ANXA* é uma proteína de ligação de fosfolipídios e da membrana reguladora de Ca^{2}. O íon Ca^{+2} é necessário para ativação da calpaína, proteína dependente de Ca^{2+} que age na degradação das fibras musculares, levando assim, ao amaciamento do produto (GOLL *et al.,* 2003).

A utilização da metodologia ssGWAS, com a matriz H (AGUILAR *et al.,* 2010), tem grandes eficiências quanto à predição dos valores genéticos, quando comparamos com a metodologia do multi-step (WANG *et al.,* 2012). Essa eficiência para a predição genômica, pode ser devido a diversos fatores, como exemplos, as informações de pedigree, informações dos marcadores genéticos podem substituir as informações de parentesco, evidencia dessas regiões que estão conectadas com as características, acurácia em relação aos parâmetros genéticos que favorecem a identificação de animais com potencial genético superior (PÉRTILE *et al.* 2016). Desse modo, a identificação dessas regiões que causam influências nas características quantitativas e qualitativas da carne é de grande valia, assim, necessita-se de estudos GWAS, para identificação dos genes que afetem diretamente e indiretamente, bem como, complementar os estudos com análises funcionais, para que, possamos

entender como os genes afetam as vias metabólicas na constituição destas características de interesse econômico.

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CAPITULO 2: GENOME-WIDE ASSOCIATION STUDY AND PATHWAY ANALYSIS FOR FAT DEPOSITION TRAITS IN NELLORE CATTLE RAISED IN PASTURE-BASED SYSTEMS

RESUMO

O Brasil é um dos principais produtores de carne bovina do mundo, sendo a raça Nelore (Zebu, Bos taurus indicus) a raça predominante, em que os animais são criados em sistemas a pasto. Apesar da competitividade do setor, a qualidade da carne é um parâmetro que precisa ser substancialmente aprimorado. Nesse contexto, o objetivo deste estudo foi identificar regiões genômicas e vias metabólicas associadas à espessura de toucinho (BFT) e espessura de gordura da garupa (RFT) em bovinos da raça Nelore. Medições baseadas em ultrassom de BFT e RFT foram coletadas em 1.440 animais aos 18 meses de idade. Esses animais foram genotipados usando o chip SNP GGP-indicus 35K, contendo 35.247 SNPs, após controle de qualidade. Um estudo de associação do genoma em uma única etapa (ssGWAS) foi realizado usando os programas da família BLUPF90. A identificação de genes candidatos foi realizada por meio do banco de dados Ensembl incorporado na ferramenta BioMart, enquanto PANTHER e REVIGO foram utilizados para identificar as principais vias metabólicas e redes de genes. Um total de 18 regiões genômicas localizadas em 10 cromossomos diferentes e abrigando 23 genes candidatos foram identificados para BFT. Para RFT, 22 regiões genômicas foram encontradas em 14 cromossomos, com um total de 29 genes candidatos identificados. Os resultados da análise da via mostraram genes importantes para BFT, incluindo TBL1XR1, AHCYL2, SLC4A7, AADAT, VPS53, IDH2 e ETS1, que estão envolvidos no metabolismo lipídico, desenvolvimento de aminoácidos celulares, transporte de solutos, transporte entre membranas do Complexo de Golgi , diferenciação celular e desenvolvimento celular. Os principais genes identificados para RFT foram GSK3β, LRP1B, EXT1, GRB2, SORCS1 e SLMAP, que estão envolvidos em vias metabólicas, como síntese de glicogênio, transporte de lipídios, metabolismo de polissacarídeos e carboidratos e homeostase de lipídios. Os genes candidatos relatados neste estudo podem ser utilizados futuramente em programas de melhoramento genético visando o melhoramento genético de características de qualidade de carcaça em bovinos Nelore.

Palavras-chave: bovino de corte, carcaça, efeitos SNP, regiões genômicas, qualidade da carne.

SUMMARY

Brazil is one of the main beef producers worldwide and Nellore (Zebu cattle, *Bos taurus indicus*) is the predominant breed, in which animals are raised in pasture-based systems. Despite the industry competitiveness, meat quality is a parameter that needs to be substantially improved. In this context, the aim of this study was to identify genomic regions and metabolic pathways associated with backfat thickness (BFT) and rump fat thickness (RFT) in Nellore cattle. Ultrasound-based measurements of BFT and RFT were collected in 1,440 animals at 18 months of age. These animals were genotyped using the GGP-indicus 35K SNP chip, containing 35,247 SNPs after quality control. A single-step genome-wide association study (ssGWAS) was performed using the BLUPF90 family programs. The identification of candidate genes was performed through the Ensembl database incorporated in the BioMart tool, while PANTHER and REVIGO were utilized to identify the key metabolic pathways and gene networks. A total of 18 genomic regions located on 10 different chromosomes and harboring 23 candidate genes were identified for BFT. For RFT, 22 genomic regions were found on 14 chromosomes, with a total of 29 candidate genes identified. The results of the pathway analysis showed important genes for BFT, including *TBL1XR1, AHCYL2, SLC4A7, AADAT, VPS53, IDH2*, and *ETS1*, which are involved in lipid metabolism, development of cellular amino acids, transport of solutes, transport between Golgi Complex membranes, cell differentiation, and cellular development. The main genes identified for RFT were *GSK3β, LRP1B, EXT1, GRB2, SORCS1*, and *SLMAP*, which are involved in metabolic pathways such as glycogen synthesis, lipid transport, polysaccharide and carbohydrate metabolism, and lipid homeostasis. The candidate genes reported in this study may be used in the future in breeding programs aiming the genetic improvement of carcass quality traits in Nellore cattle.

Keywords: Brazilian beef cattle, *carcass,* genomic regions, meat quality, SNP effects, tropical cattle.

2.1 INTRODUCTION

Brazil is a leader is the beef production and exportation in the international market (RODRIGUES *et al.,* 2019). The competitiveness of the Brazilian beef cattle industry is mainly due to the use of genetic resources that are highly adapted to tropical conditions and pasture-based systems, with over 80% of the Brazilian beef cattle population composed by the Nellore breed (Zebu cattle, *Bos taurus indicus*) or its crosses (MAGALHÃES *et al.,* 2019). Despite the high adaptive and productive performance, in general, Zebu animals produce less uniform carcasses with inferior fat deposition as compared to Taurine beef cattle (*Bos taurus taurus*, PEREIRA *et al.,* 2015). In view of the increasing worldwide demand for high-quality animal protein in recent years, there is a need to genetically improve carcass and meat quality traits in Zebu cattle populations (COOKE *et al.,* 2020, SANTANA *et al.,* 2015, SMITH *et al.,* 2018). To this end, there are producers and breeding companies investing in technologies to phenotype and breed for improved carcass quality and yield (MUELLER *et al., 2019)*.

Traits such as backfat (BFT) and rump fat (RFT) thickness are indicative of the quality and amount of fat deposited in the carcass, and therefore, key breeding goals in beef cattle breeding programs (TONUSSI *et al.,* 2015). Nevertheless, measuring carcass traits is sometimes an expensive and time-consuming task when performed at slaughter plants. This is also a challenge for phenotyping selection candidates, as they could only be accurately identified as genetically superior animals after being slaughtered (GORDO *et al.,* 2012). Alternatively, ultrasound techniques can be used to measure carcass traits in live animals, such as BFT (GRIGOLETTO *et al.,* 2020, SANTANA *et al.*, 2015). In addition to enabling data collection at an early age, this technique minimizes costs, facilitates measurement protocols, and are highly genetic correlated (> 0.85) with traits measured in slaughter plants, and therefore, useful indicators to be used in breeding programs (BONIN *et al.,* 2015, GREINER *et al.,* 2003). Grigoletto *et al.* (2020) have identified important candidate genes associated with ultrasound-based BFT and RFT in Montana Tropical Composite cattle. In Nellore, Santana *et al.* (2015) identified genomic regions associated with BFT and RFT and reported novel candidate genes for fat thickness traits not yet revealed in other studies, which indicates the need for validation studies in independent populations using larger datasets.

Hay and Roberts (2018) reported various genomic regions associated with BFT and intramuscular fat in composite beef cattle breeds (50% Red Angus, 25% Charolais, 25% Tarentaise), including regions harboring genes also associated with feed intake and growth factors (e.g., *LYN* and *LYPLA*). Júnior *et al.* (2016) reported SNPs located on several chromosomes which altogether explained 11.44% of the genetic variation for BFT in Nellore cattle, and were linked to genes associated with lipid metabolism (e.g., *SORCS2*, *AQP3*, *AQP7*, *CDC42BPA*). Silva-Vignato *et al.* (2017) also identified candidate genes for BFT in Nellore involved in 18 biological processes, including lipid metabolism pathways. Gene integration and clustering analysis through co-expression networks enable the identification of highly connected genes, which can act as regulators of significant biological pathways related to carcass traits (LANGFELDER and HORVATH, 2008, VAN DAM *et al.,* 2018). Therefore, the main objectives of this work were to: 1) perform a genome-wide association study (GWAS) based on the single-step GBLUP approach (ssGWAS) to identify (and validate) genomic regions related to BFT and RFT in Nellore cattle, and, 2) identify novel candidate genes, their biological functions, and gene networks for BFT and RFT in Nellore cattle raised in pasture-based systems.

2.2 MATERIAL AND METHODS

2.2.1 Data Availability

The data that support the findings of this study are from Katayama Ltd. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors with the permission of Katayama Ltd.

2.2.2 Phenotype, genotype, and pedigree information

No local ethical committee approval was required for this study since the data were from preexisting databases. The animals used in this study were raised in farms from the Katayama Ltd. livestock company (Guararapes, São Paulo, Brazil), spread across three Brazilian states (São Paulo, Mato Grosso do Sul, and Mato Grosso). A total of 1,440 Nellore animals (329 males and 1,111 females) born between 2009 and 2018 were genotyped using the GGP-Indicus 35K SNP panel (Neogen Company,

Lansing, Michigan, USA), which contains 35,247 SNPs. DNA extraction was performed based on hair follicle samples, following a protocol based on phenol-chloroform extraction (SAMBROOK, FRITSCH e MANIATIS, 1989). After this procedure, the DNA concentrations (ng/uL) and their degree of purity were determined using a spectrophotometer (Nanodrop - Thermo Fisher Scientific Waltham, Massachusetts, USA).

The animals were raised in pasture-based systems, receiving mineral supplementation during the winter season. The ultrasonography evaluation was performed at 18 months of age, using an Aloka SSD-500 ultrasound with a 17.2-cm, 3.5 MHz, linear array transducer (Aloka Co. Ltd., Wallingford, CT, USA). The BFT were measured between the 12th and 13th ribs in *longissimus lumborum* and RFT on the rump at the intersection of the *biceps femoris* and *gluteus medius* between ileum and ischium by ultrasound imaging. The complete pedigree file was composed of 39,903 animals, spanning over three generations. All the genotyped animals had complete genealogical information.

2.2.3 Data quality control

Quality control procedures were performed using the BLUPF90 family of programs (MISZTAL *et al.,* 2018). Individuals and SNPs with call rate lower than 0.90, non-autosomal SNPs, SNPs with duplicated or unknown position, minor allele frequency (MAF) lower than 0.05, or extreme deviation from Hardy-Weinberg equilibrium ($P \leq 10^{-5}$) were removed. After quality control of 33,613 SNPs remained in the dataset for further analyses. Additionally, phenotypic values that exceeded three standard deviations around the mean were considered outliers and were excluded from the analysis.

2.2.4 Single-step GBLUP-based genome-wide association study

The GWAS analyses were performed based on the single-step GBLUP methodology (ssGWAS, WANG *et al.,* 2012), using programs of the BLUPF90 family (MISZTAL *et al.,* 2002). The AIREMLF90 software (MISZTAl *et al.,* 2002) was used to estimate the variance components and the genetic parameters. The PREGSF90

(AGUILAR *et al.,* 2014) was used to construct a hybrid genomic relationship matrix (**H**, AGUILAR *et al.,* 2010), for both genotyped and pedigree animals. Subsequently, BLUPF90 (MISZTAL *et al.*, 2002) was used to solve the mixed model equations. Lastly, the postGSF90 package (AGUILAR *et al.,* 2014) was used to back-solve the genomic estimated breeding values and obtain SNP effects (i.e., ssGWAS). BFT and RFT were analyzed using an animal model, described as:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

where **y** is the vector of phenotypic observations, **X** is the incidence matrix linking the phenotypic records to the fixed effects, **b** is the vector of fixed effects, which included age at the measurement as linear and quadratic covariates, and the contemporary group (farm, birth season, management group, and sex), **Z** is the incidence matrix linking the phenotypic records to each animal, **a** is the vector of animal additive genetic effects, and **e** is the vector of residual effects. The model assumptions are:

$$\text{Var}[\mathbf{a}\ \mathbf{e}] = \begin{bmatrix} \mathbf{H}\sigma^2_a & 0 \\ 0 & \mathbf{I}\sigma^2_e \end{bmatrix}$$

where: σ^2_a is the direct additive genetic variance, σ^2_e is the residual variance, **H** is the relationship matrix combining pedigree and genomic relationships (AGUILAR *et al.,* 2010) and **I** is an identity matrix. The inverse of the **H** matrix can be described as (AGUILAR *et al.,* 2010):

$$\mathbf{H^{-1}} = \mathbf{A^{-1}} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G^{-1} - A^{-1}_{22}} \end{bmatrix}$$

where **A** is the pedigree relationship matrix for all animals, $\mathbf{A_{22}}$ is the relationship matrix for the genotyped animals, and **G** is the genomic relationship matrix, which was calculated as (VANRADEN, 2008).

$$\mathbf{G = WDW'}\frac{\mathbf{1}}{\mathbf{q}}$$

where **W** is a matrix of gene content adjusted for allele frequencies, **D** is a weight matrix for SNP (initially **D** = **I**), and $q = \sum_{i=1}^{M} 2p_i(1-p_i)$ is a normalizing factor.

2.2.5 Estimation of SNP effects

The SNP effects as well as their weights were calculated in three iterations. The iterations were described as proposed by Wang *et al.* (2012):

$$\mathbf{D_{(t)}} = \mathbf{I}$$

$$\mathbf{G_{(t)}} = \frac{\mathbf{WD_{(t)}\,W'}}{\sum_{i=1}^{M} 2p_i(1-p_i)}$$

where *t* is the iteration number. The SNP effects (**û**) were obtained as:

$$\mathbf{\hat{u} = \lambda DW'G^{-1}\hat{a}_g = DW'[WDW']^{-1}\,\hat{a}_g}$$

where $\mathbf{\hat{a}_g}$ is a vector of the animal effects of genotyped animals, which was represented by a function of the SNP effects ($\mathbf{\hat{a}_g} = \mathbf{Wu}$), **W** is the matrix containing the genotypes for each locus, **û** is the vector of SNP effects, λ is the variance ratio, calculated according to VanRaden (2008), **D** is the diagonal matrix of weights of the SNP variances, and **G** is the genomic relationship matrix, constructed as described above. The following model was applied to calculate the weights used for the SNPs:

$$d_{i(t=1)} = \hat{u}^2_{i(t)} 2p_i(1-p_i)$$

where *i* = SNP *i*. Lastly, the program calculates **G** with the new marker weights as:

$$\mathbf{G_{(t+1)}} = \frac{\mathbf{WD_{(t+1)}\,W'}}{\sum_{i=1}^{M} 2p_i(1-p_i)}$$

The ssGWAS results were presented as a proportion of the total additive genetic variance explained by the genomic windows of 10 adjacent SNPs (ZHANG *et al.,* 2016), as follows:

$$\frac{var(window_i)}{\alpha_a^2} \times 100\% = \frac{\sum_{j=1}^{10} var(\hat{u}_j)}{\alpha_a^2} \times 100\%$$

where $window_i$ is the additive genetic value of the genomic window i, σ^2_a is the total additive genetic variance for the trait, and $\hat{u}_j$ is the effect of SNP j within genomic window i.

2.2.6 Functional analysis and gene networks

After performing ssGWAS, the genomic windows that explained more than 0.5% of the total additive genetic variance of the trait were considered as the most relevant genomic regions. The positional candidate genes located in these regions were identified based on the Ensembl Genes 69 database incorporated in the BioMart tool (KINSELLA *et al.,* 2011). Subsequently, the PANTHER database (MI *et al.,* 2017) was used to perform the functional annotations, which pointed to which metabolic pathways the candidate genes were involved to generate the phenotypic expression of BFT or RFT. Finally, the REVIGO software (SUPEK *et al.,* 2011) was used to identify possible links between the genes found in the analyses. This analysis is important since the genes can interact with each other and their interaction could contribute to the expression of carcass quality traits in Nellore cattle.

2.3 RESULTS

2.3.1 Descriptive statistics and variance components

The descriptive statistics for carcass backfat (BFT) and rump fat (RFT) thickness measured at 18 months in genotyped Nellore animals are shown in Table 1. The mean ±SD BFT and RFT were 3.11 ± 1.47and 4.55 ± 1.95 mm, respectively. The heritabilities ± SE estimated were 0.26 ± 0.01 for BFT and 0.30 ± 0.02 for RFT, which

are, therefore, of moderate magnitude. The genetic correlation between BFT and RFT was 0.68±0.04.

2.3.2 Single-step GWAS

The results of the ssGWAS analyses are shown based on the proportion of the total additive genetic variance explained by 10 consecutive SNP windows (Figures 1 and 2). Genomic windows that explained more than 0.5% of the total additive genetic variance were considered as the most relevant. For BFT, 18 genomic regions with the greatest effect, located on BTA1, BTA4, BTA5, BTA7, BTA8, BTA17, BTA19, BTA21, BTA22, and BTA29, explained together 13.15% (range: 0.52% to 0.95%) of the total additive genetic variance and harbored 23 genes (Table 2). The genomic region with the highest peak was found on BTA5 118,784,107:119,434,691, which is associated with the *TTLL8* gene.

For RFT, 22 genomic regions with the largest effects were found on BTA1, BTA2, BTA5, BTA6, BTA7, BTA8, BTA10, BTA11, BTA14, BTA18, BTA19, BTA22, BTA26, and BTA27 (Table 2.3). These regions harbored 29 genes and individually explained from 0.51% to 0.90% of the total additive genetic variance for RFT and together accounted for 14.04% of the genetic variance (Table 3). The genomic region with the highest peak was found on BTA1 64,843,663:64,857,875, which is associated with the *GSK3β* gene.

2.3.4 Functional analysis and gene networks

After the functional enrichment, Gene Ontology (GO) analyses were carried out and revealed that seven out of the 23 positional candidate genes identified for BFT were linked to 14 GO terms, including biological processes, cellular components, and molecular functions (Table 4). Some of these genes were repeated in certain GO terms, e.g. the *AHCYL2* gene (present in seven GO terms), indicating its importance in the BFT expression.

The ontological coverage of the genes involved in the BFT and RFT expression is illustrated in blocks in Figure 3 and Figure 4, with each block representing a GO term and its size being proportional to the number of genes involved. The *FLI1* and *ETS1* genes stood out as being directly linked to differentiation and cell development

processes. Nonetheless, several of the genes were also found in significant processes for the BFT phenotypic expression, such as pathways of metabolism of amino acids, coenzymes and cofactors, as well as cytosolic transport and histone deacetylation.

For RFT, four out of the 29 positional candidate genes identified through the GWAS analysis were linked to 12 GO terms, which include biological processes and molecular functions (Table 5). In the RFT ontological coverage, *SORCS1* and *EXT1* demonstrated great representativeness in the formation of the blocks that characterize pathways such as lipid homeostasis, carbohydrate metabolism, and lipid metabolism. However, other important genes contribute to the RFT phenotypic expression and are present in key pathways, such as promoters of RNA transcription and responses to mechanical stimuli.

The STRING database enabled the identification of interactions between candidate genes that could play an important role in the phenotypic expression of each trait. As shown in Figures 5 and 6, the intensity of staining between the lines that link one gene to another is directly proportional to the strength of the interaction between these genes. The analysis of interactions of genes involved in the expression of BFT and RFT (Figures 5 and 6) revealed that there are a large number of genes that interact with each other, with fewer genes not showing any interaction.

2.4 DISCUSSION

The calculated means of BFT and RFT were 3.11 ± 1.47 mm and 4.55 ± 1.95 mm, respectively, which are similar to those reported in Nellore cattle. Bonin *et al.* (2015) reported means of 2.77 ± 1.59 mm and 3.77 ± 2.84 mm for BFT and RTF, respectively, and Bonamy *et al.* (2019) presented means of 3.68 ± 1.75 mm and 4.61 ± 2.44 mm for the same traits. Regarding the heritabilities, estimates of 0.26 for BFT and 0.30 for RFT are of moderate magnitude, indicating a good potential for selection response. Similar results were reported by Bonamy *et al.* (2019) with heritabilities of 0.21 and 0.34 for BFT and RFT, respectively, which confirms the selective potential of these traits. The genetic correlation estimates between BFT and RFT were 0.68 ± 0.04, similar to the values reported in other studies with Nellore cattle, as in Buzanskas *et al.* (2017), Ceacero *et al*. (2016) and Kluska *et al.* (2018), who obtained correlation values between BFT and RFT of 0.59 ± 0.07, 0.79 ± 0.05 and 0.73 ± 0.03, respectively. Despite the high genetic correlation, no genes in common between the two traits were

identified, as demonstrated below. The same occurred to Santana *et al*. (2015) and Silva *et al.* (2018), which also worked with BFT and RFT in beef cattle.

Several important genomic regions were identified on 29 autosomal chromosomes of Nellore cattle that harbor important genes affecting the phenotypic expression of BFT and RFT. Some of the genomic regions have not been previously reported in other cattle studies, but were identified in other mammal species (e.g. pigs and mice, BERGAMASCHI *et al.*, 2019, KABRA *et al.*, 2016). These results highlight that his study may have revealed novel genes linked to BFT and RFT in Nellore cattle.

2.4.1 Candidate genes for backfat thickness

The *EPHA6* gene on BTA1 has been previously reported to be associated with fat deposition traits in Nellore cattle (MUDADU *et al*., 2016). On the same chromosome, *TBL1XR1* gene, which was also associated with RFT, is involved in pathways such as histone (GO:0016575) and protein (GO:0006476, GO:0035601) deacetylation. *TBL1XR1* contains repetitions of WD40 proteins, which form functional complexes and act on cellular processes crucial to the organism, such as responses to DNA damage, transcription regulation, protein degradation, and histone modification (ZOU *et al.,* 2016). *TBL1XR1* encodes a protein that is one of the components of the histone deacetylase-3 complex (*HDAC3*) (CHEN; COUREY, 2000). Gene members of the *HDAC* family can induce obesity, as mice fed high-fat diets showed higher expressions of the *HDAC5* protein in the hypothalamus, having their weight as well as fat accumulation increased (KABRA *et al.,* 2016). Lundh *et al.* (2015) found that *HDAC3* inhibition improved glucose and insulin homeostasis in mice, allowing the control of obesity in the animals. Thus, the *TBL1RX1* gene may be related to a higher weight gain rate in Nellore cattle, as it is one of the components present in the *HDAC3* proteins. Furthermore, *TBL1RX1* was identified as a candidate gene for increased fat deposition in the carcasses of Large White, Duroc, and Pietran pigs, which reinforces the hypothesis of a relationship between *TBL1RX1* and weight gain (FOWLER *et al.,* 2013).

On BTA 4, the candidate genes *CACNA2D1* and *AHCYL2* were associated with the expression of BFT. *CACNA2D1* has been associated with an increase in dry-matter intake (DMI) and average daily gain (ADG) in Nellore cattle, being therefore linked to fat deposition (SHERMAN *et al.,* 2009, TIZIOTO *et al.,* 2015). Additionally,

CACNA2D1 has been reported to have a direct influence on marbling in Japanese Black cattle, reinforcing the relationship of this gene with increased fat deposition (YOKOUCHI *et al.,* 2009). Fonseca *et al.*, (2020) found that *CACNA2D2*, from the same family of *CACNA2D1*, is involved in the development of marbling in Nellore cattle. *CACNA2D1* also influences carcass composition of eight cattle breeds (Simmental, Angus, Hereford, Charolais, Limousin, Qinchuan, Luxi and Jinnan), which makes it an important candidate gene (YUAN; XU, 2011).

The *AHCYL2* gene is related to ADG in Duroc pigs *(BERGAMASCHI et al.*, 2019). Increased ADG can lead to greater carcass fat deposition in the final stage of animal growth and body maturation, thus *AHCYL2* might be an important candidate gene associated with increased BFT. *AHCYL2* is expressed in several pathways of metabolic processes of organonitrogen compounds (GO:1901564), purine-containing compounds (GO:0072521), coenzymes (GO:0006732), cofactors (GO:0051186), cellular amino acids (GO:0006520), and small molecules (GO:0044281, GO:0055086). In GO:000652, *AHCYL2* is involved in the coding of adenosylhomocysteinase-like 2 protein (SAH2), which transform adenosylhomocysteine into adenosine and homocysteine. These compounds can then be hydrolyzed and converted in the amino acid cysteine (TEIXEIRA *et al., 2019)*. Moreover, *AHCYL2* is associated with changes in the diameter of low-density lipoproteins, which plays an important role in the transport across membranes (WOOD *et al.,* 2012). Increased lipoprotein transport may be related to increased adipose tissue deposition.

The *AHCYL2* gene can also regulate the activity of the *SLC4A7* gene (YAMAGUCHI; ISHIKAWA, 2014), as shown in the network analysis (Figure 5). *SLC4A7* (BTA22) was identified in the current study as a candidate gene for BFT and it is a gene involved in the transport of solutes, such as sodium bicarbonate. Keogh *et al.* (2018) analyzed the effect of fasting and refeeding on the ruminal epithelium in cattle. The authors indicated that *SLC4A7* may be associated with greater compensatory gain after a fasting period in cattle. The greater compensatory gain can be explained by the fact that during refeeding, the animals are able to better absorb nutrients, suggesting a relationship between *SLC4A7* and better absorption of solutes. In addition, *SLC4A7* family genes are known to be essential for body lipid distribution, such as *SLC27A1*, found in skeletal muscle, whose function is to absorb and store fatty acids (MELO *et al.*, 2013).

The *SLC4A7* gene was presented as one of the central genes of the gene interaction network, which also included *AHCYL2*, as described previously, but also the *KCNJ1* and *CLCN3* genes. The latter, located on BTA8, encodes a CIC3 protein, which has chloride (Cl^-) binding sites and is located in the plasma membrane and in intracellular organelles in most tissues (CHEN and HWANG, 2008). It should be noted that CL^- is one of the fundamental elements for various activities in the animal metabolism. In carp (*Cyprinus carpio*), the same gene was reported to increase the efficiency of abdominal fat deposition (ZHENG *et al.,* 2016). *KCNJ1* (BTA29) belongs to the potassium channel family and was reported to be associated with BFT in Angus and Charolais cattle (WANG *et al.*, 2020).

The *CTNNA1* gene (BTA7) is associated with the level of myostatin expression in bovine skeletal muscle. Myostatin is a limiting growth factor for muscle tissue (PERIPOLLI *et al.,* 2018, SADKOWSKI *et al.*, 2008) and *CTNNA1* is an inhibitor of myogenesis, which can lead to a decrease in muscle differentiation (ZHAO *et al.,* 2011). Thus, the energy for muscle formation may be redirected to the formation of adipose tissue, since there is a limitation in muscle tissue.

The *AADAT* gene (BTA8) encodes the alpha-aminoadipate aminotransferase (AAT) protein, which is one of the enzymes necessary for the synthesis of L-lysine, through glutaric acid (LIU *et al.,* 2019). This corroborates with the GO findings, where *AADAT* participates in cellular amino acid metabolic pathways (GO:0006520). Moreover, AADAT in correlation with thyroid hormones (T3 and T4) by facilitating the oxidative deamination of alanine from T3 and T4, producing pyruvic acid (KUMAR and PRASAD, 2003). Thyroid hormones can influence the growth rate of the animal, since T3 and T4 are involved in the regulation and synthesis of several hormones related to production traits, such as sexual precocity in Guzerat cattle (FERNÁNDEZ *et al.*, 2017).

The *TMEM132D* gene was reported by Ali *et al.* (2013), who analyzed the composition of fat from the carcass of Brahman cattle and observed that the mentioned gene positively influenced backfat deposition, agreeing with the present findings. Additionally, Keogh *et al.* (2019) found genes of the TMEM family expressed in the gene network for compensatory gain. Fasting can improve animal performance, resulting in heavier animals while also improving fat deposition due to the compensatory gain after the fasting phase (KEOGH *et al., 2015)*. Therefore, genes

present in gene networks for compensatory gain may also play a role in the carcass fat composition in cattle.

The *VPS53* gene (BTA19) is involved in the transport of substances from the endoplasmic reticulum (ER) to the Golgi complex (GC) (PALACIOS *et al.,* 2017). The ER is the main site where the glycosylation process takes place, whereby carbohydrates from food are attached to proteins and lipids. Subsequently, in the cytoplasm of several cells, such as adipose tissues, this process results in the storage of bioavailable energy/fat (MOLINARO *et al.*, 2010). A gene from the same family as *VPS53*, *VPS51*, is also involved in lipid metabolism as well as was present in the gene interaction network associated with the meat tenderness (BRAZ *et al*., 2019). Cesar *et al.* (2018) found genes of the *TMEM*, *VPS*, *CLCN,* and *SLC* families to be related to lipid and carbohydrate metabolism, which corroborate with the present findings.

The *IDH2* gene (BTA21) encodes the isocitrate dehydrogenase 2 enzyme protein, which acts on the Krebs cycle by supplying NADPH as energy to cells (GONG *et al.,* 2019). This pathway is more important in ruminants, due to the low activity of the ATP-citrate lyase enzyme, which acts on the breakdown of citrate produced in the Krebs cycle into acetyl-COA and oxalacetate. In this context, without the action of ATP-citrate lyase, ruminants would not use glucose to form lipids, but instead the isocitrate dehydrogenase pathway to form NADPH (LEHNINGER *et al.,* 2014). Thus, *IDH2* is essential for energy production, which is converted into fatty acids and later stored as fat. Keogh *et al.* (2015) investigated the effect of fasting and refeeding on the transcriptional profile of skeletal muscle in Holstein cattle and demonstrated that *IDH2* was essential for energy production, in catalytic reactions acting on the mitochondria. This explains the presence of *IDH2* in several GO that regulate coenzymes (GO:0006732), cofactors (GO:0051186), and small molecules (GO:0055086). *IDH2* is expressed in the mitochondria, an organelle whose main function is to generate energy through the synthesis of adenosine triphosphate (ATP). In addition, the *ZNF710* gene (BTA21), which was associated with BFT, belongs to a family of genes that were previously identified to the expression of master regulators related to beef cattle carcass quality and co-expression network in multibreed Angus-Brahman (LEAL-GUTIÉRREZ *et al.,* 2020). This gene is related to the use of zinc by the body, which is one of the most active elements in the metabolic processes of cattle and may be indirectly linked to the development of carcass adiposity.

According to Li *et al.* (2019), the *ETS1* gene was expressed when fasting periods were analyzed in animal models using mice. The authors also noted that *ETS1* was one of the mediators of the *FOX01* gene, which acts on glucose homeostasis in the body, especially in a state of oxidative stress. Glucose homeostasis can be explained by the fact that the *FOX01* gene regulates fundamental enzymes for the gluconeogenesis pathway, e.g. glucose-6-phosphatase (G6pc) and phosphoenolpyruvate carboxykinase (PEPCK) *(O*H *et al*., 2013). In addition, *ETS1* also inhibits GLUT-1 (glucose transporter 1), responsible for redirecting basal glucose to cells, triggering oxidative stress, which is essential for energy production (ZHANG *et al.,* 2017).Therefore, it is suggested that the substrates that would be used in the formation of glucose will not be used and will remain stored in the form of fat.

2.4.2 Candidate genes for rump fat thickness

Several regions harboring important genes in the expression of RFT were identified (Table 3). *GSK3β* (BTA1) has been reported to influence fat deposition and adipocyte differentiation in Herefored and Angus cattle (ROMAO *et al.*, 2012). *GSK3β* controls glycogen synthesis through the activation of other genes such as *FOX01*. The latter, in turn, acts on the activation of genes, such as *G6Pase* and *PEPCK*, in a cascade effect (SAKAMAKI *et al.,* 2012). Underwood *et al.* (2007) analyzed the expression of the *GSK3β* gene in the *longissimus dorsi* muscle of Angus cattle and highlighted the greater gene activity for glycogen deposition in that muscle. As muscle glycogen is the main energy reservoir, its muscle or blood levels determine the use of fat and, later, the use of other resources, such as amino acids, which provide energy to the metabolism (FONSECA *et al.,* 2018). Thus, when *GSK3β* is activated, it can help to increase liver and muscle glycogen deposition, and when there is a shortage of glucose, glycogen can be used, thereby reducing the use of fat.

On BTA2, the *LRP1B* gene, which belongs to the family of low-density lipoprotein receptors (LDLR family), was identified as a candidate gene. *Song et al.* (2018) observed that this gene was associated with carcass quality traits in native Korean cattle (Hanwoo and Chikso). *LRP1B* mediates the lipid transport process of apolipoprotein E, which is involved in cholesterol transport, and also participates in other physiological processes, such as the transport of nutrients and vitamins, and neurological development (MAY *et al.,* 2007). This can be explained by the fact that

apopoliprotein E acts as an LDL receptor, which promotes the recognition and catabolism of triglyceride-rich lipoproteins (MAHLEY *et al.,* 1984). In a study with Holstein dairy cattle, *LRP1B* was reported as a candidate for fat deposition in milk (FLÓREZ *et al.,* 2018). In other species, such as birds, it was reported as involved in the composition of fatty acids and in fat deposition (JIN *et al.*, 2018, ZHANG *et al.,* 2012). Thus, it can be inferred that this gene acts as a mediator responsible for the transport of apolipoprotein E, which is related to fat deposition in several species. Moreover, in the gene network (Figure 5), the *GSK3β* gene, which acts on the synthesis of glycogen, interacts with *LRP1B*, which is one of the mediators of the transport of essential compounds (apolipoprotein and cholesterol) for the formation of glycogen in the metabolism of ruminants.

APAF1 (BTA5) is reported as a candidate gene for residual intake in cattle, and is involved with amino acid biosynthesis and cell apoptosis (KARISA *et al.*, 2013). On the same chromosome, *ANKS1B* (ankyrin repeat and sterile alpha motif domain-containing 1B) is associated with intramuscular fat in pigs (HAMILL *et al.,* 2013). In Hanwoo cattle, this gene has been associated with increased marbling (SEONG *et al.*, 2016). Ankyrins are structural proteins that have binding sites with membrane and cytoskeleton proteins (GALLAGHER *et al.*, 1997). The region where the ANKS1B gene is located is directly linked to the meat shear-force and levels of intramuscular fat in Charolais cattle (HORODYSKA *et al.*, 2015).

The *PKD2* gene (BTA6) was reported to increase fat deposition in beef cattle (ABO-ISMAIL *et al.,* 2014, LANSINK *et al.,* 2018). This gene is involved in the negative regulation of the transition of G1/S phases in the cell cycle, which is related to the increase in size and the duplication of components of the cytoplasm, where RNA molecules are also produced that will act on the synthesis of proteins (GUTIÉRREZ-GIL *et al.,* 2009). It is also noteworthy that the *ABCG2*, *PKD2*, *SPP1*, and *IBSP* genes are located in close locations on BTA6 and influence growth traits in Canchim beef cattle (SANTIAGO *et al.,* 2017). The *SPP1* gene has been reported in the gene network of Hanwoo cattle, as a candidate for visceral fat (LEE *et al.,* 2013). In cattle, the first adipose tissue depots are visceral, followed by subcutaneous, intermuscular and intramuscular (DU *et al.*, 2012). The average genetic correlation between visceral fat and BFT is 0.43 (GOMES *et al.,* 2012) and the current study a genetic correlation of 0.68 BFT with RFT was estimated. In analyses conducted by Kahles *et al.* (2014), the *SPP1* gene showed to be more expressed in inflamed adipocyte tissues, which

generates insulin resistance. Even though insulin does not metabolize glucose, it may be available to increase fat tissues. In this context, Hudson *et al.* (2020) reported that several genes associated with fat deposition are linked to body defense processes.

On BTA11, the *TTF1* gene, related to the RNA polymerase I transcription termination factor, was identified as a candidate for meat tenderness and carcass quality in composite breeds (Charolaise, Limousine and Blonde d'Aquitaine) (CALDAS *et al.,* 2016). According to TIZIOTO *et al.,* (2013), markers linked to the *TTF1* gene were responsible to explained the greatest additive genetic variance for the backfat thickness trait in Nellore animals. The same authors reported that TTF1 clusters with other genes (MYOD1 and ZSCAN21) linked to muscle development and meat tenderness. For instance, *MYOD1* is related to the differentiation of type-1 myosin, one of the factors regulating myogenic proliferation during cell differentiation (TATSUMI *et al.,* 2017).

Also on BTA 14, the *EXT1* gene was identified as a candidate gene for RFT, which is involved in pathways in the metabolic processes of glycogen (GO:0005977), cellular glucan (GO:0006073), polysaccharides (GO:0005976), carbohydrates (GO:0005975), and cellular polysaccharide (GO:0044264). *EXT1* encodes the exostosin glycosyltransferase 1 protein which controls the biosynthesis of heparan sulfate, involved in signal transduction and related to fibroblast growth factor 2 (*FGF2*) (NADANAKA and KITAGAWA, 2018). The interaction between growth factors and their receptors is regulated by the amount of heparan sulfate (PESSENTHEINER *et al.*, 2020). In humans, *EXT1* regulates *FGF2* and, consequently *FGF1*, which induces the proliferation of pre-adipocytes, in addition to the expression of genes related to adipogenesis (HUTLEY *et al.*, 2011). Thus, the pathways in which *EXT1* participates may be associated with increased RFT due to its link with *FGF*.

Another gene that has a connection with *FGF* is *GRB2*, present on BTA19. *GRB2* is an important candidate gene for carcass fat deposition in Angus, Charolais, Hereford, Holstein, and Simental cattle (PURFIELD *et al.,* 2019). This gene is associated with SOS (set of genes encoding guanine nucleotide-exchange factors that act on the RAS subfamily) (MAO *et al.,* 2016). Thus, the RAS proteins are activated by SOS, which recruit another protein called RAF, a serine/threonine kinase activated by RAS, activating MAPK signaling (AHN *et al.,* 2008). The MAPK (mitogen-activated protein kinase) proteins regulate cellular activities such as gene expression, mitosis, differentiation, cell survival, proliferation and apoptosis, in addition to being associated

with obesity (ZHANG *et al.*, 2012). In this respect, Oliveira *et al.* (2018) analyzed the co-expression network in the pathways of lipid metabolism in Nellore cattle and found that *GRB2* gene was not directly expressed, whereas MAPK, a protein regulated by *GRB2*, was expressed, indicating the link of *GRB2* to RFT development.

Purfield *et al.* (2019) found the *SORCS1* gene (BTA26) associated with fat deposition in beef cattle of different breeds. In addition, the *SORCS1* gene has been reported to affect feed intake and fat deposition in mice (SUBKHANGULOVA *et al.*, 2018). This gene may be associated with fat deposition, as it participates in some important GOs for the formation of the RFT trait, such as cholesterol homeostasis (GO:0042632), lipid homeostasis (GO:0055088), polysaccharide metabolic process (GO:0005976), carbohydrate metabolic process (GO:0005975), cellular response to lipopolysaccharide (GO:0071222), and lipid metabolic process (GO:0006629). *SORCS1* acts as a receptor for pancreatic b cells, which are necessary for the regulation of insulin secretion (KEBEDE *et al.,* 2014). This can also explain the role of this gene in lipid and cholesterol homeostasis, considering that these processes are regulated by insulin. Furthermore, *SORCS1* participates in the structural organization of sortilins, which are receptors that direct proteins through secretions between cells and influence protein interactions by controlling the metabolism of lipoproteins (WILLNOW *et al.,* 2011). Sortilin is expressed in adipocyte tissues and plays an important role in the GLU-T 4 pathway (insulin-responsive glucose transporter) (KANDROR, 2018).

When the expression pathways of *SORCS1* were analyzed, the gene was seen in the pathway of the lipid metabolic process (GO:0006629) together with the *SLMAP* gene, which was presented in pathways of response to mechanical stimulus (GO:009612) and detection of stimulus (GO:0051606). In this way, the *SLMAP* gene may be related to these pathways, considering that the insulin concentration is one of the stimuli for its expression. In a study with mice, Dewan (2016) reported that the *SLMAP* gene expression increased glucose uptake in the heart muscle fiber. In addition, the authors observed an increase in glucose transport through GLUT-4 (type-4 glucose transporter). Thus, *SLMAP* can act on the formation of RFT due to the increase in the GLUT-4 transporter in glucose uptake, which is necessary for the production of glycerol-3-P. It should be stressed that glycerol-3-P is an important component for the formation of triacylglycerol (TAG), which is stored in adipocytes and, therefore, acts directly on the formation of fat (FONSECA-ALANIZ *et al.,* 2006).

The identification and description of genome regions and biological process that affect BFT and RFT traits are crucial for a better understanding of the mechanisms that influence those traits. The results obtained here showed different genomic regions with genes already known as associated with BFT and/or RFT, but also several genes with previously unknown association with those traits in Nellore cattle, which should be validated in other cattle populations. The candidate genes reported in this study may be used in the future in breeding programs aiming the genetic improvement of carcass quality traits in beef cattle.

2.5 CONCLUSIONS

Both backfat thickness and rump fat thickness are moderately heritable traits of polygenic nature in Nellore cattle. A total of 18 genomic regions harboring 23 genes were identified for backfat thickness, including important candidate genes such as *TBL1XR1*, *AHCYL2*, *SLC4A7*, *AADAT*, *VPS53*, *IDH2,* and *ETS1*. These genes are involved in lipid metabolism, development of cellular amino acids, transport of solutes, transport between complex golgi membranes, cell differentiation, and cellular development. For rump fat thickness, 21 genomic regions and 29 positional genes were identified, including important candidate genes, such as *GSK3β*, *LRP1B*, *EXT1*, *GRB2*, *SORCS1*, and *SLMAP.* These genes are involved in several metabolic pathways, such as glycogen synthesis, lipid transport, polysaccharide and carbohydrate metabolism, and lipid homeostasis. Furthermore, *GRB2* and *SORCS1* act on the activation of other genes, which are important for the phenotypic expression of rump fat thickness in Nellore cattle. These findings warrant further investigation to validate the candidate genes in other Nellore populations. The candidate genes reported in this study may be used in the future in breeding programs aiming the genetic improvement of carcass quality traits in Nellore cattle.

Table 1. Descriptive statistics, variance components, and heritability for backfat thickness (BFT) and rump fat thickness (RFT) in Nellore cattle.

Trait	N.	Mean	SD	Min.	Max.	σ^2_a	σ^2_e	σ^2_p	h^2±SE	rg±SE
BFT	1,440	3.11	1.47	0.51	9.27	0.15	0.42	0.57	0.26±0.01	0.68± 0.04
RFT	1,440	4.55	1.95	0.76	9.91	0.37	0.85	1.22	0.30±0.02	

Fonte: O autor.
Abbreviations: N – number of observations, SD – standard deviation, σ^2_a – additive genetic variance, σ^2_e – residual variance, σ^2_p – phenotypic variance, h^2 – heritability, r_g additive genetic correlation, SE – standard error.

Table 2. Identification and description of genes located in genomic windows that explained more than 0.5% of the additive genetic variance for backfat thickness in Nellore cattle.

Genomic Regions	Gene Symbol	Gene Name	Var (%)
BTA1 41,521,649:41,830,647	*EPHA6*	EPH Receptor A6	0.59
BTA1 90,087,080:90,202,421	*TBL1XR1*	TBL1X Receptor 1	0.79
BTA4 38,516,487:38,670,540	*CACNA2D1*	Calcium Voltage-Gated Channel Auxiliary Subunit Alpha2delta 1	0.67
BTA4 92,663,898:92,848,894	*FAM71F2, KCP*	Family with Sequence Similarity 71 member F2, kielin cysteine rich BMP regulator	0.55
BTA4 93,226,620:93,332,291	*AHCYL2*	Adenosylhomocysteinase Like 2	0.80
BTA5 118,784,107:119,434,691	*TTLL8*	Tubulin Tyrosine Ligase Like 8	0.96
BTA7 50,183,313:50,405,133	*CTNNA1, SIL1*	Catenin Alpha 1, SIL1 nucleotide exchange factor	0.71
BTA8 1,607,879:1,635,244	*CLCN3*	Chloride Voltage-Gated Channel 3	0.89
BTA8 1,872,073: 1,977,876	*MFAP3L, AADAT*	Microfibril Associated Protein 3 Like, Aminoadipate Aminotransferase	0.72
BTA17 47,449,510:47,461,429	*TMEM132D*	Transmembrane Protein 132D	0.80
BTA19 22,100,301:22,393,674	*TLCD3A, VPS53, RPH3AL*	TLC Domain Containing 3ª, *VPS53* Subunit of GARP Complex, Rabphilin 3A Like	0.68
BTA21 21,473,508:21,620,784	*ZNF710, IDH2, CIB1*	Zinc Finger Protein 710, isocitrate dehydrogenase ($NADP^{+2}$), calcium and integrin binding 1	0.52
BTA22 1,837,980:19,876,140	*SLC4A7*	Solute Carrier Family 4 Member 7	0.68
BTA29 31,864,392:32,217,845	*ETS1,FLI1, KCNJ1*	ETS Proto-Oncogene 1, Transcription Factor, *Fli-1* Proto-Oncogene, ETS Transcription Facto, Potassium Inwardly Rectifying Channel Subfamily J Member 1	0.88

Fonte: O autor.

Table 3. Identification and description of genes located in genomic windows that explained more than 0.5% of the additive genetic variance for rump fat thickness in Nellore cattle.

Genomic Regions	Gene Symbol	Gene Name	Var (%)
BTA1 64,473,854:64,632,165	*MAATS1, NR1I2*	MYCBP Associated and testis expressed 1, Nuclear Receptor Subfamily 1 group I member 2	0.85
BTA1 64,843,663:64,857,875	*GSK3β*	Glycogen synthase kinase 3 beta	0.90
BTA2 55,950,604:56,190,829	*LRP1B*	Low-density lipoprotein receptor-related protein 1B	0.64
BTA5 62,691,496:62,842,398	*TMPO, APAF1, ANKS1B*	Thymopoietin, Apoptotic Peptidase Activating Factor 1, Ankyrin Repeat and Sterile Alpha Motif Domain Containing 1B	0.89
BTA6 36,482,509:36,889,292	*ABCG2, PKD2, SPP1, IBSP*	ATP Binding Cassette Subfamily G Member 2, Polycystin 2, Secreted Phosphoprotein 1, Integrin Binding Sialoprotein	0.81
BTA7 53,874,712:54,203,762	*ARHGAP26*	Rho GTPase Activating Protein 26	0.65
BTA8 10,933,246:10,998,893	*CDK5RAP2*	CDK5 Regulatory Subunit associated Protein 2	0.51
BTA10 80,162,749:80,274,095	*RAD51B*	RAD51 Paralog B	0.64
BTA11 83,200,393:10,266,969	*NBAS,TTF1, DCTN1*	NBAS subunit of NRZ tethering complex, Transcription Termination Factor 1, Dynactin subunit 1	0.54
BTA14 46,354,070:46,424,692	*EXT1*	Exostosin Glycosyltransferase 1	0.52
BTA18 16,621,272:17,015,232	*ABCC11,N4BP1*	ATP-binding cassette transporter sub-family C member 11, NEDD4 Binding Protein 1	0.53
BTA19 55,729,126:56,499,004	*TRIM65, GRB2, GGA3, OTOP2*	Tripartite Motif Containing 65, Growth factor receptor-bound protein 2, Golgi Associated, Gamma Adaptin Ear Containing, ARF Binding Protein 3, Otopetrin 2	0.53
BTA22 43,137,847:43,839,944	*FLNB, SLMAP, ASB14*	Filamin B, Sarcolemma Associated Protein, Ankyrin Repeat and SOCS Box Containing 14	0.65
BTA26 27,592,543:27,866,959	*SORCS1*	Sortilin Related VPS10 Domain Containing Receptor 1	0.74
BTA27 14,386,764:14,399,090	*TRAPPC11*	Trafficking Protein Particle Complex 11	0.57

Fonte: O autor.

Table 4. Relevant biological functions identified from the annotation analysis for backfat thickness in Nellore cattle.

GO	Term	Genes	P-value
GO:0016575	Histone deacetylation	*TBL1XR1*	1.86E-02
GO:1901564	Organonitrogen compound metabolic process	*AHCYL2* *AADAT* *IDH2*	1.75E-03
GO:0006476	Protein Deacetylation	*TBL1XR1*	1.86E-02
GO:0035601	Protein Deacetylation	*TBL1XR1*	2.37E-02
GO:0072521	Purine-containing compound metabolic process	*AHCYL2*	2.58E-02
GO:0042147	Retrograde transport, endosome to Golgi	*VPS53*	3.69E-02
GO:0016482	Cytoplasmic transport	*VPS53*	4.00E-02
GO:0006732	Coenzyme metabolic process	*AHCYL2* *IDH2*	3.78E-03
GO:0051186	Cofactor metabolic process	*AHCYL2* *IDH2*	1.47E-02
GO:0006520	Cellular amino acid metabolic process	*AHCYL2* *AADAT*	5.73E-03
GO:0044281	Small molecule metabolic process	*AHCYL2* *IDH2*	2.82E-02
GO:0055086	Nucleobase-containing small molecule metabolic process	*AHCYL2* *IDH2*	1.52E-02
GO:0030154	Cell differentiation	*FLI1* *ETS1*	3.85E-02
GO:0048869	Cellular developmental process	*FLI1* *ETS1*	3.85E-02

Fonte: O autor.

Table 5. Relevant biological functions identified from the annotation analysis for backfat thickness in Nellore cattle.

GO	Term	Genes	P-value
GO:0016575	Histone deacetylation	*TBL1XR1*	1.86E-02
GO:1901564	Organonitrogen compound metabolic process	*AHCYL2* *AADAT* *IDH2*	1.75E-03
GO:0006476	Protein Deacetylation	*TBL1XR1*	1.86E-02
GO:0035601	Protein Deacetylation	*TBL1XR1*	2.37E-02
GO:0072521	Purine-containing compound metabolic process	*AHCYL2*	2.58E-02
GO:0042147	Retrograde transport, endosome to Golgi	*VPS53*	3.69E-02
GO:0016482	Cytoplasmic transport	*VPS53*	4.00E-02
GO:0006732	Coenzyme metabolic process	*AHCYL2* *IDH2*	3.78E-03
GO:0051186	Cofactor metabolic process	*AHCYL2* *IDH2*	1.47E-02
GO:0006520	Cellular amino acid metabolic process	*AHCYL2* *AADAT*	5.73E-03
GO:0044281	Small molecule metabolic process	*AHCYL2* *IDH2*	2.82E-02
GO:0055086	Nucleobase-containing small molecule metabolic process	*AHCYL2* *IDH2*	1.52E-02
GO:0030154	Cell differentiation	*FLI1* *ETS1*	3.85E-02
GO:0048869	Cellular developmental process	*FLI1* *ETS1*	3.85E-02

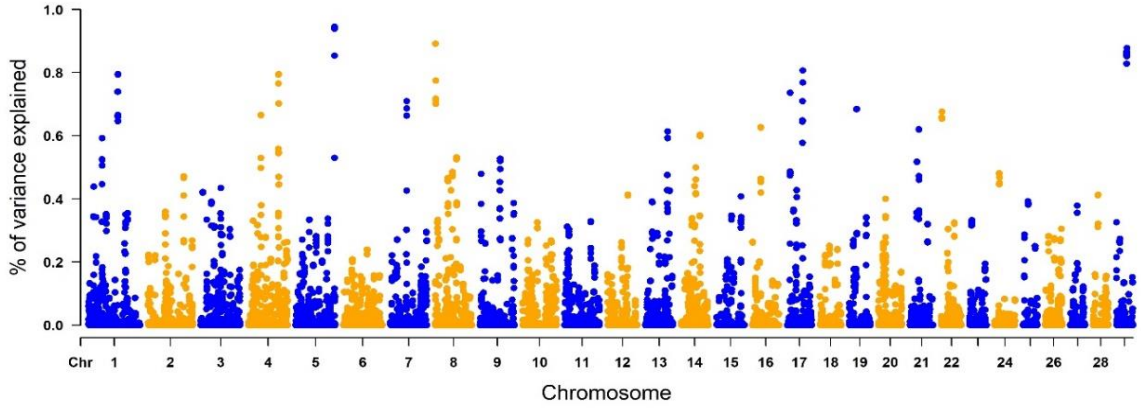
Fonte: O autor.

Table 6. Relevant biological functions identified from the annotation analysis for rump fat thickness in Nellore cattle.

GO	Term	Genes	P-value
GO:0006360	Transcription from RNA polymerase I promote	*RAD51B*	9.18E-03
GO:0042632	Cholesterol homeostasis	*SORCS1*	1.96E-02
GO:0055088	Lipid homeostasis	*SORCS1*	4.76E-02
GO:0009612	Response to mechanical stimulus	*SLMAP*	1.96E-02
GO:0051606	Detection of stimulus	*SLMAP*	2.21E-02
GO:0005977	Glycogen metabolic process	*EXT1*	3.62E-02
GO:0006073	Cellular glucan metabolic process	*EXT1*	3.62E-02
GO:0005976	Polysaccharide metabolic process	*EXT1* *SORCS1*	3.61E-03
GO:0005975	Carbohydrate metabolic process	*EXT1* *SORCS1*	2.89E-02
GO:0044264	Cellular polysaccharide metabolic process	*EXT1*	3.88E-02
GO:0071222	Cellular response to lipopolysaccharide	*SORCS1*	4.76E-02
GO:0006629	Lipid metabolism	*SLMAP* *SORCS1*	1.57E-02

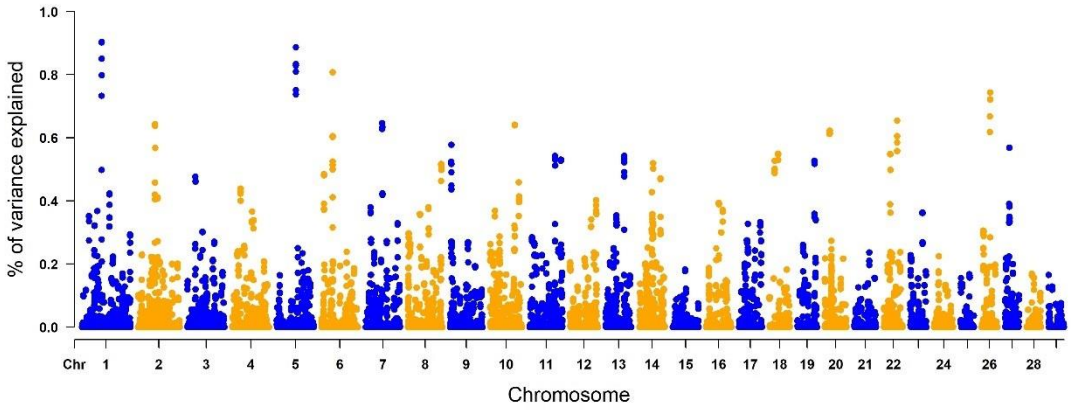
Fonte: O autor.

Figure 1. Manhattan plot of the percentage of additive genetic variation explained by windows of 10 adjacent SNPs for backfat thickness (BFT) in Nellore cattle.



Fonte: O autor.

Figure 2. Manhattan plot of the percentage of additive genetic variation explained by windows of 10 adjacent SNPs for rump fat thickness (RFT) in Nellore cattle.

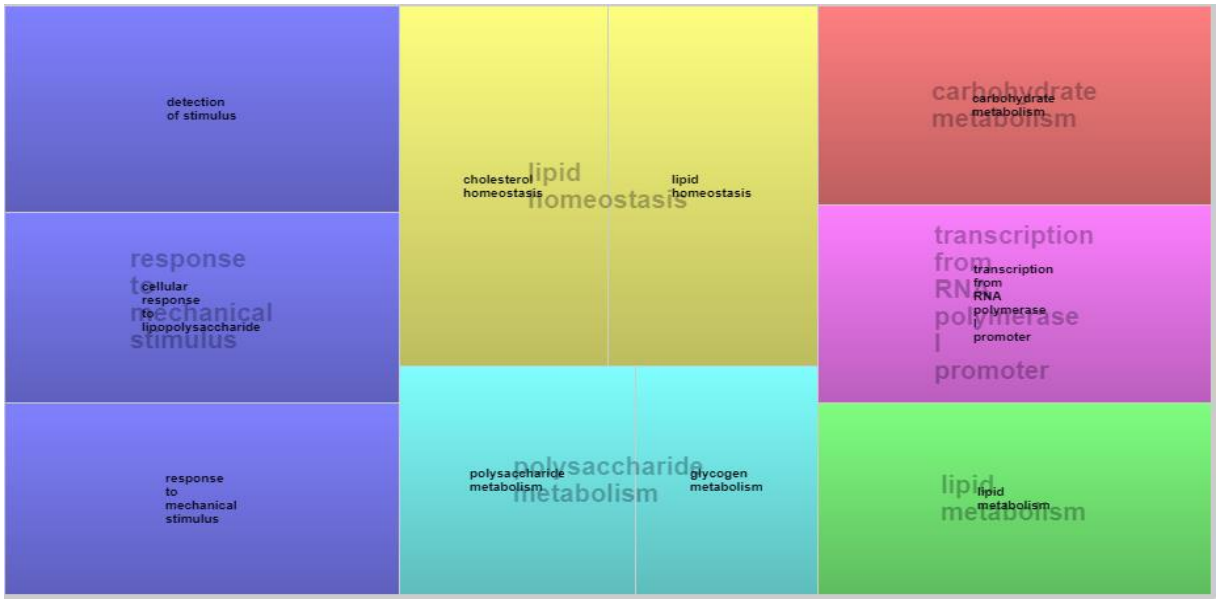


Fonte: O autor.

Figure 3. Biological process analysis of the co-association network for backfat thickness. The PANTHER overrepresentation test grouped 7 annotated genes into 14 biological process.

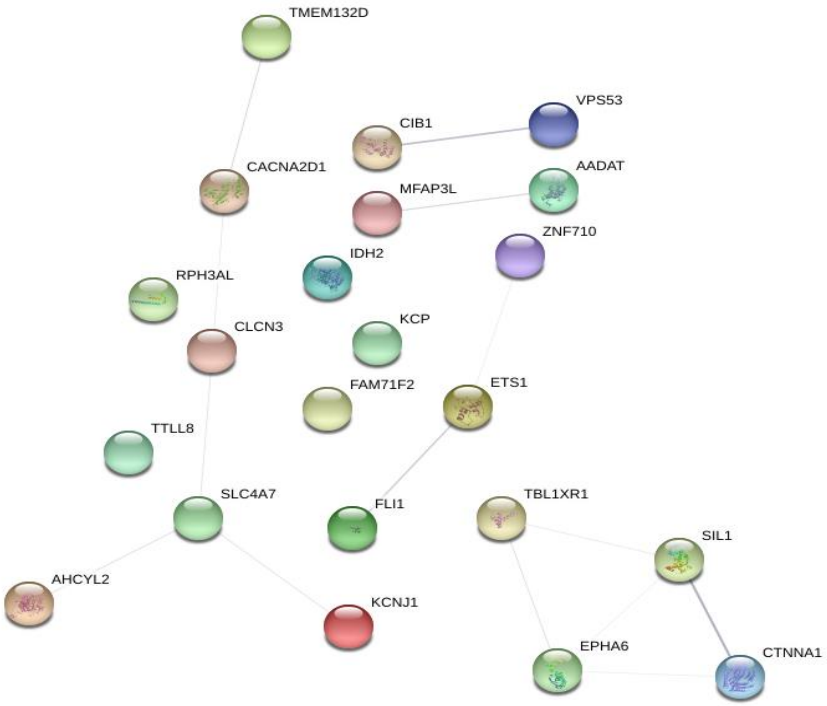
Fonte: O autor.

Figure 4. Biological process analysis of the co-association network for rump fat thickness. The PANTHER overrepresentation test grouped 4 annotated genes into 12 biological process.



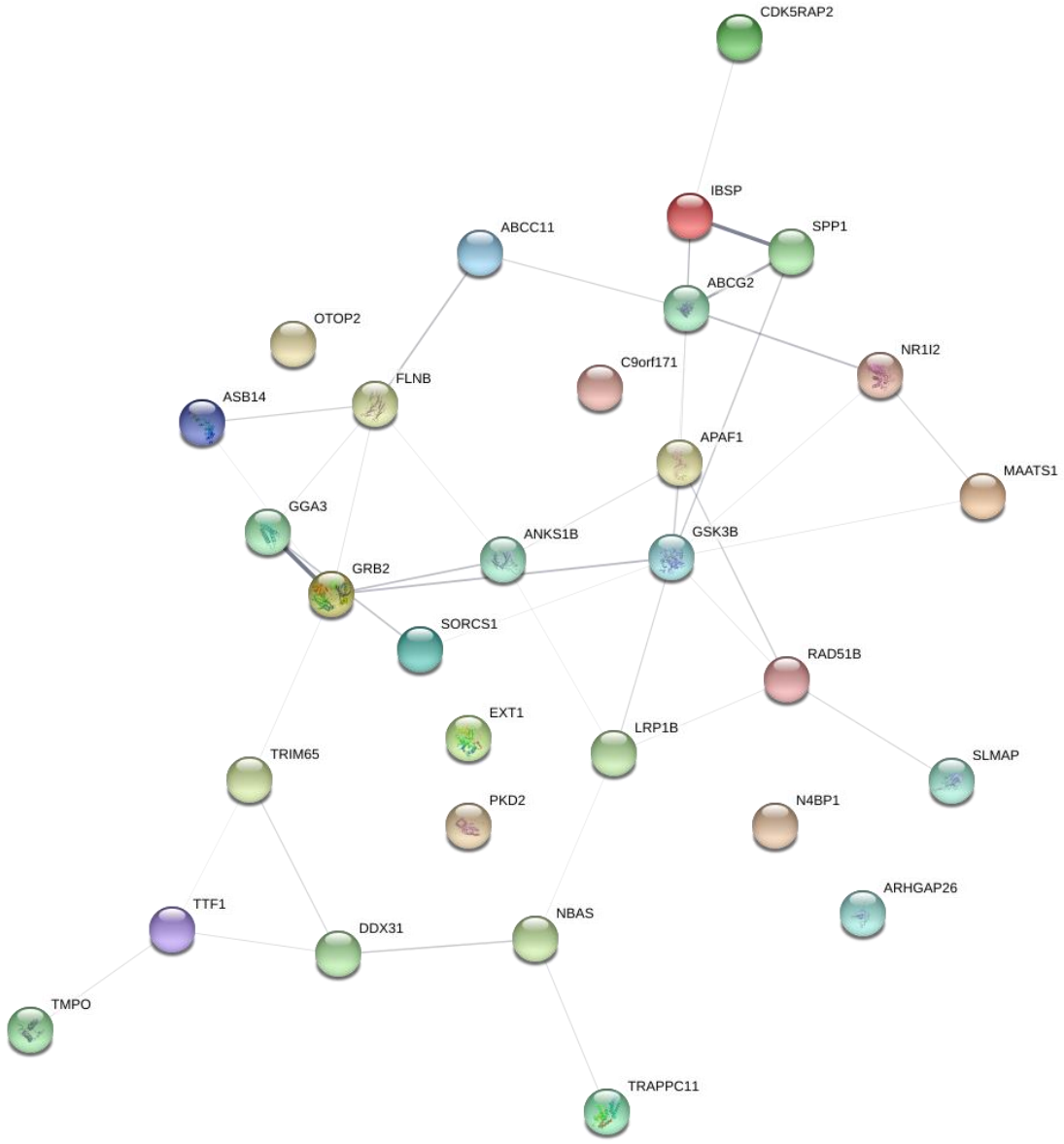
Fonte: O autor.

Figure 5. Gene interaction network for genes associated in backfat thickness in Nellore cattle.



Fonte: O autor.

Figure 6. Gene interaction network for genes associated in rump fat thickness in Nellore cattle.



Fonte: O autor.

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CAPITULO 3: GENOME-WIDE ASSOCIATION STUDY AND PATHWAY ANALYSIS FOR CARCASS FATNESS IN NELLORE CATTLE MEASURED BY ULTRASOUND

RESUMO

O Brasil possui um dos maiores rebanhos bovinos de corte comercial do mundo, em expansão devido ao aumento da demanda por carne bovina no mercado internacional. A maior parte da população brasileira de bovinos de corte é composta pela raça Nelore. Em geral, os animais Nelore têm carcaças caracteristicamente mais magras do que as raças de gado taurino. Assim, identificar os genes ou regiões genômicas que influenciam nas características de qualidade da carcaça, como acabamento (ACAB), é essencial para maximizar os processos de seleção genética. O objetivo deste estudo foi identificar as regiões genômicas associadas à característica ACAB em rebanhos bovinos Nelore, bem como elucidar as vias metabólicas e os processos biológicos subjacentes à expressão fenotípica do ACAB. Foram utilizados dados de 1440 animais genotipados com o painel GGP-Indicus 35K, para um total de 35.247 marcadores. Todos os animais genotipados também tinham fenótipos disponíveis para ACAB. As análises foram realizadas pelo método de uma etapa (ssGWAS), utilizando janelas genômicas com 10 SNPs consecutivos. Com base nos marcadores encontrados, os genes candidatos para a característica foram identificados usando o banco de dados ENSEMBL, após o que, análises de ontologia gênica foram realizadas usando o software PANTHER e análises de redes de interação gênica foram realizadas usando REVIGO. Vinte genes relacionados à espessura de gordura (BFT) foram encontrados nos cromossomos BTA1, BTA2, BTA5, BTA6, BTA7, BTA8, BTA10, BTA13, BTA14, BTA26 e BTA27. Juntos, esses genes explicaram aproximadamente 12,96% da variância genética aditiva total da característica ACAB. Para os 20 genes encontrados, a ontologia do gene revelou sete genes (ou seja, NR1L2, PKD2, GSK3β, EXT1, RAD51B, SORCS1 e DPH6) conectados a processos biológicos importantes para a característica avaliada. Os genes GSK3β, LRP1B, EXT1, SORCS1, NR1L2 e APAF1 foram destacados como estando intimamente ligados a processos relacionados à deposição de gordura. Além disso, eles estiveram presentes em importantes vias metabólicas para a ACAB, como a homeostase do colesterol e lipídios e o glicogênio, além de processos metabólicos de polissacarídeos e carboidratos. Além disso, também descobrimos várias novas regiões genômicas, bem como identificamos outras já conhecidas por estarem relacionadas a vias metabólicas que promovem a deposição de gordura nas carcaças. Espera-se, portanto, que os dados obtidos neste estudo possam contribuir para um melhor entendimento da composição genética de ACAB em bovinos Nelore.

Palavras-chave: gordura na picanha, janelas SNPs, qualidade da carne, redes de genes, ssGWAS, vias biológicas.

ABSTRACT

Brazil has one of the largest commercial beef cattle herds in the world, which is expanding due to an increasing demand for beef in the international market. The largest majority of the Brazilian beef cattle population is composed by the Nellore breed. In general, Nellore animals have characteristically leaner carcasses than taurine cattle breeds. Thus, identifying the genes or genomic regions that influence carcass quality traits such as fatness (FTN) is essential to maximize genetic selection processes. The aim of this study was to identify the genomic regions associated with the FTN trait in Nellore cattle herds, as well as to elucidate the metabolic pathways and biological processes underlying the FTN phenotypic expression. Data of 1440 animals genotyped with the GGP-Indicus 35K panel, for a total of 35,247 markers, were used. All genotyped animals also had phenotypes available for FTN. The analyses were carried out by the single-step method (ssGWAS), using genomic windows with 10 consecutive SNPs. Based on the markers found, the candidate genes for the trait were identified using the ENSEMBL database, after which, gene ontology analyses were performed using the PANTHER software and gene interaction networks analyses were undertaken using REVIGO. Twenty genes related to backfat thickness (BFT) were found on chromosomes BTA1, BTA2, BTA5, BTA6, BTA7, BTA8, BTA10, BTA13, BTA14, BTA26, and BTA27. Altogether, these genes explained approximately 12.96% of the total additive genetic variance of the FTN trait. For the 20 genes found, gene ontology revealed seven genes (i.e., *NR1L2, PKD2, GSK3β, EXT1, RAD51B, SORCS1*, and *DPH6*) connected to important biological processes for the evaluated trait. The *GSK3β*, *LRP1B*, *EXT1*, *SORCS1*, *NR1L2*, and *APAF1* genes were highlighted as being closely linked to processes related to fat deposition. Moreover, they were present in important metabolic pathways for FTN, such as cholesterol and lipid homeostasis and glycogen, as well as polysaccharide and carbohydrate metabolic processes. In addition, we also discovered several new genomic regions, as well as identified others already known to be related to metabolic pathways that promote fat deposition in carcasses. It is thus expected that the data obtained in this study can contribute to a better understanding of the genetic development of FTN in Nellore cattle.

Keywords: backfat, biological pathways, gene networks, meat quality, SNP windows, ssGWAS.

3.1 INTRODUCTION

Carcass quality is known to be associated with sensory traits such as color, flavor, and tenderness. These factors are influenced by parameters such as the degree of fatness and fat deposition (TORRICO *et al.,* 2018). In this context, despite the great participation of Nellore beef in the international market, such animals sometimes do not meet the fatness standards imposed by some of the main consumer markets in the world. In contrast, consumers in the most demanding markets are likely to pay more for a higher-quality product derived from animals with a fatter carcass and a thicker layer of subcutaneous fat (BONIN *et al.,* 2020).

One of the main factors determining carcass quality is the degree of fatness, which is necessary to maintain the sensory traits of the final product — i.e., the meat. This is, among other reasons, because the degree of fatness is related to the protection of muscle fibers in the post-slaughter process. A thicker layer of subcutaneous fat translates into a lower chilling rate, which prevents dehydration and retains the meat tenderness (BALDASSINI *et al.,* 2017). The muscle fibers of animals with fatter carcasses are generally better preserved, thus reducing muscle stiffness, compared to those of animals with a lower fat content (SILVA *et al.,* 2019).

To obtain a satisfactory degree of fatness at the time of slaughter, the animal must reach physical maturity, as only then will its muscle mass have been established and nutrients redirected for the formation of adipose tissue (FEITOSA *et al.,* 2017). In this regard, one of the main parameters that indicates the occurrence of this order of physiological growth is subcutaneous fat (“backfat”) deposition (HEDRICK, 1983). Backfat deposition is also one of the plausible parameters for use in breeding programs, since the genetic gains obtained through selection accumulate over generations (GRIGOLETTO *et al.,* 2020).

Nonetheless, difficulties are encountered in the selection of carcass quality traits when traditional selection methods are used. In this context, genomics emerges as an efficient alternative for this purpose (MAGALHÃES *et al.,* 2019). Genome-wide association studies (GWAS) are an accessible methodology for identifying candidate genes and sites associated with traits of economic interest in livestock animals (ZHANG *et al.,* 2019). Most GWAS for qualitative traits focused on taurine breeds, which suggests a dearth of studies involving Nellore cattle (SILVA *et al.,* 2019).

Several genes located in different genomic regions have been reported in Nellore cattle (JUNIOR *et al.,* 2016). For instance, *CDKN2A* and *CDKN2B* have been identified as candidates involved in cell growth and development processes, while others were related to fat deposition traits, e.g., *SORCS2*, *AQP3*, *AQP7*, *CDC42BPA*, *ASIP*, and *ACSS2*, which are associated with lipid metabolism. It is thus observed that several genes participate in the expression of the same trait. As stated by Weber *et al.* (2016), carcass traits have a polygenic nature. In addition, Pereira *et al.,* (2016) analyzed genes related to carcass and growth traits in Nellore cattle and found that the *PLAG1* gene was expressed for both traits, revealing a pleiotropic effect of the genes.

Fonseca *et al.* (2018) investigated genes for fertility in Brangus cattle and reported that some genes (e.g., *MYC*, *PPARG*, *GSK3β*, *TG*, and *IYD*) had a pleiotropic effect, with important regulatory actions associated with production- and health-related traits. Santiago *et al.* (2017) conducted a GWAS for carcass traits in Canchim cattle and found that the *ABCG2*, *PKD2*, *SPP1*, *MEPE*, and *IBSP* genes, among others, were related to body weight at 12 months. The authors also observed that some of these genes may be involved in fat deposition. These findings highlight the importance of analyzing the regions of the bovine genome, since a wide array of genes may be involved in fat deposition and have small effects, which characterizes the polygenic effect of said trait. Accordingly, GWAS analyses can identify new genes as well as validate genes already covered in the literature. On this basis, the aim of the present study was to discover genomic regions with candidate genes related to fatness and to elucidate which biological functions and processes they perform in the formation of this trait, specifically in Nellore cattle.

3.2 MATERIAL AND METHODS

3.2.1 Data Availability

The data that support the findings of this study were provided by Katayama Ltd. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors with the permission of Katayama Ltd.

3.2.2 Phenotype, genotype, and pedigree information

No local ethical committee approval was required for this study, since the data were from preexisting databases. The animals used in this study were raised on the farms belonging to the Katayama Ltd. livestock company (Guararapes, São Paulo, Brazil), spread across three Brazilian states (i.e., São Paulo, Mato Grosso do Sul, and Mato Grosso). A total of 1,440 Nellore animals (329 males and 1,111 females) born between 2009 and 2018 were genotyped using the GGP-Indicus 35K SNP panel (Neogen Company), which contains 35,247 SNPs. DNA extraction was performed based on hair follicle samples, following a protocol based on phenol–chloroform extraction (SAMBROOK *et al.,* 1989). After this procedure, the DNA concentrations (nanograms per microliter) and their degree of purity were determined using a spectrophotometer (Nanodrop, Thermo Fisher Scientific Waltham, Massachusetts, USA).

The complete pedigree file was composed of 39,903 animals, spanning more than three generations. All of the genotyped animals had complete genealogical information. The animals were raised in pasture-based systems, receiving mineral supplementation during the winter season. The ultrasonography evaluation was performed at 18 months of age, using an Aloka SSD-500 ultrasound with a 17.2 cm, 3.5 MHz, linear array transducer (Aloka Co. Ltd., Wallingford, CT, USA). The fatness (FTN) phenotype was constituted considering a composition of 35% of the backfat thickness (BFT) and 65% of the rumpfat thickness (RFT), both measured at 18 months of age by ultrasound imaging, as proposed by the National Association of Breeders and Researchers (ANCP) in Brazil.

3.2.3 Data quality control

Quality control procedures were performed using the BLUPF90 family of programs (MISZTAL *et al.*, 2018). Individuals and SNPs with a call rate lower than 0.90, non-autosomal SNPs, SNPs with duplicated or unknown positions, a minor allele frequency (MAF) lower than 0.05, or extreme deviation from the Hardy–Weinberg equilibrium ($P \leq 10^{-5}$) were removed. A total of 33,623 SNPs remained in the dataset for further analyses. Additionally, phenotypic values that exceeded three standard deviations from the mean were considered outliers and excluded from further analysis.

3.2.4 Single-step GBLUP-based GWAS (ssGWAS)

The GWAS analyses were performed based on the single-step GBLUP methodology (ssGWAS, WANG *et al.,* 2012), using the programs of the BLUPF90 family (MISZTAL *et al.*, 2018). The AIREMLF90 software (MISZTAL *et al.,* 2018) was used to estimate the variance components. The PREGSF90 (AGUILAR *et al*., 2014) was used to construct a hybrid genomic relationship matrix (**H**, AGUILAR *et al.,* 2010), for both genotyped and non-genotyped animals. Subsequently, BLUPF90 (MISZTAL *et al.*, 2018) was used to solve the mixed model equations. The postGSF90 package (AGUILAR *et al.,* 2014) was used to back-solve the genomic estimated breeding values and obtain the SNP effects (i.e., ssGWAS analyses). FTN was analyzed as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

where **y** is the vector of the phenotypic observations, **X** is the incidence matrix linking the phenotypic records to the fixed effects, **b** is the vector of fixed effects, which included age at the measurement as linear and quadratic covariates, and the contemporary group (farm, birth season, management group, and sex), **Z** is the incidence matrix linking the phenotypic records to each animal, **a** is the vector of the animal additive genetic effects, and **e** is the vector of the residual effects. The model assumptions are as follows:

$$\text{Var}\,[\mathbf{a}\ \mathbf{e}] = \begin{bmatrix} \mathbf{H}\sigma^2_a & 0 \\ 0 & \mathbf{I}\sigma^2_e \end{bmatrix}$$

where: σ^2_a is the direct additive genetic variance, σ^2_e is the residual variance, **H** is the relationship matrix combining pedigree and genomic relationships (AGUILAR *et al.*, 2010), and **I** is an identity matrix. The inverse of the **H** matrix can be described as (AGUILAR *et al.*, 2010):

$$\mathbf{H^{-1}} = \mathbf{A^{-1}} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G^{-1} - A^{-1}_{22}} \end{bmatrix}$$

where: **A** is the pedigree relationship matrix for all animals, $\mathbf{A_{22}}$ is the relationship matrix for the genotyped animals, and **G** is the genomic relationship matrix, which was calculated as (VANRADEN, 2008):

$$\mathbf{G = ZDZ'q}$$

where **Z** is a matrix of gene content adjusted for allele frequencies, **D** is a weight matrix for SNPs (initially **D** = **I**), and q is a normalizing factor.

3.2.5 Estimation of the SNP effects

The SNP effects, as well as their weights, were calculated in three iterations. The iterations are described as follows (WANG *et al.,* 2012):

$$\mathbf{D_{(t)} = I}$$

$$\mathbf{G_{(t)} = \lambda Z D_{(t)} Z'}$$

$$\lambda = \frac{1}{\sum_{i=1}^{M} 2p_i(1-p_i)}$$

where *t* is the iteration number. The SNP effects (û) were obtained as:

$$\mathbf{\hat{u} = \lambda DZ'G^{*-1}\hat{a}g = DZ'[ZDZ']^{-1}\hat{a}g}$$

where **âg** are the animal effects of the genotyped animals, represented by a function of the SNP effects (**âg** = **Zu**), **Z** is the matrix that contains the genotypes for each locus, û is the vector of the SNP effects, λ is the variance ratio, calculated according to VanRaden (2008), **D** is the diagonal matrix of the weights of the SNP variances, and **G** is the genomic relationship matrix, constructed as described above. The following model was applied to calculate the weights used for the SNPs:

$$d_{i(t=1)} = \hat{u}^2_{i(t)} 2p_i(1-p_i)$$

where *i* = SNP *i*. Lastly, the program calculates **G** with the new marker weights as:

$$\mathbf{G_{t+1}} = \frac{\mathbf{ZD_{(t+1)}\,Z'}}{\sum_{i=1}^{M} 2p_i(1-p_i)}$$

The ssGWAS results are presented as a proportion of the total additive genetic variance, considering genomic windows of 10 adjacent SNPs. This genomic window size was defined based on the levels of linkage disequilibrium captured by the SNP panel used and in the literature for other GWAS studies in Nellore cattle (e.g., CARVALHO *et al.,* 2019, SILVA *et al.,* 2019, STAFUZZA *et a*l., 2020, VARGAS *et al.,* 2020). The model was followed as proposed by Zhang *et al.* (2016) using the equation below:

$$\frac{var(a_i)}{\alpha_a^2} \text{ x } 100\% = \frac{var \sum_{j=1}^{10} \mathbf{z_j}\,\hat{u}_j}{\alpha_a^2} \text{ x } 100\%$$

where a_i is the genetic value of the genomic window *i*, σ^2_a is the total additive genetic variance for the trait, $\mathbf{z_j}$ is the vector of the genotype for SNP *i*, and $\hat{u}_j$ is the effect of SNP *j* within genomic window *i*.

3.2.6 Functional analysis and gene networks

After performing ssGWAS, the genomic windows that explained more than 0.5% of the total additive genetic variance of the trait were considered as the most relevant regions. The positional candidate genes located in these regions were identified based on the Ensembl Genes 69 database incorporated in the BioMart tool, using reference genome Cow genes (ARS-UCD1.2) (KINSELLA *et al.,* 2011*)*. Subsequently, the PANTHER database (Mi et al., 2017) was used to perform functional annotations, demonstrating in which metabolic pathways the candidate genes were involved to generate the phenotypic expression of FTN. Finally, the REVIGO software (Supek et al., 2011) was used to identify possible links between the genes found in our analyses. This analysis is important since genes can interact with one another and can contribute to the expression of this carcass trait in Nellore cattle.

3.3 RESULTS

The average FTN was 4.05 ± 1.69 mm, with minimum and maximum values of 0.93 and 11.27 mm, respectively. The estimated variance components were 0.31 for additive variance, 0.70 for residual variance, and 1.01 for phenotypic variance. The heritability estimate (± standard error) for the studied trait was 0.31 ± 0.02.

Figure 7 illustrates the genomic regions with the greatest effects for FTN, in which markers were observed on chromosomes BTA1, BTA2, BTA5, BTA6, BTA7, BTA8, BTA10, BTA13, BTA14, BTA26, and BTA27. Table 7 shows the total genetic variance explained for FTN, which was approximately 12.96% when the markers present in the analysis with an explained genetic variance greater than 0.5% were summed. Table 7 also shows the genes identified from the markers reported in the analysis of the bovine genome.

Gene ontology (GO) analyses were performed for the 20 genes reported to be involved in FTN and revealed that approximately seven of these genes were present in 16 reported terms. Some of these terms show genes in the pathways responsible for biological processes of great importance in animal metabolism, as well as for the formation of the FTN trait, as shown in Table 8 . Some genes stand out as being related to GOs, e.g., *NR1L2* and *SORCS1*, which are present in the pathways of cholesterol and lipid homeostasis and lipid metabolism, respectively. The *GSK3β* and *EXT1* genes were identified in the same GOs and were present in the metabolism of several components essential for the development of adipose tissue. Figure 8 shows the arrangements of GO analysis of FTN, such as translation regulation, response to mechanical stimuli, lipid homeostasis, and polysaccharide, carbohydrate, and lipid metabolisms. Such pathways play a direct role in the processes of the formation of carcass lipid, which corroborates the importance of GO for understanding the function of the genes found in GWAS. Figure 9 exemplifies the connections between the genes found for FTN. Edges with highly connected genes (i.e., visibility of the connection line) usually demonstrate their relation with similar biological pathways that influence the trait (BOUCHER and JENNA, 2013). As can be seen in Figure 9, the *SPP1* and *IBSP* genes are closely connected and can act together to form FTN in Nellore cattle.

3.4 DISCUSSION

The heritability estimate for the FTN trait was 0.31 ± 0.02 (i.e., moderate heritability). In general, carcass FTN is estimated by using traits that indicate fat deposition, such as BFT and RFT. Heritability estimates of 0.44 and 0.47, and 0.17 and 0.33 have been reported for BFT and RFT, respectively (Bonin et al., 2015, Kluska et al., 2018). We can observe that heritability estimates for traits that indicate fat deposition are of a moderate to high magnitude, but this can vary according to the selection intensity of each population. For example, in Guzerat cattle, for which fewer breeding programs exist, Cancino-Baier *et al.* (2019) reported heritabilities of 0.10 for BFT and 0.19 for RFT. In this respect, FTN should be introduced in breeding programs, along with traits that indicate carcass quality (e.g., BFT and RFT), given their great importance and economic implications for the beef cattle industry (CAETANO *et al.,* 2013).

Due to the economic impact of FTN, the identification and understanding of genes that make up this trait can facilitate the achievement of a fatter carcass that will also provide greater economic return for the beef chain. The regions and genes found in this study contribute to expanding the knowledge of the structure of FTN in Nellore animals. Among the genes found, *GSK3β* (BTA1), *LRP1B* (BTA2), *EXT1* (BTA14), and *SORCS1* (BTA26) stand out, which are related to glycogen metabolism and cholesterol transport, demonstrating probable associations that contribute to the development of FTN.

The *GSK3β* gene identified in our study, which is present in several metabolisms that influence carcass FTN, was also mentioned by Divari *et al.* (2020), who reported it as being involved in glycogen metabolism in Charolais cattle. The authors also identified gene expressions associated with tissues of the liver, the organ responsible for most parts of the gluconeogenesis process in ruminants. Gluconeogenesis is one of the main processes that generates energy for an organism, which is one of the main causes of the formation of adipose tissue (SAKAMAKI *et al.,* 2012).

Divari *et al.* (2020) also noted that *GSK3β* acts to control the expression of the genes involved in the gluconeogenesis cycle, such as *G6PC*, *PCK1*, *PPARG co-activator 1*, and *GYS2*. The *GYS2* gene provides information for the production of glycogen synthase of the enzyme through the hepatic cells present in the liver. This

enzyme is responsible for the formation of glycogen from dietary substrates (KUO *et al.*, 2015). The *GSK3β* gene was present in the gene network formed in this study, where it was connected to the *SORCS1*, *APAF1*, and *NR1L2* genes, among others, which are necessary for the process of gluconeogenesis in the animal organism. Gluconeogenesis is known to be the main route for the production of energy for an organism, and it is suggested that its activation may increase fat deposition in the carcasses of beef cattle. Similar genes were reported in the RFT (first article) with ACAB, where the correlation between the two was 0,98 thus, it can be said that most of the genes that form RFT also act in the FTN traits.

Underwood *et al.* (2007) observed that the *GSK3β* gene is related to the *AMPK* (adenosine monophosphate-activated protein kinase) gene, and that the two may be correlated with glycogen deposition in the skeletal muscle of Angus × Gelbvieh crossbred cattle. According to Hardie (2004), *AMPK* is related to energy metabolism, which reinforces the evidence that *GSK3β* must be associated with increased storage of glycogen, which, in excess, forms adipose tissue.

The *LRP1B* gene, already reported in several studies on carcass traits of beef cattle, encodes protein 1B, related to the low-density lipoprotein (LDL) receptor (SONG *et al.,* 2018). The gene has already been described in studies of other species, as it is crucial for the development of fat cells, e.g., in the composition of milk fat in Holstein cattle (FLÓREZ *et al.,* 2018), it has even been described in birds, in which it is associated with abdominal fat production (ZHANG *et al.,* 2015). The *LRP1B* gene positively interferes with processes related to lipid metabolism, such as the transport of apolipoprotein E, involved in physiological processes such as the formation of cholesterol and some nutrients (MAY *et al.,* 2007).

The *EXT1* gene encodes a type-1 exostosin glycosyltransferase, which catalyzes the polymerization of heparan sulfate, involved in growth factors linked to heparin, as observed in fibroblast growth factor 2 (*FGF2*). According to *Xiao et al.* (2010), *FGF2* can reduce osteoblast formation and, consequently, bone tissue formation. Thus, there may be an increase in adiposity deposition in older animals due to the increase in the number of adipocytes in the bone marrow in the long term. Corroborating this information, Abuna *et al.* (2016) reported that fat cells inhibit osteoblast differentiation and suggested that there are interactions between fat cells and bone cells. In addition, Pereira *et al.* (2016) observed the *FGF* family gene

(*FGF22*) in the gene network of carcass and growth traits and suggested that the *EXT1* gene has a direct effect on genes that act to improve FTN.

The *SORCS1* gene is related to several GOs, such as cholesterol homeostasis (GO: 0042632), lipid homeostasis (GO: 0055088), polysaccharide (GO: 0005976) and carbohydrate (GO: 0005975) metabolic processes, cellular response to lipopolysaccharide (GO: 0071222), and lipid metabolic process (GO: 0006629). Such representativeness of *SORCS1* may be related to the regulation of insulin secretion in the pancreas, which, in turn, regulates glucose homeostasis, in addition to other metabolic functions (HABER *et al.,* 2001, KEBEDE *et al.,* 2014). Due to the activities of the gene in insulin metabolism, it is possible to understand its role in response to lipopolysaccharide and lipid formation, as well as in the control of lipoprotein metabolism (WILLNOW *et al.*, 2011).

Other relevant genes associated with FTN, such as *NR1I2* (BTA1), *ANK51B*, *TMPO*, *APAF1* (BTA5), and *IBSP* (BTA6), contribute to explaining the formation of this trait in beef cattle. The *NR1L2* gene is present in several pathways, e.g., cholesterol (GO: 0042632) and lipid (GO: 0055088) homeostasis, cellular response to lipopolysaccharide (GOs: 0071222 and 0032496), and the lipid metabolic process (GO: 0006629). In addition to being a functional receptor for indole-3-propionic acid, *NR1L2* is one of the genes responsible for increasing the immunity of the intestinal epithelium, according to a study carried out in mice (VENKATESH *et al.,* 2014). In addition to its role in the action of the microbiota, the activation of this gene is related to lipid metabolism, as it induces the expression of apolipoprotein 1 (APOA1) (DE HAAN *et al.,* 2009), which is the main component of HDL cholesterol and is involved in lipid metabolism processes, as well as in cholesterol processes (TOPTAS *et al.,* 2011). Our analyses corroborate these data, as we have demonstrated that the *NR1L2* gene acts indirectly on other genes that are necessary for important processes related to lipid deposition in Nellore cattle.

The gene encoding *APOA1* was reported by Wang *et al.* (2017) as a candidate for fat deposition in Simental cattle. The authors found that the gene is directly related to the pathways of lipid–protein–complex processes. Thus, because the *NR1L2* gene is linked to the *APOA1* gene and is also present in important pathways for fat deposition, it is a promising candidate for the formation of FTN.

On BTA5, the *ANK51B* gene was identified as being related to the structuring of intramuscular fat in Large White × Landrace pigs, as demonstrated by Hamill *et al.*

(2013). Pereira *et al.* (2016) analyzed pleiotropic genes that affect carcass and growth traits in Nellore cattle and identified genes of the *ANK* family present in the gene network. The authors described that the *ANK* genes were connected to the *PHLPP1* gene (PH domain and leucine-rich repeat protein phosphatase 1). Bradley *et al.* (2015) examined the action of the *PHLPP1* gene on the growth of mice and observed that it represses the activity of protein kinases in chondrocytes, increasing *FOXO1* and reducing the expression of *FGF18* (growth factor 18). According to Sakamaki *et al.* (2012), *FOXO1* exerts a cascade effect on genes that act as substrate for the process of gluconeogenesis (e.g., *G6Pase* and *PEPCK*). Thus, *ANK* genes may be related to energy production and, consequently, to fat deposition, due to their connections with other genes involved in the important processes of energy synthesis.

The *TMPO* gene, reported in our study, codes for thymopoietin, a hormone produced in the thymus that influences the maturation of T-lymphocytes, increasing the production of defense cells for an organism (APPOLINÁRIO and MEGID, 2007). According to Hudson *et al.* (2020), who evaluated the gene expression in the intramuscular and subcutaneous fat of *Bos taurus* cattle, several genes that acted on these traits were also present in the processes related to protection of the body, which explains the action of the *TMPO* gene on both fat deposition and defense mechanisms.

Another promising gene for FTN was *APAF1* (apoptotic protease-activating factor 1), which was associated with increased feed intake in cattle as well as with cellular biosynthesis and apoptosis (KARISA *et al.,* 2013). Together with other enzymes (e.g., caspase-9 and cytochrome C), *APAF1* forms a complex called apoptosome, which is responsible for the activation of caspase enzymes. These, in turn, can initiate the pathway for the execution of apoptosis (HENGARTNER, 2000, RIGDON *et al.*, 2017). Apoptosis can be used to balance the effect of cell proliferation, where it is essential for tissue renewal and constant restructuring of adipocytes, especially for adipose tissue cells (QUEIROZ *et al.*, 2009, RAZA *et al.,* 2020).

According to Karisa *et al.* (2013), the *IBSP* gene is correlated with feed efficiency in Angus and Charolais cattle. On the same chromosome as *IBSP* and close to its location are the *ABCG2*, *PKD2,* and *SPP1* genes, also reported in Canchim cattle as influencing the trait of weight at 12 months (SANTIAGO *et al*., 2017). The literature also describes that the *IBSP* and *SPP1* genes participate in a metabolic pathway involved both in bone development (SANTIAGO *et al.*, 2017) and in adipogenesis pathways (KAHLES *et al.*, 2014). Collis *et al.* (2012) observed that the *ABCG2* gene

was related to weight at 18 months in Brahman beef cattle, in addition to being closely linked to other genes that affect FTN, such as *CAPN1* (calpain 1) and *CAST* (calpastatin). The *ABCG2* gene has also been shown to be related to lipoprotein metabolism, with its family being associated with the gene expression network responsible for the attribution of meat and carcass quality in Nellore cattle (MUDADU *et al.,* 2016, OLIVEIRA *et al.,* 2018).

Although some of the genes mentioned in the present work have already been evaluated in other studies addressing traits related to carcass fat deposition, some genes have not yet been fully elucidated. Therefore, it would be interesting to involve the complete genome of Nellore cattle to investigate these candidate genes and to further examine their involvement in carcass fat deposition, which affects the FTN trait.

3.5 CONCLUSIONS

Of the 20 candidate genes found for FTN, *GSK3β*, *LRP1B*, *EXT1*, *SORCS1*, *NR1L2,* and *APAF1* stood out, as they act directly or indirectly to form adipose tissue in beef cattle. The *GSK3β*, *EXT1*, *SORCS1,* and *NR1L2* genes were associated with metabolic pathways such as cholesterol and lipid homeostasis and glycogen, as well as polysaccharide and carbohydrate metabolic processes. The identification of these genes, which are linked to pathways and metabolisms essential for the control of FTN, can contribute to the knowledge of the genetic basis of this trait, in addition to assisting in the direction of selection programs aimed at improved carcass quality in Nellore cattle.

Table 7 Identification and description of related genes in genomic windows that explained more than 0,5% of the additive genetic variance for finishing carcass in Nellore cattle.

Genomic Regions	Gene symbol	Gene name	Var (%)
BTA1 64857875:64473854	*GSK3β, NR1L2, MAATS1*	Glycogen synthase kinase 3 beta, Nuclear receptor subfamily 1 group I member 2, MYCBP Associated And Testis Expressed	0,69
BTA2 64288572:64911519	*LYPD1*	LY6/PLAUR Domain Containing 1	0,60
BTA2 55955028:55729126	*LRP1B*	Low-density lipoprotein receptor-related protein 1B	0,52
BTA5 62691496:62906620	*TMPO, APAF1, ANKS1B*	Thymopoietin, Apoptotic Peptidase Activating Factor 1, Ankyrin Repeat And Sterile Alpha Motif Domain Containing 1B	0,95
BTA6 36889292:36634231	*IBSP, SPP1, ABCG2, PKD2*	Integrin Binding Sialoprotein, Secreted phosphoprotein 1, ATP Binding Cassette Subfamily G Member 2, Polycystin 2	0,84
BTA7 53874712:54111043	*ARHGAP26*	Rho GTPase Activating Protein 26	0,58
BTA8 109923217:109988930	*CDK5RAP2*	CDK5 Regulatory Subunit Associated Protein 2	0,55
BTA10 80215074:80274095	*RAD51B*	RAD51 Paralog B	0,56
BTA10 30868823:31326738	*DPH6*	Diphthamine Biosynthesis 6	0,51
BTA13 60193198:60759713	*C13H20orf96*	Chromosome 13 C20orf96 homolog	0,61
BTA14 46354070:46424692	*EXT1*	Exostosin Glycosyltransferase 1	0,54
BTA26 27840203:27592543	*SORCS1*	Sortilin Related VPS10 Domain Containing Receptor 1	0,85
BTA27 14386764:14399090	*TRAPPC11*	Trafficking Protein Particle Complex 11	0,60

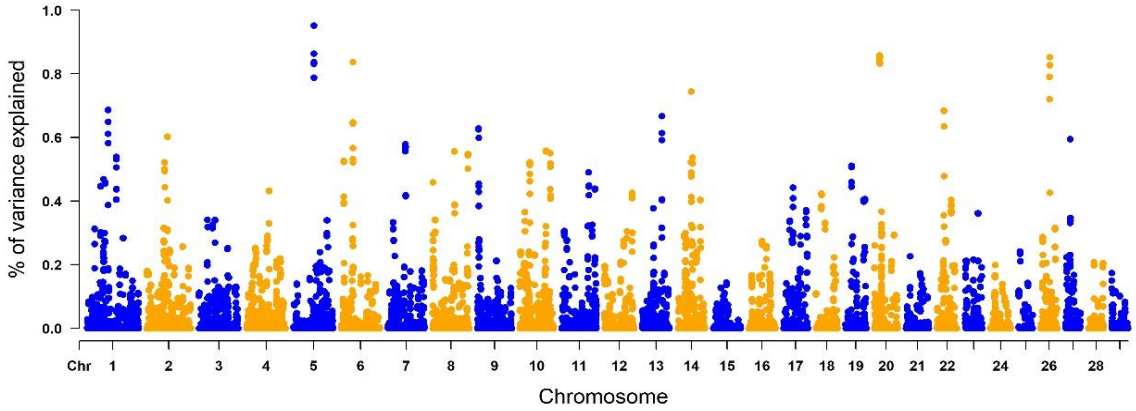
Fonte: O autor.

Table 8 Relevant biological functions identified from the annotation analysis for finishing carcass in Nellore cattle.

GO	Term	*Genes*	pValue
GO:0042632	Cholesterol homeostasis	*NR1I2*	1,35E-02
GO:0055088	Llipid homeostasis	*NR1I2*	3,31E-02
GO:0009612	Response to mechanical stimulus	*PKD2*	1,35E-02
GO:0051606	Detection of stimulus	*PKD2*	1,53E-02
GO:0005977	Glycogen metabolic process	*GSK3β*	2,51E-02
GO:0006073	Cellular glucan metabolic process	*GSK3β*	2,51E-02
GO:0005976	Polysaccharide metabolic process	*GSK3β* *EXT1*	1,72E-03
GO:0005975	Carbohydrate metabolic process	*GSK3β* *EXT1*	1,43E-02
GO:0044264	Cellular polysaccharide metabolic process	*GSK3β*	2,69E-02
GO:0071222	Cellular response to lipopolysaccharide	*NR1I2*	3,31E-02
GO:0032496	Response to lipopolysaccharide	*NR1I2*	4,44E-02
GO:0000724	Homology directed repair	*RAD51B*	4,27E-02
GO:0000725	Recombinational repair	*RAD51B*	4,27E-02
GO:0006417	Regulation of translation	*DPH6*	4,44E-02
GO:0034248	Regulation of cellular amide metabolic process	*DPH6*	4,71E-02
GO:0006629	Lipid metabolism	*SORCS1* *NR1I2*	7,67E-03

Fonte: O autor.

Figure 7 Manhattan plot for the percentage of genetic variance explained by 10 adjacent SNP windows for finishing carcass in Nellore cattle

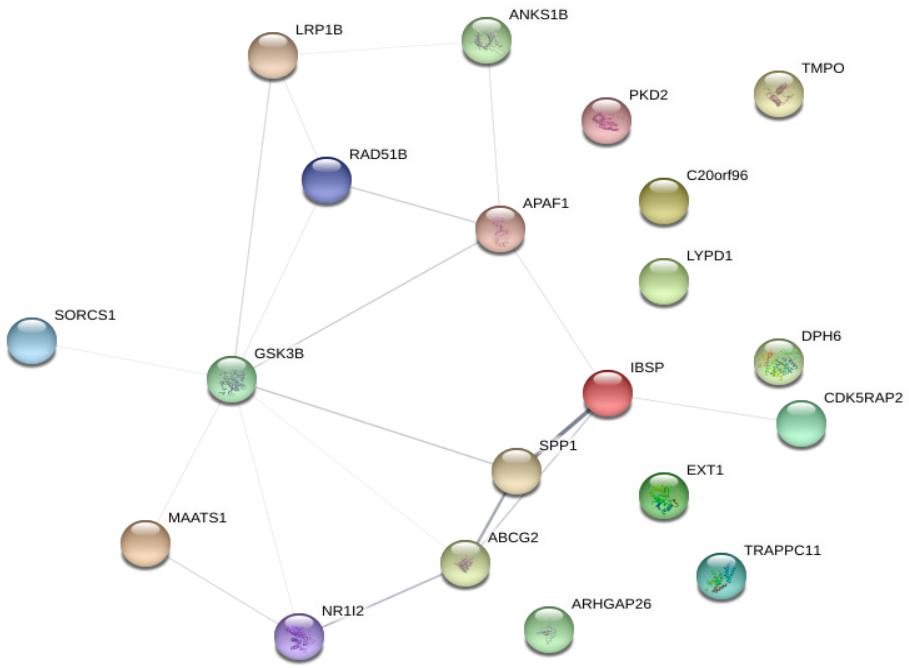


Fonte: O autor.

Figure 8 Superclusters of gene ontologies (GO) enriched with differentially expressed genes for finishing carcass. The PANTHER overrepresentation test grouped 7 annotated genes and each color indicates a main GO term.

Fonte: O autor.

Figure 9 Gene network of genes for finishing carcass. Colored circles represent genes and lines represent the predicted interactions between gene.



Fonte: O autor.

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