UNIVERSIDADE FEDERAL DE MINAS GERAIS

Genes candidatos para características de crescimento, reprodução e resistência em bovinos de corte

Virgínia Mara Pereira Ribeiro

Belo Horizonte
2020

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Tese apresentada ao Programa de Pós-Graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito parcial para a obtenção do grau de doutora em Zootecnia.

Área de concentração: Genética e Melhoramento Animal
Prof. Orientador: Dr. Fabio Luiz Buranelo Toral
Coorientador: Dr. Marcos Vinícius Gualberto Barbosa da Silva

Belo Horizonte

Ribeiro, Virginia Mara Pereira, 1990 -
R484g Genes candidatos para características de crescimento, reprodução e resistência em bovinos de corte/ Virginia Mara Pereira. -2020.

112f.:il.

Orientador: Fabio Luiz Buranelo Toral
Coorientador: Marcos Vinicius Gualberto Barbosa da Silva.
Tese (Doutorado) apresentado à Escola de Veterinária da Universidade Federal de Minas Gerais, como requisito para obtenção do grau de Doutor em Zootecnia
Área de concentração: Genética e Melhoramento Animal.

Bibliografia: f. 96 a 112.

1. Bovino - Teses – 2. Nelore – Teses – 3. Genética– Teses – 4. Melhoramento Genético – Teses – 5. Zootecnia – Teses - I. Toral, Fabio Luiz Buranelo - II. Silva, Marcos Vinicius Gualberto Barbosa da – III. Universidade Federal de Minas Gerais, Escola de Veterinária - IV. Titulo.

CDD – 636.08

Bibliotecária responsável Cristiane Patrícia Gomes – CRB2569
Biblioteca da Escola de Veterinária, Universidade Federal de Minas Gerais

UNIVERSIDADE FEDERAL DE MINAS GERAIS
ESCOLA DE VETERINÁRIA
COLEGIADO DO CURSO DE PÓS-GRADUAÇÃO EM ZOOTECNIA

FOLHA DE APROVAÇÃO

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Virgínia Mara Pereira Ribeiro

Tese de Doutorado defendida e aprovada, no dia **30 de junho de 2020**, pela Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Zootecnia da Universidade Federal de Minas Gerais constituída pelos seguintes professores:

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Belo Horizonte, 26 de agosto de 2021.

Documento assinado eletronicamente por **Angela Maria Quintão Lana, Coordenador(a) de curso de pós-graduação**, em 26/08/2021, às 00:34, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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Se, a princípio, a ideia não é absurda,

então não há esperança para ela.

Albert Einstein

Ao Melhoramento Genético Animal, dedico.

AGRADECIMENTOS

Agradeço, à Deus. A Ele, toda honra e toda glória.

À minha família, meus pais Fábio Pereira e Sandra Pereira, pelo amor incondicional, e aos meus irmãos Verônica Carvalho e Samuel Pereira, por todo apoio e incentivo.

Ao meu grande amor, Bruno Ribeiro, pelo apoio e respeito ao meu trabalho. Por ser meu porto seguro, principalmente nos momentos em que seguir em frente era quase insuportável.

Ao meu amado filho, Bento Pereira Ribeiro, que me fez renascer em todos os sentidos. Saber que você me esperava chegar todas as noites, foi o que me sustentou até o fim.

Ao professor Fabio Toral, por quem levarei para sempre comigo uma profunda admiração. Tornar fácil a caminhada nunca foi o mais importante, o primordial sempre foi desafiar e capacitar seus alunos a cumpri-la de modo independente e no mínimo brilhante. É isso que o torna um orientador excepcional. Gratidão, por todos os ensinamentos, profissionais e pessoais.

Ao professor Idalmo Garcia Pereira por não medir esforços em auxiliar os alunos do melhoramento e por todas as contribuições para conclusão deste trabalho.

Ao professor, Fabyano Fonseca e Silva, por compartilhar seu conhecimento de modo tão acessível. As contribuições dadas durante todo o processo foram essenciais para conclusão deste trabalho.

Ao professor, Renan Pedra de Souza, por ser tão disponível com aqueles que precisam e por transmitir seu conhecimento de modo inspirador. As disciplinas que tive o prazer de participar foram fundamentais para minha formação e para conclusão deste trabalho.

Ao Dr. Marcos Vinícius Gualberto Barbosa da Silva, pelo curso de “Ferramentas de controle de qualidade e análise de dados oriundos de CHIPS de DNA” e por todas as contribuições e sugestões dadas para a conclusão deste trabalho.

À Dra. Fernanda Santos Silva Raidan, pela amizade e por todas as sugestões que foram essenciais, não só para a conclusão deste trabalho, mas para a minha formação pessoal e profissional. Você é inspiradora!

Às professoras, Maria Raquel Carvalho e Ângela Maria Quintão Lana, pelas valiosas considerações, durante o processo de qualificação, e que tanto contribuíram para melhoria da qualidade deste trabalho.

Ao professor Eduardo Maldonado Turra, pelas contribuições e colaborações para conclusão do trabalho.

À minha grande amiga, Gabriela Canabrava Gouveia, por dividir tantos bons e maus momentos comigo. Nossas discussões, que aconteciam no mundo paralelo chamado LADA, sempre me faziam ficar sem dormir. Isso é ciência, é sair do lugar comum e sempre debater sobre o próximo passo a ser seguido. Obrigada por se fazer sempre presente em minha vida. Você é brilhante!

Aos companheiros de laboratório, Andresa Araújo, Clélia Assis, Íris Aganeti, Lucas Ayres, Luiza Abreu e Mariana Mamedes, pela troca de experiências, pelas conversas e por tornarem a caminhada mais amena.

Ao LADA (Laboratório de Análise de Dados), por ser uma extensão da minha casa. Por ser um ambiente que me proporcionou tantos momentos inesquecíveis, onde descobri minha paixão pelo Melhoramento Genético Animal e que ficará para sempre guardado no meu coração.

Ao Eduardo Penteado Cardoso, por permitir que o projeto fosse desenvolvido na Fazenda Mundo Novo e por sempre nos receber tão cordialmente.

Ao Daniel Resende Gonçalves pela disponibilidade e auxílio durante execução do projeto.

Ao professor Romário Cerqueira Leite por disponibilizar o Laboratório de Doenças Parasitárias para execução das análises.

Aos professores José Miguel Ortega e Tetsu Sakamoto, por todo auxílio para execução das análises no cluster HPC sagarana, CEPAD-ICB-UFMG.

A todos os professores que contribuíram para minha formação, pelos quais tenho profundo respeito e gratidão.

À Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) pela concessão da bolsa de estudos.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) pelo suporte financeiro por meio do projeto "Mapeamento de genes relacionados ao crescimento de bovinos de corte sob diferentes cargas parasitarias" (APQ- 01609-16).

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo suporte financeiro por meio do projeto "Genes candidatos e vias biológicas associados com características de resistência a parasitos em bovinos Nelore" (CNPq/ CNPQ- 461596/2014-8).

Ao Departamento de Zootecnia, Escola de Veterinária e Universidade Federal de Minas Gerais, pela estrutura e suporte para minha formação.

A todos que contribuíram para a conclusão deste trabalho

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RESUMO

Selecionar animais que possuem alelos favoráveis em genes que participam do controle genético de características de interesse econômico pode aumentar a eficiência dos programas de melhoramento de bovinos de corte. Neste sentido, dois artigos foram desenvolvidos com o objetivo principal de identificar genes candidatos funcionais (FCG) para características importantes em bovinos da raça Nelore. No primeiro artigo, "Genes underlying genetic correlations between growth, reproduction and parasite burden traits in beef cattle", estimamos a correlação genética entre características de crescimento, reprodução e carga parasitária e identificamos FCG que influenciam mais de uma dessas características. Avaliamos seis características, incluindo duas de crescimento (peso corporal - PC e ganho médio diário - GMD), uma de reprodução (circunferência escrotal - CE) e três de carga parasitária (contagem de carrapatos - CAR, ovos de nematóides gastrointestinais por grama fezes e oocistos de *Eimeria* spp. por grama de fezes - EIM). As correlações genéticas foram obtidas por meio de modelos multicaracterísticos. Um total de 21.667 marcadores SNP foram utilizados para realizar os GWAS (*Genome Wide Association Studies*) e identificar janelas genômicas que explicavam pelo menos 1% da variação genética para as características avaliadas. As correlações genéticas foram positivas e de magnitude moderada para os pares de características PC-GMD (0,64), PC-CE (0,38), PC-CAR (0,39), GMD-CE (0,27) e CAR-EIM (0,33). Somente o par GMD-EIM apresentou correlação negativa (-0,22). Para todos os outros pares, as correlações genéticas foram próximas de zero. Os efeitos dos SNPs foram calculados como proporção do desvio padrão genético e mostraram que existem SNPs mapeados próximo a FCG que promovem o melhoramento genético de ambas as características em sentido favorável. Além disso, análises funcionais foram realizadas e os FCGs foram selecionados com base no controle genético destes sobre processos biológicos para cada uma das características. As análises funcionais apontaram sete genes, SLC16A4, KCNA2, LAMTOR5, DUSP10,

MAP3K1, TPMT e KIF13A como FCGs com efeitos sobre mais de uma característica. Independentemente dos valores de correlação genética (baixo a moderado), existem FCG que podem influenciar as características de produção, reprodução ou resistência, em conjunto. No segundo artigo, "Candidate genes for longitudinal traits under selection in beef cattle", identificamos FCG que participam do controle genético do peso corporal em cinco idades diferentes idades (330, 385, 440, 495 e 550 dias), em dois arquivos de dados distintos, um com registros completos de peso corporal (AD100) e outro com uma seleção sequencial simulada de 70% dos animais mais pesados (AD70). Os parâmetros genéticos para o peso corporal foram estimados por meio de dois tipos de modelos, unicaracterísticos (UNI) e modelos de regressão aleatória com funções spline (REG) para os dois bancos de dados. Os pesos corporais foram padronizados para 330, 385, 440, 495 e 550 dias de idade para UNI. Para o REG, os nós de splines lineares foram ajustados para as idades 274, 330, 385, 440, 495, 550 e 594 dias. Os GWAS foram realizados com os resultados dos modelos UNI e REG. O GWAS e o enriquecimento funcional foram realizados conforme descrito anteriormente para o primeiro artigo. Identificamos sete FCG (DUSP10, LAMTOR5, PAFAH2, SLC30A2, TRIM63, NCAM1 e SCL16A4) para peso corporal em diferentes idades. O gene DUSP10 foi associado ao peso corporal em todas as cinco idades avaliadas, sugerindo a importância desse gene para os diferentes estágios do crescimento animal. Por outro lado, a maioria dos FCG associados ao peso corporal foram diferentes para diferentes idades, sugerindo que a importância de cada gene para o crescimento animal também pode mudar em diferentes estágios de desenvolvimento e diferentes genes podem ser mais relevantes para o peso corporal em cada estágio de crescimento. Quando a seleção sequencial foi simulada, diferentes FCG foram associadas ao peso corporal no AD100 e AD70 para cada idade, mesmo quando o REG foi utilizado. Portanto, a seleção sequencial pode influenciar os resultados de GWAS e esse pode ser mais um motivo para inconsistências frequentes verificadas nestes estudos para características de crescimento

medidas em bovinos de corte. Ressaltamos que os resultados aqui apresentados são essenciais para sugerir genes importantes que participam do controle genético de características de interesse zootécnico em bovinos de corte. Ainda, os FCG sugeridos no presente trabalho, esses poderiam ser validados para cada característica em populações maiores e outras raças, a fim de aperfeiçoar a compreensão do controle genético desses genes sobre as características avaliadas.

Palavras-chave: correlação, parâmetros genéticos, estudo de associação ampla do genoma, Nelore, seleção sequencial

ABSTRACT

Selecting animals which have favorable alleles in genes that participate in the genetic control of economic interest traits may increase the efficiency of beef cattle breeding programs. In this way, two papers were developed with the main aim of identified functional candidate genes (FCG) for important traits in Nellore cattle. In the first paper, "Genes underlying genetic correlations between growth, reproduction and parasite burden traits in beef cattle", we aim to estimate genetic correlations between growth, reproduction and parasite burden traits and identified FCG that influence more than one of these traits. We evaluated six traits, comprising two of growth (body weight - BW and average daily gain - ADG), one of reproduction (scrotal circumference - SC) and three parasite burden (counts of tick - TICK, gastrointestinal nematode eggs per gram of feaces - GIN, and *Eimeria* spp. oocysts per gram of faeces - EIM). The genetic correlations were obtained through multiple trait models. A total of 21,667 SNP markers were used to perform single-step GWAS (Genome Wide Association Studies), and to identify genomic windows that explained at least 1% of the genetic variance for the evaluated traits. The genetic correlations were positive and of moderate magnitude for the pairs of traits BW-ADG (0.64), BW-SC (0.38), BW-TICK (0.39), ADG-SC (0.27), and TICK-EIM (0.33). Only the pair ADG-EIM presented a negative correlation (-0.22). For all the other pairs, the genetic correlations were close to zero. The effects of the SNPs were calculated as genetic standard deviation and showed that there were SNPs mapped in FCG that promoted genetic improvement to both traits. Additionally, functional analyses were performed and FCGs were selected based on their roles in biological processes for each trait. The functional analyses selected seven genes, SLC16A4, KCNA2, LAMTOR5, DUSP10, MAP3K1, TPMT, and KIF13A as FCGs with effects over more than one trait. Independently of the genetic correlation (low-moderate) there are FCG that can influence both production, reproduction or resistance traits in beef cattle. In the second paper, "Candidate genes for longitudinal traits under selection in beef cattle", we

aim to identify functional candidate genes which take part in genetic control of body weight in five different ageS (330, 385, 440, 495 and 550 days) for a beef cattle population for two databases which one with complete body weight records (DB100) and another one which a sequential selection of 70% of heaviest animals (DB70). The genetic parameters for body weight were estimated by a single trait (STM) and a random regression model with spline functions (RRM) for both databases. Body weights were standardized at 330, 385, 440, 495 and 550 days of age for STM. In RRM, the knots of linear splines were fitted at 274, 330, 385, 440, 495, 550 and 594 days of age. The GWAS were performed with both STM and RRM. The GWAS and the functional enrichment were performed as previous described from the first paper. We identified seven FCG (DUSP10, LAMTOR5, PAFAH2, SLC30A2, TRIM63, NCAM1 and SCL16A4) to body weight in different ages. of each gene for animal growth can change in different development stages and different The DUSP10 gene was associated with body weight in all the five ages evaluated, appointing for the relevance of this gene for different stages of the animal growth. On the other hand, the majority of the FCG associated with body weight were different for different ages suggesting that the importance genes can be more relevant to body weight in each growth stage. When the sequential selection was simulated different FCG were associated with body weight in DB100 and DB70 for each age, even when the RRM was performed. Also, when GWAS and post GWAS are performed, the sequential selection influenced the results and this may be one more reason for frequent inconsistences in GWAS results performed for growth traits measured in beef cattle. We suggested genes as FCG which take part in the genetic control of important traits to beef cattle. Therefore, these genes could be validated in largest populations and different breeds, in order to improve the understanding about the genetic control of them over the traits here evaluated.

Key words: correlation, genetic parameters, genome wide association study, Nellore, sequential selection

1. INTRODUÇÃO GERAL

No melhoramento genético animal, informações sobre variações nas sequências de DNA entre os animais têm sido utilizadas com maior frequência para estimação de valores genômicos (GEBVs) quando comparada aos estudos dedicados a descoberta de genes e vias (Goddard e Hayes 2009). Entretanto, a identificação de genes que contribuem para o controle genético de características de importância econômica é igualmente importante, uma vez que possibilita a seleção de animais portadores de alelos desejáveis em genes que tem, de fato, associação com a característica sob avaliação (Ayuso et al., 2016). Estes genes podem ser, por definição, genes candidatos funcionais (FCG), por estarem envolvidos em vias metabólicas de interesse e codificar uma proteína relacionada com o fenótipo em questão (Tizioto, 2014).

Um passo além da identificação de FCG que contribuem para o controle genético de uma única característica é a identificação de genes que participam do controle genético de pelo menos duas características geneticamente correlacionadas. A correlação genética entre diferentes características tem sido quantificada por modelos que permitem particionar a correlação entre fenótipos em correlações genéticas e residuais (Searle, 1961; Wright, 1968). Os valores de correlações genéticas entre características importantes de bovinos de corte são comumente utilizados em programas de melhoramento animal para a definição de critérios de seleção (Simões et al., 2019). No entanto, pouco se sabe sobre quais genes estão subjacentes à correlação genética entre essas características. Neste sentido, identificar genes candidatos funcionais comuns entre as características geneticamente correlacionadas permite que SNPs (single nucleotide polimorphism) associados a genes possivelmente pleiotrópicos sejam ponderados no momento da avaliação genômica de modo a favorecer o ganho genético obtido para ambas as

características. Além disso, identificar FCG subjacentes a correlação genética pode ser um primeiro passo para a sugestão de genes importantes que participam do controle genético de pelo menos duas caraterísticas associadas a crescimento, reprodução e sanidade em bovinos de corte e que podem ser melhor investigados em estudos futuros.

Características de crescimento em bovinos de corte (ex: peso em diferentes idades) têm sido amplamente utilizadas nos estudos de associação ampla do genoma (GWAS - Genome Wide Association Study - Campos et al., 2019; Zhang et al., 2012). Uma vez que essas características podem ser medidas em diferentes estágios da vida do animal, é necessário considerar a complexidade de mecanismos metabólicos e fisiológicos envolvidos no crescimento (Owens et al., 1995). Por isso, do ponto de vista genético, é possível que a mesma característica medida em diferentes estágios do crescimento seja controlada por diferentes conjuntos de genes, como apontado por Campos et al. (2019). Esses autores encontraram janelas genômicas diferentes quando o peso de bovinos Hereford e Braford foi avaliado em diferentes idades (peso ao nascer, peso ao desmame ajustado para 205 dias, peso ao sobreano ajustado para 550 dias de idade e ganho de peso pós-desmame ajustado para 345 dias de idade).

Outro aspecto que deve ser considerado no momento da avaliação de características de crescimento em bovinos de corte é que nem todos os animais que possuem registro de peso em idades mais jovens serão novamente medidos em idades mais avançadas. Isso acontece porque, com intuito de obter-se recursos financeiros para a operação ou para oferecer melhores condições ambientais para os demais candidatos à seleção, animais são descartados e consequentemente o número de registros é reduzido o longo do processo de crescimento (Toral et al., 2019). Esses pré-descartes resultam também em pré seleção dos animais que permaneceram no rebanho e que serão medidos para outras características em idades posteriores (seleção sequencial).

A seleção pode ter efeito sobre parâmetros e valores genéticos estimados por meio de informações de fenótipo e genealogia (Kaps et al., 1999; Long et al., 1991; Schaeffer et al.,1997). Por isso, modelos que consideram uma estrutura de covariância entre efeitos aleatórios, como modelos multicaracterísticos ou modelos de regressão aleatória (RRM), foram sugeridos como uma boa alternativa com o propósito de minimizar os efeitos da seleção sobre a estimativa de parâmetros genéticos (Boligon et al., 2009, Toral et al., 2019). A utilização destes modelos pelos programas de melhoramento de bovinos de corte é extremamente pertinente, dado que a pré seleção é uma realidade dentro dos rebanhos comerciais. No entanto, os efeitos de seleção sobre a identificação de regiões genômicas associadas a características de interesse, e até mesmo sobre a identificação de FCGs que contribuem para o controle genético dessas características, ainda não foram objetos de estudos.

Assim, nesta tese foram desenvolvidos dois trabalhos, ambos, com objetivo geral de identificar FCG associados à características economicamente importantes em bovinos da raça Nelore. No primeiro trabalho, "Genes underlying genetic correlations between growth, reproduction and parasite burden traits in beef cattle", estimamos os valores de correlação genética entre características de crescimento, reprodução e resistência e identificamos FCG que participam do controle genético de mais de uma característica e que podem contribuir para a correlação genética aditiva. No segundo trabalho, "Candidate genes for longitudinal traits under selection in beef cattle", identificamos FCG para peso corporal em cinco idades (330, 385, 440, 495 e 550 dias) e posteriormente simulamos um processo de seleção sequencial entre as idades a fim de avaliar os efeitos da seleção sobre resultados de GWAS e pós-GWAS.

2. REVISÃO DE LITERATURA

2.1. Características de crescimento, reprodução e resistência em bovinos de corte

Características de crescimento como peso e ganho de peso recebem maior atenção durante o processo de seleção de bovinos de corte, pois estão diretamente associadas ao principal produto de venda, a carne (Van Melis et al., 2010; Santana Jr et al., 2012). Essas características são facilmente mensuradas, favoravelmente correlacionadas com outras características de interesse econômico e respondem à seleção individual (Boligon et al., 2010), pois apresentam herdabilidade de média magnitude (Retallick et al., 2017).

A circunferência escrotal está incluída como critério de seleção na grande maioria dos programas de melhoramento genético como indicador da idade a puberdade em machos e fêmeas, bem como fertilidade em fêmeas (Eler et al., 2006; Van Melis et al., 2010; Santana Jr et al., 2012; Loaiza-Echeverri et al., 2013). A circunferência escrotal possui alta herdabilidade e uma relação favorável com outras características reprodutivas, como idade ao primeiro parto e taxa de prenhez precoce (Eler et al., 2006).

Além de características de crescimento e reprodução, características relacionadas a sanidade animal têm ganhado cada vez mais atenção em programas de melhoramento genético de bovinos de corte (Passafaro et al., 2015; Sollero et al., 2017; Mota et al., 2018), uma vez que a infecção por parasitos promove perdas econômicas representativas por causa da perda de peso, mudanças comportamentais, infecções secundárias de pele e transmissão de patógenos (Léger et al., 2013). Características como infecção por endo e ecto parasitos têm sido avaliadas com intuito de verificar se a infecção por parasitos pode, de fato, causar danos ao desempenho produtivo e reprodutivo dos animais (Biegelmeyer et al., 2015), dado que, especialmente no Brasil, em virtude da predominância do clima

tropical a proliferação de espécies parasitárias é favorecida. Apesar das infecções por parasitos provocarem perdas produtivas significativas, a inclusão dessas caraterísticas como critérios de seleção nos principais programas de melhoramento genético ainda é modesta. Por isso, se faz necessário o estudo mais aprofundado das características associadas a infecção por parasitos, bem como da inter-relação delas com as demais características de crescimento e reprodução a fim de verificar sua relevância dentro dos programas de melhoramento genético animal de bovinos de corte.

Nos últimos anos, abordagens que fazem uso da informação genômica para otimizar o melhoramento genético animal têm sido empregadas com sucesso como, por exemplo, a seleção genômica, o GWAS e mais recentemente a identificação de genes que participam do controle genético das características. Neste sentido, a incorporação de informações genômicas nos programas de melhoramento genético animal pode auxiliar, também, na elucidação do controle genético das caraterísticas individualmente, bem como sobre as inter-relações gênicas entre caraterísticas de produção, reprodução e resistência.

2.2. Inclusão de dados genômicos nos programas de avaliação genética

Na década de 1970, o desenvolvimento de técnicas de genética molecular forneceu novas oportunidades para aperfeiçoar os programas de melhoramento genético de bovinos, permitindo o uso de marcadores de DNA para identificar genes ou regiões genômicas que controlam características de interesse (Dekkers, 2012). Em seguida, o desenvolvimento de tecnologias para o sequenciamento do genoma possibilitou a identificação de SNPs.

Os SNPs são gerados na replicação do DNA por mutação espontânea ou induzida, constituem a classe mais abundante de sítios polimórficos em qualquer genoma (The 1000 Genomes Project Consortium, 2010), são amplamente distribuídos e, de modo geral, podem estar presentes em todos os loci gênicos (Caetano et al., 2009). As vantagens do uso desses marcadores são, a baixa taxa de mutação e a relativa facilidade e custo para genotipagem, quando comparados a outros marcadores. A elaboração de chips de SNPs permitiu a genotipagem simultânea para milhares de marcadores, disponibilizando maior volume de informações para um único animal (Caetano, 2009) e, assim, os marcadores moleculares do tipo SNP passaram a ser utilizados, também, para GWAS.

Estudos de associação foram inicialmente realizados com o objetivo detectar variantes em locos genômicos que estavam relacionadas a características complexas na população e, em particular, na detecção de associações entre SNPs e doenças comuns em humanos como as cardíacas, diabetes, doenças autoimunes e transtornos psiquiátricos (Visscher et al., 2012). Posteriormente, com aumento do número de diferentes espécies que tiveram seu genoma sequenciado, os GWAS foram incorporados à diferentes espécies com intuito de mapear QTLs associados a expressão de características de importância econômica ou com a regulação de alguma rota metabólica (Bush e Moore, 2012).

O sequenciamento do genoma bovino, realizado pelo consórcio The Bovine Genome Sequencing and Analysis Consortium (2009), além de permitir a implementação de GWAS para características economicamente importantes, sugeriu que, o genoma bovino é composto por, pelo menos, 22 mil genes, apresenta altos níveis de conservação em sua estrutura, quando comparado ao genoma humano, e os genes envolvidos no metabolismo são, de modo geral, altamente conservados. Apesar de apenas uma proporção muito pequena dos marcadores (SNPs) estar localizada dentro dos genes ou sequências reguladoras, associações populacionais entre os alelos do SNP e os alelos de mutações que afetam características de interesse econômico podem ser descobertas devido ao desequilíbrio de ligação entre o marcador e o QTL (Hayes e Goddard, 2009). O desequilíbrio de ligação é, por definição, a associação não-aleatória de alelos em dois ou mais loci, não necessariamente no mesmo cromossomo (Falconer e Mackay, 1996).

De modo geral, os resultados de GWAS para bovinos são referentes, principalmente, a características de crescimento e reprodução. Para o ganho médio diário, medido em bovinos da raça Nelore, Santana Jr et al. (2014) verificaram que os dez SNPs estatisticamente mais significativos que foram associados a esta característica estavam localizados nos cromossomos 3, 6 e 10. Ainda, o SNP mais significativo estava localizado no cromossomo 3 e apresentou um efeito médio de substituição estimado de -0,27kg/dia sendo que o alelo favorável foi o T e o alelo C foi aquele que provocou efeito negativo sobre o ganho médio diário. Soares et al. (2017) realizaram um GWAS multicaracterístico para circunferência escrotal em diferentes idades de bovinos da raça Brahman e encontraram uma sobreposição de janelas genômicas no cromossomo 14 que explicou em torno de 0,8% da variação para circunferência escrotal aos 12, 18 e 24 meses de idade dos animais.

Além dos GWAS para características relacionadas ao crescimento e à reprodução, GWAS para características vinculadas à sanidade animal, como por exemplo, contagem de parasitos, também têm sido desenvolvidos. Em um GWAS para infecção por carrapatos em bovinos leiteiros foram encontraram 25 SNPs estatisticamente associados à infecção por carrapatos, distribuídos nos cromossomos 1, 2, 4, 6, 7, 8, 10, 11, 13, 14, 19, 20 e 26 (Turner et al.; 2010). Ainda, os autores verificaram que o efeito médio de substituição para 17 dos 25 SNPs significativos é negativo e positivo para os 8 demais. Mota et al. (2016) encontraram SNPs estatisticamente associados a resistência a carrapatos em bovinos das raças Hereford e Braford em 17 cromossomos e verificaram que houve interação entre os SNPs significativos quando avaliados em altas e baixas cargas parasitárias indicando que a expressão genética da resistência depende do nível de infecção parasitária.

Os GWAS têm contribuído, cada vez mais, para elucidação de fatores que associam determinada região do genoma à expressão de um caráter. Desta maneira, os resultados destes estudos podem ser utilizados como ferramenta auxiliar no momento da seleção e contribuir para maior efetividade dos programas de melhoramento genético de bovinos de corte. É importante destacar que os GWAS devem ir muito além da identificação de regiões do genoma que estão associadas à característica de interesse. É necessário identificar os genes que estão localizados dentro e nas adjacências das regiões associadas à característica sob estudo e, se possível, identificar as rotas metabólicas que justificam a influência deste gene sobre a expressão do fenótipo a fim de acrescentar informações sobre o controle genético da característica.

2.3. Genes candidatos no melhoramento genético animal

A maioria das características de interesse econômico em bovinos são quantitativas e geralmente com arquiteturas genéticas complexas (Tang et al., 2019). Até pouco tempo atrás, a seleção dos animais para tais características era baseada na obtenção e avaliação de parâmetros genéticos estimados basicamente por meio de informações de fenótipo e parentesco. Isso foi bem-sucedido, mas o processo é lento se a característica for mensurável em apenas em um sexo, após a morte ou no final da vida ou se o custo de mensuração for elevado (Goddard e Hayes, 2009).

A rápida evolução e popularização das tecnologias relacionadas à genética molecular proporcionou o desenvolvimento de diversas abordagens aplicadas ao melhoramento genético animal, dentre elas a seleção genômica, proposta inicialmente por Mewissen et al. (2001) e os GWAS (Hayes et al., 2009; Bolormaa et al., 2010). Os benefícios promovidos pela seleção genômica, como por exemplo, a redução no intervalo de gerações, aumento da intensidade de seleção e da acurácia dos valores genômicos estimados (Goddard e Hayes, 2007) tem contribuído para a maior efetividade dos programas de melhoramento genético animal.

Para que o melhoramento genético de característica complexas em animais domésticos seja mais eficiente, além da seleção genômica, seria vantajoso identificar genes que contribuem para o controle genético dessas características e selecionar animais portadores dos alelos desejáveis (Meuwissen e Goddard 1996, Ayuso et al., 2016). Assim, a abordagem de genes candidatos passou a ser aplicada aos estudos de várias características em várias espécies, na tentativa de elucidar sua base genética (Brown et al., 2013).

Um gene candidato é qualquer gene que tenha sido identificado como variação subjacente em um fenótipo específico em um organismo e, portanto, pode influenciar um

fenótipo semelhante em outro organismo (Fitzpatrick et al., 2005). Genes podem ser candidatos posicionais, quando estão localizados em uma região cromossômica associada com a característica de interesse e/ou candidatos funcionais, quando estão envolvidos na via metabólica de interesse e codificam uma proteína que está relacionada com a característica fenotípica em estudo (Tizioto, 2014).

O acesso à informação (bases públicas) de genes ligados a diversas funções em combinação com os métodos de priorização baseados em redes, possibilita caracterizar e associar determinados genes a um fenótipo de interesse. Estão, atualmente, disponíveis pacotes e servidores que atribuem índices de probabilidade para genes indicados por determinada palavra-chave (anotação funcional ou qualquer associação fenotípica) usando dados integrados dos principais repositórios de dados biológicos disponíveis ao público, como por exemplo o BIANA (Aguirre-Plans et al., 2019). Assim, é possível classificar ou priorizar a lista de genes proveniente do GWAS por similaridade da anotação funcional com o conjunto de genes provenientes das bases públicas. As pontuações de similaridade das características, obtidas por *GO terms* (termos de ontologia) individuais são combinadas em uma pontuação geral por meio de uma abordagem multivariada baseada na lógica *fuzzy* (difusa) e um valor p de cada anotação de um gene é obtido por amostragem aleatória de todo o genoma (Chen et al., 2009).

Os estudos com genes candidatos funcionais têm sido aplicados, também, a questões antes pouco elucidadas a nível molecular, como por exemplo, a pleiotropia, previamente definida como o fenômeno em que um único gene controla mais de uma característica (Wright, 1968; He e Zhang, 2006; Paaby e Rockman, 2013). Dado que a pleiotropia é uma causa permanente da correlação genética entre características (Falconer e Mackay,1996), genes candidatos funcionais poderiam ser sugeridos como pleiotrópicos quando identificados para duas características geneticamente correlacionadas.

Uma associação significativa entre genótipos de cada um dos genes CAST (cromossomo 29) e CAPN4751 (cromossomo 7) para as características força de cisalhamento e índice de fragmentação miofibrilar em bovinos da raça Nelore foi verificada por Curi et al. (2009). Para o gene CAST o genótipo AA foi favorável em relação ao AG e conferiu uma redução de -0.42kgf/cm² para força de cisalhamento e um incremento de 7,83 para o índice de fragmentação miofibrilar. Já para o gene CAPN4751 o genótipo CT heterozigoto foi favorável em relação ao TT homozigoto sendo que a substituição do alelo C por T provocou redução de -0.38kgf/cm² para força de cisalhamento e um incremento de 8,06 no índice de fragmentação miofibrilar. Os autores, sugeriram, portanto, a possibilidade de efeitos gênicos pleiotrópicos e, ou, desequilíbrio de ligação entre marcadores e polimorfismos funcionais em genes próximos.

É importante ressaltar que considerar o estágio do desenvolvimento animal para determinadas características é de fundamental relevância para a consistência dos genes apontados como candidatos para uma mesma característica em uma mesma espécie. Características associadas ao crescimento, como por exemplo, o peso medido em diferentes idades, são influenciadas por uma complexidade de mecanismos metabólicos e fisiológicos (Owens et al., 1995). Por isso, é razoável que genes candidatos associados ao peso de bovinos não sejam os mesmos quando a característica é avaliada e diferentes idades (Campos et al.; 2019). Outro aspecto que deve ser considerado quando genes candidatos são sugeridos para características de crescimento em bovinos é o efeito da seleção sobre as estimativas dos componentes de variância e a precisão das predições dos valores genéticos (Kaps et al., 1999; Long et al., 1991; Schaeffer et al., 1997, Toral et al., 2019), que pode refletir, também, sobre os genes candidatos associados a característica. Neste caso, alguns genes poderiam ser sugeridos não pela real associação com o fenótipo, mas sim pelos efeitos da seleção sobre a característica.

A aplicação do conhecimento de genes candidatos no melhoramento genético animal tem se tornado cada vez mais eficiente. Atualmente, alguns sumários das avaliações genéticas de touros trazem informação de alelos desejáveis em genes importantes, por exemplo, genes relacionados à proteína (Kappa-caseína, Beta-caseína e β-lactoglobulina) e percentual de gordura no leite (diacilglicerol O-aciltransferase 1-DGAT1) e às doenças hereditárias (Deficiência de Adesão Leucocitária Bovina- BLAD, Deficiência da Uridina Monofosfato Sintase- DUMPS e Complexo de Má Formação Vertebral - Silva et al., 2019).

É importante ressaltar que a seleção ao nível de alelos favoráveis em genes específicos requer extenso estudo e validação. Após validação, o uso desses genes pode levar ao aumento do ganho genético uma vez que permite a seleção direcionada de animais portadores dos alelos desejáveis em genes que, de fato, participam do controle de um fenótipo importante. Esse incremento no ganho genético ocorre pela possibilidade de seleção dos animais, antes mesmo que estes expressem o fenótipo, o que leva a redução do intervalo de geração, além do aumento da intensidade de seleção e da acurácia dos valores genômicos.

3. GENES UNDERLYING GENETIC CORRELATIONS BETWEEN GROWTH, REPRODUCTION AND PARASITE BURDEN TRAITS IN BEEF CATTLE

3.1. Abstract

Genetic correlation is the outcome of linkage disequilibrium and/or pleiotropic genes. As such, identifying which genes take part in the genetic control of genetically correlated traits can help us better understand the relationship between economic traits and promote more efficient breeding programs. We aim to estimate the genetic correlations between growth, reproduction and parasite burden traits and to identify functional candidate genes (FCG) underlying these correlations. Six traits were evaluated, comprising two of growth (body weight - BW and average daily gain - ADG), one reproductive trait (scrotal circumference - SC) and three related to parasite burden (tick count - TICK, gastrointestinal nematode eggs per gram of feaces - GIN, and *Eimeria* spp. oocysts per gram of faeces - EIM). The genetic correlations were estimated using a multiple-trait model. A total of 21,667 SNP markers were used to perform a single-step GWAS and to identify genomic windows explaining at least 1% of the genetic variance for the studied traits. The posterior means and highest posterior density intervals of the genetic correlations were positive and of moderate magnitudes for the pairs of traits BW-ADG (0.64; 0.52, 0.76), BW-SC (0.38; 0.26, 0.50), BW-TICK (0.39; 0.25, 0.76), ADG-SC (0.27; 0.11, 0.43), and TICK-EIM (0.33; 0.12, 0.53). Only the pair ADG-EIM presented

Artigo publicado em 6 Novembro de 2020:
RIBEIRO, V.M.P; GOUVEIA, G.C.; MORAES, M.M; ARAÚJO, A.E.M.; RAIDAN, F.S.S.; FONSECA, P.A.S.; CARDOSO, E.P.; SILVA, M.V.G.B.; TORAL, F.L.B. Genes underlying genetic correlation between growth, reproductive and parasite burden traits in beef cattle. Livestock Science, v. 242, p. 104332, 2020. https://doi.org/10.1016/j.livsci.2020.104332

a negative correlation (-0.22; -0.39, -0.05). All the other pairs showed genetic correlations close to zero. Additionally, functional analyses were performed and FCGs were selected based on their roles in biological processes for each of the traits. The effects of the SNPs were calculated as a proportion of the genetic standard deviation. Seven FCGs (SLC16A4, KCNA2, LAMTOR5, DUSP10, MAP3K1, TPMT, and KIF13A) were identified for more than one trait. Regardless of the genetic correlation values (low to moderate), there were FCGs which could influence both correlated traits. There were SNPs mapped in FCGs that might be used to promote genetic improvement in multiple traits. There are common FCGs that might control production, reproduction and parasite burden traits in beef cattle and contribute to genetic correlation values.

Key words: average daily gain, body weight, genome-wide association, Nellore, parasite, pleiotropy

3.2. Introduction

Growth and reproduction traits are, in general, genetically correlated (Abreu et al., 2018; Boligon et al., 2010; Kluska et al., 2018) and are frequently used as selection criteria in beef cattle breeding programs. On the other hand, even though the genetic correlation between growth and parasite burden traits is known to be close to zero (Biegelmeyer et al., 2015), to include the latter in a selection index would be a good alternative, since the decrease in parasite burden can lead to better conditions for cattle growth (Simões et al., 2019).

The genetic correlation between different traits can be quantified by models which allow for partitioning the correlation between phenotypes into genetic and residual correlations (Searle, 1961; Wright, 1968). The values of genetic correlations between important livestock traits have been widely used in animal breeding programs for the definition of selection criteria (Simões et al., 2019). However, little is known about which genes are underlying genetic correlation.

Identifying genes which take part in the genetic control of complex traits for domestic animals would be advantageous to animal breeding programs, since animals which carry desirable alleles could hence be selected (Meuwissen and Goddard 1996, Ayuso et al., 2016). In addition, in genomic selection, higher SNP weights could be considered for those SNPs mapped close to or in FCGs underlying each specific trait or two correlated traits. Therefore, we aim to estimate the genetic correlations between growth, reproductive and parasite burden traits and to identify common functional candidate genes across the evaluated traits.

3.3. Materials and Methods

Field management description

Phenotype data of Nellore bulls born between 2001 and 2016 were used in this study. The bull calves were raised in pasture with the predominance of Urochloa genus grass, and the stocking rate on the pastures was of approximately 0.98 AU/ha. Mineral supplementation was provided ad libitum over the year. During the cow-calf phase, calves were kept with their dams on 30-hectare pastures and were weaned at approximately 205 days of age. At weaning, management groups with 45 bulls on average were assembled and kept on the same pasture under the same rearing conditions.

The experimental data were collected during performance tests at pasture, commonly starting in August and finishing in July of the following year. These performance tests lasted 294 days, comprising 70 days of adaptation and 224 days of test. During the performance test, all bulls from a single management group were kept under the same management conditions. The bulls were weighted at the beginning and the end of the adaptation period (70th trial day), which effectively corresponds to the beginning of the trial, but follow-up weighings were conducted every 56 days until the end of the test (test days 0, 70, 126, 182, 238 and 294). The data collected at the beginning of the test (day 0) were not taken into consideration. During the performance test at pasture, the management groups were rotated among different paddocks in order to offer similar rearing conditions for all individuals from the same birth season. A thorough description of the farm can be found in Passafaro et al. (2015).

Performance traits

Two growth traits were considered for analyses, average daily gain (ADG) and body weight (BW), as well as a reproductive trait, scrotal circumference (SC) (Table 1).

Table 3.1. Summary statistics for body weight at 550 days of age, average daily gain, scrotal circumference at 550 days of age and tick, gastrointestinal nematode and *Eimeria* spp. counts for each evaluated period

Periods	N° MG[1]	N° of records	Minimum	Mean	Maximum	Sd[2]
			Body weight (Kg)			
	121	4979	162.00	320.35	456.63	39.07
			Average Daily Gain (Kg/day)			
	121	4979	-0.11	0.53	1.35	0.14
			Scrotal circumference (cm)			
	122	4714	15.50	26.00	38.00	3.17
			Tick			
First	48	1538	0	5.32	80	6.65
Second.	40	1209	0	9.09	131	11.31
Third	48	1542	0	5.34	63	7.36
Fourth	44	1453	0	6.22	80	8.20
Fifth	48	1545	0	6.49	72	8.72
			Gastrointestinal Nematodes			
First	48	1538	0	3.99	153	9.56
Second.	40	1209	0	4.50	255	11.30
Third	48	1542	0	3.39	284	13.18
Fourth	44	1453	0	3.62	182	12.80
Fifth	48	1545	0	3.29	328	13.93
			Eimeria spp.			
First	48	1538	0	4.70	80	6.80
Second.	40	1209	0	4.90	43	6.30
Third	48	1542	0	5.76	80	7.63
Fourth	44	1453	0	5.10	71	6.25
Fifth	48	1545	0	4.21	73	6.20

[1]Nº MG = number of management groups; [2]Sd = standard deviation

The average daily gain was obtained through the equation:

$$ADG = (BW_{End} - BW_{Start})/(Age_{End} - Age_{Start})$$

in which: BW_{End}, represents the weight obtained at the final weighing (test day 294); BW_{Start} the animal weight obtained at the initial weighing (test day 70); Age_{End}, the age at the end of the test, and Age_{Start}, the age at the beginning of the test.

The evaluated weight was the one corresponding to the final weighing (trial day 294) in which animals were 550 days old on average. Because age differences up to 90 days are allowed in performance tests, the weight obtained on the day 294 was standardized for 550 days through the equation:

$$BW_{550} = BW_{end} - ADG(Age_{end} - 550),$$

in which: BW_{550} represents the standardized weight for 550 days old, and the remaining terms were as previously described in equation. The scrotal circumference considered for analyses was measured at the final weighing (weighing day 294).

Parasite burden traits

Data of tick infestation (TICK), as well as gastrointestinal nematodes (GIN) and *Eimeria* spp. (EIM) infection (Table 1) were collected between 2010 and 2017 during performance tests at pasture as previously described. All data collected between the beginning (day 70) and the end of the trial (day 294) were used, with a total of up to five counts per animal, performed every 56 days within the test period.

Since the data used in the present study came from a farm with commercial purposes, parasite infestations occurred naturally, and our results were obtained based on the actual biological variability of the traits in pasture-raised cattle. In addition, approximately 65% of the bulls in the study were dewormed at weaning and at the beginning of the performance test. This procedure was always performed either in all individuals from the same management group or in none, using ivermectin at 4% (Master LP, Ouro Fino Saúde Animal, Cravinhos, SP) in a dose of 1 ml/50kg of body weight. No

preferential treatments were applied for defining the groups to be dewormed. In order to keep the parasitic load at non harmful levels for the bulls' performance, management groups were rotated among different paddocks.

Tick infestations (*Rhipicephalus microplus*) were evaluated following the method described by Wharton and Utech (1970), so that only counts of ingurgitated female ticks were registered (> 4.5 mm in length). Also, we must highlight that in the present study, only ticks on the whole right side of the animal were counted (Table 1). For GIN and EIM traits, samples of faeces were collected straight from the rectum of the bulls by using lubricated and identified plastic bags. The collected material was kept cold up to the moment of its laboratorial analysis, which was performed in the Laboratory of Parasitic Diseases of the Federal University of Minas Gerais (UFMG). This analysis consisted in counting GIN eggs and EIM oocysts following the modified Mac Master method (Ueno and Gonçalves, 1998). To perform the counts, 2g of faeces were diluted with 28 ml of drinking water. Then, after sifting the mixture, a 2 ml aliquot was mixed with 2 ml of saturated Sheater's solution (500 g of sugar, 6.5 ml of phenol and 360 ml of water). The McMaster chamber, consisting of two slides separated by a 1.5 mm space, between which there are two count chambers of 1 cm^2 (Castilho et al., 1984), was filled with a 0.15 ml aliquot of the final solution and thereafter the egg and oocyst counts were performed using a light microscope at a 10 x magnification.

All procedures and data collections were approved by The Ethics and Animal Experimentation Committee of the Universidade Federal de Minas Gerais (Protocol 255/2010).

Genotypic database

During the performance tests at pasture, samples of hair, blood or semen were collected for DNA extraction. A total of 1,230 bulls were genotyped through a low-density panel (Z-chip) with approximately 30 thousand SNP markers. A Z-chip v2 (Neogen, Lincoln, Nebraska, EUA) was especially built by its subsidiary Deoxi (Araçatuba, SP, Brazil) for the molecular genotyping of the Zebu cattle.

Quality control of samples and markers was implemented through R/ SNPStats statistical package (Clayton, 2020). In the quality control of samples, those with call rate lower than 0.90, as well as duplicated records (correlation between samples > 0.95), were excluded. In the quality control of markers, only SNPs mapped in autosomal and X chromosomes, which presented GenCall (GC score) > 0.6, call rate > 0.95 and minor allele frequency (MAF) > 0.05, were considered. After editing, a total of 21,667 SNP markers (77.12%) and 1,075 samples (88.04%) were considered for analyses.

Variance components

In order to obtain the covariance estimates for BW, ADG, SC, TICK, GIN and EIM traits, they were grouped two by two and used as response variables for analyses through a two-trait animal model. Preliminary analyses were conducted in order to ascertain which fixed effects should be included in the model for each trait. For BW, ADG, TICK, GIN and EIM, the management group was included as a fixed effect. Within each management group, the age range did not exceed 96 days and only groups consisting of at least 7 bulls which were offspring of at least 3 distinct bulls were considered as having valid records. For SC, in addition to management group, the bulls' age was also included as a covariable. For the parasite burden traits, in addition to the random additive and residual genetic effects, which were obtained too for the performance traits, the

permanent environmental effect was also estimated since each bull presented up to five repeated measurements taken over the performance test at pasture. Moreover, bulls presenting at least one count for the three parasites at the same moment (TICK, GIN, EIM) were considered.

Because TICK, GIN and EIM traits do not have a normal distribution, a logarithmic transformation was performed:

$$Trait = \left(\log_{10}(Trait + 1.001)\right),$$

and its result was used as a response variable (Ayres et al., 2013). The constant 1.001 was included since some counts were equal to zero. Coefficients of asymmetry (-0.3-TICK; 0.94-GIN and -0.19-EIM) and kurtosis (-1.0- TICK; -0.39- GIN and -1.1- EIM) were obtained and the normality of the residuals of the adjusted models was assumed (Ibelli et al., 2012; Mota et al., 2016).

The multiple-trait model can be described in a matrix notation as:

$$\begin{bmatrix} y_h \\ \underset{\sim}{y_{h'}} \end{bmatrix} = \begin{bmatrix} X_h & \Phi \\ \Phi & X_{h'} \end{bmatrix} \begin{bmatrix} \beta_h \\ \underset{\sim}{\beta_{h'}} \end{bmatrix} + \begin{bmatrix} Z_h & \Phi \\ \Phi & Z_{h'} \end{bmatrix} \begin{bmatrix} a_h \\ \underset{\sim}{a_{h'}} \end{bmatrix} + \begin{bmatrix} W_h & \Phi \\ \Phi & W_{h'} \end{bmatrix} \begin{bmatrix} p_h \\ \underset{\sim}{p_{h'}} \end{bmatrix} + \begin{bmatrix} e_h \\ \underset{\sim}{e_{h'}} \end{bmatrix}$$

in which: h and h' represent the different traits analyzed in the multiple-trait model; $\underset{\sim}{y}$ represents the vector of observations of the trait (BW, ADG, SC, TICK, EIM, GIN); X, the incidence matrix of the systematic effects (management group for all traits and ages for SC); Φ, a null matrix; $\underset{\sim}{\beta}$, the vector of solutions for the fixed effects; Z, the incidence matrix of the direct additive genetic effects; $\underset{\sim}{a}$, the vector of solutions for the direct additive genetic effects; W, the incidence matrix of the permanent environmental effects, $\underset{\sim}{p}$, the solution vector for the permanent environmental effects (only for parasite burden traits); and $\underset{\sim}{e}$, the vector of errors.

The following presuppositions were taken on for the effects included in the MULT model: $\begin{bmatrix}\beta_h & \beta_{h'}\\ \sim & \sim\end{bmatrix}^t$ ~constant; $\begin{bmatrix}a_h & a_{h'}\\ \sim & \sim\end{bmatrix}^t$ |A,G_0 ~N($[0 \quad 0]^t$,$G_0 \otimes A$), $\begin{bmatrix}p_h & p_{h'}\\ \sim & \sim\end{bmatrix}^t$|$P_0$~N($[0 \quad 0]^t$,$P_0 \otimes I$) and $\begin{bmatrix}e_h & e_{h'}\\ \sim & \sim\end{bmatrix}^t$|$R_0$~N($[0 \quad 0]^t$,$R_0 \otimes I$), in which A represents the additive genetic relationship matrix between animals (10.541); G_0, the matrix of additive genetic covariances between traits so that $G_0 = \begin{bmatrix}\sigma^2_{a_{11}} & \sigma^2_{a_{12}}\\ \sigma^2_{a_{21}} & \sigma^2_{a_{22}}\end{bmatrix}$; N refers to normal distribution; $\otimes$ is the direct product operator between matrices; P_0 is the matrix of permanent environmental for traits $P_0 = \begin{bmatrix}\sigma^2_{P_{11}} & \sigma_{P_{12}}\\ \sigma_{P_{21}} & \sigma^2_{P_{12}}\end{bmatrix}$, considering that just the parasite burden traits had permanent environmental effects adjusted for those; R_0 is the matrix of residual variances for traits so that $R_0 = \begin{bmatrix}\sigma^2_{e_{11}} & \sigma_{e_{12}}\\ \sigma_{e_{21}} & \sigma^2_{a_{12}}\end{bmatrix}$; and I is an identity matrix of order equal to the number of observations.

Inverted Wishart distributions were assumed for the covariance matrices (2 x 2) $G_0\left(_{G_0\sim IW(\Sigma^2_a,n_a)}\right)$, $P_0\left(_{P_0\sim IW(\Sigma^2_p,n_p)}\right)$ and $R_0\left(_{R_0\sim IW(\Sigma^2_e,n_e)}\right)$, where: Σ^2_a, Σ^2_p, Σ^2_e , n_a, and n_e represent the inverted Wishart distribution's hyperparameters. Information on full conditional posterior distributions was previously published by Sorensen and Gianola (2002).

Samples with full conditional posterior distributions were obtained through the Gibbs sampler using the software GIBBS3F90 (Misztal et al., 2014). Chains of 1100 000 iterations were considered, with a burn-in of 100000 iterations and samplings at every 100 iterations. The chain size was determined in a preliminary analysis following the method described by Raftery and Lewis (1992), available in the BOA package (Smith,

2005). The convergence of chains was evaluated through the criterion proposed by Geweke (1991) available in the same package, through inspection of the sampled values.

Genome-wide Association Study (GWAS)

The single-trait model was applied for each trait in analyses performed with only phenotype and pedigree information. Afterwards, the variance components obtained in these analyses were fixed and the genomic breeding values and the SNP solutions were obtained through the single-step genomic BLUP method (ssGBLUP- (Aguilar et al., 2011, 2010; Misztal et al., 2009)) and ssGWAS (genome-wide association study using a single-step BLUP (Vitezica et al., 2011; Wang et al., 2012)). In the single-trait model, we included the same fixed and random effects previously described for the multiple-trait model for each of the traits. They can be described in a matrix notation as:

$$\underset{\sim}{y} = X\underset{\sim}{\beta} + Z\underset{\sim}{a} + W\underset{\sim}{p} + \underset{\sim}{e}$$

where $\underset{\sim}{y}$ represents the vector of observations; X is the incidence matrix of the systematic effects; $\underset{\sim}{\beta}$ is the solution vector for the systematic effects; Z is the incidence matrix of the individual genetic effects; $\underset{\sim}{a}$ is the solution vector for the individual genetic effects; W is the incidence matrix of the individual permanent environmental effects (only for TICK, GIN and EIM traits); $\underset{\sim}{p}$ is the solution vector for the individual permanent environmental effects (only for TICK, GIN and EIM traits); and $\underset{\sim}{e}$ is the vector of errors.

Flat-type a priori distributions were assumed for $\underset{\sim}{\beta}\left(\underset{\sim}{\beta}\sim\text{constant}\right)$; normal distributions were assumed for $\underset{\sim}{a}\left(\underset{\sim}{a}\middle|A,\sigma_a^2\sim N\left(0,A\sigma_a^2\right)\right)$, $\underset{\sim}{p}\left(\underset{\sim}{p}\middle|\sigma_p^2\sim N\left(0,I\sigma_p^2\right)\right)$ and $\underset{\sim}{e}\left(\underset{\sim}{e}\middle|\sigma_e^2\sim N\left(0,I\sigma_e^2\right)\right)$; scaled inverse chi-squared distributions were assumed for

σ_a^2 $\left(\sigma_a^2 \sim \chi^{-2}\left(v_a, S_a^2\right)\right)$, σ_p^2 $\left(\sigma_p^2 \sim \chi^{-2}\left(v_p, S_p^2\right)\right)$ and σ_e^2 $\left(\sigma_e^2 \sim \chi^{-2}\left(v_e, S_e^2\right)\right)$; where A is the relationship matrix, I is an identity matrix of order equal to the number of animals with data, I is an identity matrix of order equal to the number of observations, and v_a, v_p, v_e, S_a^2, S_p^2 and S_e^2 are the hyperparameters of the scaled inverse chi-squared distributions. Information on full conditional distributions was previously published by Sorensen and Gianola (2002).

To estimate the genomic breeding values, the following covariance matrix of a, p and e was used:

$$\text{var}\begin{bmatrix} a \\ p \\ e \end{bmatrix} = \begin{bmatrix} H\sigma_a^2 & 0 & 0 \\ 0 & W\sigma_p^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix},$$

where, σ_a^2, σ_p^2 and σ_e^2 are the components of additive genetic, permanent environment and residual variances for each trait, respectively; I and W are identity matrices; and H, the relationship matrix comprising information of genotyped and non-genotyped animals, as described by Aguilar et al. (2010), in which the inverse of H can be described as:

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

where A^{-1} represents the inverse of the additive relationship matrix; A_{22}^{-1}, the inverse of the additive relationship matrix considering only genotyped animals; G^{-1}, the genomic relationship matrix estimated according to VanRaden et al. (2009). Subsequently, the estimated breeding values for genotyped young bulls (GEBV) were converted to SNP effects, and the equation for predicting SNP effects was as described in Wang et al. (2012). The analyses were performed using Gibbs sampling with the software GIBBS3F90 (Misztal et al., 2014). The chain length, discard and sampling were the same as those used in the multiple-trait analyses, as well as the adopted convergence criterion.

Additionally, the genomic breeding values were predicted with BLUPF90 (Misztal et al., 2014) and the effects of SNPs were calculated with POSTGSF90 (Wang et al., 2012). The results of GWAS were described as the proportion of variance explained by genomic windows with approximately 0.5 Mb. In this approach, adjacent SNPs within 0.5Mb were used, and their variance was assumed for obtaining the total variance of the window. Additionally, only non-overlapping windows which explained at least 1% of the additive genetic variance were considered, avoiding a double count.

Identification of positional and functional candidate genes

In order to recover the positional candidate genes inserted within the windows that explained at least 1% of the additive genetic variance, we used the R/GALLO package (Fonseca et al., 2020) considering the latest assembly of the bovine genome ARS-UCD1.2.

The analysis of prioritization of candidate genes was conducted using the softwares GUILDify 2.0 (Aguirre-Plans et al., 2019) and ToppGene (Chen et al., 2009). First, a list of training candidate genes associated with key-words (Table S1) was obtained for each of the six traits by using GUILD framework (Guney and Oliva, 2012) to determine the relevance of known gene products related to the given keywords. The gene products were searched at BIANA knowledge base and used to construct a species-specific network (for the present study we used Homo sapiens as model species). Then, by using a prioritization algorithm based on the network topology for classifying the genes, the top-100 classified genes obtained in this analysis were used to build a list of trained genes.

Further, this list of trained genes was taken to the software ToppGene (Chen et al., 2009) together with the list of positional candidate genes recovered through the

R/GALLO package (Fonseca et al., 2020). The software ToppGene- Gene Prioritization (Chen et al., 2009) was used to perform a prioritization analysis based on annotations, through a multivariate approach based on fuzzy (diffuse) logic. The functional information shared by the list of trained genes and the list of positional candidate genes was used to perform a multivariate analysis. These functional data were recovered from the following sources: terms of gene ontology; molecular function; biological process; cell component; human and mouse phenotypes; metabolic pathways; works in Pubmed;

Transcription factor binding site; coexpression and disease patterns. By using statistics of meta-analysis, p-values were obtained through random sampling of 5000 genes from the whole genome for each annotation information, and then combined into a global p-value. A false discovery rate (FDR) of 5% for multiple correction (p-value $\leq$ 0.05) was considered and the genes with p-values $\leq$ 0.05 were shown as functional candidate genes.

To evaluate the relationship between additive genetic correlations and important genomic regions associated with a pair of traits, we considered only SNPs that were mapped in each FCG interval. Also, we converted the SNP effects into a proportion of genetic standard deviations, which allowed the evaluation of the differences between SNP effects at the same scale.

3.4. Results

The additive variance obtained by the single-trait analysis for BW, ADG, SC, TICK, GIN and EIM were 168.06, 0.13×10^{-2}, 2.30, 1.24×10^{-2}, 0.70×10^{-2} and 2.19×10^{-2}, respectively (Table 2). The heritability values for the growth and reproductive traits were moderate for BW (0.23) and SC (0.41) and low for ADG (0.15). Also, for all parasite burden traits TICK (0.11), GIN (0.06) and EIM (0.16) the heritability values were low.

Table 3.2. Posterior means and highest posterior density intervals1 with 90% of samples (in brackets) for genetic ($\boldsymbol{\sigma_a^2}$), permanent environment ($\boldsymbol{\sigma_{pe}^2}$), residual ($\boldsymbol{\sigma_e^2}$) and phenotypic variance ($\boldsymbol{\sigma_p^2}$) and heritability (h^2) for body weight (BW), average daily gain (ADG), scrotal circumference (SC) and $\log_{10}(count + 1.001)$ of tick (TICK), gastrointestinal Nematodes (GIN) and *Eimeria* spp (EIM) in Nellore cattle

$\boldsymbol{\sigma_a^2}$	$\boldsymbol{\sigma_{pe}^2}$	$\boldsymbol{\sigma_e^2}$	$\boldsymbol{\sigma_p^2}$	h^2
		BW		
168.06 (121.70; 220.10)	-	559.83 (518.00; 604.00)	727.89 (696.90; 759.00)	0.23 (0.17; 0.30)
		ADG[1]		
0.13 (0.09;0.19)	-	0.73 (0.68; 0.78)	0.86 (0.82; 0.90)	0.15 (0.10; 0.21)
		SC		
2.30 (1.82; 2.83)	-	3.30 (2.98; 3.67)	5.60 (5.32; 5.87)	0.41 (0.33; 0.49)
		TICK[1]		
1.24 (0.55; 1.92)	1.74 (1.15; 2.32)	7.83 (7.54; 8.14)	10.81 (10.40; 11.26)	0.11 (0.05; 0.17)
		GIN[1]		
0.70 (0.34; 1.11)	0.67 (0.31; 1.04)	9.67 (9.31; 10.03)	11.05 (10.66; 11.43)	0.06 (0.03; 0.09)
		EIM[1]		
2.19 (1.34; 3.12)	1.53 (0.80; 2.23)	9.86 (9.49; 10.23)	13.59 (13.01; 0.22)	0.16 (0.10; 0.22)

[1]Trait $x10^2$

The posterior means of the additive genetic correlations showed moderate magnitudes and were positive between BW-ADG, BW-SC, BWTICK, ADG-SC and TICK-EIM but negative between ADG-EIM (Figure 1). For all the remaining pairs of traits, the high posterior density (HPD) intervals included zero (Figure 1).

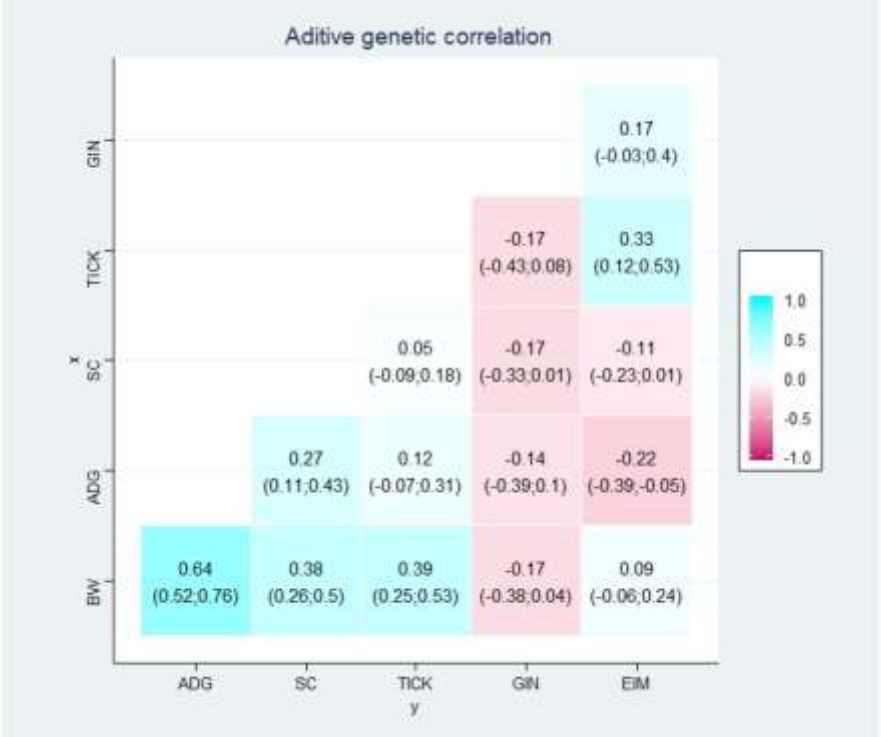

Figure 3.1. Posterior means and lower and upper limits of the high posterior density intervals (HPD), of additive genetic correlations between body weight at 550 days of age (BW), average daily gain (ADG), scrotal circumference at 550 days of age (SC) and $\log_{10}(\text{count} + 1.001)$ of ticks (TICK), gastrointestinal nematodes eggs (GIN) and *Eimeria* spp. oocysts (EIM) in Nellore cattle.

An average of 3,538 genomic windows with up to 82 SNP markers each were built throughout the 29 autosomal and X chromosomes (chr.) considered for analysis. We verified genomic windows that explained at least 1% of the additive genetic variance in

the chromosomes 3, 15, 16, 17, 18, 20, 21 and 23 (Figure 2) for the six traits. Moreover, two (chr. 3 and 23), three (chr. 3, 16 and 20), one (chr. 3), one (chr. 20), two (chr. 20 and 21), and five (chr. 3, 15, 16, 20 and 23) genomic windows explained at least 1% of the total additive genetic variance for BW, ADG, SC, TICK, GIN and EIM, respectively

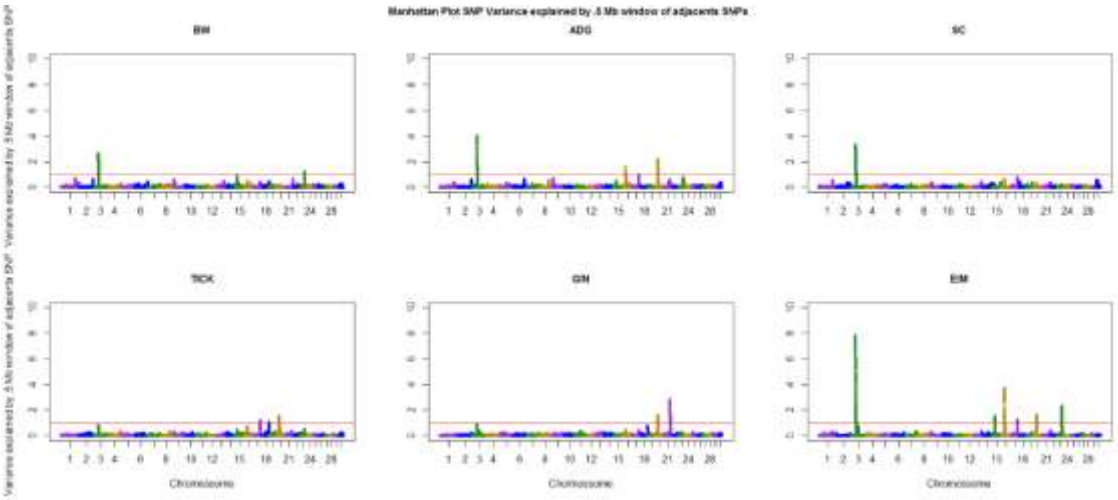

Figure 3.2. Manhattan plots for percentage of variance explained by genomic windows (0.5Mb adjacent SNPs) for body weight at 550 days of age (BW), average daily gain (ADG), scrotal circumference at 550 days of age (SC) and log10(count +1.001) of tick (TICK), *Eimeria* spp. (EIM) and gastrointestinal nematodes (GIN).

The size of the windows which explained at least 1% of the additive genetic variance varied from 0.35Mb to 0.49Mb and contained between 15 and 82 SNPs (Table 3). The genomic window present in chromosome 3 was the one that could explain the greatest percentage of additive genetic variance, representing 2.63%, 3.99%, 3.32% and 7.81% of the variance for BW, ADG, SC and EIM, respectively (Table 3). For TICK, the highest percentage of additive genetic variance was explained by the window located in the chromosome 20, representing 1.59% of the total additive genetic variance (Table 3).

For GIN, the genomic window located in the chromosome 21 explained the highest percentage (2.78%) of additive genetic variance (Table 3).

Table 3.3. Description of genomic windows which explained at least 1% of variance for body weight, average daily gain, scrotal circumference and $\log_{10}(\text{count} + 1.001)$ of tick, gastrointestinal Nematodes and *Eimeria* spp. in Nellore cattle

Chr	Pos_s	Pos_e	Size	SNPs	%Var
		Body Weight			
3	32676738	33110685	433947	82	2.63
23	39323760	39680863	357103	51	1.20
		Average daily gain			
3	32676738	33110685	433947	82	3.99
20	22126973	22480484	353511	43	2.18
16	24919996	25374981	454985	46	1.56
		Scrotal Circunference			
3	32727140	33224436	497296	79	3.32
		Tick			
20	21946439	22423318	476879	56	1.59
17	56171681	56663231	491550	19	1.18
18	64937253	65401508	464255	26	1.02
		Gastrointestinal Nematodes			
21	56675767	57165861	490094	50	2.78
20	22126973	22480484	353511	43	1.55
		Eimeria spp.			
3	32676738	33110685	433947	82	7.81
16	24913318	25374981	461663	47	3.70
23	39148251	39639650	491400	51	2.30
20	21946439	22423318	476879	56	1.62
15	23650235	24099827	449592	41	1.51
17	56464616	56962591	497975	15	1.23

Chr = Chromosome; Pos_s = start position in base pair of the window; Pos_e = end position in base pair of the window; SNPs = number of SNPs within of the window; %Var = percentage of genetic variance explained for the window

Eight, 7, 5, 5, 4 and 14 positional genes were recovered within the windows which explained at least 1% of the additive genetic variance for BW, ADG, SC, TICK, GIN and EIM, respectively, (Figure 3). In addition, 4 (SLC16A4, KCNA2, TPMT and KIF13A), 4 (LAMTOR5, KCNA2, DUSP10 and MAP3K1), 2 (LAMTOR5 and KCNA2), 1

(MAP3K1), 3 (MAP3K1, TRIP11 and SLC24A4) and 9 (SLC16A4, LAMTOR5, KCNA2, NCAM1, DUSP10, MAP3K1, TPMT, KIF13A and DEK) genes had functional information recovered from the trained list through GUILDify 2.0 and ToppGenes (ToppGene - Gene Prioritization) for BW, ADG, SC, TICK, GIN and EIM, respectively, and they were also chosen as FCGs for each of these traits (Figure 3).

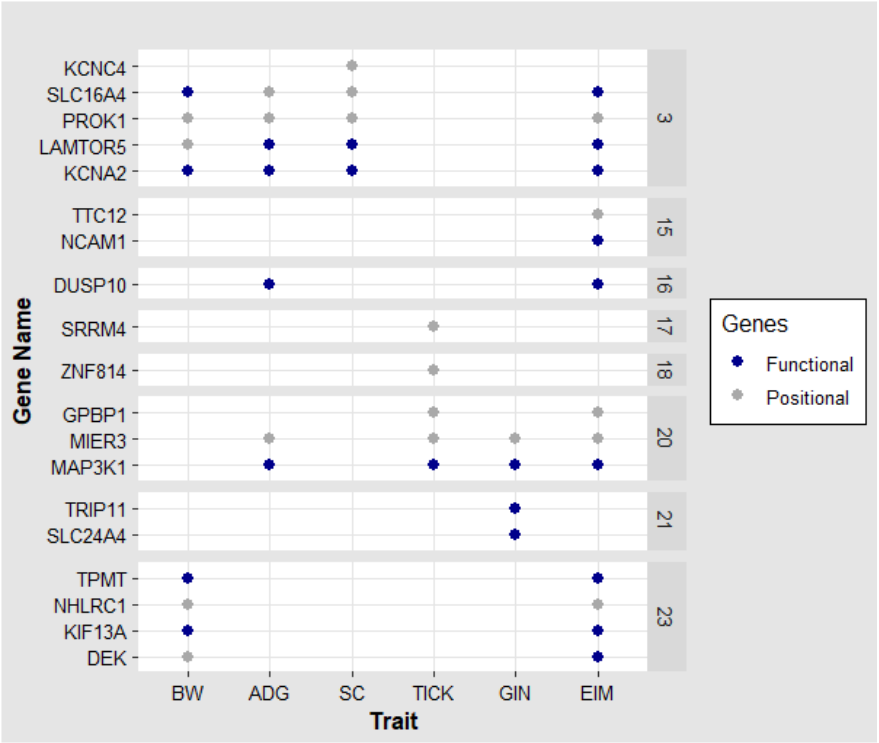

Figure 3.3. Positional and functional candidate genes for body weight at 550 days of age (BW), average daily gain (ADG), scrotal circumference at 550 days of age (SC) and $\log_{10}(\text{count} + 1.001)$ of tick (TICK), *Eimeria* spp. (EIM) and gastrointestinal nematodes (GIN).

Furthermore, seven FCGs had functional effects over more than one trait (Figure 3). The genes KCNA2, LAMTOR5, SLC16A4 (BW, ADG, SC and GIN) and MAP3K1 (ADG, TICK, GIN and EIM) were connected to four different traits, being followed by

the genes DUSP10 (ADG and EIM) KIF13A and TPMT (BW and EIM) which were associated with two different traits. In general, the pairs of traits with genetic correlation values different from zero (Figure 1) shared at least one FCG, except the pair BW-TICK. For three trait pairs (BW-GIN, SC-TICK, SC-GIN), when the genetic correlation was not different from zero (when zero is included in HPD) there were no FCGs associated with both traits in the pair. On the other hand, the majority of trait pairs that presented genetic correlation values very close to or equal to zero (BW-GIN, BW-EIM, ADG-TICK, ADG-GIN, SC-TICK, SC-GIN, SC-EIM, TICK-GIN and GINEIM) had FCGs found for both traits of the pair.

The solution of the SNPs, presented as a proportion of the standard deviation (Figure 4 and table S2), showed that there were SNPs mapped in FCGs that promoted a significant improvement in both traits of a pair, for example, the Hapmap40909-BTA-121580SNP linked with KCNA2, which simultaneously promoted an increase in BW, ADG and SC and a decrease in the EIM parasite burden trait.

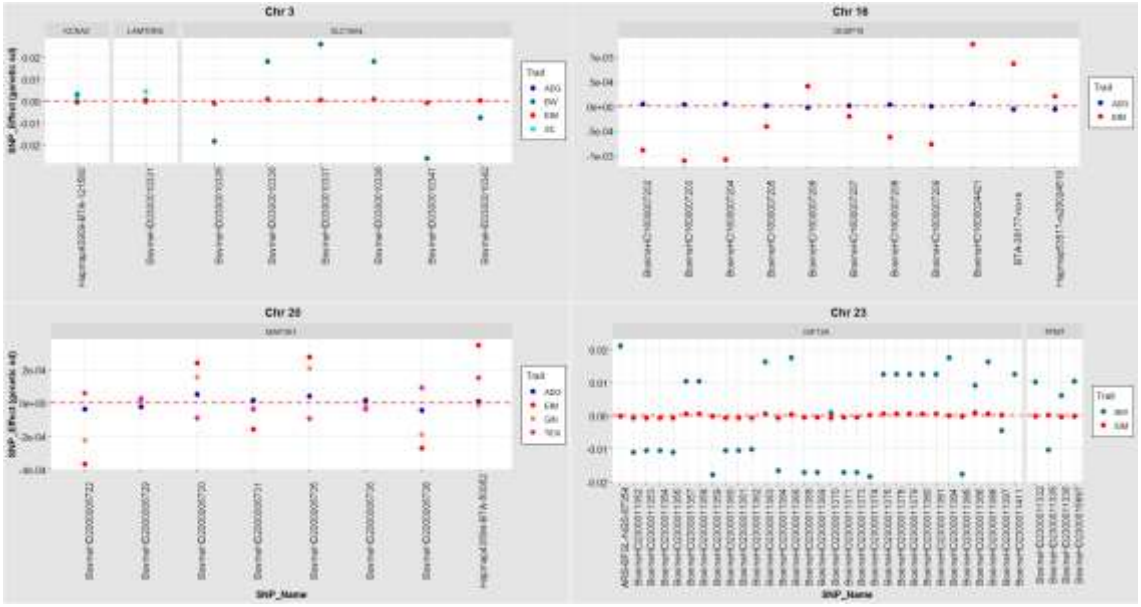

Figure 3.4. Differences in SNP effects, in genetic standard deviations (sd), according to each trait, body weight at 550 days of age (BW), average daily gain (ADG), scrotal circumference at 550 days of age (SC) and $\log_{10}(\text{count} + 1.001)$ of tick (TICK), gastrointestinal nematode (GIN) and *Eimeria* spp. (EIM).

3.5. Discussion

The variance components and the heritability values were similar to those available in the literature for growth and reproduction traits (Boligon et al., 2010), as well as for tick burden (Biegelmeyer et al., 2017). As in most traits of economic interest for beef cattle, the largest part of the phenotypic variance was explained by the environmental variance, which reflects low to moderate heritabilities.

The genetic correlation, which is a populational parameter based on genetic variants (Ni et al., 2018), could happen due to QTLs that have pleiotropic effects on multiple traits or due to closely linked QTLs, each one affecting different traits (Bolormaa et al., 2011). Previous studies associated the genetic correlations with sets of loci shared between traits (Carey, 1988; Falconer and Mackay, 1996), findings that are corroborated by our results, because we found that most pairs of genetically correlated traits showed the same FCG for both traits. On the other hand, a low genetic correlation can arise even when the same genes are involved in two traits (Carey, 1988) as found in our results, in which pairs of traits that presented genetic correlation values that were not significantly different from or close to zero also had FCGs in common.

Thus, we suggest that independently of the genetic correlation values (low-medium-high) there are FCGs that influence production, reproductive or parasite burden traits in beef cattle concurrently, and that they probably have pleiotropic effects. It is reasonable to expect a negative genetic correlation between traits of growth and parasite burden. However, a positive genetic correlation between BW and TICK (0.39) was surprisingly seen in our results, conceivably suggesting that an increase in body weight would result in an increase in the tick burden. Positive correlations between tick burden and body weight in yearlings were found by Porto Neto et al., 2011 for Tropical Composite cattle. In addition, Rocha et al. (2019) suggested that larger animals have a

wider skin surface with a denser vasculature contributing to higher tick burden in heavier animals. We believe it is possible that in our study there was an indirect selection for the reduction of the tick burden by the farmer, because they are visible to the naked eye. Therefore, we believe that if there were an effective selection for the resistance and the reduced tick burden, this selection could have been responsible for the positive correlation. On the other hand, for GIN and EIM, there may have been no selection because these parasites are not visible to the naked eye, or they were not measured in breeding programs. Therefore, the genetic correlation between them and productive traits was low or negative.

It should be highlighted that the direction of the SNP effects which were associated with FCGs can be considered in selection processes to promote better responses to selection in animal breeding. Thus, greater attention should be given to SNPs which present positive effects on growth or reproduction traits and negative effects on parasite burden simultaneously, since these SNPs can be linked and assigned to real causal variants that influence both traits.

Functional Enrichment -Chromosome 3

Three FCGs were identified on chromosome three (LAMTOR5, SLC16A4, and KCNA2). These genes were associated with four traits, comprising growth (BW and ADG), reproduction (SC) and parasite burden (EIM). The LAMTOR5 gene (Late Endosomal/Lysosomal Adaptor, MAPK and MTOR Activator 5) is associated with the regulation of TLR4 intracellular fate and immune homeostasis (Zhang et al., 2019).

Toll-like receptors (TLRs) are components that recognize conserved structures in pathogens and their function is associated with how the body recognizes a pathogen invasion, triggers innate immune responses and assembles an antigen-specific adaptive

immune response (Kawai and Akira, 2010). TLR4 is distinguished by its ability to recognize a variety of exogenous and endogenous agents and activates different signaling pathways depending on its cellular localization (Brubaker et al., 2015). A homeostatic function was suggested for LAMTOR5, specifically one of coupling pathogen insults and nutrient availability to optimize the inflammatory response, and its function might have implications for TLR4-associated inflammatory and metabolic disorders (Zhang et al., 2019). Besides the immune responses, the LAMTOR5 gene was also appointed as a candidate gene for weight gain adjusted for 345 days of age for Hereford and Braford beef cattle (Campos et al., 2019).

The SLC16A4 gene (Solute Carrier Family 16 Member 4) was also appointed as a candidate gene for post-weaning gain adjusted for 345 days of age for Hereford and Braford beef cattle (Campos et al., 2019). This gene is a member of the SLC16 gene family, known as monocarboxylate transporters and, in humans, the product of SLC16A4 is found mainly in the brain, muscle, liver, kidneys, lungs, ovaries, placenta and heart (Halestrap, 2013). In salmonid fish, the activity of this gene was shown to be influenced by a parasite infestation (Tetracapsuloides bryosalmonae) in a transcriptomics study (Sudhagar et al., 2019). In cattle, MTC5, which is an isoform of SLC16A4, was detected in both cortex and medulla of the adrenal glands, and even though these glands are associated with the metabolism of carbohydrates, fats and proteins, as well as with stress response and reproduction, few studies have been conducted on MCT5 in cattle (Kirat et al., 2009). Considering that the product of this gene is distributed among important body organs and a fundamental gland in bovines, even though our findings appointed this gene as a FCG for important animal traits (BW, ADG, SC and EIM), it is important to validate its activity in larger animal populations to improve our understanding about the genetic control of this gene over growth and parasite burden traits.

The KCNA2 gene (Potassium Voltage-Gated Channel Subfamily A Member 2) belongs to the voltage-gated potassium channel family and its main biological function is to maintain the membrane potential and to modulate electrical excitability in neurons and muscle cells (Gutman et al., 2005). But as for animal performance and parasite burden traits, we have not found any associations described in the literature

Chromosome 16

The DUSP10 gene (dual specificity phosphatase 10), identified on chromosome 16, was a FCG for the traits ADG and EIM. In a Simmental beef cattle population, a significant association was shown between a SNP for carcass weight and the DUSP10 gene (Chang et al., 2018). Also, previous studies associated this gene with infections by Mycobacterium bovis (Meade et al., 2008) and Mycobacterium avium ssp. (Kiser et al., 2018). Our results corroborate the findings in the literature, which suggest that the DUSP10 gene is associated with traits of growth and infection by parasites.

Chromosome 20

The MAP3K1 gene (Mitogen-activated protein kinase 1), identified on chromosome 20, was pointed out as a FCG mainly for parasite burden traits (TICK, GIN and EIM), but it also encodes a TLR family protein (Slawinska et al., 2011), previously characterized for LAMTOR5. A study in dairy cattle associated MAP3K1 with mastitis (Li et al., 2015). In chicken, it was suggested to be included in follow-up studies on model genetic networks of innate humoral immune response (Slawinska et al., 2011). Similarly, MAP3K1 was also appointed as a FCG for a growth trait (ADG), and it was associated with the marbling score in cattle (Ryu and Lee, 2016, 2014). Since previous studies related the MAP3K1 gene with the capacity of organisms to respond to different stressor

stimuli such as parasite infections, and to traits associated with growth in cattle, it is reasonable to suggest that this gene influences four different traits (ADG, TICK, GIN and EIM), which were associated in the present study, and that it might be a FCG to more than one trait.

Chromosome 23

Two FCGs (KIF13A and TPMT) associated with a growth trait (BW) and a parasite burden trait (EIM) were found in chromosome 23. The KIF13A gene (Kinesin-like protein) is highly expressed in all regions of the central nervous system and its transcripts are present in several tissues, with a higher expression in the pancreas, kidneys and placenta (Jamain et al., 2001). In a study with buffalos, it was suggested that this gene may be involved in the regeneration of the immune function (Singh et al., 2019); and this gene also was associated with body weight in Landrace pigs (Lee et al., 2018). Also, the TPMT gene (Thiopurine S-methyltransferase) was associated with porcine infections by Streptococcus suis, and it was suggested by the authors to be a candidate gene that may influence either the susceptibility to parasites or the parasite burden in pigs (Gaur et al., 2014).

In general, our results corroborate the current literature, which support the hypothesis that a single gene may take part in the genetic control of different traits. However, the literature findings did not always correspond to beef cattle, the focus of the present work. Therefore, it is necessary to validate these findings to each gene in each trait in both larger populations of Nellore cattle and other bovine breeds to improve the understanding about the control of genetic correlations and the architecture of growth, reproductive and parasite burden traits in cattle, especially concerning the functional candidate genes studied in the present work. In the short term, we suggest that higher

weights may be applied for the SNPs mapped in the FCGs identified by the present study when a genomic selection is performed for the same traits evaluated here.

Finally, we would like to emphasize that a high parasite infection leads to severe production losses, especially in tropical climate countries, where conditions for parasite proliferation are favorable. Therefore, despite the moderate magnitude of genetic correlation between performance and parasite burden traits, the identification of genes that can simultaneously control both parasite burden and performance traits highlight the need to evaluate more thoroughly selection criteria related with parasite burden in beef cattle breeding programs.

3.6. Conclusion

There are common functional candidate genes (SLC16A4, KCNA2, LAMTOR5, DUSP10, MAP3K1, TPMT, and KIF13A) that control growth, reproductive and parasite burden traits in beef cattle and contribute to genetic correlation values.

3.7. Supplementary Material

Table S3.1. Keywords used on GUILDify to retrieve the trained list of genes for body weight, average daily gain, scrotal circumference and $\log_{10}(\text{count}+1.001)$ of tick, gastrointestinal Nematodes and *Eimeria* spp. in Nellore cattle

Trait	Used keywords
Body weight	Body weight, protein, muscle, obesity, growth and growth factors
Average daily gain	Weight gain, protein, muscle, obesity, growth and growth factors
Scrotal circumference	Scrotal circumference, fertility, infertility and sperm
Tick	Immunity, immune response, inflammation, ectoparasite, cytokines and tick
Gastrointestinal Nematodes	Immunity, immune response, inflammation, endoparasite, cytokines and nematodes
Eimeria spp.	Immunity, immune response, inflammation, endoparasite, cytokines and *Eimeria*

Table S3.2. SNP name, allele frequency and SNP effect, in genetic standard deviation, for body weight at 550 days of age (BW), average daily gain (ADG), scrotal circumference at 550 days of age (SC) and $\log_{10}(\text{count}+1.001)$ of tick (TICK), gastrointestinal nematode (GIN) and *Eimeria* spp. (EIM).

SNP Name	Allele Frequency		SNP effect (genetic SD)					
	Common	Rare	BW	ADG	SC	TICK	GIN	EIM
Chr3 – KCNA2								
Hapmap40909-BTA-121580	1041 (A)	34 (G)	$3.1x10^{-3}$	$5.4x10^{-7}$	$1.5x10^{-3}$			$-3.6x10^{-4}$
Chr3-LAMTOR5								
BovineHD0300010331	1030 (A)	45 (G)		$5.4x10^{-5}$	$4.4x10^{-3}$			$6.7\ x10^{-4}$
Chr3- SLC16A4								
BovineHD0300010335	715(A)	360 (G)	$-1.8x10^{-2}$					$-9.4x10^{-4}$
BovineHD0300010336	905 (A)	170 (G)	$1.8x10^{-2}$					$9.4x10^{-4}$
BovineHD0300010337	1011 (A)	64 (G)	$2.6x10^{-2}$					$6.4x10^{-4}$
BovineHD0300010338	905 (A)	170 (C)	$1.8x10^{-2}$					$9.4x10^{-4}$
BovineHD0300010341	617 (G)	458 (A)	$-2.6x10^{-2}$					$-6.4x10^{-4}$
BovineHD0300010342	1049 (A)	26 (G)	$-7.3x10^{-3}$					$2.8x10^{-4}$
Chr16- DUSP10								
BovineHD1600007202	735 (A)	340 (C)		$4.5x10^{-5}$				$-8.9x10^{-4}$
BovineHD1600007203	872 (A)	203 (G)		$4.1x10^{-5}$				$-1.1x10^{-3}$
BovineHD1600007204	864 (A)	211 (C)		$4.9x10^{-5}$				$-1.5x10^{-4}$
BovineHD1600007205	1067 (A)	8 (G)		$1.7x10^{-5}$				$-4.1x10^{-4}$
BovineHD1600007206	850(G)	225 (A)		$-1.8x10^{-5}$				$4.1x10^{-4}$
BovineHD1600007207	1024 (A)	51 (G)		$1.1x10^{-5}$				$-2.1x10^{-4}$
BovineHD1600007208	618 (C)	457 (A)		$4.1x10^{-5}$				$-6.3x10^{-4}$
BovineHD1600007209	792 (A)	283 (G)		$-1.2x10^{-4}$				$-7.4x10^{-4}$
BovineHD1600024421	615 (T)	460 (C)		$4.6x10^{-5}$				$1.2x10^{-3}$

BTA-38177-no-rs	890 (A)	185 (C)	$-5.6x10^{-5}$			$8.6x10^{-4}$
Hapmap53517-rs29024619	800 (G)	275 (A)	$-5.3x10^{-5}$			$2.1x10^{-4}$
			Chr20- MAP3K1			
BovineHD2000006722	654 (A)	421 (C)	$-3.4x10^{-5}$	$6.2x10^{-5}$	$-2.2x10^{-4}$	$-3.7x10^{-4}$
BovineHD2000006729	944 (G)	131 (A)	$-2.0x10^{-5}$	$2.9\ x10^{-5}$	$2.0x10^{-5}$	$1.7x10^{-5}$
BovineHD2000006730	873 (A)	202 (G)	$5.3x10^{-5}$	$-8.5x10^{-5}$	$1.6x10^{-4}$	$2.4x10^{-4}$
BovineHD2000006731	1072 (A)	3 (G)	$1.6x10^{-5}$	$-3.7x10^{-5}$	$-3.2x10^{-5}$	$-1.5x10^{-4}$
BovineHD2000006735	884 (A)	191 (G)	$4.3x10^{-5}$	$-9.2x10^{-5}$	$2.1x10^{-4}$	$2.8x10^{-4}$
BovineHD2000006736	1069 (A)	6 (G)	$1.9x10^{-5}$	$-3.5x10^{-5}$	$-1.8x10^{-5}$	$-2.0x10^{-5}$
BovineHD2000006738	729 (A)	346 (C)	$-4.4x10^{-5}$	$9.2x10^{-5}$	$-1.9x10^{-4}$	$-2.7x10^{-4}$
Hapmap43594-BTA-50052	777 (G)	298 (A)	$9.8x10^{-6}$	$1.5x10^{-4}$	$-1.1x10^{-5}$	$3.5x10^{-4}$
			Chr23- KIF13A			
ARS-BFGL-NGS-87354	726 (G)	349 (A)	$2.1x10^{-2}$			$-6.8x10^{-5}$
BovineHD2300011352	790 (G)	285 (A)	$-1.0x10^{-2}$			$-6.8x10^{-4}$
BovineHD2300011353	790 (G)	285 (A)	$-1.0x10^{-2}$			$-6.9x10^{-4}$
BovineHD2300011354	731 (B)	352 (A)	$2.2x10^{-2}$			$-6.8x10^{-3}$
BovineHD2300011355	790 (G)	285 (A)	$-1.0x10^{-2}$			$-6.8x10^{-4}$
BovineHD2300011357	1056 (A)	19 (G)	$1.0x10^{-2}$			$6.7x10^{-4}$
BovineHD2300011358	1056 (A)	19 (C)	$1.0x10^{-2}$			$-6.7x10^{-4}$
BovineHD2300011359	1034 (A)	41 (G)	$-1.8x10^{-2}$			$-8.0x10^{-5}$
BovineHD2300011360	789 (C)	286 (A)	$-1.0x10^{-2}$			$-6.7x10^{-4}$
BovineHD2300011361	790 (G)	285 (A)	$-1.1x10^{-2}$			$-6.9x10^{-4}$
BovineHD2300011362	788 (G)	287 (A)	$-1.0x10^{-2}$			$-6.9x10^{-4}$
BovineHD2300011363	1046 (A)	29 (C)	$1.6x10^{-2}$			$5.9x10^{-4}$
BovineHD2300011364	736 (G)	339 (A)	$-1.7x10^{-2}$			$-6.0x10^{-4}$
BovineHD2300011366	1038 (A)	37 (G)	$1.7x10^{-2}$			$4.2x10^{-4}$
BovineHD2300011368	694 (G)	381 (A)	$-1.7x10^{-2}$			$-4.3x10^{-4}$
BovineHD2300011369	694 (G)	381 (A)	$-1.7x10^{-2}$			$-4.3x10^{-4}$
BovineHD2300011370	581 (A)	494 (G)	$1.1x10^{-2}$			$-6.3x10^{-4}$
BovineHD2300011371	694 (C)	381 (A)	$-1.7x10^{-2}$			$-4.3x10^{-4}$

BovineHD2300011373	694 (C)	381 (A)	$-1.8x10^{-2}$	$-4.3x10^{-4}$
BovineHD2300011374	1059 (A)	16 (G)	$-1.8x10^{-2}$	$1.9x10^{-4}$
BovineHD2300011375	1044 (A)	31 (G)	$1.3x10^{-2}$	$5.6x10^{-4}$
BovineHD2300011378	1044 (A)	31 (G)	$1.3x10^{-2}$	$6.2x10^{-4}$
BovineHD2300011379	1044 (A)	31 (G)	$1.3x10^{-2}$	$6.0x10^{-4}$
BovineHD2300011380	1044 (A)	31 (C)	$1.3x10^{-2}$	$6.2x10^{-4}$
BovineHD2300011381	1044 (A)	31 (G)	$1.3x10^{-2}$	$6.2x10^{-4}$
BovineHD2300011384	698 (G)	377 (A)	$1.8x10^{-2}$	$7.0x10^{-5}$
BovineHD2300011385	1034 (A)	41 (C)	$-1.7x10^{-2}$	$7.0x10^{-5}$
BovineHD2300011386	968 (A)	94 (C)	$9.2x10^{-3}$	$9.4x10^{-3}$
BovineHD2300011388	1046 (A)	29 (G)	$1.6x10^{-2}$	$5.9x10^{-4}$
BovineHD2300011397	652 (A)	423 (G)	$-4.3x10^{-3}$	$3.0x10^{-4}$
		Chr23- TPMT		
BovineHD2300011332	1058 (A)	17 (G)	$1.0\ x10^{-2}$	$-1.7x10^{-4}$
BovineHD2300011335	818 (G)	257 (A)	$-1.0\ x10^{-2}$	$1.7x10^{-4}$
BovineHD2300011336	1056 (T)	19 (C)	$6.1x10^{-3}$	$-2.2x10^{-4}$
BovineHD2300015697	1062 (A)	13 (g)	$1.0x10^{-2}$	$-1.6x10^{-4}$

4. CANDIDATE GENES FOR LONGITUDINAL TRAITS UNDER SELECTION IN BEEF CATTLE

4.1. Abstract

Animal growth includes a complexity of metabolic events that occur along time. Therefore, it may be possible that different sets of loci take part in the genetic control of body weight at different stages of life. Further, the selection effects, also, should be considered in the growth traits evaluations, given that the majority of animal records are preselected. Thus, we aim to identify functional candidate genes which take part in the genetic control of body weight in five different ages in a beef cattle population with and without sequential selection simulation. The genetic parameters were estimated by a single trait (STM) and a random regression model (RRM) with spline functions for two databases, one with complete records of body weights (DB100) and another one in which a sequential selection of 70% (DB70) of heaviest animals was applied. Body weights (BW) were standardized at 330, 385, 440, 495 and 550 days of age for the STM. In the RRM, the knots of linear splines were fitted at 274, 330, 385, 440, 495, 550 and 594 days of age. The genome wide association studies (GWAS) were performed with results from both STM and RRM. A total of 21,667 SNP markers were used to perform the single-step GWAS, and to identify genomic windows that explained at least 1% of the genetic variance for the evaluated traits. Additionally, functional analyses were performed and functional candidate genes (FCGs) were selected based on their roles in biological processes for each of the traits. We associated seven FCG (DUSP10, LAMTOR5, PAFAH2, SLC30A2, TRIM63, NCAM1 and SCL16A4) to body weight in different ages. The DUSP10 gene was associated with body weight in all the five ages evaluated, appointing for the relevance of this gene for different stages of animal growth. On the

other hand, the majority of the FCG associated with body weight were different for different ages, suggesting that the importance of each gene for animal growth can change in different development stages and different genes can be more relevant to body weight in each growth stage. The genetic parameters for DB100 and DB70 were, in general, similar. On the other hand, different FCG were associated with body weight in DB100 and DB70 for each age when the sequential selection was simulated, even when the RRM was performed. Therefore, the sequential selection can affect the GWAS and post GWAS results, and this may be one more reason for frequent inconsistencies in GWAS results performed for growth traits measured in beef cattle.

Keywords body weight, genome-wide association, growth, random regression model, Nellore

4.2. Introduction

Genome wide association studies (GWAS) have been widely developed for complex traits in domestic animals (Zhang et al., 2012), mainly for growth traits in beef cattle (Cesar et al., 2014; Zhang et al., 2012), since these traits are extensively improved by breeding programs. Considering the complexity of the metabolic and physiologic mechanisms involved in animal growth (Owens et al., 1995), it is possible that the same trait, measured in different stages of the animal growth, may be controlled by different sets of loci, as suggested by Campos et al. (2019), which verify different genomic regions and consequently, different candidate genes, associated with Hereford body weight in different ages.

Another point that should be consider when GWAS are performed to bovine growth traits is the animal culling in order to have financial resources for operation, or to offer better environmental conditions for the remaining candidates to selection (Toral et al., 2019). These may result in a decrease of animal records number, since animals which has body weight records in younger are discarded and, consequently, not to be weighed again in older ages. Further, the animals that remained in the herd, will be selected again at later ages. Consequently, the majority of the genetic and genomic evaluations of important post-weaning growth traits (age at first calving, body weight for different ages and residual feed intake) are performed with phenotypic records of pre-selected animals. It must be said that the selection process can influence the estimates of variance components and the accuracy of breeding values predictions (Kaps et al., 1999; Long et al., 1991; Schaeffer et al., 1997, Toral et al., 2019). Thus, the effects of pre-selection should be considered in both GWAS and genetic/genomic evaluations.

The use of models which consider a covariance structure between random effects, as multiple trait models or random regression models (RRM), has been shown as a good

alternative for evaluation of traits which are under sequential selection (Boligon et al., 2009, Toral et al., 2019). Recently, the use of a RRM with linear splines adjusted in different stages of the growth for beef cattle, submitted to sequential selection by simulation, was appointed as a good alternative to be used by beef cattle breeding programs (Toral et al., 2019). Until now, the better adjustment of the previously cited models for traits which have been under sequential selection were performed to phenotypic databases. On the other hand, the effects of sequential selection have not been elucidated neither for genomic regions associated with traits under selection nor over genes which contribute to genetic control of these traits. The incorporation of genomic information at genetic evaluations can allow us to verify the effects of sequential selection on traits at a genomic level.

There have already been genes identified as functional candidates which take part in genetic control of carcass (Santana et al., 2015; Chang et al., 2019) and growth (Horodyska et al., 2018; Campos et al., 2019) traits. In this way, identifying genes which contribute for body weight genetic control in different ages can allow to verify whether the same trait may be controlled by different groups of genes depending on animal growth stage. Also, the selection effects over the GWAS results may be verified by identification of candidate genes for the same trait which is under sequential selection or not. Thus, we aim to identify FCG associated with body weight in five different ages in a beef cattle herd with and without simulated sequential selection.

4.3. Materials and Methods

Field management description

Phenotype data of Nellore bulls born between 2001 and 2016 were used in this study. The bull calves were raised in pasture with the predominance of Urochloa genus grass, and the stocking rate on the pastures was of approximately 0.98 AU/ha. Mineral supplementation was provided ad libitum over the year. During the cow-calf phase, calves were kept with their dams on 30-hectare pastures and were weaned at approximately 205 days of age. At weaning, management groups with 45 bulls on average were assembled and kept on the same pasture under the same rearing conditions.

The body weights were recorded during performance tests at pasture, commonly starting in August and finishing in July of the following year. These performance tests lasted 294 days, comprising 70 days of adaptation and 224 days of test. During the performance test, all bulls from a single management group were kept under the same management conditions. The bulls were weighted at the beginning and the end of the adaptation period (70th trial day), which effectively corresponds to the beginning of the trial, but follow-up weighings were conducted every 56 days until the end of the test (test days 0, 70, 126, 182, 238 and 294). The data collected at the beginning of the test (day 0) were not taken into consideration. At weaning, approximately 20% of the lightest calves have been discarded. During the performance test at pasture, the management groups were rotated among different paddocks in order to offer similar rearing conditions for all individuals from the same birth season. A thorough description of the farm can be found in Passafaro et al. (2015).

Because age differences up to 96 days are allowed in the performance tests, it was necessary to standardize the body weights for ages 330 (BW330), 385 (BW385), 440

(BW440), 495 (BW495) and 550 days (BW550) for the single trait model (STM). The standardized weight at age d, was obtained by

$$\mathrm{BW_d} = (\mathrm{ABW_d} + \mathrm{ADG}(\mathrm{d} - \mathrm{Age_d})),$$

where BW represents the standardized weight at age d; ABW represents the actual weight obtained near age d; ADG represents the mean daily gain for the period of 56 days prior to age d; and Age represents the actual age. For BW330, the ADG for standardization was obtained after the reference age.

Two database were considered, the first one with complete records for all animals (DB100) and the second was constructed from the former file, but with simulation of a selective recording procedure. This file was formed by selective data recording of the heaviest (standard body weight) animals at weighing. The percentage of individuals who were selected was 70% (DB70). If an animal did not satisfy the established criteria, the weights of the animal that were obtained at subsequent ages were excluded. It is important to highlight that, all the analyses were performed to two databases independently and after that, the results of both were compared. The descriptive statistics of the files are presented in Table 1.

Table 4.1. Summary statistics for body weight (BW) at five different ages (330, 385, 440, 495, 550 days) for complete database (DB100) and select database (DB70).

Trait	n.	Body Weight		Age	
		Mean (Min-Max)	sd	Mean (Min- Max)	sd
DB100					
BW330	3783	212.07 (131-359)	30.81	318.62 (274-361)	19.70
BW385	3783	224.53 (133-398)	34.48	376.77 (330-417)	18.17
BW440	3783	251.71 (146-460)	37.68	432.19 (386-472)	18.47
BW495	3783	289.98 (155-474)	39.37	487.98 (414-532)	18.82
BW550	3783	325.92 (162-520)	39.15	547.47 (506-594)	16.11
DB70					
BW330	3783	212.07 (131-359)	30.81	318.62 (274-361)	19.70
BW385	2633	233.36 (154-398)	32.62	377.09 (330-417)	18.10
BW440	1832	267.49 (169-460)	35.76	432.82 (386-471)	18.40
BW495	1263	313.23 (196-474)	36.19	488.45 (414-532)	18.78
BW550	868	356.11 (234-520)	36.19	547.75 (507-588)	16.09

Genotypic database

During the performance tests at pasture, samples of hair, blood and semen were collected for DNA extraction. A total of 1,230 male individuals were genotyped through a low-density panel (Z-chip) with approximately 30 thousand SNP markers. A Z-chip v2 (Neogen, Lincoln, Nebraska, EUA) was especially built by its subsidiary Deoxi (Araçatuba, SP, Brazil) for the molecular genotyping of Zebu cattle.

Quality control of samples and markers was implemented through the R/SNPStats statistical package (Clayton, 2017). In the quality control of the samples, those with call rate lower than 0.90 and duplicated records (correlation between samples > 0.95) were excluded. In the quality control of markers, only SNPs mapped in autosomal and X chromosomes, which presented GenCall (GC score) > 0.6, call rate > 0.95 and minor allele frequency (MAF) > 0.05 were considered. After editing, a total of 21,667 SNP markers (77.12%) and 1,075 samples (88.04%) were kept for analyses. Out of the 1,075 animals with genotypic information, 644 had phenotypic information.

Variance components

The variance estimates for body weight in each standard age were obtained by a single trait model (STM) and a random regression model with linear splines (RRM). Preliminary analyses were conducted in order to ascertain which systematic effects should be included in the model. For the STM, the standard body weight at each age (BW330, BE385, BW440, BW495 and BW550) was considered as a trait and only the management group was included as systematic effect. Also, two random effects, genetic and residual, were considered. Within each test, the age range did not exceed 96 days and a total of 97 groups with at least 25 animals each were considered as having valid records. The single-trait model can be described in matrix notation as:

$$\underset{\sim}{y} = X\underset{\sim}{\beta} + Z\underset{\sim}{a} + \underset{\sim}{e},$$

where $\underset{\sim}{y}$ represents the vector of observations; X and Z, the incidence matrices of the systematic and genetic effects, respectively; $\underset{\sim}{\beta}$ and $\underset{\sim}{a}$, the solution vectors for the systematic and genetic effects, respectively; and $\underset{\sim}{e}$ is the vector of errors.

The following assumptions were assumed for the effects included in the STM: flat-type a priori distributions were assumed for $\underset{\sim}{\beta}$ $\left(\underset{\sim}{\beta} \sim \text{constant}\right)$; normal distributions were assumed for $\underset{\sim}{a}$ $\left(\underset{\sim}{a} \middle| A,\sigma_a^2 \sim N\left(0, A\sigma_a^2\right)\right)$ and $\underset{\sim}{e}$ $\left(\underset{\sim}{e} \middle| \sigma_e^2 \sim N\left(0, I\sigma_e^2\right)\right)$; scaled inverse chi-squared distributions were assumed for σ_a^2 $\left(\sigma_a^2 \sim \chi^{-2}\left(v_a, S_a^2\right)\right)$ and σ_e^2 $\left(\sigma_e^2 \sim \chi^{-2}\left(v_e, S_e^2\right)\right)$; where A is the relationship matrix, I is an identity matrix of order equal to the number of animals with data, I is an identity matrix of order equal to the number of observations, and v_a, v_e, S_a^2, and S_e^2 are the hyperparameters of the scaled inverse chi-squared distributions.

For adjustment of mean trajectory in RRM, the management group was included as a systematic effect and seven knots were adjusted at seven ages (274, 330, 385, 440, 495, 550 and 594) as covariables. Two knots were adjusted in the lower and upper extremities of ages (274 and 594) in order to delineate the entire age range with available weights. Additive and residual effects were considered as random, also the permanent environmental effect, because each animal presented up to five repeated measurements taken over the management group at pasture. The heterogeneous residual variance was formed by five classes one to each age.

To adjust the linear splines (Misztal, 2006; Toral et al., 2019), the age of the animal (k) was converted into a covariate of function $(\varphi_i(P_1))$. Considering n knots in T_i points ($i = 1,...,n$), and $T_i \leq t < T_i + 1$, the covariables can be obtained at i and $i+1$ knots, through the equations: $\varphi_i(t) = \frac{T_{i+1} - t}{T_{i+1} - T_i}$ and $\varphi_{i+1}(t) = 1 - \frac{T_{i+1} - t}{T_{i+1} - T_i}$, respectively. For other values out of the T_i and T_{i+1} interval, $\varphi_i(t) = 0$. Assuming that the observed value for the standard age corresponds to the fitted knot, $\varphi_i(T_k) = \begin{cases} 1 & se \quad i = k \\ 0 & se \quad i \neq k \end{cases}$. The RRM with seven knots adjusted in different ages can be described as:

$$y_{ijk} = test_j + \sum_{h=1}^{7} \varphi_h(S_k) b_h + \sum_{h=1}^{7} \varphi_h(S_k) a_{i_h} + \sum_{h=1}^{7} \varphi_h(S_j) p_{i_h} + e_{ijk}$$

where y_{ijk} represents the weight of animal i in test j at age k; 7 denotes the number of knots; $\varphi_h(S_k)$ represents the linear spline h that refers to age k; b_h, is the h^{th} coefficient of regression that is associated with t age k on weight; a_{i_h}, is the h^{th} coefficient of the additive genetic random regression h for animal i; p_{i_h}, is the h^{th} individual permanent

environment random regression coefficient h for animal i; and e_{ijk}, the error associated with each observation.

For the RRM model: $\underset{\sim}{\beta}\sim$constant ,$\underset{\sim}{a}|A, G_0 \sim N(0, G_0 \otimes A)$, $\underset{\sim}{p}|P, \sim N(0, P_0 \otimes I)$ and $e|R \sim N(0, R)$. Inverted Wishart distributions were assumed for genetic covariance matrices (7 x 7), being $G_0\left(G_0 \sim IW(\Sigma_a^2, n_a)\right)$ and $P_0\left(P_0 \sim IW\left(\Sigma_p^2, n_p\right)\right)$, where: Σ_a^2, Σ_p^2, $n_a$and n_p represent hyperparameters of the inverted Wishart distributions. It was considered heterogeneity of the residual variance with $R = \text{diag}\{\sigma_e^2\}$, where σ_e^2, is the residual variance for BW330, BW385, BW440, BW495 and BW550. The residual variance presented in this paper corresponded to the sum of the permanent effect and the residual environment effect.

Samples with full conditional posterior distributions were obtained through the Gibbs sampler using the software GIBBS3F90 (Misztal et al., 2014). Chains of 610000 iterations were considered, with a burn-in of 10000 iterations and samplings at every 200 iterations. The chain size was determined in a preliminary analysis following the method described by Raftery and Lewis (1992), available in BOA package (Smith, 2005). The convergence of chains was evaluated through the criterion proposed by Geweke (1991) available in the same package and through inspection of the sampled values.

Genome-wide Association Study (GWAS)

The genomic breeding values and the SNPs solutions were obtained by the same animal model (STM or RRM) which the covariance components were estimated and then, the variance components were fixed as obtained in previous analyses. The single-step genomic BLUP method (ssGBLUP- (Aguilar et al., 2011, 2010; Misztal et al., 2009)) and

the ssGWAS (genome-wide association study using a single-step BLUP (Vitezica et al., 2011; Wang et al., 2012)) were used.

To estimate the genomic breeding values the covariance matrix of a and e can be described as:

$$\text{var}\begin{bmatrix} a \\ p \\ e \end{bmatrix} = \begin{bmatrix} H\sigma_a^2 & 0 & 0 \\ 0 & W\sigma_p^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

where, σ_a^2, σ_p^2 and σ_e^2 are the components of additive genetic, permanent environment and residual variances for each trait, respectively; I, and W an identity matrix; and H, the relationship matrix comprising information of genotyped and non-genotyped animals, as described by Aguilar et al. (2010). Here, it is necessary to highlight that, the permanent environment effect was adjusted only to RRM. In addition, for RRM these procedures were performed for the solutions of the regression coefficients, which for linear spline are given directly without need any prior transformation (Misztal, 2006).

The inverse of H can be described as:

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

where A^{-1} represents the inverse of the additive relationship matrix; A_{22}^{-1}, the inverse of the additive relationship matrix considering only genotyped animals; G^{-1}, the genomic relationship matrix estimated according to VanRaden et al. (2009). Subsequently, the estimated breeding values for genotyped animals (GEBV) were converted to SNP effects, and the equation for predicting SNP effects was as described in Wang et al. (2012). The analyses were performed through Gibbs sampler with the software GIBBS3F90 (Misztal et al., 2014). The chain length, discard and sampling were the same as used in the multi-trait analyses, as well as the adopted convergence criterion. Additionally, the genomic breeding values were estimated by BLUPF90 (Misztal et al., 2014) and the effects of

SNPs were calculated through POSTGSF90 (Wang et al., 2012). The results of GWAS were described with the proportion of variance explained by genomic windows with approximately 0.5 Mb. In this approach, adjacent SNPs within 0.5Mb were used, and their variance was assumed for obtaining the total variance of the window. Additionally, only non-overlapping windows which explained at least 1% of the additive genetic variance were considered, avoiding a double count.

Identification of positional and functional candidate genes

In order to recover the positional candidate genes inserted within the windows that explained at least 1% of the additive genetic variance, we used the R/GALLO package (Fonseca et al., 2020) considering the latest assembly of the bovine genome ARS-UCD1.2.

The analysis of prioritization of candidate genes was conducted through the software GUILDify 2.0 (Aguirre-Plans et al., 2019) and ToppGene (Chen et al., 2009). First, a list of training candidate genes associated with keywords (Body weight, protein, muscle, obesity, growth and growth factors) was obtained for each of the six traits by using GUILD framework (Guney and Oliva, 2012) to determine the relevance of known gene products related to given keywords. The gene products were searched at BIANA knowledge base and used to construct a species-specific network (for the present study we used *Homo sapiens* as the model species). Then, by using a prioritization algorithm based on the network topology for classifying the genes, the top-100 classified genes obtained in this analysis were used to build a list of trained genes.

Further, this list of trained genes was taken to the software ToppGene (Chen et al., 2009) together with the list of positional candidate genes recovered through the R/GALLO package (Fonseca et al., 2020). The software ToppGene- Gene Prioritization

(Chen et al., 2009) was used for performing a prioritization analysis based on annotations through a multivariate approach based on fuzzy (diffuse) logic. The functional information shared between the list of trained genes and the list of positional candidate genes was used to perform a multivariate analysis. These functional data were recovered from the following sources: terms of gene ontology; molecular function; biological process; cell component; human and mouse phenotypes; metabolic pathways; works in Pubmed; Transcription factor binding site; coexpression and disease patterns. by using statistics of meta-analysis, p-values were obtained in a random sampling of 5000 genes from the whole genome for each annotation information, and then combined in a global p-value. A false discovery rate (FDR) of 5% of multiple correction (p-valor ≤ 0.05) was enforced and the genes with p-values ≤ 0.05 were shown as functional candidate genes.

4.4. Results

For STM in DB100, there was overlapping of the highest density interval with 90% of samples (HPD) between all ages for additive variance and for heritability (Table 4.2). For residual variance, there was an increase of the values in the two last ages, BW495 and BW550 and HPD overlap between them. The values of phenotypic variance increased since BW385 until BW550 and there was a short HPD overlap between BW385 and BW440 and between BW495 and BW550.

When RRM was performed for DB100, the additive variances were similar between ages BW330, BW385 and BW440 and there was an increase between BW330 and the last two ages, BW495 and BW550 (Table 4.2). The residual variances for BW330 and BW385 were similar, but it increased from BW440 until BW550. However, residual HPD for BW495 and BW550 overlapped. Heritability HPD of body weight at all ages overlapped.

The variance components for STM estimated by DB100 and DB70 were different after the first selection was performed (Table 4.2). On the other hand, in general, the variance components were similar between the DB100 and DB70, when the RRM was performed (Table 4.2).

Table 4.2 Posterior means and lower and upper limits of the highest posterior density intervals with 90% of samples (in brackets) for genetic ($\boldsymbol{\sigma_a^2}$), residual ($\boldsymbol{\sigma_e^2}$) and phenotypic ($\boldsymbol{\sigma_p^2}$) variances and heritability (h^2) for body weight at 330, 385, 440, 495 and 550 days of age (BW330, BW385, BW440, BW495 and BW550, respectively) estimated by single trait and random regression models for complete database (DB100) and selection database (DB70)

	Genetic parameters			
	Single trait		Random regression	
Ages	DB100	DB70	DB100	DB70
			σ_a^2	
BW330	84.29 (51.33, 118.10)	84.29 (51.33, 118.10)	131.44 (88.29, 180.60)	120.13 (82.25, 157.90)
BW385	98.08 (60.07, 135.80)	35.27 (10.60, 57.36)	157.12(114.80, 207.70)	144.83 (98.29, 190.20)
BW440	113.23 (70.25, 155.80)	24.57 (14.45, 46.94)	200.80 (143.30, 259.80)	159.33 (108.90, 213.30)
BW495	145.75 (90.47, 200.50)	36.61 (6.23, 74.70)	285.69 (209.70, 367.20)	282.83 (185.40, 392.20)
BW550	172.55 (110.30, 236.90)	56.37 (15.92, 100.30)	429.50(330.30, 540.10)	357.23 (217.00, 484.50)
			σ_e^2	
BW330	299.82 (270.50, 328.40)	299.82 (270.50, 328.40)	338.16 (298.24, 375.67)	314.92 (105.00, 162.10)
BW385	327.90 (297.60, 362.90)	226.51 (203.60, 248.50)	366.65 (328.29, 399.27)	268.00 (277.60, 349.60)
BW440	398.76 (361.50, 435.90)	264.67 (237.80, 291.90)	589.92 (536.17, 643.48)	597.68 (227.98, 306.50)
BW495	475.62 (431.00, 523.50)	254.02 (218.10, 291.70)	710.83 (643.79, 785.68)	605.42 (523.19, 668.05)
BW550	542.04 (489.60, 594.60)	209.45 (166.80, 251.30)	752.29 (669.36, 837.76)	658.41 (505.71, 712.05)
			σ_p^2	
BW330	384.12 (365.57, 403.98)	384.12 (365.57, 403.98)	469.60 (439.97, 499.86)	435.05 (407.80, 463.00)
BW385	425.98 (405.60, 448.60)	267.78 (247.15, 276.52)	520.76 (490.68, 555.17)	412.83 (377.86, 447.24)
BW440	512.00 (487.10. 537.20)	289.24 (270.56, 307.57)	790.72 (744.51, 841.81)	757.02 (694.67, 820.86)
BW495	621.39 (587.86, 650.50)	290.63 (267.14, 315.15)	996.51 (934.69, 1053.76)	888.25 (800.66, 971.22)
BW550	714.60 (678.90, 751.30)	265.83 (237.74, 291.54)	1181.79 (1115.68, 1248.29)	1015.64 (897.00, 1135.10)
			h^2	

BW330	0.22 (0.14, 0.30)	0.22 (0.14, 0.30)	0.28 (0.20, 0.35)	0.28 (0.21, 0.34)
BW385	0.23 (0.14, 0.31)	0.13 (0.05, 0.22)	0.30 (0.23, 0.37)	0.35 (0.27, 0.43)
BW440	0.22 (0.13, 0.27)	0.08 (0.07, 0.16)	0.25 (0.19, 0.31)	0.21 (0.16. 0.26)
BW495	0.23 (0.15, 0.32)	0.13 (0.01, 0.24)	0.27 (0.23, 0.35)	0.31 (0.23, 0.40)
BW550	0.24 (0.16, 0.32)	0.21 (0.06, 0.36)	0.36 (0.30, 0.43)	0.35 (0.25, 0.44)

The correlation between both estimated breeding values (EBV) and genomic estimated breeding values (GEBV) for DB100 or DB70, when STM was performed, were positive and high to moderate for body weight at 330 days old (BW330 – Figure 4.1). For the others evaluated periods, the correlations were of low magnitude and with values ranging from positive to negative in the different ages. On the other hand, when RRM were used the correlations were positive and varied from moderate to high magnitudes for all the ages

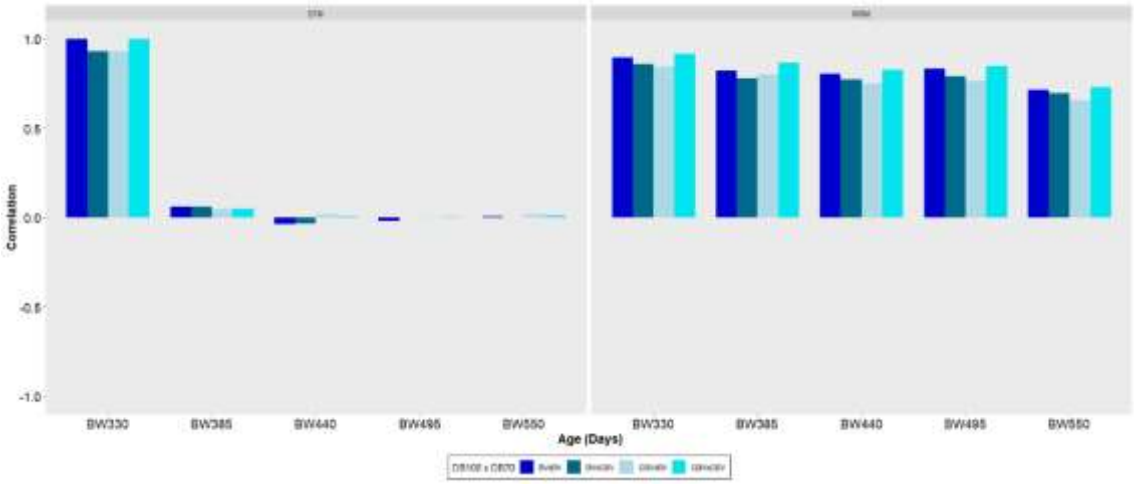

Figure 4.1: Correlation between estimated breeding values (EBV) and genomic estimated breeding values (GEBV) for body weight in complete (DB100) and selected (DB70) databases when single trait model (STM) or random regression model (RRM) were used in the analysis.

There was difference between genomic regions associated with body weight over the animal growth for DB100 when STM was performed (Figure S4.1 and Table S4.1). The same is true for RRM (Figure 4.2). After the selection simulation (DB70), the genomic regions associated with body weight for each age were, in general, different from

those associated to the same age when selection was not simulated to both models STM (Figure S4.1 and Table S4.1) and RRM (Figure 4.2).

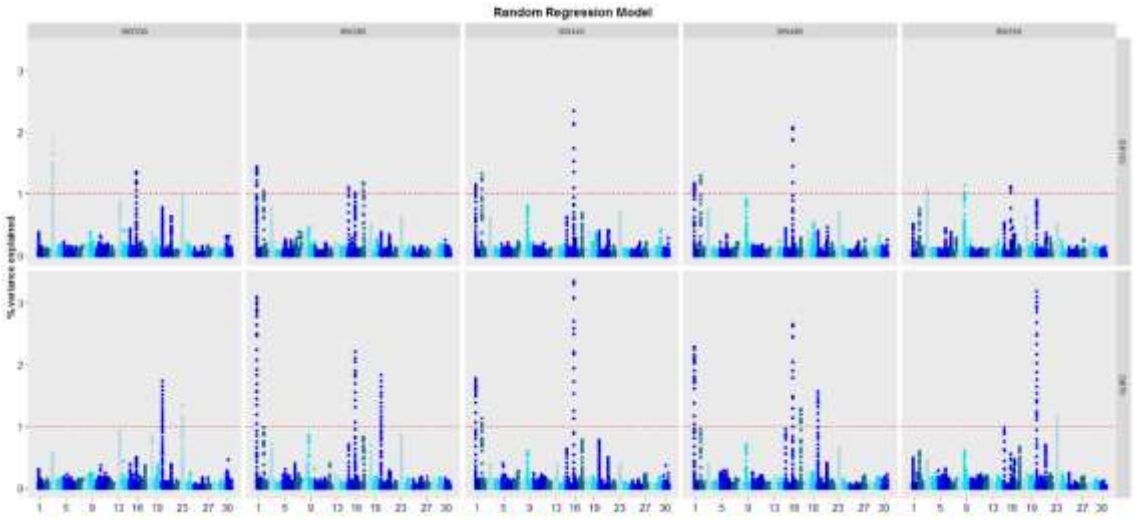

Figure 4.2. Manhattan plots for percentage of variance explained by genomic windows (0.5Mb) for body weight in five different ages (330, 385, 440, 495, and 50 days) for complete (DB100) and selected databases (DB70), when the random regression models were performed. The red line indicates the threshold (windows that explained at least 1% of the additive genetic variance), for which the windows were considered associated to the body weight.

As it shown on the previous results the STM was not suitable to develop both genetic evaluations and GWAS to longitudinal traits under sequential selection, given the inconsistencies in the results when the sequential selection was simulated. Thus, the following results related to the gene prioritization analyses were presented just to the RRM.

For DB100, there were different genomic windows that explained at least 1% of the genetic variance for body weight along the animal's growth, since some of these

windows were specific of each age (Table 4.3). For example, there were genomic windows associated only to BW385 in chromosomes 15 and 17. The same is true for chromosome 9 which contained a significant window only for BW550. Thus, it could be suggested that different genes are more relevant to growth in different stages of animal's life. On the other hand, in chromosome 16 there were similar genomic windows that explained more than 1% of genetic variance for all ages. It is important to highlight that, even though the window of chromosome 16 was not exactly the same for the different ages, there is an overlap between them, and the SNPs contained within these windows were, in general, the same. This can indicate that an important gene can contribute to control of body weight in all stages, here evaluated, of the growth of the animal.

Most of the genomic windows appointed as explicative for a portion of the genetic variance in the complete database (DB100) were not found in the selection database DB70 (Table 4.3). For example, for BW550 no genomic window that explained, at least, 1% of the genetic variance were similar between DB100 and DB70. For BW330, of the three genomic windows associated to body weight, only one was similar between DB100 and DB70. For BW385 and BW495 only two windows (chromosome 1 and 16) were similar between DB100 and DB70. On the other hand, for BW440, all three windows associated with body weight were similar between DB100 and DB70. It is important to highlight that, new genomic windows were associated with body weight in different ages when the selection was simulated, likely as a consequence of the simulated selection effect.

Table 4.3. Genomic Windows (0.5Mb) description, which explained at least 1% of variance for body weight at five different ages (BW330, BW385, BW440, BW495, and BW550 days) for complete database (DB100) and select database (DB70) estimated by random regression model

Chr	Pos_s	Pos_e	SNPs	%Var	Pos_s	Pos_e	SNPs	%Var
	DB100				DB70			
				BW330				
3	24735772	25234801	82	1.90				
16	32676738	33110685	44	1.35				
20					21946439	22423318	56	1.73
23	39323760	39680863	51	1.00	39323760	39680863	51	1.35
				BW385				
1	131320440	131804990	40	1.44	131198050	131617330	46	3.10
2	126650991	127124043	25	1.05				
15	23650235	24099827	41	1.11				
16	24913318	25374981	47	1.05	24913318	25374981	47	2.21
17	56171681	56663231	19	1.17				
20					21946439	22423318	56	1.83
				BW440				
1	131198050	131617330	46	1.15	131198050	131617330	46	1.78
2	126650991	127124043	25	1.33	126650991	127124043	25	1.13
16	24735772	25234801	44	2.35	24913318	25374981	47	3.35
				BW495				
1	131198050	131617330	46	1.18	131310669	131804990	41	2.29
2	126650991	127124043	25	1.29				
16	24735772	25234801	44	2.08	24913318	25234801	47	2.65
17					56171681	56663231	19	1.28
				BW550				
3	32676738	33110685	82	1.10				
9	10384530	10817832	58	1.14				
16	24913318	25374981	47	1.12				
20					21946439	22423318	56	3.16
23					39323760	39680863	51	1.16

Chr = Chromosome; Pos_s = position in base pair of start of the window; Pos_e = position in base pair of end of the window; SNPs = number of SNPs within of the window; %Var = percentage of genetic variance explained for the window

As a consequence of the differences in the associated genomic regions to body weight for each age in DB100, the FCG associated with body weight along the animal growth also can be different between different ages (Table 4.4). For example, whereas only the FCG DUSP10 was associated to weights in all of the measured ages, the other

FCG appear to be specific to each age. The LAMTOR5 gene was appointed as FCG to BW330 and BW550. The genes PAFAH2, SLC30A2 and TRIM63 were FCG for three different ages (BW385, BW440 and BW495) and the FCG SLC16A4 only for BW550.

Different FCG were associated with body weight, to each age, after the simulation of sequential selection (DB70 x DB100). Only the FCG DUSP10 was associated with BW385, BW440 and BW495 in both databases, while all other FCG were different between both databases.

Table 4.4. Positional and functional* candidate genes for body weight at five different ages (BW330, BW385, BW440, BW495, BW550) when the random regression model was performed

	DB100	DB70
Chr	Candidate genes	Candidate genes
	BW330	
3	CYM, KCNA2, LAMTOR5*; PROK1, SLC16A4	-
16	DUSP10 *	-
20	-	MAP3K1*, MIER3, GPB1
23	KIF13A, NHLRC1, TPMT, DEK	DEK, KIF13A*, NHLRC1, TPMT
	BW385	
2	EXTL1, PAFAH2*, SLC30A2*, SRRM4, TRIM63*	-
15	NCAM1*, TTC12	-
16	DUSP10*	DUSP10*
20	-	GPB1, MAP3K1*, MIER3
	BW440	
2	CEP85, EXTL1, PAFAH2*, PDIK1L, SLC30A2*, TRIM63*, ZNF683	CEP85, EXTL1, PAFAH2*, PDIK1L, SLC30A2*, TRIM63*, ZNF683
16	DUSP10*	DUSP10
	BW495	
2	CEP85, EXTL1, PAFAH2*; PDIK1L, SLC30A2*; TRIM63*, ZNF683	-
16	DUSP10*	DUSP10*
17	-	SRRM4
20	-	GPB1, MAP3K1*, MIER3
	BW550	
3	CYM, KCNA2, LAMTOR5*, PROK1, SLC16A4*	-
9	OGFRL1	-
16	DUSP10*	-
20	-	GPB1, MAP3K1*, MIER3
23	-	DEK, KIF13A*, NHLRC1, TPMT

*Functional Enrichment FDR ≤0.05

4.5. Discussion

Animal growth was previously defined as an increase of protein, fat, and bone over time and depends on several factors including genetic, nutritional and hormonal (Owens et al., 1995). Given the complexity of metabolic events that occur along the growth, it is reasonable that there was some difference in the set of loci that control the body weight when evaluated in different ages. Here, we showed that the sets of FCG which contribute to genetic control of body weight over time are not the same. Similar results were found for growth traits in Hereford and Braford cattle on what four growth traits (birthweight, weaning weight adjusted to 205 days, yearling weight adjusted to 550 days age and postweaning weight gain adjusted for 345 days of age) were evaluated and the majority of the genomic windows and consequently the candidate genes associated to each trait were different between the traits (Campos et al., 2019). Further, our results suggested that, even for a short period of animal growth, the set of loci which take part of the growth genetic control may be different.

On the other hand, there are important genes which can contribute to genetic control of body weight along all five ages here evaluated, such as the DUSP10 gene. This gene was functionally associated with body weight for all ages in DB100. The DUSP10 (dual specificity phosphatase 10) gene was associated with the control of brown adipocytes. Overexpression of this gene resulted in lower lipid accumulation than that in cells overexpressing the inactive mutant DUSP10 (Choi et al., 2013). The amount of brown adipose tissue in adult humans has been found to be highly correlated with their degree of obesity (Frühbeck et al., 2009). Taken together with other important genes, the gene DUSP10 can contribute to higher capability of adipogenesis and proliferation in Wagyu than in Holstein cattle (Huang et al., 2017). In Simmental beef cattle, the DUSP10 gene was also associated with carcass weight (Chang et al., 2018). Therefore, we suggest

that the DUSP10 gene may be considered an important gene which contributes to genetic control of important growth stages in beef cattle.

After the sequential selection simulation, it was shown that the genomic regions associated to body weight may not be the same as those appointed when no selection was simulated, even with RRM was performed. The inconsistencies between GWAS results for the same growth trait evaluated under selection or not can be a consequence of the selection effect and not of the real association with phenotype. This may be one more factor that also contributes to frequent inconsistencies in GWAS results performed for the same trait measured in animal production under sequential selection.

There has been great progress in GWAS in domestic animals and some genes for economically important traits have been identified. However, the main problem lies in the inconsistencies among the results of these GWAS reports for the same trait (Zhang et al., 2012). The replication studies show that only a small portion of associated loci in the GWAS can be replicated, even within the same populations, and factors such as inconsistencies between SNP arrays and between genotype calling algorithms are potential sources for the lack of reproducibility in GWAS results (Hong et al., 2010). It is important to highlight that, here, we suggest that the sequential selection is only one more factor which can contribute to these inconsistences and lack of reproducibility in animal herds which are under sequential selection.

The majority of FCG that were associated with body weight in some age for DB100, as DUSP10 previously characterized, were not associated with body weight at the same age after the selection was simulated. For example, the DUSP10 gene was functionally associated with body weight for all ages when there was no selection. On the other hand, after sequential selection, this gene was not associated with BW330 and BW550. The same is true for LAMTOR5 associated with BW330 and BW550 in DB100

but not associated with body weight at the same ages in DB70, for the genes PAFAH2, SLC30A2 and TRIM63 not associated in DB70 with BW385 and BW495, for the gene NACAM1 with BW385 and for the gene SLC16A4 with BW550.

Most of the genes which were FCG for body weight at some age in DB100 and were FCG in DB70 (DUSP10, LAMTOR5, PAFAH2, SLC30A2, TRIM63, NCAM1 and SCL16A4) were associated, in previous studies, to some postweighing growth trait. The PAFAH2 (Platelet Activating Factor Acetylhydrolase 2) gene was related to lipid metabolism and fatty acid composition and associated, by differential expression, with intramuscular beef fatty acids in Nellore (Berton et al., 2016). The TRIM63 (Tripartite Motif Containing 63) gene was also identified by differential expression in the muscle of Nellore bulls, and was indicated as a positional candidate for beef tenderness and related directly with skeletal muscle functions and muscle constituents (Muniz et al.; 2020). The TRIM63 gene was used as a reference gene of target transcripts for study of RNA-seq of muscle from pigs divergent in feed efficiency and, as a result, it presented significant differences in mRNA abundances (Horodyska et al., 2018).

Signatures of selection overlapped with QTL terms "meat and carcass" were associated to the NCAM1 gene (Neural cell adhesion molecule 1) in dual purpose Gyr cattle (Maiorano et al., 2018). In Holstein Friesian bulls, the same gene was differentially expressed in M. longissimus dorsi following compensatory growth and re-alimentation (Keogh et al 2016). The SLC16A4 (Solute carrier family 16 member 4) gene was associated with carcass merit traits after an enrichment of biological functions for a beef cattle population (Wang et al., 2020). For Hereford and Braford beef cattle, the genes SLC16A4 and LAMTOR5 (Late endosomal/lysosomal adaptor, MAPK and MTOR activator 5) were appointed as candidate gene for post weaning gain adjusted for 345 days of age (Campos et al., 2019).

In this way, we would like to highlight two points. First, there are important genes which contribute to genetic control of body weight along all five ages here evaluated, as exemplified by the DUSP10 gene. On the other hand, the importance of each gene for animal growth can change in different development stages and different genes can be more relevant to body weight in each growth stage for beef cattle. Second, the use of models that consider a covariance structure are still an alternative for genetic evaluations of longitudinal traits under sequential selection, since the estimated genetic parameters are no different between DB100 and DB70. Also, moderate to high correlation values between EBV and GEBVs are shown here. However, when GWAS and post GWAS are performed, we showed that the sequential selection can influence results, and this may be one more reason for frequent inconsistences in GWAS results performed for growth traits measured in beef cattle. Furthermore, it is necessary to validate these findings for each gene in each trait and age in larger populations and other breeds to improve the understanding about the selection effects for growth traits, especially the functional candidate genes suggested in the present work.

4.6. Conclusion

There are important genes which contribute to genetic control of body weight along all five ages here evaluated, as the DUSP10 gene. On the other hand, the importance of each gene for animal growth can change in different development stages and different genes can be more relevant to body weight in each growth stage for beef cattle, even for a short period of animal growth.

The pre-selection which often occurs in commercial beef cattle can contribute to inconsistencies in GWAS results and consequently on the identification of FCG between studies, even when models that consider a covariance structure are used.

4.7. Supplementary Material

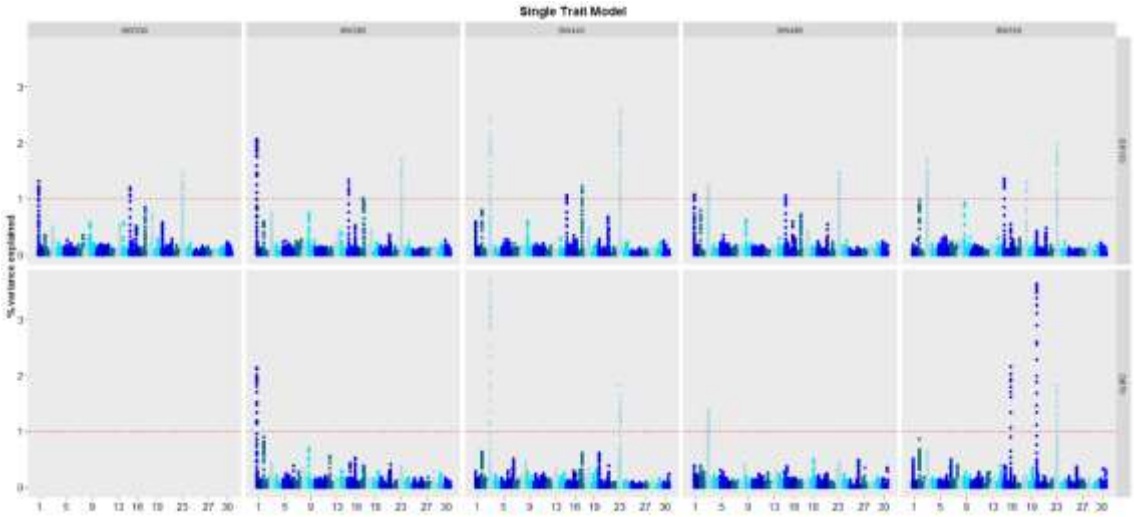

Figure S4.1. Manhattan plots for percentage of variance explained by genomic windows (0.5Mb) for body weight in five different ages (BW330, BW385, BW440, BW495, BW550) for complete (DB100) and select database (DB70) when the single trait model was performed. The red line indicates the threshold from which the windows were considered as significantly associated to the body weight. For BW330, the parameters' estimates of DB100 and DB70, so the Manhattan plots for both DB100 and DB70.

Table S4.1. Description of Genomic Windows (0.5Mb) that explained at least 1% of genetic additive variance for body weight in five different ages (BW330, BW385, BW440, BW495, BW550) for complete database (DB100) and select database (DB70) estimated by the single trait model

Chr	Pos_s	Pos_e	SNPs	%Var	Pos_s	Pos_e	SNPs	%Var
	DB100				DB70			
				BW330				
1	131310669	131804990	41	1.32				
15	23650235	24099827	41	1.20				
23	39323760	39680863	51	1.47				
				BW385				
1	131310669	131804990	41	2.06	131198050	131617330	46	2.13
15	23650235	24099827	41	1.34				
17	56219805	56717042	20	1.00				
23	39323760	39680863	51	1.72				
				BW440				
3	32676738	33110685	82	2.46	32676738	33110685	82	3.70
15	23650235	24099827	41	1.07				
17	56219805	56717042	20	1.23				
23	39323760	39680863	51	2.58	39148251	39639650	51	1.80
				BW495				
1	131198050	131617330	46	1.08				
3	32676738	33110685	82	1.24	32727140	33224436	79	1.39
15	23650235	24099827	41	1.05				
23	39323760	39680863	51	1.46				
				BW550				
3	32676738	33110685	82	1.70				
15	23650235	24099827	41	1.37				
16					24735772	25234801	44	2.15
18	64937253	65401508	26	1.31				
20					21989825	22480484	53	3.62
23	39323760	39680863	51	2.00	39323760	39680863	51	1.81

Chr = Chromosome; Pos_s = position in base pair of start of the window; Pos_e = position in base pair of end of the window; SNPs = number of SNPs within of the window; %Var = percentage of genetic variance explained for the window

5. CONSIDERAÇÕES FINAIS

Nossos resultados contribuem para darmos um passo na direção da seleção de animais que possuem alelos favoráveis em genes que, de fato, participam do controle genético de características importantes. Em um primeiro momento, os genes aqui apontados como candidatos funcionais, poderiam ser utilizados na seleção genômica de modo que, pesos mais elevados seriam atribuídos aos SNPs mapeados próximos ou nos genes candidatos funcionais. Assim, associar informação de genes candidatos funcionais ao conhecimento prévio de componentes de covariância e valores genéticos poderia levar ao incremento da eficiência dos programas de melhoramento genético.

A utilização de informação genômica no estudo das inter-relações de caraterísticas de produção, reprodução e de resistência contribuiu para aprofundarmos o conhecimento e esclarecermos teorias que antes não poderiam ser exploradas, como por exemplo, quais são os genes possivelmente pleiotrópicos que contribuem para a correlação genética entre características. No primeiro trabalho, além de apontarmos para genes que são candidatos funcionais para características importantes na bovinocultura de corte (SLC16A4, KCNA2, LAMTOR5, DUSP10, MAP3K1, TPMT e KIF13A), sugerimos que, independente dos valores de correlação genética obtidos exclusivamente por meio de informações fenotípicas e de pedigree, existem genes candidatos funcionais iguais que influenciam as características de produção, reprodução ou resistência dos bovinos de corte. Sugerimos ainda que os genes designados como candidatos funcionais comuns a mais de uma característica, e possivelmente pleiotrópicos, podem ser utilizados como informação auxiliar na composição de índices pelos programas de melhoramento gado de corte.

Nossos resultados apontam ainda para uma questão a respeito dos efeitos da seleção sobre parâmetros genéticos. No segundo trabalho verificamos que, de fato,

modelos que consideram uma estrutura de covariância entre efeitos aleatórios minimizam os efeitos da seleção sobre as estimativas de parâmetros genéticos. Entretanto, quando estudos de associação genômica ampla (GWAS) são realizados por meio desses modelos, ainda sim, pode haver um viés nas regiões genômicas apontadas como associadas à característica em questão e, consequentemente, os genes candidatos funcionais apontados como aqueles que participam do controle genético da característica também são diferentes. Assim, os resultados do GWAS seriam reflexo também dos efeitos da seleção sobre a característica e não apenas da real associação com o fenótipo. Este fato poderia contribuir para inconsistências, muitas vezes verificadas, entre os GWAS para uma mesma característica mesurada em bovinos de corte, uma vez que, a grande maioria dos dados utilizados nas pesquisas e nos programas de melhoramento são impactados pela pré seleção.

Os resultados apresentados no presente trabalho são essenciais para sugerir genes importantes que participam do controle genético de características de interesse zootécnico em bovinos de corte. Além disso, os genes apontados como candidatos funcionais para cada uma das características aqui avaliadas poderiam ser validados em populações maiores e outras raças, a fim de aperfeiçoar a compreensão do controle genético desses genes sobre as características de crescimento, reprodução e sanidade de bovinos de corte.

6. REFERÊNCIA BIBLIOGRÁFICA

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