

UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

**ASSOCIAÇÃO GENÔMICA AMPLA DE PRODUÇÃO DE
LEITE E CARACTERÍSTICAS LINEARES DE
CONFORMAÇÃO EM VACAS DA RAÇA GIR DA COLÔMBIA
E O BRASIL**

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Médico Veterinario Zootecnista

2023

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E O BRASIL**

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Orientador: Prof. Dr. Josineudson Augusto Il Vasconcelos Silva

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Ciências Agrárias e Veterinárias – Unesp,
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Impacto potencial desta pesquisa

As estimativas de herdabilidade neste estudo indicaram que as características de conformações poderiam responder à seleção. Além disso, as correlações entre essas características e produção de leite ressaltaram a importância de considerar a resposta correlacionada à seleção, sinergias e compensações entre essas variáveis. Finalmente, foram identificadas regiões no genoma e genes candidatos associados à produção de leite e às características de conformação. Esta informação é relevante para a criação seletiva de gado Gir, visando o aumento da produção de leite em ambientes tropicais.

Potential impact of this research

The heritability estimates of this study indicated that body conformations traits could respond to selection. Moreover, the correlations between these traits and milk yield highlighted the importance of considering the correlated response to selection, synergies, and trade-offs between these variables. Finally, genomic regions and candidate genes associated with milk yield and conformation traits were identified. This information is relevant for the selective breeding of Gir cattle, aiming to increase milk yield in tropical environments.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: ASSOCIAÇÃO GENÔMICA AMPLA DE PRODUÇÃO DE LEITE E CARACTERÍSTICAS LINEARES DE CONFORMAÇÃO EM VACAS DA RAÇA GIR DA COLÔMBIA E O BRASIL

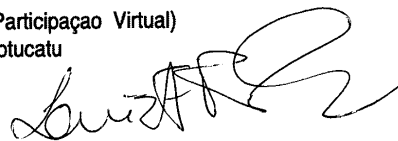
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DADOS CURRICULARES DO AUTOR

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ASSOCIAÇÃO GENÔMICA AMPLA DE PRODUÇÃO DE LEITE E CARACTERÍSTICAS LINEARES DE CONFORMAÇÃO EM VACAS DA RAÇA GIR DA COLÔMBIA E O BRASIL

RESUMO – A Gir é uma raça de gado *Bos indicus* adaptada às regiões tropicais que tem sido intensamente selecionada para a produção de leite no Brasil. Seus programas de seleção também consideram características lineares de conformação corporal, pois elas têm sido associadas à saúde, ao bem-estar e à longevidade das vacas, afetando assim a produtividade. Por tanto, o objetivo deste estudo foi estimar parâmetros genéticos para características lineares de conformação e produção de leite, além de realizar um estudo de associação genômica ampla (GWAS) para identificar regiões genômicas e genes candidatos associados a essas características em gado Gir leiteiro da Colômbia e o Brasil. As características lineares de conformação foram classificadas como características de "dimensão corporal", "pés e pernas" e "sistema mamário". Após a imputação do genótipo e o controle de qualidade, 42.105 SNPs estavam disponíveis para análise e 24.489 vacas com informações de pedigree tinham fenótipos. Modelo animal univariado e bivariado utilizando inferência bayesiana foram usados para estimar os componentes de variância, utilizando informação do pedigree e genótipos. Para o GWAS, foi empregado o método WssGBLUP uni-característica, utilizando janelas genômicas móveis de 0,11Mb, definidas com base no desequilíbrio de ligação. As 10 principais janelas que explicam a maior porcentagem de variação genética aditiva foram selecionadas para anotação de QTL e genes. As estimativas de herdabilidade para as características lineares de conformação variaram de 0,08 a 0,35, e para produção de leite foi de 0,23. A altura na garupa e características do sistema mamário como profundidade do úbere e largura do úbere posterior foram geneticamente correlacionadas com produção de leite. As regiões genômicas identificadas em nosso trabalho se sobrepuseram com QTLs previamente descritos como relacionados à produção de leite, altura do corpo, largura do úbere e profundidade do úbere. No total, 16 genes foram anotados como possíveis genes candidatos. Desses genes, o PIK3R5 e o PIK3R6 já haviam sido relacionados ao tamanho do corpo do gado. Vários dos genes identificados estão envolvidos em características de metabolismo de lipídios, sobrevivência, reprodução e produção de leite. Com base nas estimativas de herdabilidade e correlações obtidas, é importante considerar a possível resposta correlacionada à seleção, sinergias entre as características lineares de conformação e a produção de leite no gado Gir. Além disso, as regiões genéticas identificadas e os genes candidatos estabelecem uma plataforma fundamental para estudos futuros que ajudam a entender as vias biológicas que afetam as características estudadas em bovinos da raça Gir.

Palavras-chave: *Bos indicus*, características morfológicas, gado de leite, genes candidatos, GWAS, parâmetros genéticos

GENOME WIDE ASSOCIATION OF MILK YIELD AND LINEAR CONFORMATION TRAITS IN GIR COWS FROM COLOMBIA AND BRAZIL

ABSTRACT - The Gir is a breed of *Bos indicus* cattle adapted to tropical regions that have been intensively selected for milk production in Brazil. Its selection programs also consider linear body conformation traits, as these have been associated with the health, welfare, and longevity of cows, thus affecting productivity. Therefore, the objective of this study was to estimate genetic parameters for linear conformation and milk yield, in addition to carrying out a genome-wide association study (GWAS) to identify genomic regions and candidate genes associated with these traits in Gir dairy cattle from Colombia and Brazil. Linear conformation traits were classified as "body size", "feet and legs" and "mammary system". After genotype imputation and quality control, 42,105 SNPs were available for analysis and 24,489 cows with pedigree information had phenotypes. Univariate and bivariate animal models using Bayesian inference were used to estimate variance components, using pedigree and genotype information. For GWAS, the single-trait WssGBLUP method was used, using moving genomic windows of 0.11Mb, defined based on linkage disequilibrium. The top 10 windows explaining the highest percentage of additive genetic variation were selected for QTL and gene annotation. Heritability estimates for linear conformation traits ranged from 0.08 to 0.35, and for milk production, it was 0.23. Hip height and traits related to the mammary system, as udder depth and rear udder width were genetically correlated with milk yield. The genomic regions identified in our work overlapped with QTLs previously described to be related to milk yield, body height, udder width, and udder depth. In total, 16 genes were noted as possible candidate genes. Of these genes, PIK3R5 and PIK3R6 have previously been linked to body size in cattle. Several of the identified genes are involved in traits of lipid metabolism, survival, reproduction, and milk production. Based on the heritability estimates and correlations obtained, it is important to consider the possible correlated response to selection and synergies between linear conformation traits and milk yield in Gir cattle. Furthermore, the identified genetic regions and candidate genes establish a fundamental platform for future studies that help to understand the biological pathways that affect the studied traits in Gir cattle.

Keywords: *Bos indicus*, morphological traits, dairy cattle, candidate genes, GWAS, genetic parameters

LISTA DE ABREVIATURAS

AC	Ângulo do casco
AG	Ângulo da garupa
ALT	Altura na garupa
BTA	<i>Bos taurus</i> autosomes
CT	Comprimento de tetos
DT	Diâmetro de tetos
ESS	Effective sample size
FA	Foot angle
GWAS	Estudo de associação ampla do genoma
HG	Heart girth
HH	Hip height
LD	Linkage disequilibrium
LU	Largura do úbere
MAF	Minor allele frequency
MY	305-day cumulative milk yield
PL	Produção de leite acumulada aos 305 dias
PLT	Pernas traseiras-vista lateral
PT	Perímetro torácico
PU	Profundidade do úbere
QTL	Quantitative trait loci
RA	Rump angle
RLS	Rear legs-side view
RU	Rear udder width
TD	Teat diameter
TL	Teat length
UD	Udder depth
WssGBLUP	Weighted single-step genomic approach

CAPÍTULO 1 - Considerações gerais

1 Introdução

Na pecuária leiteira em áreas tropicais e subtropicais, como o Brasil e a Colômbia, onde predominam os sistemas de produção extensivos e com forragens de baixa qualidade, as raças taurinas (*Bos taurus taurus*) não conseguem expressar seu potencial produtivo que apresentariam se estivessem produzindo em clima temperado. Entretanto, as raças zebuínas (*Bos taurus indicus*), por seus ancestrais terem desenvolvidos em áreas semelhantes, apresentam adaptação tanto ao clima quanto ao sistema de produção, tornando-se necessárias na produção de leite nos trópicos (Santana Jr et al., 2014).

Entre os zebuínos, a raça Gir tornou-se relevante na cadeia produtiva do Brasil por sua rusticidade, longevidade produtiva, e produção leiteira (Santana Jr et al., 2014). De acordo com Toro et al. (2023), a raça Gir e seus cruzamentos são material genético consolidado para a sustentação da pecuária de países tropicais e subtropicais, demonstrando a importância do estudo da raça, visando aumentar a eficiência de produção de alimento para a população.

O Gir leiteiro brasileiro é o resultado de processo de seleção que começou após as primeiras importações de gado Gir no ano de 1870 provenientes da Índia. A seleção dos reprodutores por mais de 50 anos foi realizada de forma empírica, no entanto, em 1985 a Associação Brasileira de Criadores Gir Leiteiro (ABCGIL) junto com a Embrapa Gado de Leite começaram “Programa Nacional de Melhoramento do Gir Leiteiro”, com o objetivo principal de promover o melhoramento da produção, conformação e manejo no Gir leiteiro (Fernandes et al., 2019). Esta constatação fundamenta o crescente interesse por distintos países pela genética produzida no Brasil.

Inicialmente os programas de melhoramento genético na raça Gir foram direcionados para aumentar a produção de leite (Prata et al., 2015). No entanto, para otimizar a produção, incluindo a produção de leite e a vida produtiva, faz necessário que o indivíduo tenha estrutura morfológica e condição corporal capaz de manter a produção e permanecer no rebanho (Almeida et al., 2017; Saowaphak et al., 2017).

De acordo com Saowaphak et al. (2017) e Fernandes et al. (2019), em programas de melhoramento genético de gado leiteiro, as características de conformação são extremamente importantes, desde estejam focadas na promoção de animais mais eficientemente produtivos.

As diferentes medidas corporais em bovinos tem sido objeto de estudo durante anos e estas variáveis classificadas como quantitativas, estão estritamente ligadas à saúde do indivíduo, produtividade e vida útil (An et al., 2019). Além disto, características de conformação estão correlacionadas com caracteres de importância econômica como facilidade de parto, longevidade, laminites e eficiência produtiva (Abo-Ismael et al., 2017). A avaliação de características lineares de conformação em bovinos leiteiros visa selecionar indivíduos com harmonia entre produção e funcionalidade, permitindo aumentar a permanência dos indivíduos no rebanho e facilitando o manejo. Neste sentido, variáveis de tipo são necessárias em programas de melhoramento com o fim de identificar indivíduos melhor adaptados às condições nos sistemas de produção, evitando o aumento dos custos na substituição dos animais.

A estimativa dos parâmetros genéticos e a relação entre as características de conformação e produção de leite podem apresentar variações entre raças. A maior parte da pesquisa disponível sobre essas estimativas concentra-se em raças taurinas, sendo a raça Holandês, provavelmente a mais estudada. Portanto, entender como as características de conformação corporal e a produção de leite se correlacionam geneticamente, é importante para os programas de melhoramento em gado Gir.

Com o advento de tecnologias como a genotipagem e o sequenciamento completo do genoma bovino, tem surgido oportunidades na produção animal por meio das análises genômicas. Essas abordagens oferecem a possibilidade de compreender mecanismos subjacentes às manifestações de características complexas (Scott et al., 2007). De acordo com Carreño et al. (2019), o sequenciamento do genoma bovino disponibilizou informação que pode ser utilizada na predição de valores genéticos por meio da seleção genômica e na localização de regiões ou genes associados com fenótipos de interesse por meio de estudo de associação ampla do genoma (GWAS). Conhecer as regiões genômicas influenciando a expressão de características lineares

de conformação em bovinos Gir leiteiro favoreceria a seleção de reprodutores permitindo explorar a pecuária de maneira mais eficiente.

Estudos de GWAS fornecem abordagem robusta para descrever efeitos fenotípicos ou mapear características de loci quantitativos (QTL) de variáveis leiteiras importantes no genoma, utilizando para isto marcadores de polimorfismo de nucleotídeo único (SNP) (Cole et al., 2011). A detecção de QTL fornece informação relevante que pode facilitar a compreensão dos mecanismos genéticos subjacentes aos fenótipos, além disto, fornece a detecção de polimorfismos causais que permitem acelerar o processo de melhoramento genético usando a seleção genômica (Meuwissen et al., 2001; Vanraden, 2008)

Em bovinos, estudos de associação têm focado nas variáveis de maior importância econômica, incluindo características de produção, reprodução e resistência a doenças (Wu et al., 2013). Diversos estudos de GWAS tem sido reportados em bovinos leiteiros, incluindo o estudo de 17 características funcionais e de tipo em touros holandeses do Canada e utilizando SNPs de genes candidatos (Kolbehdari et al., 2008); o estudo de associação de 18 variáveis de conformação corporal, classificadas em seis grupos, tamanho corporal, forma corporal, úbere, tetos, pernas e pés, em vacas holandesas dos Estados Unidos com painel de 50k (Cole et al., 2011); o estudo em vacas de primeiro parto da raça Holandês na China, avaliando 21 características de conformação linear (Wu et al., 2013); o trabalho realizado com fêmeas Simental na Alemanha, associando 12 QTLs relacionados na conformação da glândula mamária (Pausch et al., 2016); e associação ampla do genoma de 16 variáveis de conformação em vacas holandesas no México (Gonzalez et al., 2017).

Estes estudos forneceram informações consideráveis sobre QTLs, contribuindo para construir um consenso sobre os efeitos em bovinos leiteiros (Cole et al., 2011). No entanto, é importante notar que a maioria dos estudos até o momento tem se concentrado na raça Holandesa. Devido à distância genética entre essa raça e as raças zebuínas, como a Gir, existe preocupação de que os conhecimentos adquiridos possam não ser diretamente aplicáveis ao gado Gir. Além disso, é relevante observar a ausência de estudos de associação genômica relacionados às características lineares de conformação específicas da raça Gir leiteira.

O objetivo deste estudo foi estimar parâmetros genéticos para características de conformação e produção de leite, e realizar um GWAS para identificar regiões genômicas e genes candidatos associados a essas características em gado Gir leiteiro da Colômbia e do Brasil.

2 Revisão de Literatura

2.1 A raça Gir

A raça Gir (*Bos taurus indicus*) destaca-se pela sua capacidade de produção de leite a pasto em ambientes tropicais. Devido à sua origem na Índia, esta raça apresenta alta tolerância ao estresse térmico e resistência a diversas parasitas e doenças tropicais, o que a tornou bem aceita no Brasil após o início das importações em 1870 (Santana Jr et al., 2014).

Em 1985, foi estabelecido o Programa Nacional de Melhoramento do Gir Leiteiro (PNMGL), com o objetivo de promover o melhoramento genético da raça por meio da identificação e seleção de animais geneticamente superiores para as características de produção de leite, conformação e manejo (Fernandes et al., 2019). Em 1993 foi publicado o primeiro resultado da avaliação genética de leite e gordura, envolvendo um grupo de nove touros Gir (Santana Jr et al., 2014). No ano seguinte (1994), iniciou-se a avaliação de características lineares de conformação nas vacas Gir puras, e em 2001, iniciou-se a coleta de sangue e sêmen com o objetivo de construir um banco de DNA.

A partir de 2016, a seleção de touros jovens para o teste de progênie foi auxiliada pela incorporação de informações genômicas. Em 2018, a seleção genômica foi incluída no programa de melhoramento genético do Gir Leiteiro. Segundo Fernandes et al. (2019) a utilização de touros com base nas avaliações genéticas desde 1993 (ano em que o primeiro resultado da avaliação genética foi disponibilizado) tem levado à melhoria da produção leiteira, largura do úbere, facilidade de ordenha e temperamento.

A base genética da raça Gir da Colômbia é de origem brasileiro. Os primeiros exemplares da raça foram importados do Brasil há mais de três décadas por meio das

associações de criadores (ASOCEBU, 2023). Nas importações mais recentes, há uma preferência por touros e matrizes de alto valor genético, com o objetivo de aprimorar a produção nas fazendas colombianas. Devido a que não existe um programa de melhoramento genético nacional para a raça. Atualmente, a associação de criadores de zebu na Colômbia (ASOCEBU) está envolvida em atividades como controle leiteiro, mensuração de características de conformação e acompanhamento na seleção de animais por parte dos criadores de Gir na Colômbia. Devido á origem da raça Gir na Colômbia existe uma forte relação com os rebanhos Brasileiros (Toro Ospina et al., 2021). Estes autores avaliaram o parentesco entre rebanhos Gir na Colômbia e o Brasil, utilizando a matriz de parentesco genômico. Eles identificaram uma forte associação entre as populações, justificando que essa relação decorre do uso de sêmen de touros Gir brasileiros pelos criadores da raça na Colômbia.

2.2 Características lineares de conformação

A produção de leite tem sido considerada como a característica de maior relevância em programas de melhoramento de bovinos leiteiros. No entanto, a seleção exclusiva para esta variável pode acarretar impactos desfavoráveis em outras características funcionais, como a fertilidade e saúde do rebanho (Pérez-Cabal et al., 2006; Lagrotta et al., 2010).

As características lineares de conformação influenciam características economicamente importantes, como produção de leite, saúde e longevidade. Por exemplo, as características de conformação relacionadas ao úbere estão associadas com a saúde da vaca (Dube et al., 2009). Características de conformação relacionadas à capacidade corporal, como altura e perímetro torácico, estão associadas à eficiência alimentar e ao balanço energético (Søndergaard et al., 2002). Além disso, o ângulo da garupa e profundidade do úbere foram associadas à longevidade (Lund et al., 1994; Vollema et al., 2000). Portanto, mensurar características lineares de conformação em rebanhos leiteiros pode contribuir na rentabilidade dos rebanhos.

A avaliação linear em bovinos leiteiros teve origem na América do Norte, concentrando-se inicialmente na raça holandesa. Inicialmente, esse método envolvia avaliação subjetiva para identificar os animais mais produtivos no rebanho, com base

em medidas de conformação e características corporais. No entanto, maior ênfase foi dada no controle leiteiro. Com o decorrer dos anos, a avaliação linear progrediu, incluindo atribuição de pontuações para descrever as características de forma mais precisa, e mais tarde, foi integrada à avaliação genética (Short e Lawlor, 1992; McManus e Saueressig, 1998). No Brasil, a avaliação linear teve início em 1994, começando com a raça Gir Leiteiro (Panetto et al., 2022).

A avaliação linear em vacas de leite é realizada de forma individual, abrangendo observações e medidas corporais que são confrontadas com um padrão considerado como ideal para cada raça. Essa abordagem tem grande benefício para os criadores e técnicos, viabilizando a avaliação de variáveis que podem influenciar no bem-estar, saúde, e longevidade dos animais.

As características lineares de conformação podem ser classificadas como: dimensão corporal, estrutura de pés e pernas e sistema mamário. No contexto da raça Gir, as características, medidas e critérios de avaliação apresentados a seguir têm como embasamento o padrão utilizado no Programa Nacional de Melhoramento do Gir Leiteiro Brasileiro, que se fundamenta no trabalho de Panetto et al. (2022).

As características de conformação como altura na garupa (ALT), perímetro torácico (PT), e ângulo da garupa (AG) fazem parte do grupo de variáveis relacionadas à dimensão corporal. ALT e o PT são mensurados em centímetros. ALT corresponde ao comprimento do animal desde o topo da garupa até o chão (Figura 1a). São desejadas matrizes com mais de 136 cm de altura (Panetto et al., 2022). O PT está relacionado à capacidade torácica e abdominal (Figura 1b), envolvendo importantes órgãos como o coração, pulmões e sistema digestivo que são de grande importância para o desempenho do animal. Animais com medida para PT superiores a 175 cm são desejáveis (Panetto et al., 2022). Por outro lado, o AG é mensurado por meio da inclinação entre ílios e ísquios e são atribuídos escores (de 1 até 9) para avaliar esta variável (Figura 1c). Valores intermediários são desejados.

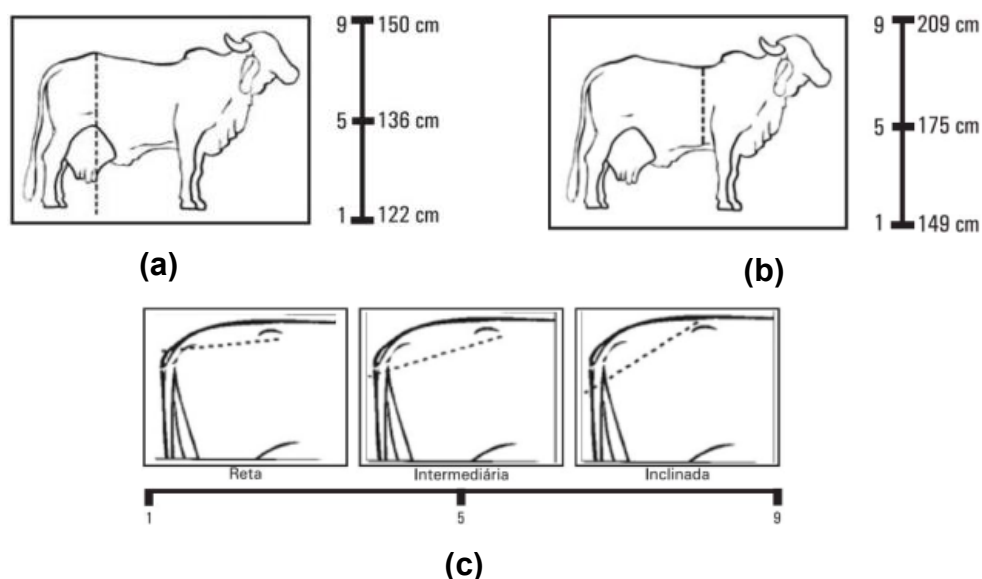


Figura 1. Características de conformação relacionadas à dimensão corporal: altura na garupa (a), perímetro torácico (b), e ângulo da garupa (c). Adaptado de Panetto et al., (2022).

Dentro da categoria estrutura de pés e pernas, podem ser incluídas as características ângulo do casco (AC) e as pernas traseiras-vista lateral (PTL). Ambas as características são avaliadas por meio de escores. O AC é medido pelo ângulo formado entre duas linhas imaginárias, a primeira paralela à superfície do solo e a segunda à parede do casco (1, baixo e 9, inclinado; Figura 2a). Quanto a PTL corresponde à angulação formada entre os ossos da tíbia e do metatarso (1, reto e 9, curvo; Figura 2b). Valores intermediários para a categoria de pés e pernas são desejáveis. Estas características têm sido relacionadas com lesões no casco e tempo de permanência no rebanho (Sewalem et al., 2004; Chapinal et al., 2013).

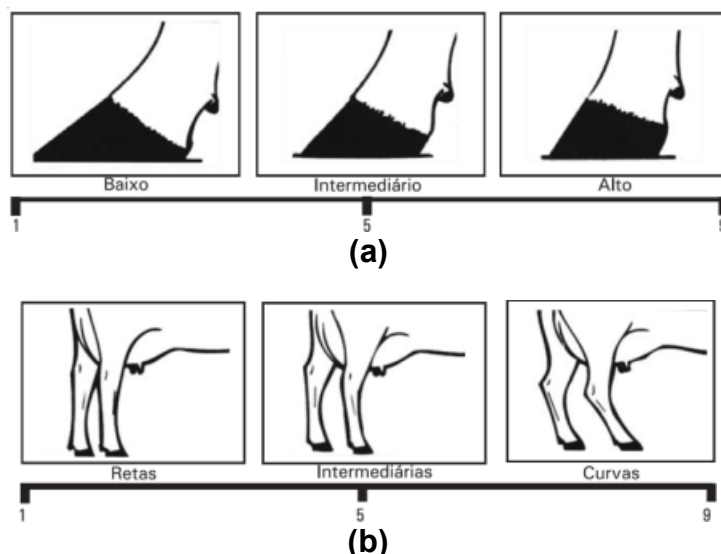


Figura 2. Características de conformação relacionadas à estrutura de pés e pernas: ângulo do casco (a) e pernas traseiras-vista lateral (b) (Panetto et al., 2022)

A categoria sistema mamário está composta por variáveis relacionadas ao úbere e aos tetos. Entre estas podem ser destacadas características como: largura do úbere posterior (LU), profundidade do úbere (PU), comprimento de tetos (CT) e diâmetro de tetos (DT). A LU é a distância entre a junção da perna e o úbere do lado esquerdo até a mesma junção no lado direito (1, estreito e 9, largo; Figura 3a). Para mensurar o PU é preciso observar a distância entre a articulação tíbio-társica ou jarrete e o ponto mais baixo do assoalho do úbere (1, raso e 9, profundo; Figura 3b). O úbere ideal apresenta o seu assoalho a aproximadamente 10 cm acima do jarrete.

Quanto a características relacionadas aos tetos, são desejáveis tetos de comprimento intermediários (aproximadamente 7,5 cm; Figura 3c) e de diâmetro intermediário para baixo (Figura 3d). Características do sistema mamário têm sido associadas com incidência de mastites (Dube et al., 2009; Bharti et al., 2015). Da mesma forma, características relacionadas aos tetos desempenham papel importante na prevenção da mastite e na ordenha mecânica. Tetos longos e grossos apresentam dificuldades durante a ordenha mecânica e para a alimentação dos bezerros, além de serem suscetíveis a lesões, o que pode comprometer a saúde do úbere (Dube et al., 2009).

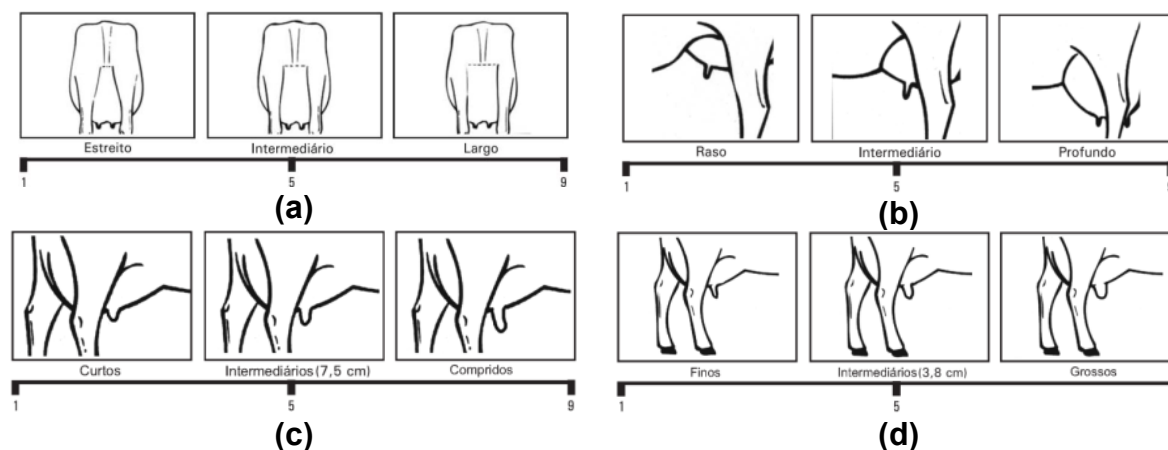


Figura 3. Características de conformação relacionadas ao sistema mamário: largura do úbere posterior (a), profundidade do úbere (b), comprimento de tetos (c) e diâmetro de tetos (d) (Panetto et al., 2022)

2.3 Parâmetros genéticos para produção de leite e características lineares de conformação

Ao longo dos anos, houve interesse considerável em investigar os parâmetros genéticos relacionados as características lineares de conformação em vacas de leite, bem como suas interconexões com a produção de leite. A principal literatura sobre essas estimativas provém das raças *Bos taurus taurus*, principalmente da raça Holandesa. Por exemplo, estudos foram conduzidos nas raças, Holandesa (DeGroot et al., 2002; Berry et al., 2004; Campos et al., 2015), Pardo Suíço (De Haas et al., 2007; Samoré et al., 2010) e Jersey (Dube et al., 2009). No entanto, a literatura referente a essas características em gado *Bos taurus indicus* é escassa, sendo o Gir a principal raça utilizada para conduzir essas pesquisas (Lagrotta et al., 2010; Wenceslau et al., 2000; Carvalho et al., 2021).

Compreender como as características lineares de conformação respondem a seleção e como estas estão associadas com a produção de leite é uma ferramenta fundamental para os programas de melhoramento genético. Para esse propósito, são estimados os parâmetros genéticos como herdabilidade e correlação genética. Tais estimativas podem apresentar variações consideráveis dependendo da raça, populações e do modelo estatístico. Portanto, a avaliação destes parâmetros dentro da população-alvo é fundamental para determinar os critérios de seleção em programas de melhoramento genético.

Na categoria de características relacionadas à dimensão corporal, estudos têm indicado estimativas de herdabilidade mais elevadas em comparação com os demais grupos de características lineares de conformação. Dentro dessa categoria, a altura na garupa geralmente apresenta a estimativa de herdabilidade mais elevada, embora a estimativa apresente variação consideravelmente entre estudos (variando de 0,31 a 0,74) (De Haas et al., 2007; Campos et al., 2015; Carvalho et al., 2021). Outra característica que também apresenta variação entre as estimativas reportadas na literatura é o ângulo da garupa, com valores variando de 0,13 a 0,36 (Abo-Ismael et al., 2017; Olasege et al., 2019; Wu et al., 2013). Por outro lado, baixa variação foi obtida nas estimativas de herdabilidade quando comparados resultados entre estudos anteriores que investigaram a característica PT na raça Gir (estimativas de 0,23 e 0,30; Carvalho et al., 2021; Lagrotta et al., 2010). Estes valores mostram que as estimativas de herdabilidades para características relacionadas a dimensão do corpo tendem a ser de moderadas a altas, sugerindo que estas características devem responder à seleção de forma mais rápida.

As características que compõem a categoria de estrutura de pés e pernas apresentam estimativas de herdabilidade de baixa magnitude com valores variando de 0,06 a 0,14 para AC (Abo-Ismael et al., 2017; Carvalho et al., 2021; Olasege et al., 2019) e de 0,10 a 0,16 para PTL (Carvalho et al., 2021; Čítek et al., 2022; Djedović et al., 2023), indicando alta influência dos efeitos ambientais na sua expressão.

Para as características da categoria do sistema mamário, observa-se variabilidade nas estimativas de herdabilidade na literatura, com valores distintos entre as diversas características. Por exemplo, para PU e CT, constata-se faixa mais ampla de variação, com valores variando de 0,15 a 0,41 e 0,18 a 0,44, respectivamente, como reportado em estudos anteriores (Abo-Ismael et al., 2017; Carvalho et al., 2021; Olasege et al., 2019; Wu et al., 2013). No caso da LU, embora exista variação, está apresenta escala mais restrita, situando-se entre 0,13 e 0,21 (Carvalho et al., 2021; Olasege et al., 2019; Wu et al., 2013). Devido a que o DT não é uma característica de interesse em raças taurinas, escassa informação foi observada para esta variável na literatura. No entanto, avaliando a raça Gir no Brasil, foram obtidas estimativas de herdabilidade variando de 0,07 e 0,24 para o DT (Carvalho et al., 2021; Lagrotta et al., 2010; Wenceslau et al., 2000); evidenciando ampla variação entre as estimativas

reportadas para esta variável. É válido salientar que, mesmo ao se considerar a mesma raça e o mesmo país, as discrepâncias nos resultados podem ser atribuídas à variação no tamanho das amostras e modelo estatístico utilizado nas análises.

Características relacionadas com a produção de leite tem sido enfatizada na seleção de bovinos leiteiros devido a sua destacada importância econômica. As estimativas de herdabilidade para produção de leite acumulada aos 305 dias (PL) em gado Gir variam de 0,24 a 0,45 (Lagrotta et al., 2010; Toro-Ospina et al., 2022; Wenceslau et al., 2000). Estas estimativas indicam possibilidade de obter ganhos genéticos para produção de leite no Gir.

As estimativas de herdabilidade para características lineares de conformação relacionadas ao sistema mamário e dimensão corporal, bem como para produção de leite, sugerem que é possível obter mudanças por meio da seleção de animais. Por outro lado, no caso de características da categoria estrutura de pés e pernas, devido à sua baixa estimativa de herdabilidade, as mudanças resultantes da seleção seriam menos pronunciadas. Portanto, para estas variáveis, mudanças no ambiente teriam impacto maior do que a seleção genética.

A estimativa de correlação genética entre características lineares de conformação e produção de leite indica associação entre as variáveis. A correlação genética tem implicações para o uso de características de conformação em programas de melhoramento de gado de leite as quais podem variar entre raças. Estimativas de correlação genética entre PL e características relacionadas à dimensão corporal em raças taurinas, revelam coeficientes positivos que variam em magnitude, com valores variando de 0,21 a 0,61 para ALT, de 0,16 a 0,39 para PT e de 0,12 a 0,62 para AG (Campos et al., 2015; DeGroot et al., 2002; De Hass et al., 2007). Isto indica que animais com maiores medidas de dimensão corporal (maiores valores para ALT e PT) tendem a produzir mais leite. Por outro lado, estudos em gado Holandês e Gir apresentaram estimativas de correlação genética nulas entre PL com as características ALT (valores entre -0,08 e -0,02) e PT (valores entre -0,17 e -0,11) (Campos et al., 2015; Lagrotta et al., 2010), sugerindo que os genes para dimensão corporal não influenciam a produção de leite.

Em relação às características da categoria estrutura de pés e pernas, estudos tem indicado estimativas de correlação genética com a PL variando consideravelmente. Por exemplo, em animais da raça Holandesa, as estimativas variaram de -0,05 a 0,39 para PL e AC, e de 0,04 a 0,83 entre PL e PTL (Campos et al., 2015; DeGroot et al., 2002; Djedovic et al., 2023). Na raça Gir, Lagrotta et al. (2010) descreveram estimativas com valores de 0,11 para PL e AC e 0,30 para PL e PTL.

A correlação genética entre produção de leite e características lineares de conformação relacionadas ao sistema mamário varia consideravelmente na literatura, com valores entre -0,46 (entre PL e PU) a 0,61 (entre PL e LU), como evidenciado em estudos realizados em raças Holandês e Sahiwal (Campos et al., 2015; Khan et al., 2016). Em relação ao gado Gir, Lagrotta et al. (2010) reportaram estimativas de correlação entre PL e as características do sistema mamário CT e DT, com valores de 0,13 e 0,26, respectivamente. Essas associações genéticas são indesejáveis, pois sugerem que a manutenção da ênfase na seleção de animais para produção de leite pode resultar em tetos grossos e alongados, dificultando o processo de ordenha e a amamentação dos bezerros.

Dessa forma, a estimativa das correlações genéticas entre a produção de leite e as características lineares de conformação assume grande importância no desenvolvimento de programa eficaz de melhoramento genético para a população alvo. Portanto, considerar os parâmetros genéticos e, incluí-los como critérios de seleção, poderiam possibilitar alcançar os objetivos de seleção adequados em populações.

2.4 Estudo de Associação Genômica Ampla (GWAS)

As avaliações genéticas em gado leiteiro vêm incluindo informação genômica há mais de uma década (Hayes et al., 2009). Os dados genotípicos permitiram implementar a metodologia GWAS para identificar SNPs associados a características de interesse econômico (Sahana et al., 2023). O GWAS assume que genes ou mutações não identificadas, responsáveis por características de relevância econômica, estão em desequilíbrio de ligação com um SNP ou um grupo de SNPs (Cantor et al., 2010). Isso implica que estas duas regiões estão fisicamente conectadas, e como resultado, tendem a ser segregadas juntas na população.

O principal propósito do GWAS é identificar associações significativas entre variantes genéticas e os fenótipos de cada característica estudada, estimando os efeitos de cada variante, visando detectar as variantes candidatas (Sahana et al., 2023). Os resultados obtidos através do GWAS podem viabilizar a aplicação de modelos complexos que vão além dos efeitos aditivos, elucidar a arquitetura genética de características específicas e, finalmente, decifrar a biologia subjacente de características complexas (Sahana et al., 2023).

Duas metodologias comuns para analisar todas as variantes genéticas simultaneamente em GWAS são a metodologia BLUP genômica de etapa única (ssGBLUP) e a abordagem Bayesiana. Os métodos Bayesianos utilizando abordagem não-linear, podem considerar que o SNP explica diferentes proporções da variação genética (por exemplo, BayesA) ou que a maioria dos SNPs não tem efeito, deixando a variação genética total ser compartilhada entre alguns SNPs principais (por exemplo, BayesB) (Lourenco et al., 2017). Em contraste, o método GBLUP utiliza uma abordagem linear que pressupõe que todos os SNPs explicam a mesma quantidade da variação genética (Lourenco et al., 2017). No entanto, Wang et al. (2012) propuseram uma maneira de calcular e atribuir variâncias diferentes para cada SNP (WssGBLUP), fornecendo estimativas de valores genéticos com precisão semelhantes aos que usam o procedimento bayesiano (Su et al., 2014).

Devido à sua facilidade de uso, o ssGBLUP, é cada vez mais adotado para realizar análises de associação em bovinos e suínos (Bernal Rubio et al., 2016; Aguilar et al., 2019). Estudos de GWAS tem sido relatados na literatura para diferentes espécies de animais de produção. Em bovinos leiteiros, esses estudos foram conduzidos para identificar variantes ou genes associados a características de conformação linear (Schnabel et al., 2005; Wu et al., 2013; Wang et al., 2022) e produção de leite (Bennewitz et al., 2004; Cole et al., 2011; Ning et al., 2018). No entanto, é importante destacar a necessidade desses estudos também em gado zebuino, já que a maioria das pesquisas tem sido realizadas em gado taurino.

Por meio da aplicação da abordagem GWAS na raça Gir, diversos estudos identificaram e anotaram potenciais genes candidatos associados à produção de leite (Toro-Ospina et al. 2023), bem como as características reprodutivas, como o número

de oócitos e embriões (Rocha et al. 2023). Recentemente, Braga et al. (2023) identificaram regiões de variação do número de cópias, que potencialmente afetam genes relacionados a características de produção, saúde e reprodução em bovinos Gir. Apesar desses esforços recentes, até o momento, não tem sido publicados estudos de GWAS para características de conformação corporal na raça Gir.

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CAPITULO 2 - Genetic parameters for milk production and body conformation traits in Dairy Gir cattle¹

ABSTRACT - Gir is a tropically adapted breed of *Bos indicus* cattle that have been intensively selected for milk production in Brazil. Their selection programs also consider body conformation traits as these have been associated with health, welfare, and cow longevity, thus affecting productivity. This study focused on Gir cattle to estimate genetic and phenotypic parameters for body conformation traits as well as their correlations with milk production traits. Body conformation traits were classified as “frame”, “feet and leg”, or “mammary system” traits for this study. After genotyping imputation, and quality control, 42,105 polymorphisms were available for analyses and 24,489 cows with pedigree information had phenotypes. Univariate and bivariate animal models were used to estimate genetic parameters, benefiting from both genotypes and pedigree data. The heritabilities for body conformation traits ranged from a low of 0.08 to a medium value of 0.35, and the heritability for milk production was 0.23. We identified genetic correlations between pairs of body conformation traits and between them and milk production. Height and udder traits were genetically correlated with milk production. Given the heritabilities and correlations reported here, it is important to consider the possible correlated response to selection, synergies, and trade-offs between body conformation traits and milk production in Gir cattle. This information is relevant to selective breeding of Gir to enhance tropical milk production.

Keywords: body conformation traits, bovine, genetic correlation, heritability, milk yield, tropical dairy cattle, phenotypic correlation, selection.

1 Introduction

The search for productive and efficient animals in dairy cattle leads to selecting cows by milk yield and morphological structure suitable to maintain production and permanence in the herd. Morphological structure or body conformation traits have been found to be related to health, welfare, and longevity (Sewalem *et al.* 2004; Kern *et al.* 2015). Deficiencies in body conformation traits steer to lower production and health problems, therefore causing premature removal of cows from the herd and decreased profitability. In addition, body conformation traits have medium to moderately high estimates of heritability (Wiggans *et al.* 2004; De Haas *et al.* 2007; Carvalho *et al.* 2021). Their heritability adds to the relevance of body conformation traits in the selective breeding of dairy cattle.

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Body capacity traits, such as stature and heart girth, were related to body weight (Heinrichs *et al.* 1992; Mäntysaari 1996), which is an important functional trait associated with feed efficiency and energy balance (Søndergaard *et al.* 2002). Traits that describe the cow's rump have been associated with calving ease and uterus prolapse (Nogalski and Mordas 2012; Sawa *et al.* 2013). Feet and leg traits were genetically associated with hoof lesions (Sewalem *et al.* 2004; Chapinal *et al.* 2013). Conformation traits of the mammary system have been genetically correlated with mastitis incidence and somatic cell counts (Dube *et al.* 2009; Bharti *et al.* 2015). Altogether, these associations with production and health traits demonstrate that body conformation traits can influence lifespan and milk yield in dairy cattle, and therefore they are of economic importance (Abo-Ismael *et al.* 2017).

Estimating genetic parameters and the relationship between conformation and production traits might be different for different breeds of cattle. Genetic correlations have implications for the use of body conformation traits in dairy cattle breeding programs that might differ with the breed. The main body of literature on these estimates is from *Bos taurus taurus* breeds, mainly the Holstein breed, which does not adapt to tropical climates. Indicus dairy breeds such as the Gir cattle are relevant because of their thermal tolerance and resistance to parasites, which are desirable to produce milk under tropic conditions. Therefore, understanding how body conformation traits and milk yield correlate, including the potentially correlated response to selection, is important for all breeds and need to be studied in each one. This study focused on Gir cattle and aimed to estimate genetic and phenotypic parameters for body conformation traits as well as their correlations with milk production traits.

2 Materials and Methods

The study was conducted in accordance with animal welfare guidelines that follow the State Law No. 11.977 of the State of São Paulo, Brazil. All animal procedures were approved by the Ethics and Animal Handling Committee of the São Paulo State University (UNESP), Botucatu, SP, Brazil.

2.1 Dataset and editing

According to definitions set by the Brazilian Dairy Gir National Breeding Program (Panetto et al. 2022), seven linear body conformation traits were scored from 1 to 9, and two were measured in centimeters. In the present study, body conformation traits were divided in three groups, namely the “frame”, the “feet and leg”, and the “mammary system” groups. The frame group included three descriptors: hip height (HH), heart girth (HG), and rump angle (RA). The feet and leg group included two traits, foot angle (FA) and rear legs-side view (RLS). The mammary system traits were four: rear udder width (RU), udder depth (UD), teat diameter (TD), and teat length (TL). The production trait 305-day cumulative milk yield was recorded in kilograms. The details and descriptive statistics of the traits analysed are presented in Table 1. For more details about how body conformation traits are measured, see Table 1A.

Table 1. Descriptive statistic for body conformation traits and 305-day cumulative milk yield

Trait	Abbr	Units/Ideal Score*	N	Min	Max	Mean	SD	Median	Mode
Production									
Milk yield	MY	kg	38,996	209	15,355	3,566.5	1,975.5	3,183.5	2,087
Frame									
Hip height	HH	cm	1,062	103	180	134.1	7.08	135	135
Heart girth	HG	cm	1,055	134	210	176.6	9.71	176	170
Rump angle	RA	≤ 5	1,062	2	9	-	-	6	6
Feet and leg									
Foot angle	FA	5	1,060	2	9	-	-	5	5
Rear legs-side view	RLS	5	1,060	2	8	-	-	5	5
Mammary system									
Rear udder width	RU	9	916	2	9	-	-	5	5
Udder depth	UD	5	1,043	1	8	-	-	5	5
Teat diameter	TD	≤ 5	1,043	2	9	-	-	5	5
Teat length	TL	5	1,044	1	9	-	-	5	5

Abbr: abbreviations; Units: Units of measurement; N: number of records; Min: minimum value for the evaluated trait; Max: maximum value for the evaluated trait; SD: mean standard deviation by trait. *Ideal score according to (Panetto *et al.* 2022).

The dataset contained 1,105 body conformation traits' evaluations (cow age at scoring ranged between 20 and 263 months) and 38,996 records of 305-day cumulative milk yield (cows aged at calving ranged between 21 and 262 months) from

24,489 Gir cows born between 1969 and 2020. The pedigree file used in the analyses for all traits was constituted by 38,571 animals, with each animal traced back to its ancestor as many generations as possible. Body conformation traits were measured on 1,105 cows (one measure per animal) from October 2019 until January 2022 by three expert technicians with previous training in fourteen herds (twelve from Brazil and two from Colombia).

A total of 1,593 animals of both sexes were genotyped using six different commercial SNPs panels (Table 2A). For purposes of this study, the reference population will refer to the animal genotypes with the GGP_Indicus_50k_A1 panel (50K) used to create the library of haplotypes for the imputation from other panels to 50K genotypes. A genotyping quality control (QC) on 50K genotypes was performed in accordance with the following exclusion criteria: a call rate less than 0.9 (for animals or individual SNPs), minor allele frequency (MAF) less than 0.01 and a maximum difference between observed and expected frequency >0.15 (i.e., extreme departure from Hardy-Weinberg equilibrium) and no pedigree conflicts. A total of 51,657 SNPs from the 50K panel distributed over the 29 autosomes passed all QC criteria and were used for the imputation of the target population. These same QC criteria were applied to the other genotype panels. All raw genotypes were mapped to the ARS-UCD1.2 reference genome.

Genotype imputation was performed with the FImpute program (Sargolzaei *et al.* 2014) using the pedigree file. First, the SNPs in the HD panel that were not in common with the reference panel (50K) were eliminated. Low-density genotypes and HD common SNPs to the reference panel were imputed to the 50K using a reference set of 100 animals (Table 2A). After imputation, an additional QC was performed on imputed genotypes using the preGSf90 software (Aguilar *et al.* 2010). The filters applied were the same as used in the previous QC, with the difference that the MAF criteria was <0.05 . After QC, 42,105 SNPs and 1,593 animals were kept for further analysis.

2.2 Statistical analysis

Before the analyses, the effects and interactions that could influence production and body conformation traits were tested using the least square method with the GLM procedure available in the R software (R 2022). If the effects of its interactions were significant at a significance level equal to 5% ($P < 0.05$), they were included in the mixed model to evaluate the traits studied.

For body conformation traits, the effects of year and season of birth, year and season of evaluation, evaluator, and herd were tested. However, the year and season of evaluation were not significant, probably due to the distribution of the number of observations in relation to the year and season of measure. As for the effect of the evaluator, it was probably not significant because three experienced and trained technicians performed the body conformation measurements on the cows. Therefore, these effects were not included in the model. On the other hand, the interaction of calving year, calving season and herd were statistically significant for the production trait 305-day cumulative milk yield. Therefore, these effects were considered to generate the contemporary groups. Records above or below 3.5 standard deviations from their contemporary group mean were removed, and contemporary groups with less than three observations were discarded from the dataset.

Variance and covariance components of production and body conformation traits were estimated through univariate and bivariate animal models, respectively, using GIBBSF90+ software (Misztal *et al.* 2018), which uses a Bayesian approach via the Gibbs sampling algorithm. The model can be represented in matrix notation as follow:

$$y = Xb + Za + Wpe + e$$

where y is a vector of observations for the analysed production and body conformation traits, b is a vector of systematic effects [for MY: contemporary groups (2,165 levels) and age at calving as linear and quadratic covariate; for body conformation traits: herd (fourteen levels), year of birth (fifteen classes), and age at scoring as linear covariate]; a is the vector of random additive animal effects, pe is the vector related to permanent environment random effects (that vector and its incidence matrix were used only for

MY), $\mathbf{e}$ is the vector of random residual effects, and $\mathbf{X}$, $\mathbf{Z}$ and $\mathbf{W}$ are incidence matrices relating records to their respective effects. Due to the structure of the MY data, it was possible to include the permanent environment effect in the model (the average lactation per cow was 1.6).

Posterior means and standard deviations for (co) variance components, heritabilities, and genetic and phenotypic correlations were obtained using the POSTGIBBSF90 (Misztal *et al.* 2018). The analysis consisted of a single chain with a length of 200,000 samples was defined for Gibbs sampling, discarding the first 66,000 samples (about a third of the sample) and collecting samples at every 20 cycles, resulting in 6,700 samples for inference. The convergence was verified with the graphical evaluation of sampled values and by assessing the effective chain size and the Geweke diagnosis. The genotype dataset was included in analyses using the specific option (OPTION SNP_file) of the GIBBSF90+ software. The genotypes were used to include another source of genetic information and thus contribute to obtaining estimates more accurately.

3 Results

3.1 Variance components and heritability

Variance components and heritability estimates in univariate analyses are presented in Table 2. Only those estimates from univariate analyses are shown; because parameters estimated in bivariate analyses, averaged over nine estimates for each of the analysed traits, gave similar values, with one exception. For MY, the estimated heritability with the bivariate model was higher (0.33, instead of 0.23) than univariate analyses (Table 3A). According to the samples of variance posterior densities, the central tendency measures such as mean, mode and median of the later marginal densities of heritability estimates showed similarity for most of the variance components, demonstrating symmetry and convergence (Table 2). The exception was for the additive genetic variance (σ_a^2) of feet and legs traits, where the mode was smaller than the mean and median. The effective sample size (ESS) for σ_a^2 ranged from 14.8 to 16 samples for RLS and FA (Table 2), respectively, which suggested convergence since the minimum ESS expected to reach convergence should be ten

samples (Misztal *et al.* 2018). In addition, the values obtained by the Geweke test were lower than one, indicating that the total chain samples stipulated were adequate for the convergence for all variance components estimated.

The posterior mean estimate of heritability obtained for single trait animal model of the body conformation traits ranged from 0.23 ± 0.07 to 0.33 ± 0.08 for the group of frame traits, 0.08 ± 0.04 and 0.09 ± 0.06 for the feet and legs traits, and from 0.25 ± 0.06 to 0.35 ± 0.07 for the mammary system group (Table 2). The h^2 estimate for MY was 0.23 ± 0.01 . Standard errors for the h^2 of traits studied ranged from 0.01 to 0.08. In general, the lowest estimates of heritability were associated with feet and legs traits.

Table 2. Variance components and heritability estimated in single trait analyses for production and body conformation traits in dairy Gir cattle.

Trait	Parameter	Mean	SE	Median	Mode	HPD 95%	ESS	Geweke diagnosis
Production trait								
MY	σ_a^2	411,760	26,269	411,200	408,170	360,400 - 453,000	2,701.2	0.01
	σ_{ep}^2	502,500	21,840	502,700	505,240	460,700 - 546,400	3,510.3	-0.03
	σ_e^2	857,550	10,514	857,500	855,330	836,900 - 878,000	24,428.9	0.01
	h^2	0.23	0.01	0.23	0.23	0.210 - 0.260	2643.1	0.02
Body conformation trait								
Frame								
HH	σ_a^2	10.51	2.82	10.34	9.33	5.113 - 15.610	46.40	0.04
	σ_e^2	21.12	2.22	21.12	20.37	16.670 - 25.310	64.20	-0.03
	h^2	0.33	0.08	0.33	0.33	0.178 - 0.476	45.90	0.04
HG	σ_a^2	20.41	6.70	19.97	20.42	7.479 - 33.020	17.60	-0.20
	σ_e^2	56.88	5.70	57.01	56.87	45.490 - 64.700	24.70	0.18
	h^2	0.26	0.08	0.26	0.25	0.118 - 0.425	17.30	-0.21
RA	σ_a^2	0.18	0.06	0.17	0.17	0.077 - 0.295	30.00	0.14
	σ_e^2	0.61	0.05	0.61	0.61	0.502 - 0.710	45.20	-0.13
	h^2	0.23	0.07	0.22	0.21	0.101 - 0.360	23.00	0.15
Feet and leg								
RLS	σ_a^2	0.06	0.03	0.06	0.02	0.009 - 0.126	14.80	0.33
	σ_e^2	0.72	0.04	0.72	0.72	0.636 - 0.801	43.20	-0.19
	h^2	0.08	0.04	0.07	0.02	0.010 - 0.158	14.70	0.33
FA	σ_a^2	0.08	0.05	0.07	0.02	0.008 - 0.165	16.00	0.27
	σ_e^2	0.74	0.05	0.74	0.73	0.633 - 0.831	30.80	-0.20
	h^2	0.09	0.06	0.09	0.02	0.010 - 0.200	15.50	0.28
Mammary system								
RU	σ_a^2	0.49	0.11	0.48	0.43	0.305 - 0.700	58.20	-0.19
	σ_e^2	0.91	0.09	0.91	0.93	0.731 - 1.085	98.30	0.15
	h^2	0.35	0.07	0.35	0.38	0.227 - 0.481	60.80	-0.19
UD	σ_a^2	0.35	0.08	0.35	0.36	0.186 - 0.507	52.10	0.04
	σ_e^2	0.81	0.07	0.81	0.81	0.663 - 0.938	93.20	-0.03
	h^2	0.30	0.06	0.30	0.31	0.175 - 0.426	53.20	0.04
TL	σ_a^2	0.34	0.09	0.34	0.38	0.152 - 0.512	28.70	-0.01
	σ_e^2	1.00	0.09	1.00	0.98	0.836 - 1.171	47.70	-0.01
	h^2	0.25	0.06	0.25	0.26	0.127 - 0.381	29.20	-0.01
TD	σ_a^2	0.30	0.08	0.30	0.33	0.138 - 0.444	30.80	-0.01
	σ_e^2	0.83	0.07	0.83	0.84	0.692 - 0.968	57.80	-0.01
	h^2	0.27	0.06	0.27	0.29	0.129 - 0.380	31.10	-0.01

MY: 305-day cumulative milk yield; HH: hip height; HG: heart girth; RA: rump angle; RLS: rear legs-side view; FA: foot angle; RU: rear udder width; UD: udder depth; TL: teat length; TD: teat diameter; σ_a^2 : additive genetic variance; σ_{ep}^2 : permanent environment variance; σ_e^2 : residual variance; h^2 : heritability.

3.2 Genetic and phenotypic correlation among conformation traits

The estimates of genetic and phenotypic correlation between conformation traits are presented in Table 3. The genetic correlation between the group of frame traits showed moderate estimates for HH with RA (0.48) and high estimates for HH with HG (0.71). The phenotypic correlation between them ranged from moderate 0.37 (between HH and HG) to weak 0.01 (between HG and RA). Positive correlations were observed for the mammary system set traits, with the strongest genetic correlation estimated between UR and UD (0.72) and the strongest phenotypic correlation between TL and TD (0.50). On the other hand, estimates of the correlation between feet and leg traits (RLS and FA) were negative, with values from -0.05 and -0.91 for phenotypic and genetic correlation, respectively.

For the correlation between groups of body conformation traits, a range of positive and negative genetic correlations were observed among all the individual traits, with the strongest positive genetic correlation estimated between RLS and TL (0.86) and the strongest negative correlation between FA and UD (-0.48) (Table 3).

Table 3. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) and their respective standard error (parentheses) estimated in bivariate analyses between milk production and body conformation traits in dairy Gir cattle.

Trait	Producti on	Frame			Feet and leg		Mammary system			
	MY	HH	HG	RA	RLS	FA	RU	UD	TL	TD
MY		0.31 (0.13)	0.17 (0.14)	0.24 (0.16)	-0.31 (0.17)	-0.16 (0.16)	0.61 (0.14)	0.63 (0.11)	0.22 (0.15)	0.29 (0.13)
HH	0.15 (0.05)		0.71 (0.15)	0.48 (0.16)	-0.37 (0.19)	0.28 (0.23)	0.25 (0.15)	0.24 (0.16)	-0.12 (0.18)	-0.02 (0.18)
HG	0.15 (0.05)	0.37 (0.03)		0.10 (0.27)	-0.25 (0.21)	-0.06 (0.21)	0.31 (0.24)	0.32 (0.23)	-0.16 (0.19)	0.07 (0.25)
RA	0.09 (0.05)	0.14 (0.03)	0.01 (0.04)		0.20 (0.24)	-0.22 (0.20)	0.51 (0.13)	0.28 (0.17)	-0.25 (0.19)	0.14 (0.19)
RLS	-0.06 (0.05)	-0.08 (0.03)	-0.02 (0.03)	0.02 (0.03)		-0.91 (0.11)	0.08 (0.24)	-0.10 (0.26)	0.86 (0.14)	0.25 (0.27)
FA	-0.04 (0.05)	0.02 (0.03)	0.05 (0.03)	-0.01 (0.03)	-0.05 (0.03)		-0.33 (0.23)	-0.48 (0.23)	-0.26 (0.25)	-0.10 (0.29)
RU	0.27 (0.05)	0.04 (0.04)	0.07 (0.04)	0.08 (0.03)	0.09 (0.03)	-0.03 (0.03)		0.72 (0.11)	0.22 (0.15)	0.53 (0.15)
UD	0.25 (0.05)	0.02 (0.03)	0.06 (0.04)	0.06 (0.03)	0.07 (0.03)	-0.01 (0.03)	0.48 (0.03)		0.02 (0.19)	0.54 (0.13)
TL	0.15 (0.05)	0.03 (0.03)	0.01 (0.03)	-0.01 (0.03)	0.07 (0.03)	-0.03 (0.03)	0.24 (0.03)	0.32 (0.03)		0.33 (0.19)
TD	0.16 (0.05)	0.04 (0.03)	0.08 (0.04)	0.01 (0.03)	0.07 (0.03)	-0.01 (0.03)	0.34 (0.03)	0.43 (0.03)	0.50 (0.03)	

Production traits, MY: 305-day cumulative milk yield; frame traits, HH: hip height; HG: heart girth; RA: rump angle; feet and legs traits, RLS: rear legs-side view; FA: foot angle; mammary system traits, RU: rear udder width; UD: udder depth; TL: teat length; TD: teat diameter

3.3 Genetic and phenotypic correlations between milk yield and conformation traits

The estimates of genetic correlations between MY and body conformation traits revealed positive coefficients of low to high magnitude, ranging from 0.17 (between MY and HG) to 0.63 (between MY and UD) for the set of frame and mammary system traits (Table 3). However, the genetic correlation between MY and the feet and leg traits FA (-0.16) and RLS (-0.31) was negative (Table 3). The phenotypic correlation estimates between MY and all the body conformation traits were weak, except RU (0.27) and UD (0.25).

4 Discussion

Estimating genetic parameters and genetic correlations help to understand how traits respond to selection and, in this information builds or adjusts the selection indices in breeding programs. Our results showed that body conformation traits could respond to selection, except for the feet and leg traits that had lower heritability and might respond better to environmental improvement. Genetic correlations between milk production, udder conformation traits (UD and RU) and HH are strong, suggesting that some of the same genes will play a part in the genetics of these traits. A genetic correlation between MY and RU is desirable; however, genetic correlations observed between MY with UD and HH is undesirable because it suggests that the selection for more milk could affect udder health and mature size in Gir cows. These synergies and trade-offs need to be considered when formulating a breeding program.

The heritability estimates of the feet and legs traits were of low magnitude (aprox. 0.09), as reported in the literature (Olasege *et al.* 2019; Carvalho *et al.* 2021; Djedović *et al.* 2023), indicating a high influence of environmental factors on their expression. Cows with extreme score classification for FA and RLS are more susceptible to be discarded from the herd due to locomotion problems (Sewalem *et al.* 2004). Thus, based on the low heritability estimates for those traits, fewer locomotor problems can be expected if environmental improvement were implemented.

The frame category showed moderate heritability estimates, and HH presented the highest estimate (0.33), which is in agreement with values ranged 0.31 to 0.39 reported in Holstein (Wu *et al.* 2013; Olasege *et al.* 2019; Djedović *et al.* 2023) and Gir breeds (Carvalho *et al.* 2021). The heritability estimate for HG was similar to the reported values of 0.23 (Carvalho *et al.* 2021) e 0.30 (Lagrotta *et al.* 2010) in Gir cattle. Finally, the RA trait showed a moderate heritability estimate, with a value close to what was reported by Wu *et al.* (2013). However, some reports in the literature for RA ranged from 0.07 to 0.35 (Lagrotta *et al.* 2010; Carvalho *et al.* 2021) in Gir cattle and 0.13 to 0.36 in Holsten cattle (Abo-Ismael *et al.* 2017; Olasege *et al.* 2019; Čítek *et al.* 2022). The differences in the estimates may be due to breed differences, sample sizes or the statistical model applied.

The magnitudes of the heritability estimates for the mammary system traits indicate that a considerable proportion of the phenotypic variation occurs due to differences in the genome that can be modeled as additive effects. The heritability estimate for RU was higher than most of the results in the literature, which ranged from 0.19 to 0.28 for different dairy breeds such as Holstein, Ayrshire, Brown Swiss, Guernsey, Jersey, Shorthorn, and Gir (Wiggans *et al.* 2004; Campos *et al.* 2012; Carvalho *et al.* 2021). On the other hand, the heritability estimate for UD and TD were similar in magnitude to values reported in previous studies, such as from 0.29 to 0.32 for UD (Němcová *et al.* 2011; Carvalho *et al.* 2021; Čítek *et al.* 2022) and from 0.19 to 0.21 for TD (Lagrotta *et al.* 2010; Carvalho *et al.* 2021).

The TL and TD play an important role in preventing mastitis and in mechanical milking. Long and thicker teats are an issue for mechanical milking, and calf feeding, and are more susceptible to injuries, which can compromise udder health (Dube *et al.* 2009). The heritability estimate for TL was lower than previously published studies in Gir cattle (Lagrotta *et al.* 2010; Carvalho *et al.* 2021). However, some studies in *Bos taurus taurus* cattle estimated heritability of 0.21 for TL (Campos *et al.* 2012) and 0.29 (Abo-Ismael *et al.* 2017) in Holstein breed, 0.30 and 0.28 in Ayrshire and Shorthorn breeds (Wiggans *et al.* 2004), respectively. These values are similar to that found in this study. The heritability estimates for teat traits indicate that genetic gains could be obtained when these traits are included as selection criteria.

Traits related to milk production have been emphasized in the dairy cattle selection process due to obvious economic relevance. The heritability estimate for MY was similar to magnitude values reported in previous studies, ranging from 0.18 to 0.21 in Holstein (Campos *et al.* 2015; Dominguez-Castaño *et al.* 2021; Djedović *et al.* 2023) and from 0.20 to 0.24 in Gir cattle (Lagrotta *et al.* 2010; Silva *et al.* 2016; Hortolani *et al.* 2022). It is possible to obtain genetic gains in response to selection for MY in Gir, as reported by Fernandes *et al.* (2019).

The heritability estimates can vary between studies according to the classification system (measure or score), breed, number of records, statistical model, consistency among evaluators, as well as data editing procedures (Campos *et al.* 2012), so comparisons among studies should be undertaken with caution. The

knowledge of this genetic parameter for conformation and production traits into a population allows breeders to orient their selection criteria.

The choice of Bayesian methodology for this study was because this approach allows accurate estimates and robust results, even with small sample populations and complex models, providing posterior distribution, credible intervals, convergence diagnostics and graphical visualization for validation of parameter estimations (Yadav et al. 2023). Additionally, it doesn't require normality and linearity assumptions of the data (Gianola and Fernando 1986). This methodology has not been used to estimate genetic parameters for body conformation in Gir cattle.

In studies conducted by Lagrotta et al. (2010) and Carvalho et al. (2021), which involved larger populations of Gir cattle (approximately 3,000 and 9,000 animals with phenotypes, respectively), likelihood-based methods were employed to estimate variance components in body conformation traits. It should be noted that these analyses did not incorporate genomic information. Despite the difference in population size when compared to our study, the fact that the standard errors of the heritability estimates were similar, ranging from 0.03 to 0.09, lends greater reliability to our results. Standard errors for correlations between traits were not estimated in the study by Carvalho et al. (2021), and in the Lagrotta et al. (2010) study, they were not reported.

The sample size can influence the standard error of the estimates, with larger samples typically resulting in lower standard errors (Snijders and Bosker 1993). As a result of the number of observations utilized in this study, certain genetic and phenotypic correlation estimates exhibited a pronounced magnitude of standard error. Consequently, these estimates should be observed with caution. Correlation estimates were considered significant when the standard error was less than half of the size of the correlation (Olasege *et al.* 2022).

The genetic correlation estimates between body conformation traits evidenced high magnitudes in each group of traits (Table 3). For the frame group, HH was strongly genetically correlated with HG (0.71) and moderate with RA (0.48), indicating that the genes responsible for height also play a major role in perimeter thoracic. This result agrees with values reported ranging from 0.34 to 0.69 for the genetic correlation between HH and HG (De Haas *et al.* 2007; Lagrotta *et al.* 2010). The traits that conform

feet and leg category showed a negative and high genetic correlation (-0.91). In agreement with our findings, previous studies reported a negative correlation between rear legs and foot angle, with values ranging from -0.20 to -0.67 in six taurus dairy breeds (Wiggans *et al.* 2004; Němcová *et al.* 2011; Olasege *et al.* 2019). The result suggests that cows with straight rear legs tend to have steeper foot angles and vice versa. However, the low heritability estimates and consider standard error for feet and leg traits suggest that more phenotypes are necessary to obtain accurate estimates. Thus, the genetic correlation between these traits has to be observed with caution.

The genetic correlation estimates between mammary system traits evidenced that udder traits were strongly related. The high magnitude estimate between UD and RU (0.72) indicates that selecting a wide rear udder can lead to a deeper udder. Wide rear udders are desired due to their associated with increased milk production (Fernandes *et al.* 2019). On the other hand, deeper udders are more likely to get dirty, increase somatic cell count and develop clinical mastitis (Bharti *et al.* 2015). Accordingly, it is necessary attention when selection is applied to increase the mean of RU because is expected an increase in the mean of UD. Lower values for the genetic correlation estimate between UD and RU were reported in studies with six different taurus breeds, ranging from -0.25 to 0.16 (Wiggans *et al.* 2004; Němcová *et al.* 2011). There are no reports in the literature on the Gir breed. Divergence in results can be assigned to the breed, number of observations, statistical model, and other factors.

The udder traits UD and RU also were highly correlated genetically with TD (Table 3), suggesting a strong association between the genes responsible for udder conformation and TD. The TD is not a trait ordinarily evaluated in the *Bos taurus taurus* breeding programs. However, given the great genetic variability in indicus breeds, there is an ongoing need to select for decreased teat thickness (Fernandes *et al.* 2019). Overly thick teats impair the calf feeding and mechanical milking process, making it an undesirable trait for the daily Gir cattle breeders. Our results suggest that cows with a wide udder tend to have a deep udder and thick teats. As a result, necessary attention is required when selection is applied to increase the mean of some of these traits because they are genetically correlated. For example, selection to increase one trait will cause an expected increase in the other trait.

The UR trait showed a high genetic correlation with the frame group trait RA (0.51). Fewer values were reported for the correlation between those traits in taurus cattle (Wiggans *et al.* 2004; Němcová *et al.* 2011). In dairy Gir cattle, the primary morphological trait that affects rear udder height is the RA (Fernandes *et al.* 2019). Given that rear udder height is strongly genetically correlated with rear udder width (Wiggans *et al.* 2004; Němcová *et al.* 2011), it is reasonable to expect a significant genetic correlation between RU and RA, as observed in our study.

The genetic correlation between milk production and body conformation traits showed moderate to high and positive correlation between MY with HH, UD and UR, indicating that considerable common gene action exists between those traits, and it can be expected, to a greater or lesser degree, that the progeny of cows will show higher MY, wide and deep udders, and high hip height, leading to that have higher producing capacity. It is important to highlight that selection for stature can generate an increase in mature size in cattle that may not necessarily be advantageous. Changes in the mature size may consequently affect nutritional requirements. Therefore, caution should be taken when selecting traits related to stature. Similar results have been previously reported in the literature with estimates ranging between 0.21 (DeGroot *et al.* 2002) to 0.47 (De Haas *et al.* 2007) for genetic correlation among MY and HH in Holstein cattle. In contrast, some studies in Gir and Holstein breeds express no genetic correlations between those traits (Lagrotta *et al.* 2010; Djedović *et al.* 2023).

The high and positive genetic correlation found between MY with UD (0.63) suggest that the continued emphasis on the selection of animals for MY, would leave in deep udders as a correlated response, in other words, udders below the line of the hock, which could lead to health problems in the cow, such as mastitis leading to increased premature and involuntary removal from the herd. This result agrees with previous studies that found an undesirable genetic correlation between those traits (DeGroot *et al.* 2002; Campos *et al.* 2015). On the other hand, the high, positive and favourable genetic correlation observed between MY and UR (0.61) are indicative that considerable common gene action exists between these traits and if selection is applied to increase rear udder width, it can be expected that mean milk production will

increase. Deep and large rear udders are related with more storage areas and more capacity for milk production (Fernandes *et al.* 2019).

The estimates obtained for the phenotypic correlation between most conformation traits, especially those groups related to the frame and feet and leg, were of low magnitude, and most values were close to null (Table 3). An exception was observed between HH and HG (0.37) traits, suggesting that taller cows tend to have a bigger perimeter thoracic. This result agrees with previous studies that found moderate and positive phenotypic correlation between those traits in dairy (Koenen and Groen 1998; Zhang *et al.* 2017) and beef cattle (Kamprasert *et al.* 2019). In the same way, phenotypic correlations between mammary system group had positive coefficients of moderate magnitude, suggesting that changes in one trait will alter the expression of the other traits.

The phenotypic correlations between production and conformation traits were weak except for correlation among MY with UD (0.25) and RU (0.27), indicating that cows with deep and wider posterior udder could produce more milk.

5 Conclusions

The heritability estimates confirmed the possibility of selecting animals for milk yield and body conformations traits related to the frame and mammary system categories. Many of the traits analyzed were strongly correlated, indicating redundancy among traits. Based on the estimated genetic correlations, considerable importance should be given to the emphasis on selection for increased MY, because in the long term, this may promote undesirable changes in some type traits as HH and UD (increasing body height and udder depth of the cows).

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APÊNDICE A

Table 1A. Description of the body conformation traits evaluated in dairy Gir cattle.

Trait	Description
Hip height	Length of the animal from the top of the rump to the ground
Heart girth	The thoracic perimeter
Rump angle	It is the difference between the height of the pin and the hip bones (1-high pins and 9-low pins)
Foot angle	The angle formed between two imaginary lines, the first parallel to the ground surface and the second to the wall of the hoof (1-low and 9-steep)
Rear legs-side view	Angulation formed between the tibia and metatarsus bones (1-straight and 9-curved)
Rear udder width	The distance between the junction of the leg and the udder from the left side to the same junction on the right side (1-narrow and 9-wide)
Udder depth	The distance from the hock to the floor of the udder (1-shallow and 9-deep)
Teat diameter	The teat thickness (1-thin and 9-thick)
Teat length	Teat size (1-short and 9-long)

Table 2A. The number of animals genotyped, number of SNPs by chip, and SNPs in common with reference GGP_Indicus_50k_A1 chip to HD and low-density SNPs chips

SNP Chip	N_SNPs	N_SNPs_29	SNPs_S	N_Animals
GSGT Version 2.0.4 GGP_Indicus_50K_A1	54,791	51,975	-	100
Illumina® BovineHD	777,962	735,965	51,038	36
GSGT Version 2.0.2 GGP_Indicus_35K	35,339	33,516	33,206	166
GSGT Version 1.9.4 GGP_SuperLDv4_Public_ D	30,108	28,260	15,047	290
GSGT Version 1.9.4 GGP_SuperLDv4_C	30,105	28,260	15,008	155
Zoetis_BR_LD_ZL5	29,842	27,843	9,047	846

N_SNPs, number of SNPs; N_SNPs_29, number of SNPs location until chromosome 29; SNPs_S, number of SNPs in common with reference GGP_Indicus_50k_A1 chip; N_Animals; the number of animals.

Table 3A. Average of the heritability estimate (h^2) and standard error (SE) using bivariate analyses for milk yield and body conformation traits in dairy Gir cattle.

Trait	h^2	SE
MY	0,33	0,01
HH	0,36	0,08
HG	0,27	0,07
RA	0,25	0,07
RLS	0,11	0,04
FA	0,14	0,05
RU	0,36	0,07
UD	0,29	0,07
TL	0,25	0,07
TD	0,28	0,07

MY: 305-day cumulative milk yield; HH: hip height; HG: heart girth; RA: rump angle; RLS: rear legs-side view; FA: foot angle; RU: rear udder width; UD: udder depth; TL: teat length; TD: teat diameter.

CAPITULO 3 - Genome-wide association study (GWAS) for milk yield, frame and udder conformation traits of Gir dairy cattle¹

ABSTRACT - Identifying significant genomic regions and candidate genes associated with performance and productivity traits may enhance animal selection strategies for Gir dairy cattle. This study aimed to perform a GWAS (genome-wide association study) to identify genomic regions and candidate genes associated with milk yield, frame, and udder conformation traits in Gir dairy cattle. Body conformation traits were classified as “frame” and “udder” traits for this study. After genotyping imputation and quality control, 42,105 polymorphisms were available for analyses and 24,489 cows with pedigree information had phenotypes. Weighted single-step genome-wide association methodology was performed using genomic moving windows defined based on linkage disequilibrium. The top 10 windows explaining the highest percentage of additive genetic variance were selected and used for QTL and gene annotation. The genomic regions identified in our work overlapped with QTLs from the animal QTL database on chromosomes 1 to 23, except for chromosome 4. The Gir breed is less studied than the Holstein breed, so the animal QTL database is biased towards Holstein results. Hence, it is noteworthy that our GWAS had similarities with previously described QTLs. These previously known QTLs were related to milk yield, body height, udder width, and udder depth. In total, 16 genes were annotated as potential candidate genes. Of these genes, *PIK3R5* and *PIK3R6* had been previously related to cattle body size. Several identified genes are implicated in lipid metabolism, survival, reproduction, and milk production traits. These findings establish a foundational platform for further studies that help understand the biological pathways that affect milk yield, frame, and udder conformation traits in Gir cattle.

Keywords: body conformation traits, *Bos indicus* cattle, candidate genes, dairy cattle, milk production, SNP.

1 Introduction

Breed programs often use body conformation or type traits in dairy cattle. Producers use these traits as indicators of a cow's performance, productivity, and ability to stay in the herd. In other words, body conformation traits are related to economically important traits, such as milk yield, overall health, and longevity. For example, udder conformation traits were related to cow's health (Dube et al. 2009). Frame conformation traits, such as stature and heart girth, were associated with feed efficiency and energy balance (Søndergaard et al. 2002). Conformation traits such as rump angle and udder depth were associated with longevity (Lund et al. 1994; Vollema et al. 2000). Additionally, body conformation traits have been genetically correlated with

milk yield in Holstein dairy cattle (Short and Lawlor 1992; De Haas et al. 2007). Therefore, studies on the genetics of body conformation traits benefit dairy cattle breeding and the milk industry.

Gir dairy cattle (*Bos indicus*) are an important genetic resource for tropical dairy industries. Gir cattle are capable of milk production in pasture-based systems and have lower nutritional requirements and higher resilience to heat and parasites when compared to Holstein cattle (Santana Jr et al. 2014). The genetics of Holstein cattle have been far more studied than the genetics of Gir cattle, especially in terms of applying genomics to identify variants and genes or QTL (quantitative trait loci) that affect production traits. Employing a genome-wide association approach (GWAS) within the Gir breed, some studies have identified and annotated potential candidate genes associated with milk yield (Toro-Ospina et al. 2023) as well as reproductive traits (Rocha et al. 2023). Braga et al. (2023) recently identified high confidence copy number variation regions potentially affecting genes related to complex traits, such as production, health, and reproduction in Gir cattle. Despite these recent efforts, GWAS for body conformation traits in the Gir breed have yet to be reported.

The Holstein breed is probably the most studied breed of dairy cattle. Many genome-wide association studies have documented QTL, variants and genes associated with body conformation traits (Schnabel et al. 2005; Wu et al. 2013; Wang et al. 2022), as well as milk production traits (Bennewitz et al. 2004; Cole et al. 2011; Ning et al. 2018). In short, studies have reported the heritability and the underpinning genetics of Holstein phenotypes. This knowledge may or may not apply to other dairy cattle breeds. Some QTLs are breed specific, as studies in beef cattle reported (Hawken et al. 2012; Fortes et al. 2020). Therefore, it is important to investigate the genetics of these traits within the Gir breed to verify which variants are relevant in *Bos indicus* dairy cattle. The objective of this study was to perform GWAS to identify genomic regions and candidate genes associated with milk yield, frame and udder conformation traits in Gir dairy cattle.

2 Materials and methods

2.1 Data, genotypes, and heritability estimates

The traits used to perform GWAS are a subset of traits from our previous study (Dominguez-Castaño et al., 2023). The subsets were used because our data had sufficient statistical power to detect genetic (co)variance using genome-wide SNPs, according to the online GCTA-GREML power calculator (Visscher et al. 2014). For the selected traits, the estimated statistical power was equal to 1.0 and the standard error ranged from 0.03 to 0.04. In our previous paper, univariate animal models were used to estimate variance components by the Bayesian approach for milk yield and nine body conformation traits using pedigree and genotype files (Dominguez-Castaño et al., 2023). The body conformation traits selected were four traits associated with the udder conformation: rear udder width (RU), udder depth (UD), teat diameter (TD), and teat length (TL); and three conformation traits related to the frame: hip height (HH), heart girth (HG), and rump angle (RA). The dataset consists of ~1050 individuals measured for conformation traits, according to definitions set by the Brazilian Dairy Gir National Breeding Program (Panetto et al. 2022). We also had 38,996 records of 305-day cumulative milk yield (MY) from 24,489 Gir cows born between 1969 and 2020. A total of 1,593 animals of both sexes were genotyped using six different commercial SNPs panels (Table S1). Genotypes were imputed to the moderate density panel known as 50K, with approx. 50 thousand DNA markers. The heritability estimates of each trait and their corresponding standard error, number of records, and descriptive statistics are provided in Table 1.

Table 1. Descriptive statistic for 305-day cumulative milk yield and body conformation traits related to udder and frame in Gir dairy cattle.

Trait	Units/Ideal Score**	N	Mean $\pm$ SD	Min	Max	$h^2 \pm SE$
Production						
Milk yield	kg	38,996	3,566.5 $\pm$ 1,975.5	209	15,355	0.23 $\pm$ 0.01
Frame						
Hip height	cm	1,062	134.1 $\pm$ 7.1	103	180	0.33 $\pm$ 0.08
Heart girth	cm	1,055	176.6 $\pm$ 9.7	134	210	0.26 $\pm$ 0.08
Rump angle	≤ 5	1,062	6*	2	9	0.23 $\pm$ 0.07
Udder						
Rear udder width	9	916	5*	2	9	0.35 $\pm$ 0.07
Udder depth	5	1,043	5*	1	8	0.30 $\pm$ 0.06
Teat length	≤ 5	1,044	5*	1	9	0.27 $\pm$ 0.06
Teat diameter	5	1,043	5*	2	9	0.25 $\pm$ 0.06

Units: Units of measurement; N: number of records; Min: minimum value for the evaluated trait; Max: maximum value for the evaluated trait; Mean: mean value for the evaluation trait; SD: mean standard deviation by trait; h^2 : heritability estimate; SE: heritability standard error. *Mode. **Ideal score according to (Panetto *et al.* 2022).

2.2 Genome-wide association study

The estimated variance components were used in the GWAS carried out using weighted single-step genomic approach (WssGBLUP; (Wang *et al.* 2012)), where same to ssGBLUP the **A** inverse is replaced by the **H** inverse (Aguilar *et al.* 2010), which is a relationship matrix that combines both pedigree (**A**) and the genomic relationships (**G**). The WssGBLUP is an iterative process that involves: (i) initializing **D** equal to an identity matrix (**I**); (ii) constructing **G**, as $\mathbf{G} = \frac{\mathbf{Z}\mathbf{D}\mathbf{Z}'}{\sum_{i=1}^M 2p_i(1-p_i)}$, in which **Z** is an incidence matrix adjusted for allele frequencies, in where **D** is a diagonal matrix of weights for SNP variances, *M* is the number of markers, and p_i represents the MAF of the *i*th SNP; (iii) calculating GEBV; (iv) converting DGV into SNP effects by $\hat{u} = \lambda \mathbf{D}\mathbf{M}'\mathbf{G}^{-1}(\mathbf{D}\mathbf{G}\mathbf{V})$, in where $\hat{u}$ is a vector of estimated SNP effects, λ is the ratio of SNP variance to genetic variance and DGV is the direct genomic value; (v) calculating SNP weights for the next interaction by nonlinearA procedure according (VanRaden 2008), $d_i = 1.125 \frac{|\hat{u}_i|}{\sigma(\hat{u})}^{-2}$, where 1.125 is a constant that describes the deviation of the SNP effect from a normal distribution, $|\hat{u}_i|$ is the absolute estimated SNP effect for marker *i*, and $\sigma(\hat{u})$ is the standard deviation of the vector of the estimated SNP effects; (vi) normalizing **D** to maintain a constant total genetic variance; (vii) iterating from step (ii) (Lourenco *et al.* 2017).

We used results from each of two iterations of WssGBLUP because two iterations of weights are sufficient to maximize genomic accuracy and to correctly identify major SNP (Zhang et al. 2016). The WssGBLUP was conducted using the BLUPF90 software family (Miszta et al. 2018), and the GWAS results were reported as the percentage of genetic variance explained by a moving window of 0.11-megabase, in which the window size was determined by the calculating of linkage disequilibrium by chromosome (Figure 1S) using the PREGSF90 software (Miszta et al. 2018). In this study, using a 0.11-megabase window size allowed us to achieve an r^2 value above 0.3 for linkage disequilibrium. This level of linkage disequilibrium among markers proved to be highly effective in GWAS (Ardlie et al. 2002). Manhattan plots with the percentage of genetic variance were created using the 'CMplot' package (Yin et al. 2021) in R software (R 2022).

2.3 QTL regions and gene search

The top 10 windows explaining the highest percentage of additive genetic variance were selected to QTL annotation. The identification of putative candidate genes was carried based on the genomic windows that were previously associated to QTLs related to the traits of interest. QTL annotation and identification of candidate genes were based on the initial and final coordinate of each window on the ARS-UCD1.2 assembly of the bovine genome using the GALLO package (Fonseca et al., 2020) in the software R (R 2022). Which uses the databases Animal QTLdb (<https://www.animalgenome.org/QTLdb>) and Ensemble (<http://www.ensembl.org/>), for QTL annotations and gene search, respectively.

3 Results

The percentage of genetic variance explained by each window of 0.11-megabase is displayed in Manhattan plots for milk production, frame, and udder conformation traits (Figure 1 and 2). As seen in the figures, there is a key region on BTA 3 that seems relevant to most of the studied traits. The additive genetic variance explained by the top 10 windows was 4.95 % for RU, 4.43 % for UD, 5.98 % for TL, 4.27 % for TD, 4.08 % for MY, 7.86 % for HH, 7.91 % for HG and 6.60 % for RA (Tables 2-4).

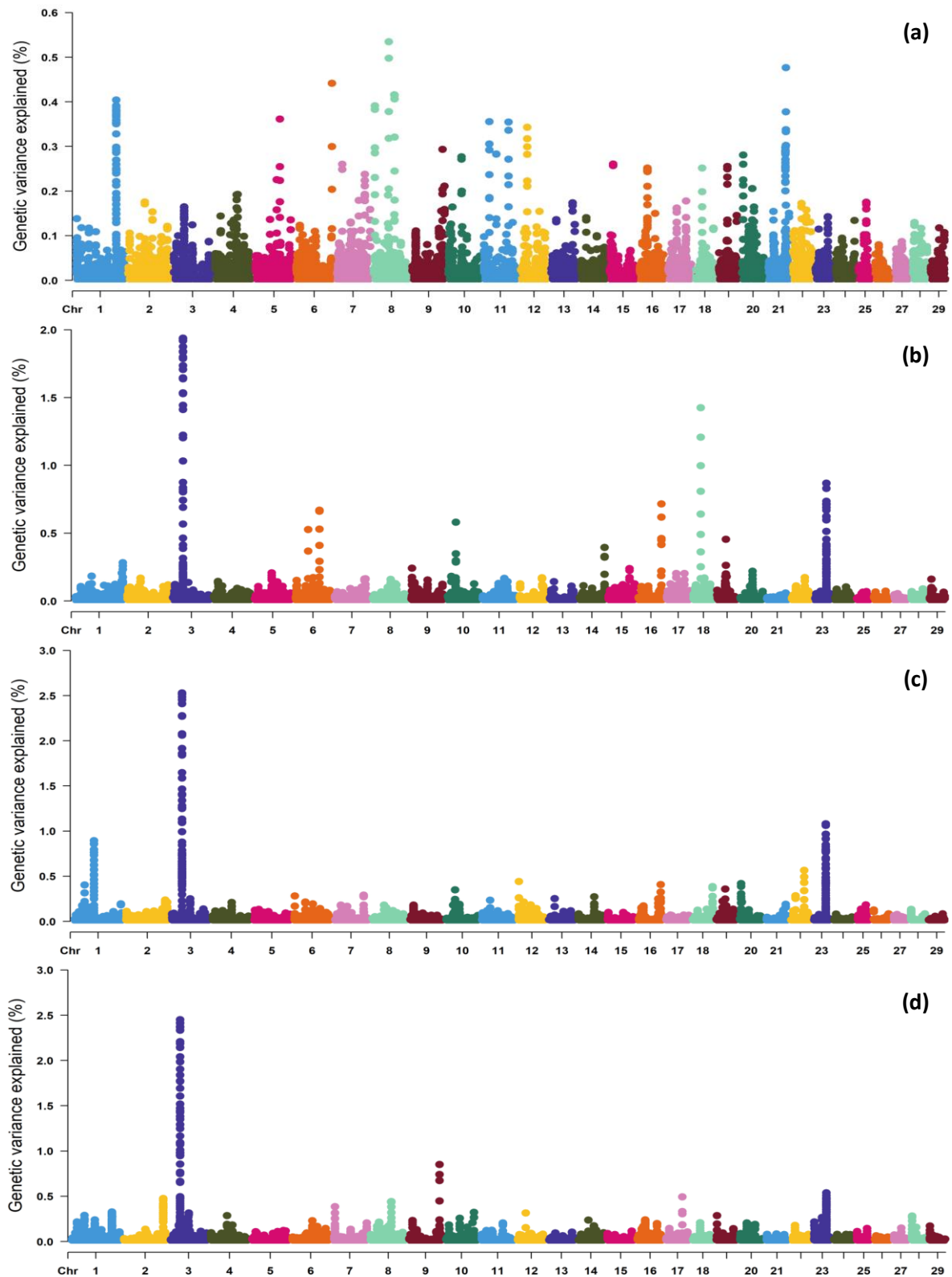


Figure 1. Manhattan plot of proportion of additive genetic variance explained (%) by 0.11-megabase window for milk yield (a), hip height (b), heart girth (c) and rum angle (d) in Gir dairy cattle.

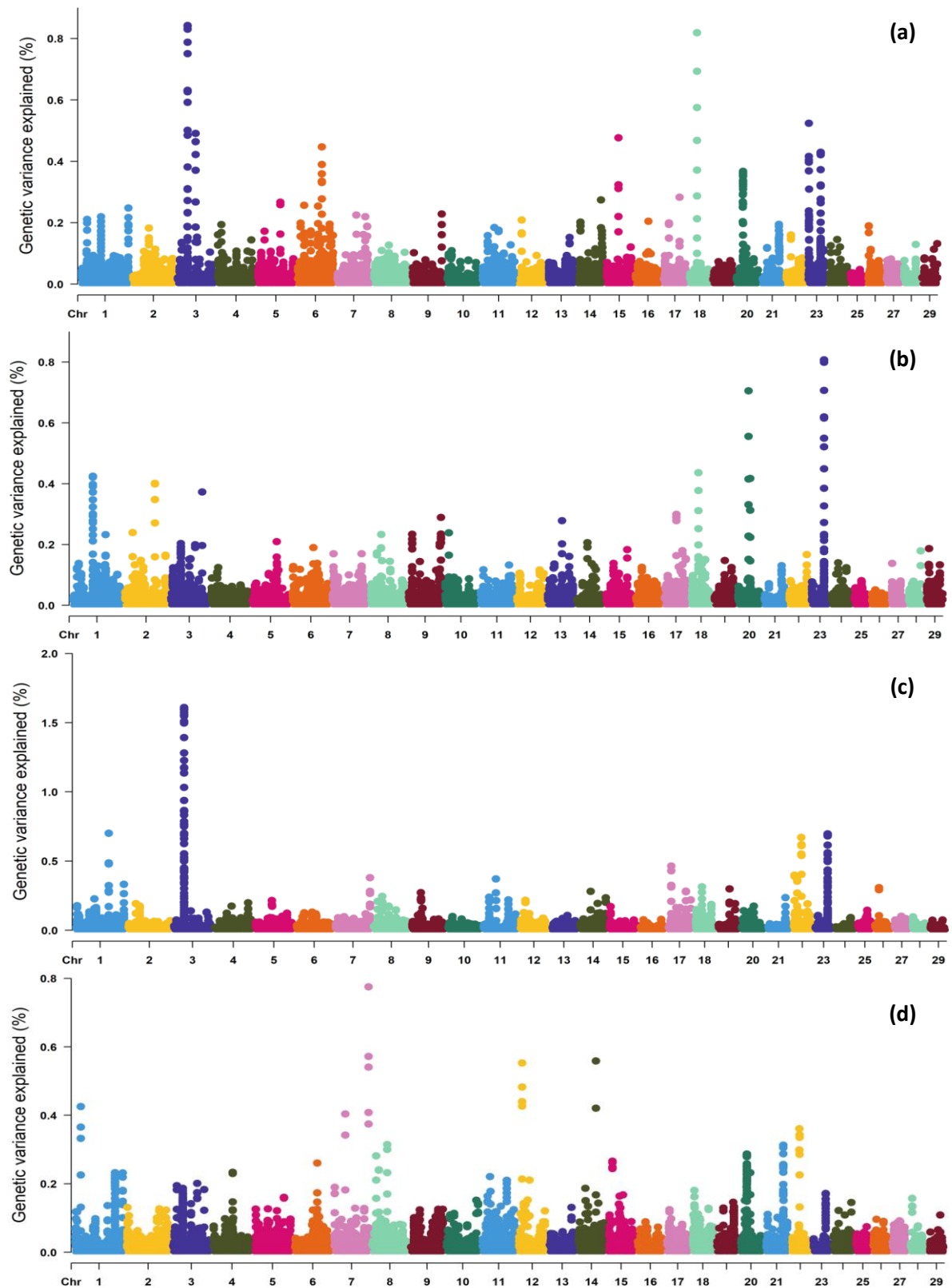


Figure 2. Manhattan plot of proportion of additive genetic variance (%) explained by 0.11-megabase window for rear udder width (a), udder depth (b), teat length (c) and teat diameter (d) in Gir dairy cattle.

Table 2. Genomic regions of the top 10 windows for the udder traits udder depth (UD), teat length (TL), and teat diameter (TD) in Gir dairy cattle.

Trait	BTA	Start position (bp)	End position (bp)	Var (%)
UD	23	39,355,450	39,505,270	0.8073
	20	33,359,561	33,451,856	0.7054
	18	24,230,415	24,346,129	0.4361
	1	65,891,201	66,033,633	0.4244
	20	38,940,148	39,088,930	0.4173
	2	96,702,992	96,806,227	0.4013
	3	101,923,791	102,018,821	0.3724
	17	38,109,427	38,230,789	0.2991
	9	104,016,649	104,110,205	0.2887
	13	46,685,126	46,824,650	0.2781
TL	3	32,792,333	32,941,843	1.6098
	1	107,058,263	107,202,385	0.7006
	23	39,369,304	39,517,761	0.6962
	22	27,234,189	27,379,399	0.6714
	17	10,102,391	10,222,640	0.4637
	22	5,230,295	5,345,306	0.3976
	7	110,325,671	110,452,975	0.3791
	11	39,555,856	39,703,103	0.3709
	22	13,781,757	13,907,734	0.3638
	1	156,146,437	156,292,071	0.3323
TD	7	110,325,671	110,452,975	0.7755
	14	55,700,820	55,803,198	0.5589
	12	10,622,131	10,724,266	0.5527
	1	19,827,553	19,957,793	0.4255
	7	34,847,278	34,967,857	0.4041
	22	27,234,189	27,379,399	0.3605
	8	48,347,706	48,496,148	0.3144
	21	57,016,245	57,164,772	0.3126
	20	22,158,626	22,307,782	0.2868
	8	12,429,392	12,532,915	0.2815

BTA: *Bos taurus* autosomes; bp: base pair; Var: percentage of additive genetic variance explained by each window.

Table 3. Genomic regions of the top 10 windows for the frame traits hip height (HH), heart girth (HG), and rump angle (RA) in Gir dairy cattle.

Trait	BTA	Start position (bp)	End position (bp)	Var (%)
HH	3	32,769,633	32,915,329	1.9389
	18	24,230,415	24,346,129	1.4257
	23	39,479,425	39,625,358	0.8682
	16	74,849,194	74,955,352	0.7161
	6	79,743,662	79,875,430	0.6685
	10	28,247,513	28,300,839	0.5802
	6	42,761,005	42,870,371	0.5271
	19	28,249,637	28,380,621	0.4547
	14	82,172,085	82,309,539	0.3941
	1	156,146,437	156,292,071	0.2828
HG	3	32,769,633	32,915,329	2.5292
	23	39,479,425	39,625,358	1.0803
	1	65,881,852	66,021,770	0.8940
	3	32,967,546	33,110,685	0.7888
	22	42,574,390	42,709,094	0.5656
	12	1,014,664	1,146,901	0.4412
	20	3,508,119	3,628,845	0.4183
	16	74,849,194	74,955,352	0.4086
	1	35,575,565	35,702,194	0.4039
	18	65,164,063	65,308,095	0.3842
RA	3	32,767,893	32,915,329	2.4508
	9	97,912,180	98,002,752	0.8504
	23	39,483,485	39,625,358	0.5378
	17	51,866,183	52,015,673	0.4925
	2	126,976,378	127,125,968	0.4748
	8	66,699,044	66,833,492	0.4405
	7	7,232,660	7,350,133	0.3847
	1	131,359,310	131,506,371	0.3277
	10	92,051,721	92,133,831	0.3228
	3	60,541,269	60,676,331	0.3156

BTA: *Bos taurus* autosomes; bp: base pair; Var: percentage of additive genetic variance explained by each window.

Table 4. Genomic regions of the top 10 windows for milk yield (MY) and rear udder width (RU) in Gir dairy cattle.

Trait	BTA	Start position (bp)	End position (bp)	Var (%)
MY	8	48,347,706	48,496,148	0.5347
	21	58,557,857	58,707,834	0.4765
	6	116,395,476	116,502,046	0.4417
	8	66,685,400	66,833,492	0.4157
	1	131,389,856	131,531,825	0.4041
	8	3,153,165	3,300,895	0.3913
	5	78,689,623	78,796,160	0.3615
	11	17,393,875	17,520,483	0.3556
	11	79,757,994	79,881,907	0.3549
	12	21,201,868	21,318,244	0.3428
RU	3	32,709,470	32,859,001	0.8427
	18	24,230,415	24,346,129	0.8186
	23	956,245	1,099,333	0.5238
	3	59,416,883	59,562,857	0.4909
	15	40,601,546	40,715,768	0.4765
	6	79,743,662	79,875,430	0.4468
	23	39,355,450	39,505,270	0.4295
	20	22,158,626	22,307,782	0.3671
	17	51,866,183	52,015,673	0.2827
	14	77,918,152	78,045,230	0.2739

BTA: *Bos taurus* autosomes; bp: base pair; Var: percentage of additive genetic variance explained by each window

The genomic regions from the top 10 windows identified overlapped with QTL from the QTL database. The database QTLs were classified as Conformation, Health, Meat and Carcass, Milk, Production, or Reproduction traits. Approximately, 39% of the overlapping QTLs are associated with Milk traits, 13% with Production traits, which include HH and HG, and approximately 5% with Conformation traits, which include body conformation traits and udder traits (Figure 3).

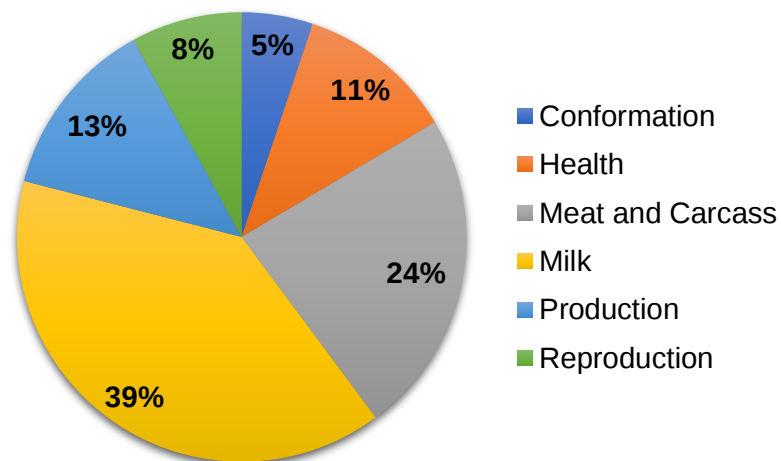


Figure 3. Pie plot for the Quantitative Trait Loci from QTL database overlapped with the genomic regions identified in the top 10 windows for milk yield, frame, and udder conformation traits of Gir dairy cattle.

A total of 50 QTLs related to milk production and milk composition traits, were found mapping against the genomic regions from the top 10 windows identified on BTA1, BTA5, BTA8, BTA11 and BTA12 for MY trait. Six of them are specifically described as related to milk yield (Figure 4). Furthermore, 18 QTLs were identified for RU, 13 QTLs for UD, and eight QTLs for TL, all of which are linked to external traits for QTL-type. Among the QTLs related to RU, UD, and TL, two each were specifically attributed to udder width, udder depth, and front teat placement, respectively (Figure 4). In addition, 38 QTLs related to production traits were annotated for HH trait. Six of these QTLs are specifically described as related to body height. The results for HG, RA and TD traits did not overlap with previously reported QTLs related to these traits (Figure 5) and consist of new QTL identified herein.

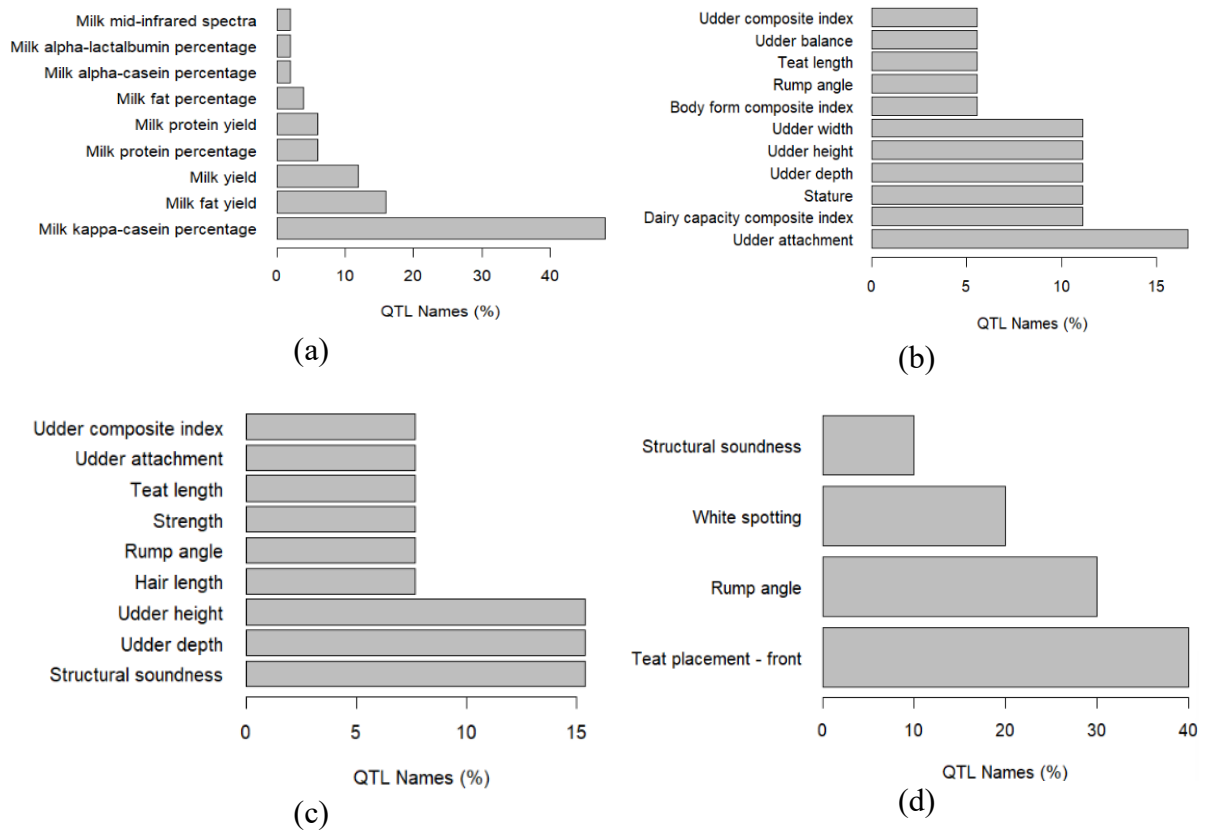


Figure 4. (a) Percentage of each trait annotated as a Milk QTL in the top 10 windows for Milk Yield. (b) Percentage of each trait annotated as a Conformation QTL in the top 10 windows for rear udder width, (c) udder depth and (d) teat length.

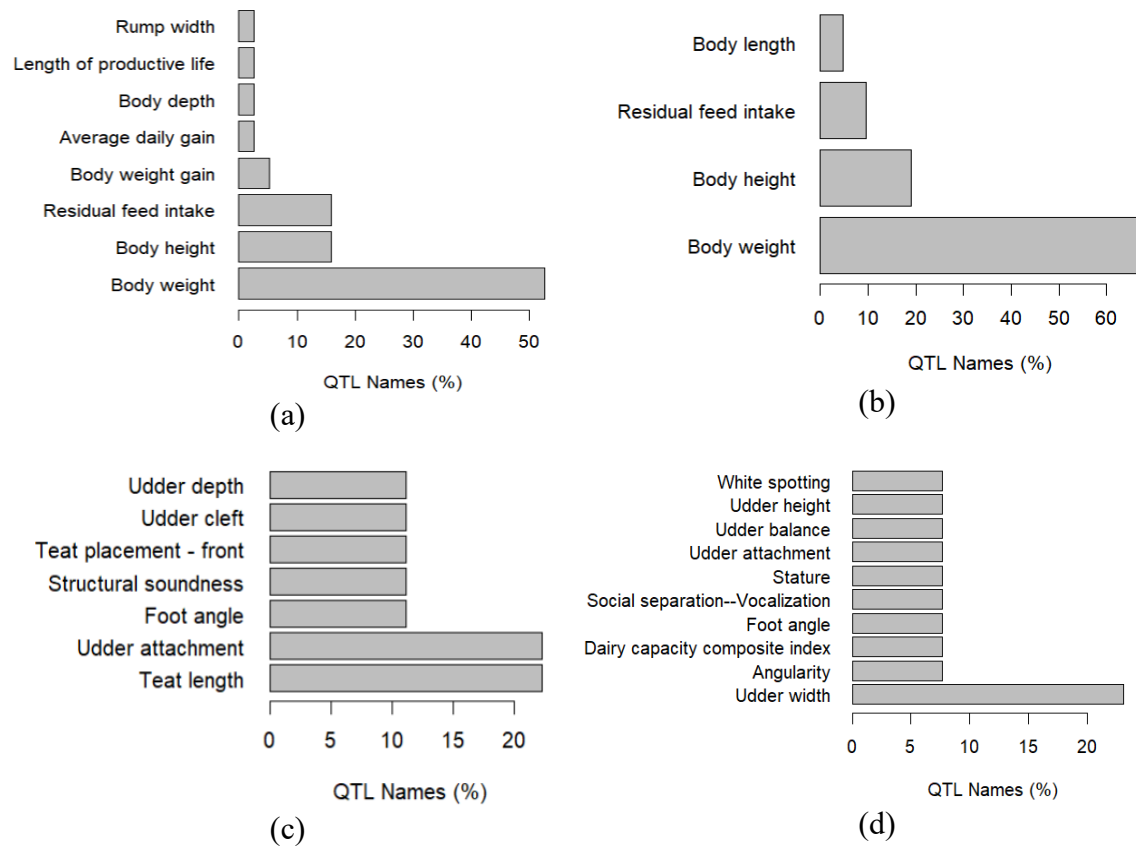


Figure 5. (a) Percentage of each trait annotated as a Production QTL in the top 10 windows for hip height and (b) heart girth. (c) Percentage of each trait annotated as a Conformation QTL in the top 10 windows for rump angle, and (d) teat diameter.

In total, 16 candidate genes were present in the windows corroborated by the QTL database with overlaps to specific QTL for each studied trait (Table 5). Eight of these candidate genes were associated with HH, three with RU, three with UD, and two with MY. All of them were protein-coding genes.

Table 5. QTL annotation and gene identification in genomic windows associated with hip height (HH), rear udder width (RU) udder depth (UD) and milk yield (MY) in Gir dairy cattle.

Trait	BTA	Start position (bp)	End position (bp)	Var (%)	QTL_Name	Pubmed_ID	Candidate gene
HH	6	42761005	42870371	0,53	Body height	12605852 20477797	
	6	79743662	79875430	0,67	Body height	20477797	TECRL
	10	28247513	28300839	0,58	Body height	20477797	LPCAT4 NUTM1 NOP10
	16	74849194	74955352	0,72	Body height	20477797	
	19	28249637	28380621	0,45	Body height	33176675	CDC42 MFSD6L PIK3R6 PIK3R5
RU	14	77918152	78045230	0,27	Udder width	16167984	RALYL ENSBTAG00 000053138
	20	22158626	22307782	0,37	Udder width	16167984	MIER3
UD	2	96702992	96806227	0,40	Udder depth	17433017	PTH2R
	18	24230415	24346129	0,44	Udder depth	16230715	AMFR GNAO1
MY	5	78689623	78796160	0,36	Milk yield	19793271 15514072 19793271 12778594 12729552	
	12	21201868	21318244	0,34	Milk yield	12778594	DHRS12 CCDC70

BTA: *Bos taurus* autosomes; bp: base pair; Var: percentage of additive genetic variance explained by each window

4 Discussion

Identifying genomic regions and potential candidate genes influencing traits of economic interest holds the potential to enhance animal selection strategies for dairy cattle. Breed specific QTLs may be present, so it is important to study each dairy breed and confirm (or reject) known QTLs from other studies. This study focused on the Gir breed, which is a *Bos taurus indicus* breed genetically distant from the Holstein *Bos taurus taurus* cattle that is often studied (Decker et al. 2014).

Herein, we detected QTL previously described for milk yield (on BTA5 and 12), body height (on BTA6, 10, 16 and 19), udder width (on BTA14 and 20) and udder depth

(on BTA2 and 18). These QTLs overlapped with some of the top 10 windows with the highest percentage of additive genetic variance explained for MY, HH, RU and UD. Additionally, our analysis has identified 16 genes as potential candidate genes associated with these traits in Gir cattle. Of these genes, PIK3R5 and PIK3R6 had been previously related to cattle body size (Kominakis et al. 2017; Vanvanhossou et al. 2020). Several of the identified genes are implicated in lipid metabolism, survival, reproduction, and milk production traits, among others. Notably, certain genomic regions and candidate genes unveiled by this study have not been associated with milk yield, frame structure, or udder conformation traits before. This suggests the existence of QTL and candidate genes that might be unique to Gir cattle and are yet to be fully characterized. Further exploration of *Bos indicus* breeds might unveil distinct potential candidate genes linked to traits of economic interest for the dairy industry.

The percentage of genetic variance explained by each one of 10 top windows identified for the studied traits ranged from 2.5% (for HG) to 0.27% (for RU). These small genetic variances confirm the common assumption that the evaluated traits are polygenic, and that the importance of each QTL, gene or associated SNP is small. Therefore, these traits will probably benefit from whole genome approaches, such as genomic selection, and are not likely to be selected using a reduced panel of specific markers.

Gir QTLs discovered here for MY and HH are corroborated by previous literature. A genomic region on BTA 5 was detected for MY, overlapping QTLs previously reported as associated to milk yield in different dairy breeds such Holstein (Bennewitz et al. 2003; Bennewitz et al. 2004), Fleckvieh (Awad et al. 2010), and Finnish Ayrshire (Viitala et al. 2003). Additionally, in the study performed by Viitala et al. (2003), they described another QTL related to milk yield on BTA 12 in the same region identified in this current study for MY (Table 5). The windows on BTA 6, BTA 10, BTA 16 and BTA 19 associated to HH is within QTLs significantly associated with body height in Angus (McClure et al. 2010), French Holstein, Normande, and Montbéliarde (Boichard et al. 2003), and Beninese indigenous cattle (Vanvanhossou et al. 2020). These QTL for MY and HH seem to be important across breeds. Knowing that at least some of the genetics for MY and HH are relevant across breeds may encourage multi-

breed, or crossbreed, selection programs. The Gir crossed Holstein ‘Girolando’ cattle is an important resource for tropical dairy industries that may benefit from this study.

Regarding udder traits, the regions on BTA 14 and BTA 20 associated with RU are located within QTL regions previously reported to influence udder width in Holstein cattle (Schnabel et al. 2005). Similarly, the windows on BTA 2 and BTA 18 related to UD overlap with QTLs that have demonstrated a significant effect on udder depth (Ashwell et al., 2005; Leyva-Baca et al., 2007). These findings show that a proportion of the top 10 identified windows overlap with QTL regions that have been previously associated with milk yield and some of the frame and udder traits in dairy cattle. Of note, MY is one of the most studied traits, which bias any GWAS results for identifying overlapping results with QTLs for MY.

Teat traits, such as TD and TL, can impact udder health, mechanical milking and calf feeding processes. Long and thicker teats generate issues for mechanical milking and calf feeding (Fernandes et al. 2019). These problems with teat traits are often reported in indicus cattle, and not so much in Holstein cattle, so there are not many reports in the literature for these traits. Our results showed that TL significant SNP overlapped with a known QTL associated with teat placement, in a region on BTA 22. Also, TD top windows were overlapping with a QTL for udder conformations traits, on BTA 20 and BTA 21. We might consider this overlap between teat and udder QTLs as an expected result because TD is genetically correlated with udder traits, such as RU and UD (0.53 and 0.54 respectively; Dominguez-Castaño et al 2023). The top regions reported in this study for BTA 20 and BTA 21 should be considered in future studies investigating the genes that underpin the known genetic correlation between teat and udder traits in Gir dairy cattle.

The HG top windows discovered here did not overlap with known QTL for HG. However, approximately 66% of known QTLs that overlapped with the top 10 windows for HG were QTL for body weight (Figure 5). This result aligns with the strong genetic correlation described between HG and body weight (Koenen and Groen 1998; Abbasi and Ghafouri-Kesbi 2011). Therefore, selection targeting the HG trait in Gir cattle, including using markers in these top regions, might lead to heavier body weights on average.

Candidate genes were identified by examining only the windows that overlapped known QTLs corresponding to the specific traits being studied (i.e., genomic regions with supporting evidence from the results and the literature). Several candidate genes were identified, and the full list was provided (see supplementary materials). This discussion focuses on genes with a biological function that should be relevant to the studied traits.

In discussing candidate genes associated with HH, we focused on genes related to animal growth, tissue development, and metabolism. In the window on BTA19 which was associated with HH, we identified *PIK3R5* and *PIK3R6* genes. These genes are a regulatory subunit of the phosphatidylinositol 3-kinase (*PI3K*) gamma complex and its signaling pathway has been implicated in the action of insulin and many growth factors (Winnay et al. 2016). *PIK3R5* gene was previously identified in a region associated with body size in sheep (Kominakis et al. 2017) and *PIK3R6* showed direct functional associations with height and body size in Beninese indigenous cattle breeds (Vanvanhossou et al. 2020). Some of the other candidate genes for HH are involved in the accumulation and metabolic regulation of fatty acid, like the *TECRL* gene (Xie et al. 2021), lipid biosynthesis, such as the *LPCAT4* gene (Banliat et al. 2019), and visceral and subcutaneous fat depots, as the *CDC42* gene (Hishikawa et al. 2005). In short, these genes are relevant to fat deposition. According to Nascimento et al. (2018), early fat deposition in Nelore cattle can have a decreased growth rate. Indicating a relationship between fat deposition and growth, which could possibly explain the association of these candidate genes with HH in the current study.

Three potential candidate genes were associated with UD, two of them (*AMFR*, and *GNAO1*) on BTA 18 and one (*PTH2R*) on BTA 2. *AMFR* gene was found to be significantly associated with fat percentage and protein percentage in Holstein cows (Cole et al. 2011), *PTH2R* was described as candidate gene for days open in Japanese Black cattle (Sasaki et al. 2016), and *GNAO1* was related to reproduction, participating in ovarian follicle development in cross-breed beef heifers (Zielak-Steciwko et al. 2014) and in the puberty in *Bos indicus* cattle (Nguyen et al. 2022). Also, the *GNAO1* gene was involved in enriched pathways of biological relevance for milk yield, fat yield and age at first calving in Thai multibreed dairy cattle (Laodim et al. 2019). Two more genes, *RALYL* (BTA 14) and *MIER3* (BTA 20), were annotated as potential candidate genes

for RU. The gene *MIER3* was previously associated with survival in Holstein and Jersey cattle (Raven et al. 2014). The gene *RALYL* was related to persistent bovine viral diarrhea virus infection in cross-bred cattle and with pre-weaning calf mortality in Nellore cattle (Casas et al. 2015; Marín-Garzón et al. 2021). No reports were found in the literature on the association of *RALYL* and *MIER3* specifically with udder traits, so our results require confirmation and further research.

The genomic region identified on BTA12 for MY was associated with the potential candidate genes *DHRS12* and *CCDC70*. The gene *DHRS12* encodes a member of the dehydrogenase-reductase short-chain family. Members of this family are enzymes that play a pivotal role in the metabolism of a wide array of compounds, including steroid hormones, prostaglandins, retinoids, lipids, and xenobiotics. Notably, *DHRS12* was associated with oleic acid levels in Nelore cattle (Lemos et al. 2016), as well as with serum albumin concentrations in both Irish beef-suckler and dairy calves (Johnston et al. 2020). Furthermore, *DHRS12* plays a role in bovine mammary epithelial cells, specifically in its response to mastitis infections (Wang et al. 2020), underscoring its significance in diverse biological contexts. *CCDC70* was associated with semen traits in pigs (Zhang et al. 2023) and in mice (Chen et al. 2016). However, despite its importance for the present study, *DHRS12* and *CCDC70* have not been reported in studies directly investigating milk production traits in cattle.

This study did not detect *DGAT1*, a well-known and previously reported gene for milk traits in *Bos taurus taurus* cattle (Grisart et al. 2002; Thaller et al. 2003; Ning et al. 2018). Likewise, other studies conducted on *Bos indicus* cattle such Gir breed (Toro-Ospina et al. 2023) and crossbreed *Bos taurus indicus* x *Bos taurus taurus* cattle, as Girolando breed (Gir x Holstein) (Otto et al. 2020; Da Cruz et al. 2021), did not detect *DGAT1* as related to milk yield. In the study by Toro-Ospina et al. (2023), two distinct Gir cattle populations were examined, leading to the identification of separate sets of candidate genes associated with milk yield within each population. In Girolando cattle, Da Cruz et al. (2021), described that *NUB1* and *SLC24A2* are related to milk production. Different results were shown by Otto et al. (2020) using the same breed. The authors suggested seven genes related to milk yield on BTA1, 4, 6 and 26, which did not coincide with those found by Da Cruz et al. (2021). These results collectively indicate that genomic regions related to MY in Gir cattle are not necessarily the same

as those described in *Bos taurus taurus* or crossbred cattle. In short, more studies should be conducted in Gir cattle to identify genomic regions related to MY in this breed.

Pleiotropy is an important phenomenon for polygenic traits. Pleiotropy describes the circumstances in which a single genetic locus influences two or more separate phenotypic traits. For example, *LCORL* and *PTGER4* were related as pleiotropic genes for body conformation traits in Holstein cows (Wang et al. 2022). In the same way, the *DGAT1* gene was associated with milk yield (Ning et al. 2018) and fat yield (Ibeagha-Awemu et al. 2016). This study reported a genomic region on BTA3 in common between the traits HH, HG, RA, RU and TL (Tables 2-4; Figure 1 and 2). Indicating the relevance of this region for conformation traits, hence, the region on BTA3 should be considered in designing SNP chips to enhance genomic selection in Gir cattle.

The top 10 regions from our study potentially harbor new QTLs and previously unknown candidate genes. Some QTLs and candidate genes identified in this study were not previously associated with milk yield, frame, or udder conformation traits. These inconsistencies might be due to differences in breed or particularities of the studied herd, such as the extent of LD, allelic frequencies, sample size, or discrepancy in the statistical approaches (Wang et al. 2022). This also indicated that there are many important genetic markers or candidate genes in the bovine genome that are yet to be discovered. The study of *Bos indicus* breeds could reveal different potential candidate genes linked to traits of economic interest.

5 Conclusion

The association study provided the top 10 windows explaining the highest percentage of additive genetic variance for milk yield, frame and udder traits in Gir dairy cattle. Some of these windows were located within QTL regions previously reported as related to milk yield, body height, udder width and udder depth. Additionally, 16 genes were identified as potential candidate genes for MY, HH, RU and UD. Of these genes, *PIK3R5* and *PIK3R6* had been previously related to cattle body size. Some of the windows were novel genomic locations that could be used to

better understand the complexity of the genetic basis of the studied traits. These findings establish a foundational platform for further studies that aim to understand the biological pathways that affect milk yield, frame and udder traits in dairy cattle, particularly in Gir cattle. This research is relevant to *Bos indicus* cattle and the tropical industries that breed these adapted animals.

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APÊNDICE B

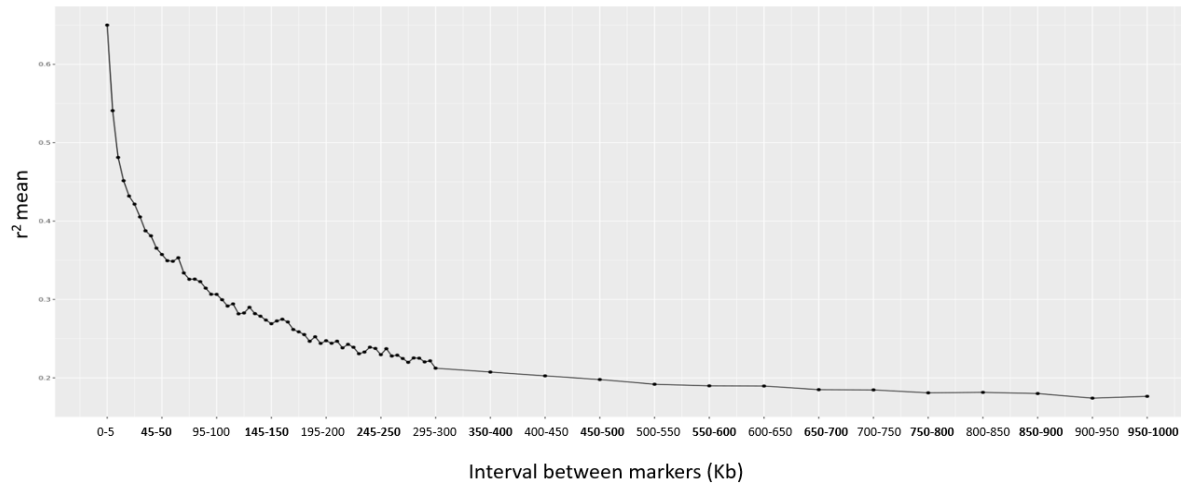


Figure 1B. Linkage disequilibrium decay calculated as the r^2 mean within interval of 5kb between markers from 0 to 300kb and within a 50kb of interval between markers from 300 to 1000kb.