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CÂMPUS DE JABOTICABAL

GENETIC PARAMETERS AND GENOME-WIDE ASSOCIATION STUDY FOR LINEAR CONFORMATION TRAITS, MILKING EASE AND TEMPERAMENT IN DAIRY GIR

Samla Marques Freire Cunha
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“Devo lembrá-lo que a verdade pode ser vital, mas, sem amor, ela é insuportável.”

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GENETIC PARAMETERS AND GENOME-WIDE ASSOCIATION STUDY FOR LINEAR CONFORMATION TRAITS, MILKING EASE AND TEMPERAMENT IN DAIRY GIR

ABSTRACT – Conformation and handling traits can be used in the indirect selection to improve longevity, health, and welfare of dairy cattle. To optimize genetic gains for these traits it is necessary use statistical models that will best fit the phenotypic data and allow the understanding on how these traits respond to selection and their genetic association with milk yield. The knowledge about genetic variants that may be associated with these traits can also contribute to the selection process. The objective of this study was to determine how fixed and random effects should be included in the statistical models for conformation traits, milking ease, temperament, and 305-days cumulative milk yield for Dairy Gir cattle and estimate genetic and phenotypic parameters. Besides, aimed identify genes and quantitative trait loci (QTL) associated with udder conformation traits, milking ease, and temperament of the breed. The genotype and phenotype records used in this study was provided by Embrapa Dairy Cattle (Embrapa Gado de Leite). To identify the model that fitted the data better, four models were tested in one-trait analyses performed to estimate the variance components using the Average Information Restricted Maximum Likelihood (AIREML) within linear and threshold model. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to identify which model fitted the data better. Two-trait analyses were performed using AIREML methodology to estimate genetic and phenotypic parameters for the studied traits. The weighted single-step genome-wide association study methodology was used to identify moving windows of 20 adjacent SNP that explained more than 1% of the total genetic variance. Candidate genes were annotated inside the identified widows using the ARS-UCD1.2 bovine genome assembly, and QTL annotation was performed using the UMD3.1 bovine genome assembly. Functional information and biological pathways were identified using the candidate genes. Linear models performed better than threshold models based on their AIC and BIC values. Among the linear models, the one that included only significant ($p<0.05$) fixed effects and contemporary group (CG) as random effect fitted better the phenotypic data, based on the AIC and BIC values. Heritability estimates for the studied traits ranged from 0.07 (0.01) to 0.50 (0.03). Heritability and repeatability estimates for continuous traits were higher when compared to heritability estimates from traits measured in categorical scale. The selection to increase rump length, rear udder width, udder depth, and teat diameter can contribute to increase the mean of milk yield due to genetic correlation estimates between them. The genomic association study identified important genes and relevant QTL for udder conformation and handling traits. Fore udder attachment was associated with three genomic windows (BTA 1, 21, and 28). Among the identified genes within these regions, the *KLC1* and *XRCC3* (BTA21) were found express in mammary gland tissue which make them candidates genes related to the studied trait. For rear udder width, one genetic window located on BTA1 was found explained more than 1% of the genetic variance. Genomic windows on BTA1, were identified for fore udder attachment and rear udder width overlapping. For both windows, the *URB1* was found which can make it a candidate gene; however, little in know about its function. Five windows were located on BTA 5, 6, 9, 14, and 16 for udder depth. On BTA14, two candidate genes were identified (*CYP7B1* and *BHLHE22*) and both genes were found related to mammary gland tissue. The *CYP7B1* gene was related to onset the puberty, estrogenic functions, and reproductive organs and mammary gland development. For milking ease and temperament, two (BTA 5 and 19) and three (BTA 4, 18, and 29) windows was associated with the traits, respectively. One gene (*CA10*) was identified for milking ease within BTA 19 which is related with synapses function in the cerebrum and plays a role in secretory pathways. Two genes were identified for temperament located on BTA4 and BTA18 (*TFEC* and *CDH11*, respectively) and they are related with behavioral traits. Based on these results, the statistic model with the best fit for the phenotypic data included only statistically significant ($p<0.05$) fixed effects and CG as random effect. Heritability estimates showed that it is possibly change the studied traits through selection. The traits with the high response to selection will be stature, teat length, and navel length. The results obtained from the genomic association study can be helpful to understand the genetic structure of the udder conformation traits, milking ease, and temperament; specially, fore udder attachment, udder depth, milking ease, and temperament. All results found here are very important in the breed genetic development and can be applied in the Gir breeding program.

Keywords: Dairy cattle, selection, SNP, welfare, WssGWAS, zebu cattle

PARÂMETROS GENÉTICOS E ASSOCIAÇÃO GENÔMICA AMPLA PARA CARACTERÍSTICAS LINEARES DE CONFORMAÇÃO, FACILIDADE DE ORDENHA E TEMPERAMENTO EM GIR LEITEIRO

RESUMO – Características lineares de conformação e manejo podem ser utilizadas na seleção indireta para melhorar longevidade, saúde e bem-estar dos bovinos leiteiros. Para se otimizar os ganhos genéticos para essas características é necessário utilizar modelos estatísticos que melhor ajustarão os dados fenotípicos e que permitam a compreensão sobre como essas características respondem à seleção e a sua associação genética com a produção de leite. O conhecimento sobre variantes genéticas que podem ser associadas a essas características também pode contribuir para o processo de seleção. Assim, o objetivo desse estudo foi determinar como os efeitos fixos e aleatórios devem ser incluídos nos modelos estatísticos para as características de conformação, facilidade de ordenha, temperamento e produção de leite acumulada até os 305 dias no Gir Leiteiro e estimar parâmetros genéticos e fenotípicos. Além disso, objetivou-se identificar genes e QTL associados com as características de conformação de úbere, facilidade de ordenha e temperamento da raça. Os registros fenotípicos e genotípicos utilizados nesse estudo foram cedidos pela Embrapa Gado de Leite. Para identificar o modelo estatístico que melhor ajustaria os dados, quatro modelos foram testados em análises uni-características, realizadas para estimar os componentes de variância utilizando o algoritmo AIREML em modelos lineares e multinomiais. O Critério de Informação Akaike (AIC) e o Critério de Informação Bayesiano (BIC) foram utilizados para identificar qual modelo ajustou melhor os dados. Análises bi-características utilizando a metodologia AIREML foram realizadas para estimar parâmetros genéticos e fenotípicos das características estudadas. A metodologia de estudo de associação genômica ampla de passo único ponderada (WssGWAS) foi utilizada para identificar janelas deslizantes com 20 SNP adjacentes que explicassem mais que 1% de toda variação genética. Os genes candidatos foram anotados nas janelas identificadas utilizando a montagem de referência do genoma bovino ARS-UCD1.2 e a anotação de QTL foi realizada utilizando a montagem de referência do genoma bovino UMD3.1. As informações funcionais e vias metabólicas foram identificadas utilizando os genes candidatos encontrados. Os modelos lineares ajustaram melhor os dados fenotípicos do que os modelos multinomiais, com base nos valores de AIC e BIC. Entre os modelos lineares, aquele que inclui somente os efeitos fixos significativos ($p<0.05$) e grupo de contemporâneos (GC) como efeito aleatório ajustou melhor os dados fenotípicos, baseado nos valores de AIC e BIC. Estimativas de herdabilidade para as características estudadas variaram entre 0,07(0,01) a 0,50 (0,03). Estimativas de herdabilidade e repetibilidade para as características medidas em escala contínua foram mais altas quando comparadas com aquelas para as características medidas escala categórica. A seleção para aumentar o comprimento de garupa, largura posterior do úbere, profundidade de úbere e diâmetros dos tetos pode contribuir para aumentar a média de produção de leite devido as estimativas de correlação genética entre as mesmas. O estudo de associação genômica ampla identificou genes importantes e QTL relevantes para as características de conformação de úbere e comportamentais. Três janelas genômicas foram identificadas associadas com a característica de ligamento do úbere anterior (BTA 1, 21 e 28). Entre os genes identificados dentro dessas regiões, o *KLC1* e o *XRCC3* (BTA 21) foram encontrados expressos em tecidos da glândula mamária, o que faz desses genes candidatos para a característica estudada. Para largura posterior do úbere, uma janela genômica foi encontrada no BTA1 explicando mais que 1% da variância genética aditiva. Janelas genômicas no BTA 1 foram identificadas sobrepostas para as características ligamento do úbere anterior e largura posterior do úbere. Para ambas as janelas, o *URB1* foi identificado, o que faz desse gene candidato, porém pouco se sabe sobre suas funções. Cinco janelas foram localizadas nos BTA 5, 6, 9, 14 e 16 para a característica profundidade de úbere. No BTA 14, dois genes candidatos foram encontrados (*CYP7B1* e *BHLHE22*) e ambos foram identificados relacionados à glândula mamária. O gene *CYP7B1* foi relacionado com o início da puberdade, funções estrogênicas e desenvolvimento dos órgãos reprodutivos e desenvolvimento da glândula mamária. Para facilidade de ordenha e temperamento, duas (BTA 5 e 19) e três (BTA 4, 18 e 29) janelas foram associadas com as características, respectivamente. Um gene (*CA10*) foi identificado para facilidade de ordenha no BTA19, o qual está relacionado com as funções sinápticas no cerebelo e participa em vias secretoras. Dois genes foram identificados para temperamento no BTA4 e BTA18 (*TFEC* e *CDH11*, respectivamente) e estão relacionados com características comportamentais. Pelos resultados, o modelo estatístico que melhor ajustou os dados fenotípicos incluiu somente efeitos fixos estatisticamente significativos (p<0,05) e GC como efeito aleatório. A partir das estimativas de herdabilidade, é possível melhorar as características estudadas por meio da seleção. As características com maior resposta à seleção serão altura de garupa, comprimento dos tetos e comprimento do umbigo. Os resultados obtidos do estudo de associação genômica podem ser úteis no entendimento da estrutura genética das características de conformação do úbere, facilidade de ordenha e temperamento, principalmente para ligamento anterior, profundidade do úbere, facilidade de ordenha e temperamento. Os resultados encontrados são relevantes e podem ser aplicados no programa de seleção da raça Gir.

Palavras-chave: bem-estar, bovinos de leite, seleção, SNP, WssGWAS, zebuíno

ABBREVIATION LIST

27HC	27-hydroxycholesterol
ABCGIL	Brazilian Association of Dairy Gir Breeders
ABCZ	Brazilian Association of Zebu Breeders
ACTH	adrenocorticotrophic hormone
AIC	Akaike information criterion
AIREML	Average Information Restricted Maximum Likelihood
ASD	Autism spectrum disorder
BHLHE22	Basic Helix-Loop-Helix Family Member E22
BIC	Bayesian information criterion
BL	Body length
BLAD	Bovine Leukocyte Adhesion Deficiency
BLUP	Best linear Unbiased prediction
BTA	*Bos taurus* chromosome
CA10	Carbonic Anhydrase 10
CDH11	Cadherin 11
CG	Contemporary group
CVM	Complex Vertebral Malformation
CYP7B1	Cytochrome P450 Family 7 Subfamily B Member 1
D	Diet
DNA	Deoxyribonucleic acid
DUMPS	Deficiency of Uridine Monophosphate Synthase
E	Evaluator
E_2	Estrogen
EBV	Estimated breeding values
EMT	Epithelial to mesenchymal transition
ERα	Estrogen receptor α
ERβ	Estrogen receptor β
FA	Foot angle
FAM227A	Family with Sequence Similarity 227 Member A

FAO	Food and Agriculture Organization of the United Nations
FDZ8	Frizzled Class Receptor 8
FUA	Fore udder attachment
GBLUP	Genomic best linear unbiased prediction
GEBV	Genomic estimated breeding value
GLM	General linear model
GO	Gene Ontology
GWAS	Genome-wide association study
H	Herd
HD	High Density
HG	Hearth girth
HW	Hook width
KEGG	Kyoto Encyclopedia of Genes and Genomes
KLC1	Kinesin Light Chain 1
KLHL2	Kelch-like family member 2
KM	Knockout mice
KRT5	Keratin 5
L	Linear effect of dam age
LD	Linkage disequilibrium
LRV	Rear legs – rear view
LSV	Rear legs – side view
M1	Model 1
M2	Model 2
M3	Model 3
M4	Model 4
MAF	Minor allele frequency
mb	Mega base pairs
ME	Milking ease
MeSH	Medical Subject Headings
NL	Navel length
NMHC-A	Non-muscle myosin II heavy chain A
PGBD5	PiggyBac Transposable Element Derived 5

PNMGL	Brazilian Dairy Gir National Breeding Program
PPP1R13B	Protein Phosphatase 1 Regulatory Subunit 13B
pre-mRNA	precursor messenger RNA
PTA	Predicted transmitting ability
PW	Pin width
Q	Quadratic effect of dam age
QTL	Quantitative trait loci
RA	Rump angle
RL	Rump length
RNA5S1	RNA, 5S Ribosomal 1
rRNA	ribosomal RNA
RUW	Rear udder width
SCS	Somatic cell score
SE	Season of evaluation
SNP	Single nucleotide polymorphisms
snRNA	small nuclear RNA
ssGBLUP	Single-step genomic best linear unbiased prediction
ssGWAS	Single-step genome-wide association study
STA	Stature
TD	Teat diameter
TEM	Temperament
TFEC	Transcription Factor EC
TGF-β	Transforming Growth Factor β
TL	Teat length
U6	U6 gene
UD	Udder depth
URB1	URB1 Ribosome Biogenesis Homolog
WGBLUP	Weighted genomic best linear unbiased prediction
WM	Wilder mice
WNK	With-no-lysine kinase
WssGWAS	Weighted single-step Genome Wide Association Study
XRCC3	X-ray Repair Cross Complementing 3

YE	Year of evaluation
ZFYVE21	Zing Finger FYVE-Type Containing 21

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CHAPTER 1 – General Considerations

1. Introduction

The dairy industry in Brazil is an important economic sector and it is responsible on the employment generation direct and indirect through the milk production chain (Perobelli et al., 2018). According to FAO (2020), in 2019, Brazil occupied the fifth position in the world milk production rank, with 35,169 million tons which demonstrated an increase of 3.1% when compared to previous year. The most challenge condition for farmers in Brazil is the tropical climate that covers most of the country. The combination of hot air temperature and high humidity can lead to heat stress in dairy cows which is a problematic situation. Thus, the use of Dairy Zebu breeds can be recommended whereas they are characterized by its thermo-tolerance, as well as rusticity, resistance to parasites, and great capacity of gross roughage utilization (Vercesi-Filho et al., 2010; Rewe et al., 2015).

Breeding programs of Zebu breeds in Brazil are important in the maintenance of crossbreeding schemes, widely practiced with European cattle. Crossbreed animals are responsible for around 80% of the total milk production in the country (Vercesi-Filho et al., 2010). The Girolando breed (which is formed by Dairy Gir x Holstein cross) and the Gir breed, a Zebu cattle, is the second and fourth, respectively, most used breeds in the 100 largest milk farmers in Brazil which demonstrates the importance of the Dairy Zebu cattle in the Brazilian milk production (Carvalho et al., 2019).

In Brazil, milk yield still is the most important trait in a dairy milk farm. However, the exclusive selection for this trait over the years can negatively affect health, productive lifespan, corporal composition, and reproductive performance which leads to a premature culling of animals and consequently increases the expenses with animal replacement in commercial herds (Rennó et al., 2003). In this context, conformation and handling (milking ease and temperament) traits can be used in the indirect selection to improve longevity, health, and welfare, because they are favorable genetic correlated with functional longevity, have moderate heritability estimates, are suitable indicators of an animal's health, are easy to evaluate, and measurement are obtained relatively early in the animal's life, normally in the first lactation (Sewalem et al., 2004; Daliri et al., 2008; Kern et al., 2014).

Kern et al. (2014) showed that mammary system conformation traits, such as udder depth, udder height, and rear teat placement and corporal dimension traits, such as stature, chest width, body depth, and loin strength had higher heritability estimates than longevity and presented considerable genetic correlation with measurements of functional longevity. In the same way, Sewalem et al. (2004) concluded that mammary system and feet and legs traits had strong relationship with functional longevity. Selection for conformation traits can help improve general health, for example, animals with better udder conformation are less susceptible to mastitis and trauma (Bharti et al., 2015) and animals with better conformation of feet and legs have less chance of develop hoof lesions (Chapinal et al., 2013).

Handling traits have a higher impact in the daily management when compared with conformation traits. Reactive or aggressive animals and with slow milking can have their productive performance affected and delay the milking process. Besides, reactive animals also increase teat cups risk of failing which lead to contamination and affects milk quality as well as enhance the occurrence of mastitis throughout the flock (Costa et al., 2015). It is a well-known fact that *Bos indicus* animals are more reactive than *Bos taurus*, which make more necessary understand the animal’s behavior in herds with Zebu cattle, mainly if the animals’ milking is automatic, without the calf and in primiparous cows (Fordyce et al., 1988; Costa et al., 2015).

Based on the importance of conformation and handling traits, their inclusion in a breeding program relies on knowing how they respond to selection and the correlation between them and other traits, such as milk yield (Sewalem et al., 2004). However, there is a lack of studies aiming to estimate genetic parameters of conformation and handling traits and milk yield for Gir breed, since most of the studies are with European breeds in temperate countries (Lagrotta et al., 2010). Besides, these estimates differ a lot among studies which may be due to several reasons, such as different breeds analyzed, number of animals evaluated, and statistical models’ definitions (Nemcova et al., 2011). Therefore, it is necessary define optimized statistical models, as well as estimate genetic parameters for each breed, specifically, which will lead to higher gains in the selection process.

Another valuable resource that can contribute to the dairy cattle improvement is the genomic information, which can be applied in the genomic selection and in

Genome-wide association studies (GWAS). The GWAS, allows us to identify quantitative trait loci (QTL) and genes that are responsible for the phenotypic expression of important economic traits. These results can be used posteriorly to increase genetic gain faster, through genomic selection or to a better understand of the genetic structure of these traits (Hirschhorn and Daly, 2005; Korte and Farlow, 2013). However, association studies between genotype and phenotype for conformation and handling traits were not found for Dairy Gir cattle.

Therefore, the objective of this study was defined optimized statistical models for the conformation traits, milking ease, and temperament in the Dairy Gir cattle. It was estimated genetic and phenotypic parameters for conformation and handling traits and 305-days cumulative milk yield to contribute with the breed selective breeding process. Besides, identified genes and QTL associated with udder conformation traits, temperament and milking ease for the Gir breed.

2. Literature Review

2.1. Gir breed history in Brazil

The Gir breed is originated from India, more specifically from the hills and forest of Kathiawar including Junagadh, Bhavnagar, Rajkot, and Amreli districts of Gujarat. In India, the breed is known with other names but the name Gir is in reference to the Gir forest the natural habitat of the breed. The Gir breed is well adapted to the tropical climate, with high tolerance to heat stress and resistance to various tropical diseases which made the breed well accepted in Brazil after its importation (Gaur et al., 2003).

With coffee plantations in decay in Brazil between 1864 and 1870, the farmers needed to change their agricultural activity. Thereby, Brazil started importing Zebu cattle from India. With world war I, Brazil increased meat exports which favored the continued import of Zebu. The migration of people to more hot regions inside Brazil and most of the European cattle being devastated because some tropical disease resulted in the first Gir cattle import in 1921 (Araújo et al., 2012; Santana Jr et al., 2014).

In the middle of 1930, a civil revolution changed the economic scenario in Brazil. With low investments from the government, the crossbreed (*Bos indicus* x *Bos taurus*) was put aside and the farmers opted for the pure zebu breed. A few years later, Getúlio

Vargas, who was the president at the time, encourage the establishment of the Herd Book for Zebu breeds by the Brazilian Association of Zebu Breeders (ABCZ) which contributed to spread the pure zebu including the Gir breed (Araújo et al., 2012; Santana Jr et al., 2014).

Between 1950 and 1960, some farmers started to milk Gir cows which divided the breed in two ability: The Dairy Gir and the Beef Gir. The beef Gir started to lose space for the Nelore cattle and the Dairy Gir received the last importation of animals from India, after 1955 (Araújo et al., 2012).

In 1985, the Brazilian Dairy Gir National Breeding Program (PNMGL) was created and the first result of the genetic evaluation of milk and fat was published in 1993 for a group of nine Gir bulls (Santana Jr et al., 2014). In 1994, the linear evaluation began in the pure Gir cows and in 1999 the measurement of protein, lactose, total solids, and somatic cell score (SCS) began. In 2001, the blood and semen collection started with an objective to build a DNA bank. In 2006, the first molecular results about the kappa-casein and beta-lactoglobulin was published. In 2013, results about the hereditary diseases such as Deficiency of Uridine Monophosphate Synthase (DUMPS), Complex Vertebral Malformation (CVM), and Bovine Leukocyte Adhesion Deficiency (BLAD) were introduced in the PNMGL. In 2016, the molecular information about the beta-casein gene was included in the same breeding program (Panetto et al., 2019).

Since 2016, genomic information has been used to help the selection of young bulls indicated to the progeny test. In 2018, the genomic selection was included in the Dairy Gir breeding program. In august of 2018, the first Dam Summary using genomic information for the Dairy Gir was published (Panetto et al., 2019). According to Fernandes et al. (2019) the use of genetically-testes bulls since 1993 (year when the first genetic evaluation result was made available) has taken the improvement of milk yield, udder width, milking ease, and temperament.

2.2. Linear evaluation

The linear evaluation in dairy cattle began in 1925 in Canada and in 1929 in the United States in pure breed Holstein. At first, the linear evaluation was a subjectively way to identify more productive animals in the herd through conformation and body measurements which was later replaced by dairy control. Over the years, the linear evaluation has been improved by using scores to describe traits and this information has been included in the genetic evaluation (Short and Lawlor, 1992; McManus and Saueressig, 1998). In Brazil, the linear evaluation began to be conducted in the Dairy Gir breed in 1994 (Panetto et al., 2019).

In dairy cows, the linear evaluation is performed individually, and each cow is evaluated by observation and body measures and then compared to a type standard considered ideal for each breed. Linear evaluation is useful for producers and technicians to measure traits that may be related to animal health, welfare and lifespan. In the Brazilian Dairy Gir National Breeding Program for the Dairy Gir cattle, 18 traits composed the linear evaluation and these traits can be separate in four categories. Corporal dimension, feet and legs, and mammary system are categories that describes the animal’s body conformation and measures. The handling category describes the animal’s daily management (Panetto et al., 2019). Traits description, measurements and scores recommendations described below are for the Gir breed pattern, applied in the Brazilian Dairy Gir National Breeding Program and was based on Panetto et al. (2019).

Traits belonging the corporal dimension category are stature, heart girth, body length, rump length, pin width, hook width, rump angle, and navel length. The stature is measured in centimeters and is the length of the animal from the top of the rump to the ground. The recommended is animals higher than 136 cm. The heart girth is measured in centimeters and is expected measures above 175 cm. The body length is the distance between the end of the shoulder blade and the ilium. It is measured in centimeters, and the optimal size is above than 102 cm. Navel length is measured in scores between 1 (short) to 9 (long), and the pattern are animals with shorter navel close to 1 cm.

The rump length is the distance between the tip of the ilium and the tip of the ischium in the same side of the animal, is measured in centimeters and the recommended are values above 40 cm. The pin width is the distance between the tip of the ischium from the left to the right side, the pattern are animals with sizes above 18 cm. The hook width is the distance between the tip of the ilium on the left side until the right side of the animal, measurements bigger than 48 cm are expected. The rump angle is measured in scores (1 – 9) and it is the difference between the height of the pin and the hip bones, if this value is close to zero, the rump is considered straight (score 1), but if this difference is large (close to 45° - score 9) the rump is considered low. The pattern for the breed are scores between 4 and 6.

Feet and legs category are composed by foot angle, rear legs – side view, and rear legs – rear view. In the Brazilian Dairy Gir, the foot angle can be measured as the angle formed between two imaginary line, the first parallel to the ground surface and another in the wall of the hoof. This trait is measured in scores (1 – 9) where score 1 (15°) represents a low foot angle and 9 (65°) represents a steep foot angle. Scores near to 5 (45°) are the pattern for the breed.

Rear legs - side view and rear view are both measured in scores (1 – 9). In the side view the angulation between the tibia and metatarsus bones can be wide and the animal show a straight leg (score 1) or the animal can have a small angulation with a curved leg (score 9). In the rear view, it is evaluated the proximity of the hocks, where the hocks can go inside and be closer (score 1 – hocked-in) and the hocks can go outside and be too far away (score 9). Therefore, the pattern are animals with an intermediary curvature in the side view (score 4 – 6) and a straight leg in the rear view (score 5).

Five conformation traits are evaluated in the mammary system category (fore udder attachment, rear udder width, udder depth, teat length, and teat diameter). The fore udder attachment can be measured drawing to imaginary lines. The first will be parallel to the ventral region of the animal and the second will be parallel to the lateral of the udder, forming an angle. The higher the angle, better is the fixation of the anterior udder. This trait is evaluated in scores between 1 (weak) with small angle to 9 (strong) with higher angle and the pattern are animals with score close to 9 as possible.

Rear udder width measures the rear udder, seen from behind, and represents the distance between the junction of the leg and the udder from the left side until the same junction on the right. This trait is also evaluated in score from 1 (narrow) to 9 (wide) and the pattern are animals with score equal to 9. Udder depth is the distance from the hock to the floor of the udder. This trait is measured in scores from 1 (shallow) that represents an udder floor close to the abdomen to 9 (deep) an udder floor that exceeds the hock, the pattern is an udder floor that is 10 cm above the hock (score 5).

Teat length and diameter are both measured in scores (1 – 9). The pattern are animals with scores close to 5, that are approximately 7.5 cm long and 3.8 cm in diameter.

The handling category is composed by the traits milking ease and temperament. Milking ease is characterized as the time and effort spent at the cows' milking. The evaluation is in scores from 1 to 9 made by the farmer, where animals with score close to 1 are easy to milking and desirable to keep in the herd. The temperament trait is measured in scores that vary between "very tame" (score 1) and "very aggressive" (score 9). The desired are animals with scores close to 1, indicating docile animals with easy handling.

2.3. Importance of the conformation and handling traits

Conformation traits can be a proper indicator of an animal's health in the future life in the herd, thereby avoiding early culling in a commercial herd and increasing the cow's longevity. In addition to help in the indirect selection to longevity, conformation traits can assist selection of animals with better conformation body to improve general health and welfare. Besides that, these traits are easy to evaluate, and measurement usually are done in the first lactation (Sewalem et al., 2004; Daliri et al., 2008).

Several studies were done to understand how conformation and handling traits relate to health, welfare, and daily management in the Holstein breed (Wenceslau et al., 2000; Sewalem et al., 2004; Daliri et al., 2008; Onyiro and Brotherstone, 2008; Sawa et al., 2013). Although, most of the results are about European cattle, it is possible to make an overall of the importance and impact of these traits in a dairy milk herd, whereas the traits describe the same phenotypes across different breeds.

In the corporal dimension category, traits that describes the animal's rump are very important in a dairy farm. Rump length, pin width, hook width, and rump angle describe the size and inclination of the rump and are related with the udder back support and the easy of parturition. Cows with long and little inclined rump is favorable for the udder extension and the correct fixation of the elastic suspensory ligament supporting the udder at the pelvic symphysis and ischiatic bones (Nogalski and Mordas, 2012).

Sawa et al. (2013) showed that Holstein cows with sloping or raised rump were required most frequently human intervention at calving. Cows with raised rump presented more dystocia parturition and needed for caesarean sections and cows with sloping rump required much more force than normal to deliver the calf. The rump angle can change the size of the pelvic inlet and outlet, which are the most critical places at the calving moment. Cows with more difficult on calving has more frequently placenta retention because of the previous stress from the parturition and the difficult course of the birth canal. Besides that, too sloping rump could lead for more incidence of uterus and vaginal prolapse (Nogalski, 2002; Nogalski and Mordas, 2012).

Nogalski and Mordas (2012) concluded that beyond the calf size, the pelvic area also contributes to difficult calving, where animals with larger pelvis diameter has less incidence of dystocic parturition. Dadati et al. (1985) described that rump score, which is a general evaluation of the rump, including hips and pins width (rump width) and rump angle among other measurements, had a favorable genetic correlation with ease calving. The authors also concluded that width of ischia is probably the most important conformation trait for ease calving.

Besides the rump's traits, the navel length can be a very important trait in the corporal dimension category. Animals with long navel are more susceptible to pathologies, such as foreskin prolapse, due to constant contact with grass. As well as, longer navel can hinder or disable the copulation and lead to loss of libido (Boligon et al., 2016; Romero et al., 2018; Romero et al., 2018a). Rabelo et al. (2008) studied the epidemiological aspects of surgical diseases in the genital tract of bulls in Brazil and reported that almost 83% of the affected animals were zebus and of those almost 36% were Gir animals. As highlighted by the authors, the higher frequency of affected Zebu animals when compared to the European cattle may be associated to the anatomical conformation, such as pendulous prepuce, which in the Gir breed, is measured by the navel length in the dams.

The feet and legs category are an important group of linear traits, that can determine the animal's lifespan in the herd. Problems in the feet and legs of the animals (lameness, infections, inflammations, lesions, horn diseases) are the most common cause for culling in a Dairy farm (Fatehi et al., 2003). Sewalem et al. (2004) concluded that cows with extreme score classification (1 to 3 and 7 to 9) for foot angle and rear legs – side view are more susceptible to be discarded from the herd, reducing their lifespan.

Besides reducing the animal’s lifespan bad feet and legs conformation can increase the occurrence of hoof lesions. Chapinal et al. (2013) founded phenotypic correlation between low foot angle (score = 1 to 3), curved rear legs – side view (score = 9), hocked-in legs (score = 1 to 2, rear view), and low score for locomotion (score = 1 to 2) with a high incidence of horn lesions. Locomotion trait recorded in different lactations in the animal life, besides cheap and easy to record, is high genetically correlated with roof lesions such as interdigital dermatitis, heel horn erosion, digital dermatitis, interdigital hyperplasia, and chronic laminitis. It also can be a good indicator of latter developing of claw disorders in the animal’s life (van der Waaji et al., 2005). However, the frequency of hoof lesions can be reduced by genetic selection and better environment management (Chapinal et al., 2013).

Traits that describe feet and legs are highly related with the environment that the animals are living. According to Onyiro and Brotherstone (2008), cows that lived in pasture had better score for feet and legs conformation and less locomotive problems which can be due to the softness of the grass to lying and walking. By that, the indication is no slippery, level floor, and cleaned paving to reduce locomotion disorders. Likewise, Onyiro et al. (2008) concluded that cows living in pasture had reduced incidence of digital dermatitis when compared with other housing systems.

Seemingly that feet and legs conformation are not the only reason for locomotor problems. Onyiro and Brotherstone (2008) estimated genetic correlation of 0.30 and 0.48 between locomotor trait with foot angle and with mammary system, respectively. Implying that cows with low foot angle and sagging or pendulous udders are more likely to present locomotor problems.

The mammary system is the most important group of traits for the milk production system, because it impacts directly the cow’s milk yield. So, cows less susceptible to develop udder diseases will reduce costs with treatments, keep the milk production constant, have a longer lifespan, be healthier, and will have a better welfare. Zwald et al. (2004) positively correlated sire predicted transmitting ability (PTA) for clinical mastitis with PTA for udder traits (fore udder attachment, rear udder height, rear udder width, udder cleft, udder depth, front teat placement and teat length) for the US dairy cattle meaning that an udder with an ideal conformation is more likely to reduce mastitis incidence, because they are less likely to injury, to soiled, and are completely milked.

Bharti et al. (2015) found a high somatic cell count in crossbred Jersey cows under hot-humid climate with deep udder (close to the floor), which indicate a larger probability of developing mastitis. This can happen because deeper udder has larger tendency to get dirty and to get injury. The authors also found a significantly positive correlation ($p<0.01$) between somatic cell count and thick and long teat which can probably be attributed to milking-machine incompatibilities, which leads to frequent liner slips increasing the risk for intra-mammary infections.

Swedish Holstein and Swedish Red cows had significantly negative genetic correlation estimates (ranging from low to moderate) between udder health (lactation average SCS and risk of clinical mastitis) with udder depth and with fore udder attachment suggesting that shallow udders with strong fore attachment are associated with lower risk of clinical mastitis. In addition to this Swedish Holstein cows with thin teat were positive genetic correlated with udder health, where thinner teat is associated with lower risk of clinical mastitis (Carlström et al., 2016).

Investigating the South African Jersey cattle (Dube et al., 2008), Holstein cows (Juozaitiene et al., 2006), Danish Holstein (Lund et al., 1994), and Danish Red, Danish Holstein, and Danish Jersey (Sorensen et al., 2000), the authors concluded that cows with deeper udder, week fore udder attachment, and long teat are associated with high probability of clinical mastitis.

Besides all the associations between mastitis and udder conformation, anterior udder with strong attachment was also associated with cows that had lower risk for udder cleft dermatitis, which is a skin lesion located at the anterior junction between the udder and the abdominal wall or between the front quarters of the udder (Waller et al., 2014). The udder and teat conformation traits can be considered to decide which animals can be removed from the herd and which ones will continue their productive life, whereas they had high influence in the animals susceptible to mastitis and udder and teat trauma (Sewalem et al., 2004).

The two traits that belong to the handling category are milking ease and temperament. Milking ease, also called milking speed, sometimes can be measured using two different approaches. In the first, the farmer evaluated the animals using a scale from 1 to 5 or 1 to 9. The second method is more objective, and the trait is measured as kilograms of milk per minute during the complete milking event or only in the main milking phase (Kramer et al., 2013). Cows to slow to be milked cows can delay the milking process what becomes undesirable (Luttinen and Juga, 1997) and cows to fast to be milked tend to have a high somatic cell score, which increase the probability of developing mastitis (Rupp and Boichard, 1999).

Temperament is defined as an animal's response to interaction with humans (Grandin, 1996). Several behavioral traces combined can compose an animal's temperament, such as fear, stubbornness, aggressiveness, shyness, curiosity, and nervousness. The combination of these can result in an animal more aggressive, reactive or calm, which can affect its productive performance (Costa et al., 2015). In a dairy system with Zebu animals, like Gir cows, the temperament trait can be more important than usual. Whereas, *Bos indicus* cattle are more aggressive and reactive than *Bos taurus* (Fordyce et al., 1988). At the milking, the zebu cattle can be more difficult to deal with, mostly if the milking is automatic, without the calf and in primiparous cows (Costa et al., 2015).

In a milk farm, the interaction between animal and men are daily; thus, an animal's non-aggressive response to the milking can maximize the process and reduce residual milk. (Fordyce et al., 1988; Haskell et al., 2014; Costa et al., 2015). Additionally, cattle with calmer temper are easily adapted to daily handling and manifest less stress with less cortisol liberation during the management (Grandin, 1996). Differently, aggressive animals can hurt other animals, farmer, and broke installations. Besides, it can increase teat cups risk of failing leading to contamination which directly affects the milk quality and enhance the probability of mastitis throughout the flock (Costa et al., 2015).

Carlström et al. (2016a) estimated genetic correlation between linearly scored temperament and teat cup attachment failures of -0.44 for Swedish Holstein and -0.71 for Swedish Red which means that in the automatic milking system calmer cows will have less problem with teat cup attachment and less risk of kicking off the teat cups. Temperament was also found genetic correlated with health. Lund et al. (1994) estimated genetic correlation of -0.96 between diseases and temperament showing that more reactive cows are more predisposed to develop health problems.

Temperament and milking ease can be correlated physiologically, and temperament can interfere with milking ease. During the milking, animals more aggressive or reactive to the milking process will be more stimulated to liberate high quantities of cortisol in the blood flow. The cortisol will inhibit the central nervous system release oxytocin which is responsible for the continuous milk ejection in the milking process. The high levels of cortisol will inhibit the oxytocin liberation by the central nervous system or its peripheral action at the mammary gland which will interrupt the milk flow. The milk flow interruption can happen also by the peripheral action of catecholamines, that will act directly in the mammary gland, increasing the duct resistance and reducing the milk flow during milking (Bruckmaier and Blum, 1998).

As concluded by Hemsworth et al. (1989) primiparous cows that had human contact at the time of the calving had less response (flinch, step, and kick) during milking time. As well as, these cows had a significantly smaller ($p<0.05$) overall mean of cortisol concentration in the milk. Fear can promote the cortisol production in the animal’s body, but with more contact with human, these animals display less fear and less cortisol levels (Hemsworth et al., 1989). However, for the selection and breeding of easier milking and calmer cows it is necessary initially understand the genetic and environmental factor that regulate these traits and how they interact.

2.4. Genetic parameters for conformation traits, milking ease, and temperament and their correlation with milk yield

Estimate genetic parameters for conformation traits, milking ease, and temperament and their correlation with milk yield has been aim of study by several researches over the years. Mostly of the studies were done with European breeds in temperate countries, such as the Holstein-Friesian (Berry et al., 2004; Nemcova et al., 2011), Brown Swiss (De Haas et al., 2007; Samoré et al., 2010; Kramer et al., 2013), and Red and White, (De Haas et al., 2007). Studies with the same objectives were performed for the Holstein breed in Brazil (Esteves et al., 2004; Campos et al., 2012; Campos et al., 2015), Brown Swiss (Rennó et al., 2003) and Dairy Gir (Lagrotta et al., 2010; Wenceslau et al., 2000).

Understand how these traits respond to selection is an important tool in a breeding program and for that, heritabilities are estimated. These estimates can differ a lot between reports, but in some cases follow the same tendency. The corporal dimension category has the highest estimates among all groups of traits and within this group, stature normally has the highest heritability estimate, but it differs a lot between studies (0.37 to 0.74) (Berry et al., 2004; De Haas et al., 2007; Duru et al., 2012; Campos et al., 2015). Other traits that differ a lot between results were pin width (or rump width) and rump angle, which the variation was from 0.16 to 0.49 and 0.09 to 0.41, respectively (Daliri et al., 2008; Nemcova et al., 2011; Duru et al., 2012; Campos et al., 2012).

For traits in the mammary system category, the range of heritability estimates in the literature for some traits were bigger than for others. Fore udder attachment, udder depth, and teat length had the largest variation and ranged from 0.00 to 0.36, 0.09 to 0.33, 0.007 to 0.45, respectively (Berry et al., 2004; Daliri et al., 2008; Campos et al., 2012; Duru et al., 2012). Whilst, rear udder width had a smaller variation ranging from 0.17 to 0.28 (Campos et al., 2012; Campos et al., 2015). Heritability estimates of 0.07 and 0.24 for teat diameter was found for the Gir breed in Brazil (Wenceslau et al., 2000; Lagrotta et al., 2010). Besides, the results were found for the same breed and same country, differences in results may be due to the number of animals used in both analyses.

Feet and legs category has the lowest heritability estimates, according to Pérez-Cabal and Alenda (2002) who estimated heritabilities for corporal dimension, mammary system, and feet and legs categories. Between the traits inside this category, rear legs – rear view had less estimates in the literature. Duru et al. (2012) and Nemcova et al. (2011) estimated heritabilities of 0.11 and 0.10 for this trait, for Holstein cattle in Turkey and Czech Republic, respectively. Several heritability estimates were found for foot angle, varying from 0.09 to 0.31 (Mrode et al., 1999; Lagrotta et al., 2010; Duru et al., 2012) and for rear legs – side view, ranging from 0.14 to 0.27 (Mrode et al., 1999; Lagrotta et al., 2010; Campos et al., 2012).

Heritability estimates for traits that describes the animal’s behavior are scarce. Furthermore, the methodologies for the evaluation of temperament and milking ease can differ among reports which makes difficult the comparison between results (Kramer et al., 2013). Berry et al. (2004) estimated heritability of 0.11 for milking ease and 0.12 for temperament in Holstein cattle. Both measurements were made in a scale from 1 – 9 by the herdsman where 1 represents slow to milking/nervous animals and 9 to fast to milking/calm animals. Resing and Ruten (2005) estimated heritability of 0.10 for milking ease in Holstein cattle using a scale from 1 (slow to milk) to 9 (fast to milk).

Another important genetic parameter in a breeding program is the genetic correlation between conformation and handling traits and milk yield. This knowledge can help in the selection of more productive animals, still considering more healthier animals that will stay longer in the herd (Daliri et al., 2008; Kern et al., 2014). Comparison between genetic correlation estimates in the literature need to be done very carefully, whereas traits description and evaluation methodology may be different between studies (Khan and Khan, 2016). As an example, udder depth (distance from the hock to the udder floor – evaluated in scores from 1 to 9). In the Brazilian Dairy Gir cattle, the national breeding program considers 1 shallow udder and 9 deep udder. For the Holstein in Canada, the breeding association consider 1 as deep and 9 as shallow udder, which can change in different direction the estimates (Holstein Association of Canada, 2018; Panetto et al., 2019).

Genetic correlation estimates between all conformation and handling traits varied in the literature and can range from positive to negative as well as between conformation and handling traits and milk yield. Genetic correlation estimates between stature and milk yield varied from -0.62 to 0.61 for Dairy Gir cattle and Holstein, respectively (Wenceslau et al., 2000; De Haas et al., 2007). Between pin width and milk yield, genetic correlation estimates ranged from 0.46 to -0.19 in Holstein and Dairy Gir cows, respectively (Berry et al., 2004; Lagrotta et al., 2010). De Hass et al. (2007) estimated genetic correlation between heart girth and milk yield of 0.39, 0.16, and 0.35 for Holstein, Brown Swiss, and Red and White cattle. Differently, Wenceslau et al. (2000) estimated genetic correlation of -0.37 for the same traits in Brazilian Gir cattle.

Genetic correlation between fore udder attachment and milk yield ranged from -0.50 to 0.32 (Berry et al., 2004; Lagrotta et al., 2010). As well as, genetic correlation between teat length and milk yield ranged from -0.16 to 0.13 (Rennó et al., 2003; Lagrotta et al., 2010). Genetic correlation between udder depth and milk yield varied from -0.05 to -0.46 (Berry et al., 2004; Campos et al., 2015) and for rear udder width and milk yield the estimates ranged from 0.40 to 0.61 (Alphonsus et al., 2010; Khan and Khan, 2016).

Genetic correlation estimates between milk yield and feet and legs traits ranged from negative to positive as well. Foot angle was the trait with most estimates found in the literature and the largest range, varying from -0.18 to 0.58 (Duru et al., 2012; Khan and Khan, 2016). In the same way, genetic correlation estimates between rear legs – side view and milk yield ranged from -0.08 to 0.30 (Lagrotta et al., 2010; Campos et al., 2015). Duru et al. (2012) and Khan and Khan (2016) estimated genetic correlation of 0.05 and -0.06 for Holstein and Sahiwal cows, respectively, between rear legs – rear legs and milk yield.

Genetic correlation estimates between conformation traits can be used to help optimize the number of selected traits and to understand how a second trait will respond to the selection process performed in a primary trait in a breeding program of dairy cows. Usually, estimates between traits within the same category were higher than between traits from different categories. Corporal dimension traits such as stature, body length, pin width, hook width, heart girth, and rump length had genetic correlation estimates varying from 0.15 to 0.81 for the Gir breed (Lagrotta et al., 2010). For the mammary system category, Dube et al. (2008) and Rénno et al. (2003) estimated genetic correlation between udder depth and fore udder attachment of 0.81 and 0.91 in Holstein and Brown Swiss cattle. Esteves et al. (2004) estimated genetic correlation of 0.42, 0.65, and 0.48 for fore udder attachment and udder depth, teat length and rear udder width, and rear udder width and udder depth, respectively.

For the feet and legs category, Nemcova et al. (2011) estimated genetic correlation between rear legs – rear view and rear legs – side view and foot angle of -0.40 and 0.39, and among rear legs – side view and foot angle of -0.67 for Holstein cattle. Esteves et al. (2004) estimated genetic correlation between foot angle and rear legs – side view of -0.44 for Holstein cattle.

Between the traits in the handling category, Berry et al. (2004) estimated genetic correlation between temperament and milking ease of 0.27 for Holstein cattle and Kramer et al. (2013) estimated genetic correlation of 0.18 for Brow Swiss cattle.

Differences in genetic parameters estimates may be due to several reasons, such as selection strategies, different levels of production between populations, scales used for score the traits, statistical model definitions, breeds analyzed, number of records, data editing, accuracy and consistency of classifiers, and trait definition (Samoré et al., 2010; Nemcova et al., 2011). For handling traits more reasons can be considered such as measurements made with different methodologies and in some cases those measures are subjectively made by the farmer in scales (Kramer et al., 2013). These elements difficult the comparison between results and the understanding of the traits.

Several studies aiming to estimate genetic parameters for conformation traits, milking ease, temperament, and milk yield are available in the literature. However, there is still a lack of studies with Dairy Gir cattle. Although Lagrotta et al. (2010) and Wenceslau et al. (2000) estimated genetic parameters for the Dairy Gir cattle, some traits were not considered in the analyzes and the relationship between these traits can differ a lot even in the same breed, which explain why it is necessary study these relationship for each breed and population over the years (De Haas et al., 2007).

2.5. Genome-wide association study (GWAS)

The first manuscripts published using the GWAS methodology were conducted by Klein et al. (2005) e Dewan et al. (2006) in humans. However, according to Visscher et al. (2012) the first assignment well delineated using GWAS was publish in 2007 (WELLCOME TRUST CASE CONTROL CONSORTIUM, 2007). The Genome-wide Association Study (GWAS) was initially used in human genetics for the identification of genes or regions in the genome that were related to diseases for a posterior study of the region, function, and metabolic pathways. In the animal breeding, the primally aim of the use of GWAS methodology is the association between single nucleotide polymorphisms (SNP) and quantitative trait loci (QTL) of economic interest (Rosa et al., 2013; Hirschhorn and Daly, 2005).

The GWAS methodology assumes that genes or unidentified mutations, responsible for relevant traits are in linkage disequilibrium (LD) with a SNP or group of SNP, meaning that these two regions are physically connected forming haplotype blocks that will segregate together in the population (Cantor et al., 2010; Rosa et al., 2013). The LD strongly depends on the population history, specifically the effective population size, which can change over time (Goddard and Hayes, 2009; Kruglyak, 1999). The LD patterns can differ among different populations from the same species can differ (O’Brien et al., 2014), as well as the QTL effects and allele frequencies among breeds (Raven et al., 2014).

Some requirements must be fulfilled to apply the GWAS methodology. First, it is necessary identify an important trait in the population which has reliable phenotypic records and genetic variation (Korte and Farlow, 2013). The number of samples with genotype and phenotype information need to be large enough to be representative of the population which will allow to identify the smallest associations (Cantor et al., 2010). Another requirement that needs to be performed is quality control of the SNP, obtained through genotyping panels and samples. The quality control aims remove SNPs or samples that are responsible for bias in the estimates. For example, SNP with low gene call score, low minor allele frequency (MAF), and deviating from Hardy-Weinberg equilibrium and samples with low call rate are excluded (Gondro et al., 2013).

Currently, two approaches normally are used in genome-wide association studies: Genomic best linear unbiased prediction (GBLUP) and Bayesian methods (Zhang et al., 2016). Bayesian methods, such as BayesA, BayesB, and BayesC are non-linear methods which assume heterogeneous variances of SNP effects and SNP related to major effects has emphasis in this approach which is an advantage from this methodology over GBLUP (Meuwissen et al., 2001; Zhang et al., 2016). Differently, GBLUP, a linear methodology, commonly assumes equal weights for all SNP (Meuwissen et al., 2001). However, GBLUP has undergone changes over time. The inclusion of different weights for SNPs is one way to account for *locus*-specific variance in GBLUP methods (Zhang et al., 2016). Su et al. (2014) concluded that a proper weighted GBLUP (WGBLUP) provides genomic breeding values with accuracies similar to those using Bayesian procedure.

Misztal et al. (2009) and Christensen and Lund (2010) propose the single-step GBLUP (ssGBLUP or ssGWAS) to improve the GWAS methodology where information from genotypes were combined with all phenotypes and pedigree information from all animals (including those without genotype) for a higher detection and more precise estimation of the relationship between phenotype and genotype. However, Wang et al. (2012) proposed a modified version for the ssGWAS, where the authors converted the genomic estimated breeding values (GEBV) obtained in the ssGBLUP to marker effect and marker weights which are used in an iterative approach to update solutions. This methodology can be applied to any statistical model that has GEBV estimated by BLUP.

Differences among GWAS results for the same trait can occur due to several reasons such as the applied methodology, linkage disequilibrium differences, genes or regions that controls phenotype expression, DNA mutation, measurement of traits, statistical model, selection strategies, genotype panel size, effective population size, sample size, breed or population studied, population structure (Goddard and Hayes, 2009; Raven et al., 2014).

Association studies between genotype and phenotype is well explored in the animal breeding and help increase genetic gain through genomic selection (Korte and Farlow, 2013). Some of the application of GWAS in the animal breeding are in: chicken meat quality (Sun et al., 2013), growth traits and meat quality in sheep (Zhang et al., 2013), pH and meat coloration in pigs (Zhang et al., 2015), milk production in Chinese Holstein cattle (Jiang et al., 2010), lactation persistency trait in German Holstein dairy cattle (Harder et al., 2006), feet and legs conformation in Nelore cattle (Vargas et al., 2018), carcass traits in Korean beef cattle (Kim et al., 2011), intramuscular fat deposition in cattle (Ramayo-Caldas et al., 2014) and scrotal circumference in Nellore cattle (Utsunomiya et al., 2014). However, few association studies are available for Dairy Gir cattle and no study was found for conformation and handling traits in the breed

3. References

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CHAPTER 2 – Dairy Gir conformation traits, milking ease, and temperament: a comparison of statistical models

ABSTRACT – Statistical models used to adjust phenotypes for conformation and handling traits can have an impact on estimated breeding values and their accuracies. Therefore, the objective of this study was to identify between linear and threshold models which performed better for categorical traits and how fixed and random effects should be considered in the statistical model for the genetic parameters estimates of conformation traits, milking ease, and temperament in Dairy Gir cattle. The data set used in this study was provided by Embrapa Dairy Cattle (Embrapa Gado de Leite). Records were collected between 1992 and 2018 from herds located in southeast region of Brazil. The number of records considered for further analyses ranged from 5,539 to 10,802 from 4,762 to 7,795 cows for 16 conformation and two handling traits from first to seventh lactation. Four models were tested in this study. For model 1 (M1), only the controlled fixed effects (contemporary group (CG) composed by herd and year of evaluation or herd (H) or year of evaluation (YE), season of evaluation (SE), diet (D), evaluator (E), and linear (L) and quadratic (Q) effects of age of dam at the evaluation) that were statistically significant ($p<0.05$) in the general linear model (GLM) were included in the model. For model 2 (M2), all controlled fixed effects (CG, SE, D, E, L, Q) were included in the model. For both models, were considered as random effects animal, permanent environmental of animal, and residual. For model 3 (M3), the controlled fixed effects (SE, D, E, Q, L) that were statistically significant ($p<0.05$) in the GLM were included in the model. For model 4 (M4), the controlled fixed effects (SE, D, E, L, Q) were included in the model. For M3 and M4, CG, animal, permanent environmental of animal, and residual were included as random effect in the model. One-trait analyses were performed to estimate the variance components using the Average Information Restricted Maximum Likelihood (AIREML) under a repeatability model. Traits evaluated by scores were also analyzed using threshold model (for M1 and M2). Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to compare all models. The impact of each model in the genetic evaluation was evaluated by Spearman's rank correlation between estimated breeding values for the TOP 5% sires, and the accuracy average of sires with at least 20 phenotyped daughters. Linear M1 and M2 showed smaller AIC and BIC values when compared to threshold M1 and M2, respectively. Among all four linear models the M3 performed better (smallest AIC and BIC values). Genetic additive variance estimates for threshold models were higher when compared with linear models. However, heritability estimates were similar, although the models were in different scale. Additive genetic variance and heritability estimates among all four linear models were similar. However, body length and teat diameter had higher estimates of additive genetic variance and heritability for M1 when compared with M2. Models with CG as random effect showed smaller heritability estimates. Estimates of additive genetic variance and heritability for rear legs – rear view for all linear and threshold models were low and poorly reliable. The Spearman's rank correlation was high for almost all comparisons (from 0.79 to 1) showing fewer changes in the sires reranking between models. However, when M1 was compared to M2 and M3, the traits body length, rear legs – side and rear view, and teat diameter had small value of rank correlation (from 0.397 to 0.744) which indicate greater differences between the ranks. Average accuracies for linear M1 and M2 was higher than threshold M1 and M2, respectively (except for rear legs – side view). Among linear M1 and M2 average accuracies were similar for almost all traits. Models with CG as random effect had average accuracies higher for most of traits. Linear models performed better than threshold models. Linear models should consider CG as random effect and only statistically significant fixed effects.

Keywords: Genetic parameters estimation, linear model, threshold model, Zebu cattle

1. Introduction

In the Brazilian Dairy Gir National Breeding Program, eighteen linear traits are routinely evaluated using scores (from 1 to 9), centimeters, or angles. These traits can be separated into four categories: corporal dimension, feet and legs, mammary system, and handling. Some studies showed that traits related to rump have an impact on calving ease, uterus and vaginal prolapse, and udder fixation (Nogalski and Mordas, 2012; Sawa et al., 2003). Mammary system traits, mainly anterior udder, udder depth, and teat length, are related to mastitis and susceptibility to traumas (Bharti et al., 2015). Handling traits are important in the daily dairy management because reactive animals can injure themselves and other animals, induce accidents and consequently, delay the milking process (Costa et al., 2015; Luttinen and Juga, 1997).

In an animal breeding program, the structure and quality of the data can impact the prediction of animal breeding values. Improving different sources of information, such as the data set and pedigree, can help improve prediction accuracy (Tosh and Wilton, 1994; Meijering, 1985). Besides the data structure, statistic models used to adjust phenotypic data can impact the estimated breeding values (EBV) prediction and accuracy.

Considering that some of the conformation and handling traits are categorical traits (evaluated by scores) it is important define whether threshold models could fit these traits better than linear models. In theory, threshold models would be a better choice for categorical data than linear models (Gianola, 1982). Linear models assume that residuals are normally distributed, which usually does not happen when the data is evaluated using scores (Kadarmideen et al., 2000).

Another relevant question is the use of contemporary group as fixed or random effect. In dairy cattle, models used for genetic evaluation usually compare animal performance within groups, which assumes that animals are raised in similar environments. These groups, known as contemporary groups (CG), can be defined by the combination of herd-year-season, herd-year, or their interactions (Van Bebber et al., 1997). Since 1973, when Henderson introduced BLUP for sire evaluation, CG have been treated as a fixed effect to avoid bias in situations where good sires were used in well-managed herds, but this idea can change among evaluation systems (Schaeffer, 2009; Schaeffer et al., 2001). If CG is treated as random effect, bias can happen whether there was dependence between genetic and environmental effects. Dependence among these effects can arise from selection and management improvement practices (Babot et al., 2003; Ugarte et al., 1992). If CG are treated as fixed, it is assumed that all differences among CG are due to management (Schaeffer et al., 2001).

Some simulation studies were performed to better understand this effect. Ugarte et al. (1992) compared models using CG as fixed and as random effect. The authors concluded that when sires are randomly distributed among CG, and this effect is considered as random effect, better predictions will be estimated with a better animal ranking, especially when CG is small. However, the population studied were simulated and according to the authors these results could change in a real population with selection happening over time. Visscher and Goddard (1992) also tested the use of CG as fixed and random effect in a simulated population and stated that if sires and CG had a nonrandom association, CG should be treated as random effect because more information is included in the evaluation which increases accuracy of selection. Therefore, the objective of this study was to identify between linear and threshold models which performed better for categorical traits and how fixed and random effects should be considered in the statistical model for the genetic evaluation of conformation traits, milking ease, and temperament in Dairy Gir cattle.

2. Material and Methods

2.1. Data description

The data set used in this study was provided by Embrapa Dairy Cattle (Embrapa Gado de Leite) and it is part of the Brazilian Dairy Gir National Breeding Program (Programa Nacional de Melhoramento do Gir Leiteiro – PNMGL). Records were collected by the Brazilian Association of Dairy Gir Breeders (Associação Brasileira dos Criadores de Gir Leiteiro – ABCGIL) and Brazilian Association of Zebu Breeders (Associação Brasileira dos Criadores de Zebu), between 1992 and 2018, in herds located in southeast region of Brazil.

Traits evaluated in this study included 16 conformation traits and two handling traits. Stature (STA), heart girth (HG), body length (BL), navel length (NL), rump length (RL), pin width (PW), hook width (HW), teat length (TL), and teat diameter (TD) were measured and recorded in centimeters. Foot angle (FA), rear legs – side view (LSV), rear legs – rear view (LRV), fore udder attachment (FUA), rear udder width (RUW), udder depth (UD), milking ease (ME), and temperament (TEM) were recorded using a visual score (9-point scale). Rump angle (RA) was recorded in angle degrees, using a slope inclinometer protractor or, in part of the cases, approximated from a visual linear score. To a better understand how the traits were measured, see Panetto et al. (2019).

The raw data set contained 10,956 linear type evaluations, from first to seventh, of 7,913 Gir cows. The pedigree file used in the analysis for all traits was constituted by 18,402 animals, which considered up to 15 generations. In the first data edition, data with possible annotation errors and values outside the biological possible range for traits evaluated in cm and angles degrees were removed from the data file. Due to differences in the models, the data set needed to be edited in two different ways creating the data set 1 and 2. The descriptive statistics for data sets 1 and 2 are presented in Table 1 and Table 2, respectively. Despite the differences in the number of animals used in both data sets, mean and standard deviation were similar between the datasets.

2.2. Data distribution and transformation

Skewness and Kurtosis tests were performed using the R-package “moments” (Komsta and Novomestky, 2015) to verify the normality of the data. Several data transformations were tested when the traits distribution deviation from normality including log transformation, square-root transformation, cube root transformation, Yeo-Johnson transformation, and Ordered Quantile normalizing transformation. All analyses were performed using the R software (R Core Team, 2008). The Ordered Quantile normalizing method (Peterson and Cavanaugh, 2019) was chosen to transform records measured by score because it presented the best Skewness and Kurtosis values after the transformation. The transformed data were used only in the analyses of the linear models. For the threshold models analyses, the original data recorded in scores was used.

Table 1. Descriptive statistics for traits evaluated using the data set 1

Trait	N	Min	Max	Mean	SD	Median	Mode
Stature (cm)	9677	119	155	136.90	4.92	137	136
Hearth girth (cm)	9529	141	210	175.90	8.91	175	180
Body length (cm)	10504	86	127	103.50	5.95	103	102
Rump length (cm)	9652	31	59	42.76	4.30	43	45
Pin width (cm)	9520	10	29	18.25	2.62	18	18
Hook width (cm)	9501	33	62	46.27	4.37	47	48
Rump angle (degree)	5936	1	50	24.42	7.73	25	30
Foot angle (score)	8746	1	9	-	-	5	5
Rear legs – side view (score)	7200	1	9	-	-	5	5
Rear legs – rear view (score)	7194	1	9	-	-	5	5
Fore udder attachment (score)	5617	1	9	-	-	5	5
Rear udder width (score)	5822	1	9	-	-	5	5
Udder depth (score)	6003	1	9	-	-	5	5
Teat length (cm)	9690	3	14	7.51	1.92	7	7
Teat diameter (cm)	10802	1	8	3.89	0.91	4	4
Milking ease (score)	9486	1	9	-	-	3	5
Temperament (score)	9672	1	9	-	-	4	5
Navel length (cm)	5539	1	20	10.20	3.16	10	10

N: number of records; **Min**: minimum value for the evaluated trait; **Max**: maximum value for the evaluated trait; **Mean**: mean; **SD:** standard deviation from the mean; **Median**: median by trait; **Mode**: mode by trait.

Table 2. Descriptive statistics for traits evaluated using the data set 2

Trait	N	Min	Max	Mean	SD	Median	Mode
Body length (cm)	9387	86	127	103.50	5.98	103	102
Rear legs – side view (score)	6035	1	9	-	-	5	5
Rear legs – rear view (score)	6029	1	9	-	-	5	5
Teat diameter (cm)	9666	1	8	3.87	0.87	4	4

N: number of records; **Min**: minimum value for the evaluated trait; **Max**: maximum value for the evaluated trait; **Mean**: mean; **SD:** standard deviation from the mean; **Median**: median by trait; **Mode**: mode by trait.

2.3. Data edition

The first edition removed records from evaluators with less than 5 evaluations in both data sets. CG with less than 5 records within group were removed from posterior analyses. The number of CG removed by this constrain varied between data sets and traits. For the first data set, fourteen traits were edited, and the number of CG removed ranged from 542 to 570, depending on the trait. For the second data set, all the eighteen traits were edited, with the number of removed CG also ranging from 542 to 570.

Connectedness between CG was checked using the AMC program (Roso and Schenkel, 2006), in which the degree of connectedness is measured through the existence of genetic links attributed to a common ancestor. The program used ten or more genetic links to consider a CG connected. Four to eight CGs were disconnected and removed from the both datasets. The final number of CG ranged from 350 to 462.

The data set 1 was used in the analyses for model 1, and the data set 2 was used for models 2, 3, and 4. The difference between the two data sets was only for the traits body length, rear legs – side view and rear view, and teat diameter (as showed in Table 1 and Table 2) in which CG was not included as fixed or random effect in the model. Thus, CG with less than 5 records within group and disconnected CG were not removed for these traits.

2.4. Model description

Four different models were used in the analyses. The general statistical models were defined below:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{Wpe} + \boldsymbol{\varepsilon} \qquad \text{(M1 and M2)}$$

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{Wpe} + \mathbf{Sd} + \boldsymbol{\varepsilon} \qquad \text{(M3 and M4)}$$

where: $\mathbf{y}$ is the observation vector for the linear conformation traits, milking ease or temperament; $\mathbf{X}$ is the incidence matrix of fixed effects; $\mathbf{Z}$ is the incidence matrix for the animal additive genetic effect; $\mathbf{W}$ is the incidence matrix for the permanent environment effect of animal; $\boldsymbol{\beta}$ is the vector of fixed effects; $\mathbf{a}$ is the vector of animal random effects; $\mathbf{pe}$ is the vector related to permanent environment random effects of animal; $\mathbf{S}$ is the incidence matrix for the CG effect; $\mathbf{d}$ is the vector of CG and $\boldsymbol{\varepsilon}$ is the vector of random errors or residual effects associated with each observation.

For the proposed models mentioned above, it was assumed that $\mathrm{E}[\mathbf{y}] = \mathbf{X\beta}$; $\mathrm{Var}(\mathbf{a}) = \mathbf{A} \otimes \mathbf{G}$, $\mathrm{Var}(\mathbf{d}) = \mathbf{I} \otimes \mathbf{S}$, $\mathrm{Var}(\mathbf{pe}) = \mathbf{I} \otimes \mathbf{W}$, and $\mathrm{Var}(\mathbf{e}) = \mathbf{I} \otimes \mathbf{R}$, where $\mathbf{G}$ is the additive genetic covariance matrix; $\mathbf{R}$ is the residual covariance matrix; $\mathbf{A}$ is the numerator matrix of additive genetic relationships; $\mathbf{I}$ is the identity matrix; $\boldsymbol{S}$ is the contemporary group covariance matrix, $\mathbf{W}$ is the permanent environmental covariance matrix, and $\otimes$ is the direct product or the Kronecker product between two matrices. Genetic and residual effects were assumed to be independent.

2.4.1. Model 1 (M1) and 3 (M3)

For M1 and M3, only the fixed effects that were statistically significant ($p<0.05$) were included in the models. A general linear model (GLM) was performed considering $p<0.05$ to determine which fixed effects would be included in the genetic analyses. The effects tested were herd (H), year of evaluation (YE), season of evaluation (SE), evaluator (E), diet (D), age of the dam as covariate (with linear, l; and quadratic, Q effects), and the interaction between H and YE. The CG was composed by H and YE.

For M1, all the fixed effects described above were tested, and those included in the model for each trait are described in Table 3. The random effects considered in this model were effect of animal, permanent environment, and residual.

For M3, CG was not considered as a fixed effect but as a random effect along with animal, permanent environment, and residual. Thus, it was tested the fixed effects: season of evaluation, evaluator, diet, and the age of the dam as covariate (with linear and quadratic effects) and those statistically significant ($p<0.05$) were included in the model (Table 3).

2.4.2. Model 2 (M2) and 4 (M4)

All fixed effects were considered in M2 and M4 with no need to be statistically significant ($p<0.05$). For M2, were considered as fixed effects: CG (composed by herd and year of evaluation), season of evaluation, evaluator, diet, and the age of the dam as covariate (with linear and quadratic effects). The random effects considered in this model were effect of animal, permanent environment, and residual.

For M4 the fixed effects considered in the model were: season of evaluation, evaluator, diet, and the age of the dam as covariate (with linear and quadratic effects). Random effects considered in this model were effects of animal, permanent environment, contemporary group, and residual for all traits.

Table 3. Fixed effects included in the statistical models used for the genetic evaluation of linear conformation and handling traits in Dairy Gir cattle

Trait	Model 1	Model 3
Stature (cm)	CG + E + D + I + Q	E + D + I + Q
Hearth girth (cm)	CG + SE + E + D + I + Q	SE + E + D + I + Q
Body length (cm)	YE + SE + E + D + I + Q	SE + E + D + I + Q
Rump length (cm)	CG + SE + E + D + I + Q	SE + E + D + I + Q
Pin width (cm)	CG + SE + E + I + Q	SE + E + I + Q
Hook width (cm)	CG + D + I + Q	D + I + Q
Rump angle (degree)	CG + SE + E + D + I + Q	SE + E + D + I + Q
Foot angle (score)	CG + SE + D + I + Q	SE + D + I + Q
Rear legs – side view (score)	SE + E + D + I	SE + E + D + I
Rear legs – rear view (score)	E + D	E + D
Fore udder attachment (score)	CG + SE + D + I	SE + D + I
Rear udder width (score)	CG + SE + D + I	SE + D + I
Udder depth (score)	CG + E + D + I + Q	E + D + I + Q
Teat length (cm)	CG + SE + E + D + I + Q	SE + E + D + I + Q
Teat diameter (cm)	YE + E + I + Q	E + I + Q
Milking ease (score)	CG + E + I + Q	E + I + Q
Temperament (score)	CG + SE + E + I + Q	SE + E + I + Q
Navel length (cm)	CG + SE + E + D + I + Q	SE + E + D + I + Q

CG: contemporary group (herd + year of evaluation); **H**: herd; **YE**: year of evaluation; **SE**: season of evaluation; **E**: evaluator; **D**: diet effect; **I**: linear effect of age of the dam; **Q**: quadratic effect of age of the dam.

2.5. Data analyses

Variance components were estimated using the Average Information Restricted Maximum Likelihood (AIREML) method (Gilmour et al., 1995) under a single-trait repeatability animal model for all four models. Traits evaluated by scores (1-9) were also analyzed using a threshold model (for the statistical models 1 and 2). All analyses were conducted using the ASREML 4.1 software (Gilmour et al., 2009).

For the multinomial analyses the !MULTINOMIAL qualifier and the !LOGIT function were used. The logit function is defined as:

$$\eta = \log\left(\frac{\mu}{1-\mu}\right)$$

where: μ is the mean on the data scale and η is the linear predictors on the underlying scale. On the logit scale the residual variance is:

$$\sigma_e^2 = \frac{\pi^2}{3} \approx 3.29$$

2.6. Model evaluation criteria

Models were compared using the Akaike information criterion (AIC) (Akaike, 1973) and the Bayesian information criterion (BIC) (Schwarz, 1978). The Akaike information criterion (AIC) was calculated as:

$$\mathrm{AIC} = 2\mathrm{k} - 2\log\left(\hat{\mathrm{L}}\right)$$

where*:* k is the number of parameters and $\hat{\mathrm{L}}$ is the full log-likelihood. The Bayesian information criterion (BIC) was calculated as:

$$\mathrm{BIC} = \log(\mathrm{n})\mathrm{k} - 2\log\left(\hat{\mathrm{L}}\right)$$

where: n is the number of observations, k is the number of estimated parameters and $\hat{\mathrm{L}}$ is the full log-likelihood. The lowest AIC and BIC values indicate the best fitted model.

2.7. Impact of models in the animal evaluation

Heritability (h^2) and repeatability (r) estimates for the threshold models were estimated as:

$$\mathrm{h}^2 = \frac{\sigma_{\mathrm{a}}^2}{\sigma_{\mathrm{a}}^2 + \sigma_{\mathrm{pe}}^2 + \frac{\pi^2}{3}} \quad \text{and, } \mathrm{r} = \frac{\sigma_{\mathrm{a}}^2 + \sigma_{\mathrm{pe}}^2}{\sigma_{\mathrm{a}}^2 + \sigma_{\mathrm{pe}}^2 + \frac{\pi^2}{3}}$$

where: σ_{a}^2 is the additive genetic variance and σ_{pe}^2 is the permanent environmental variance (Gilmour et al., 2015). And the heritability (h^2) and repeatability (r) for the linear models were calculated as:

$$\mathrm{h}^2 = \frac{\sigma_{\mathrm{a}}^2}{\sigma_{\mathrm{p}}^2} \quad \text{and, } \mathrm{r} = \frac{\sigma_{\mathrm{a}}^2 + \sigma_{\mathrm{pe}}^2}{\sigma_{\mathrm{p}}^2}$$

where: σ_{a}^2 is the additive genetic variance, σ_{p}^2 is the phenotypic variance, and σ_{pe}^2 is the permanent environmental variance.

To understand the impact of using different models in the animal genetic evaluation, the Spearman's rank correlation (ρ) were computed between estimated breeding values for the TOP 5% sires (114 sires) from the first model against all sires from the second model and the average accuracy of sires with at least 20 phenotyped daughters within trait was calculated. The accuracy (r) of the estimated breeding values for each sire was estimated as:

$$\mathrm{acc} = \sqrt{\left(1 - \frac{\mathrm{SEP}^2}{(1 - \mathrm{f_i})\,\sigma_{\mathrm{a}}^2}\right)}$$

where: SEP is the standard error of prediction estimated by ASREML; $\mathrm{f_i}$ is the inbreeding coefficient; and σ_a^2 is the population additive genetic variance (Gilmour et al., 2015). A two-tailed student's t-test was used to verify statistically differences ($p<0.05$) between the accuracies of sires with at least 20 phenotyped daughters between the models for each trait.

3. Results and Discussion

For all categorical traits in this study, linear models (for both M1 and M2) showed the smallest AIC (Table 4) and BIC (Table 5) values when compared with threshold models (M1 and M2, respectively). Among the four linear models, M3 (CG was considered as a random effect and only fixed effects statistically significant with $p<0.05$ were included in the model) presented the smallest AIC and BIC values for all traits studied. However, for hearth girth, body length, rump length, teat length, and navel length, linear M3 and M4 presented the same AIC and BIC values because the models were the same. Similarly, hearth girth, rump length, teat length, and navel length presented the same AIC and BIC values for linear M1 and M2, because the models were the same.

Table 4. Akaike information criterion (AIC) estimated for all models evaluated

Trait	M1L	M2L	M3L	M4L	M1T	M2T
Stature	1001.58	1003.58	145.55	147.55	-	-
Hearth girth	981.33	981.33	143.29	143.29	-	-
Body length	203.33	955.46	139.43	139.43	-	-
Rump length	995.84	995.84	147.80	147.80	-	-
Pin width	970.03	980.03	135.98	145.99	-	-
Hook width	917.71	993.73	15.66	147.69	-	-
Rump angle	803.80	805.80	123.76	125.76	-	-
Foot angle	891.00	967.02	19.02	145.05	903.33	978.82
Rear legs – side view	141.14	817.28	125.33	127.33	153.53	831.25
Rear legs – rear view	137.19	817.32	121.39	127.38	149.64	830.07
Fore udder attachment	713.50	763.52	17.50	121.58	725.81	775.12
Rear udder width	751.97	802.04	17.91	128.10	762.09	812.33
Udder depth	809.37	811.37	125.41	127.41	821.27	823.54
Teat length	1012.23	1012.23	150.22	150.22	-	-
Teat diameter	203.61	1010.03	138.17	150.16	-	-
Milking ease	1005.37	1017.37	139.41	151.41	1016.62	1028.31
Temperament	1003.20	1013.20	141.24	151.23	1014.67	1024.56
Navel length	768.21	768.21	120.19	120.19	-	-

M1L: model 1 using linear methodology; **M2L:** model 2 using linear methodology; **M3L:** model 3 using linear methodology; **M4L:** model 4 using linear methodology; **M1T:** model 1 using threshold methodology; **M2L:** model 2 using threshold methodology.

The AIC and BIC criteria can be used to compare different models in order to select which one will fit better the data when parameters were estimated using

AIREML. However, it is necessary to use the standard full likelihood to estimate such criteria (Verbyla, 2019). Besides AIC and BIC pointed to a better fit by linear M3, it is important to understand the impact of using different models on the genetic parameters estimates, average accuracy, and animal's ranking since these can be really important factors in the genetic evaluation.

Table 5. Bayesian information criterion (BIC) estimated for all models evaluated

Trait	M1L	M2L	M3L	M4L	M1T	M2T
Stature	2004.38	2008.37	298.45	302.44	-	-
Hearth girth	1960.96	1960.96	293.70	293.70	-	-
Body length	417.59	1906.22	285.40	285.40	-	-
Rump length	1992.11	1992.11	302.60	302.60	-	-
Pin width	1937.58	1957.47	278.45	298.34	-	-
Hook width	1833.42	1984.60	39.40	301.96	-	-
Rump angle	1523.84	1527.62	240.81	244.59	-	-
Foot angle	1762.88	1912.68	44.26	292.62	1788.80	1938.08
Rear legs – side view	278.58	1550.92	242.85	242.85	303.97	1577.36
Rear legs – rear view	270.89	1550.78	235.32	235.32	296.34	1576.00
Fore udder attachment	1343.32	1437.08	38.49	233.55	1367.88	1460.93
Rear udder width	1420.93	1515.13	39.09	246.36	1443.41	1537.77
Udder depth	1534.95	1538.72	242.78	246.56	1559.30	1563.34
Teat length	2025.26	2025.26	307.14	307.14	-	-
Teat diameter	417.13	2018.53	281.11	281.11	-	-
Milking ease	2005.78	2029.63	283.74	307.60	2030.86	2054.42
Temperament	2005.88	2025.81	288.17	308.09	2031.25	2051.07
Navel length	1444.66	1444.66	231.77	231.77	-	-

M1L: model 1 using linear methodology; **M2L:** model 2 using linear methodology; **M3L:** model 3 using linear methodology; **M4L:** model 4 using linear methodology; **M1T:** model 1 using threshold methodology; **M2L:** model 2 using threshold methodology.

Additive genetic, permanent environmental, and residual variances for all models evaluated are showed in Table 6. Heritability and repeatability for all models evaluated are showed in Table 7. The estimated standard errors for the variances are showed in Appendix A on appendix and standard error for heritability and repeatability estimates are in Appendix B on appendix.

For teat diameter, estimate of additive genetic variance was higher for M1 (0.16) than for M2 (0.09) and estimate of permanent environmental variance was higher for M2 (0.12) than for M1 (0.09). These differences may be due to the inclusion of CG (interaction among herd and year of evaluation) and season of evaluation for M2 while only year of evaluation was considered in M1, as fixed effects. The difference in the genetic additive variance reflect in a higher heritability estimate for M1 (0.30 ± 0.03) than M2 (0.21 ± 0.03). Repeatability estimates were similar for both models (0.47). Besides the differences among variance components and heritability estimates between models for body length and teat diameter, AIC and BIC values for M1 were smaller. Small difference in the repeatability estimate for hook width between M1 (0.35) and M2 (0.45) was noted, which can be attributed to inclusion of season of evaluation and evaluator in M2.

Estimates of heritability for rear legs – rear view were low and poorly reliable for all six models evaluated which can be attributed to the fact that the data have not been able to capture all the phenotype variation. Traits regarding feet and legs are highly influenced by environment and management (Onyiro and Brotherstone, 2008; Onyiro et al., 2008). Estimates of heritability in the literature for rear legs – rear view is scarce, however few studies reported heritability approximately of 0.10 (Ptak et al., 2011; Duru et al., 2012). Duru et al. (2012) reported heritability of 0.11; however, with a standard error of 0.18 and Ptak et al. (2011) reported heritability equal to 0.09 with a standard error of 0.02.

Genetic additive and permanent environmental variances for threshold M1 and M2 were higher than the same parameters for linear M1 and M2 (Table 6), respectively. Variations in genetic parameters can occur due to multinomial analyses be performed on a different scale from linear models. Besides the differences in the variance components and the differences in scales for linear and threshold model, estimates of heritability among linear and threshold M1 and linear and threshold M2 were similar (Table 7). Therefore, a comparison between the results from linear and threshold models are not recommended.

Heritability and repeatability estimates were similar between models with the same random effects. M3 and M4, which included CG as random effect, showed estimates of heritability and repeatability smaller than M1 and M2, respectively (Table 7). This result might be due to the inclusion of CG variance in the phenotypic variance, leading to a higher denominator in the fraction resulting in a small heritability and repeatability estimate.

Table 6. Additive genetic, permanent environmental, and residual variances estimated for all models evaluated

Trait	**M1L**			**M2L**			**M3L**			**M4L**			**M1T***		**M2T***	
	σ_a^2	σ_{pe}^2	σ_e^2	σ_a^2	σ_{pe}^2	σ_e^2	σ_a^2	σ_{pe}^2	σ_e^2	σ_a^2	σ_{pe}^2	σ_e^2	σ_a^2	σ_{pe}^2	σ_a^2	σ_{pe}^2
STA	8.54	5.00	3.54	8.54	5.00	3.53	8.90	4.87	3.54	8.90	4.87	3.54	-	-	-	-
HG	12.44	18.53	17.49	12.44	18.53	17.49	14.35	17.39	17.54	14.35	17.39	17.54	-	-	-	-
BL	7.65	2.74	15.76	4.46	4.12	14.18	5.00	3.67	14.27	5.00	3.67	14.27	-	-	-	-
RL	1.16	1.55	2.53	1.16	1.55	2.53	1.32	1.40	2.62	1.32	1.40	2.62	-	-	-	-
PW	0.65	0.67	1.77	0.65	0.66	1.77	0.66	0.63	1.82	0.66	0.63	1.82	-	-	-	-
HW	1.33	1.35	5.01	1.37	1.95	4.06	1.39	1.32	5.00	1.55	1.82	4.11	-	-	-	-
RA	3.54	8.76	17.66	3.54	8.76	17.66	3.43	8.70	17.86	3.43	8.71	17.86	-	-	-	-
FA	0.05	0.10	0.56	0.05	0.11	0.53	0.05	0.09	0.56	0.05	0.10	0.55	0.31	1.16	0.33	1.29
LSV	0.07	0.22	0.50	0.07	0.20	0.45	0.06	0.20	0.45	0.06	0.20	0.45	0.47	1.68	0.71	2.50
LRV	0.001	0.27	0.47	0.004	0.26	0.42	0.005	0.26	0.42	0.006	0.26	0.42	0.00	1.76	0.04	2.51
FAU	0.04	0.32	0.29	0.05	0.31	0.28	0.04	0.32	0.29	0.04	0.31	0.29	0.54	5.70	0.64	5.74
RUW	0.03	0.14	0.29	0.04	0.13	0.28	0.03	0.15	0.29	0.04	0.14	0.28	0.50	3.09	0.58	3.18
UD	0.08	0.16	0.44	0.08	0.16	0.44	0.07	0.16	0.44	0.07	0.16	0.44	0.82	2.99	0.83	2.99
TL	0.93	0.57	0.76	0.93	0.57	0.76	0.93	0.56	0.77	0.93	0.56	0.77	-	-	-	-
TD	0.16	0.09	0.29	0.09	0.12	0.24	0.10	0.11	0.24	0.10	0.11	0.24	-	-	-	-
ME	0.08	0.13	0.33	0.09	0.13	0.33	0.09	0.13	0.34	0.09	0.13	0.34	0.90	1.24	0.90	1.25
TEM	0.07	0.19	0.35	0.07	0.19	0.35	0.06	0.19	0.35	0.06	0.19	0.35	0.66	1.70	0.65	1.72
NL	3.17	2.89	1.93	3.17	2.89	1.93	3.25	2.80	1.95	3.25	2.80	1.95	-	-	-	-

M1L: model 1 using linear methodology; **M2L**: model 2 using linear methodology; **M3L**: model 3 using linear methodology; **M4L**: model 4 using linear methodology; **M1T**: model 1 using threshold methodology; **M2T**: model 2 using threshold methodology.

σ_a^2: additive genetic variance; σ_{pe}^2: permanent environmental variance; σ_e^2: residual variance.

STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length.

*****: residual variance assumed for the threshold model was 3.29 (in the logit scale). Standard error ranged from 0.01 to 1.74.

Table 7. Heritability (h^2) and repeatability (r) estimated for all models

Trait	M1L		M2L		M3L		M4L		M1T		M2T	
	h^2	r	h^2	r	h^2	r	h^2	r	h^2	r	h^2	r
STA	0.50	0.79	0.50	0.79	0.43	0.67	0.43	0.67	-	-	-	-
HG	0.26	0.64	0.26	0.64	0.20	0.45	0.20	0.45	-	-	-	-
BL	0.29	0.40	0.20	0.38	0.18	0.31	0.18	0.31	-	-	-	-
RL	0.22	0.52	0.22	0.52	0.17	0.35	0.17	0.35	-	-	-	-
PW	0.21	0.43	0.21	0.43	0.12	0.24	0.13	0.24	-	-	-	-
HW	0.17	0.35	0.19	0.45	0.08	0.16	0.13	0.29	-	-	-	-
RA	0.12	0.41	0.12	0.41	0.09	0.33	0.09	0.33	-	-	-	-
FA	0.07	0.21	0.07	0.22	0.05	0.16	0.06	0.18	0.07	0.31	0.07	0.33
LSV	0.09	0.37	0.10	0.37	0.08	0.34	0.08	0.33	0.09	0.40	0.11	0.49
LRV	0.001	0.37	0.007	0.38	0.006	0.36	0.008	0.36	0.00	0.35	0.01	0.44
FAU	0.06	0.56	0.07	0.56	0.04	0.38	0.06	0.48	0.06	0.65	0.07	0.66
RUW	0.07	0.38	0.08	0.37	0.03	0.19	0.06	0.30	0.07	0.52	0.08	0.53
UD	0.11	0.35	0.11	0.35	0.09	0.30	0.09	0.30	0.12	0.54	0.12	0.54
TL	0.41	0.66	0.41	0.66	0.35	0.56	0.35	0.56	-	-	-	-
TD	0.30	0.47	0.21	0.47	0.18	0.38	0.17	0.38	-	-	-	-
ME	0.15	0.40	0.15	0.40	0.13	0.31	0.13	0.32	0.17	0.39	0.17	0.40
TEM	0.11	0.43	0.11	0.42	0.09	0.35	0.09	0.35	0.12	0.42	0.12	0.42
NL	0.40	0.76	0.40	0.76	0.37	0.70	0.37	0.70	-	-	-	-

M1L: model 1 using linear methodology; **M2L**: model 2 using linear methodology; **M3L**: model 3 using linear methodology; **M4L**: model 4 using linear methodology; **M1T**: model 1 using threshold methodology; **M2T**: model 2 using threshold methodology.
STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length.
-: not a categorical trait; Standard error ranged from 0.01 to 0.05.

Heritability estimates for conformation and handling traits varied among results in the literature, which can be due to several factors, including statistical model, breed studied, fixed and random effects considered in the models, data edition, among others (Nemcova et al., 2011). Besides the variation in the literature and in this study (due to different models used), heritabilities coefficients estimated here were similar to results in the literature (Wenceslau et al., 2000; Rennó et al., 2003; Resing and Ruten, 2005; De Hass et al., 2007; Daliri et al., 2008; Lagrotta et al., 2010; Duru et al., 2012; Kramer et al., 2013).

Spearman's rank correlations (ρ) among EBV for the TOP 5% sires (i.e. 114 animals) ranged from 0.397 to 1 (Table 8). The rank correlation between EBV using linear M1 and M2 for almost all traits were higher than 0.92, which means that the top rank 114 animals for both models were similar. Fore udder attachment presented smaller ρ (0.857) than other traits but still high, which means that the rank animal list between both models were similar. Lowest ρ were found between EBV based on M1 and M2 of body length (0.533), rear legs – side (0.656) and rear view (0.403), and teat diameter (0.397), implying that the animal's position between the rankings sharply changed.

When linear M1 and M3 were compared the ρ for body length (0.573), rear legs – side (0.744) and rear view (0.551), and teat diameter (0.496) were also small which point for a bigger difference between the two ranks for each of these traits. For the other traits, ρ ranged from 0.811 to 0.974 indicating slight reranking of animals based on linear M1 and M3. Correlations between EBVs using linear M2 and M4 ranged from 0.81 to 0.97 for almost all traits, except for rear legs – rear view (0.79). Thus, a large reranking of animals were observed for rear legs – rear view when compared to the other traits.

We found no difference in reranking using linear M3 and M4 due to high ρ between the EBV (ranging from 1 to 0.972). However, hook width and fore udder attachment had smaller ρ values of 0.881 and 0.704, respectively. Thus, it is expected some difference in the reranking of the sires. High-rank correlations were estimated (from 0.86 to 0.985) between linear and threshold models, suggesting slight differences in the animal's reranking.

Table 8. Spearman's rank correlation estimated for the TOP 5% bulls

Trait	M1M2	M1M3	M2M4	M3M4	M1LT	M2LT
STA	0.999	0.869	0.866	1.000	-	-
HG	+	0.895	0.895	+	-	-
BL	0.533	0.573	0.848	+	-	-
RL	+	0.811	0.811	+	-	-
PW	0.989	0.836	0.846	0.989	-	-
HW	0.970	0.963	0.867	0.881	-	-
RA	1.000	0.932	0.933	0.999	-	-
FA	0.985	0.974	0.940	0.978	0.964	0.961
LSV	0.656	0.744	0.874	0.998	0.963	0.950
LRV	0.403	0.551	0.790	0.972	0.860	0.947
FAU	0.857	0.869	0.898	0.704	0.985	0.985
RUW	0.920	0.966	0.899	0.886	0.953	0.948
UD	1.000	0.966	0.891	1.000	0.965	0.964
TL	+	0.940	0.940	+	-	-
TD	0.397	0.496	0.949	0.996	-	-
ME	0.999	0.861	0.869	0.996	0.942	0.964
TEM	0.997	0.958	0.965	0.997	0.946	0.947
NL	+	0.882	0.882	+	-	-

M1M2: comparison among linear models 1 and 2; **M1M3**: comparison among linear models 1 and 3; **M2M4**: comparison among linear models 2 and 4; **M3M4**: comparison among linear models 3 and 4; **M1LT**: comparison among linear model 1 and threshold model 1; **M2LT**: comparison among linear model 2 and threshold model 2.
STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length.
-: not a categorical trait; +: equal model in the comparison.

Regardless of which model will be used, the best choice relies on which model will optimize the trait selection measured by the estimated accuracy. Average accuracies were calculated for sires with more than 20 phenotyped daughters by trait

(Table 9). After that, a paired Student's t-test was performed to verify if the sires' accuracy from one model showed a statistically significant difference when compared to the accuracy for the same sires from another model (Table 10).

Similar average accuracies were estimated for linear M1 and M2, except for body length and teat diameter, which showed highest averages for M1. This result was expected since the additive genetic variance which impacts on the accuracy value, estimated for these traits (BL and TD) was higher for M1. Average accuracies for M3 (CG as random effect) were higher than for M1, except for body length, rear legs – side view, fore udder attachment, and teat diameter. Average accuracies from linear M4 (CG as random effect) were higher when compared with M2, for almost all traits (except for rear legs – side view, fore udder attachment, rear udder width, temperament, and navel length).

Table 9. Average accuracy (acc) estimated for each trait using bulls with more than 20 phenotyped daughters

Trait	M1L	M2L	M3L	M4L	M1T	M2T
STA	0.863	0.863	0.871	0.871	-	-
HG	0.774	0.774	0.797	0.797	-	-
BL	0.827	0.741	0.766	0.766	-	-
RL	0.754	0.754	0.777	0.777	-	-
PW	0.751	0.752	0.756	0.757	-	-
HW	0.724	0.729	0.733	0.751	-	-
RA	0.657	0.657	0.661	0.660	-	-
FA	0.541	0.547	0.556	0.557	0.514	0.519
LSV	0.642	0.631	0.624	0.624	0.637	0.634
LRV	-0.014	0.114	0.162	0.177	-0.052	0.103
FAU	0.537	0.566	0.532	0.557	0.518	0.548
RUW	0.563	0.592	0.561	0.590	0.553	0.574
UD	0.662	0.662	0.663	0.663	0.658	0.658
TL	0.842	0.842	0.847	0.847	-	-
TD	0.824	0.744	0.764	0.762	-	-
ME	0.698	0.698	0.710	0.710	0.695	0.695
TEM	0.635	0.633	0.631	0.630	0.628	0.625
NL	0.842	0.863	0.854	0.854	-	-

M1L: model 1 using linear methodology; **M2L**: model 2 using linear methodology; **M3L**: model 3 using linear methodology; **M4L**: model 4 using linear methodology; **M1T**: model 1 using threshold methodology; **M2T**: model 2 using threshold methodology.
STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length.
-: not a categorical trait.

When linear and threshold M1 were compared, linear M1 had higher accuracies average. However, in both models, rear legs – rear view had negative accuracy average. For almost all traits, linear M2 had higher accuracy mean than threshold M2, except for rear legs – side view but the difference was 0.003. The negative accuracy average estimated for rear legs – rear view in the linear and threshold M1 may be due to the data structure that do not describe the variation of the phenotype and could lead to biased estimates.

Differences among accuracies for sires with more than 20 phenotyped daughters were statistically different (p<0.05) for almost all comparisons made in this study. For stature using linear M1 and M2, for udder depth using linear M1 and M3 and linear M2 and M4, and foot angle using linear M2 and M4, average accuracies were not statistically different (p<0.05).

Table 10. Student's t-test among accuracies of bull with more than 20 phenotyped daughters for pair of models

Trait	M1M2	M1M3	M2M4	M3M4	M1LT	M2LT
STA	NS	**	**	**	-	-
HG	+	**	**	+	-	-
BL	**	**	**	+	-	-
RL	+	**	**	+	-	-
PW	**	**	**	**	-	-
HW	**	**	**	**	-	-
RA	**	**	**	**	-	-
FA	**	**	**	NS	**	**
LSV	**	**	**	**	**	**
LRV	**	**	**	**	**	**
FAU	**	**	**	**	**	**
RUW	**	**	**	**	**	**
UD	**	NS	NS	**	**	**
TL	+	**	**	+	-	-
TD	**	**	**	**	-	-
ME	**	**	**	**	**	**
TEM	**	**	**	**	**	**
NL	+	**	**	+	-	-

M1M2: comparison among linear models 1 and 2; **M1M3**: comparison among linear models 1 and 3; **M2M4**: comparison among linear models 2 and 4; **M3M4**: comparison among linear models 3 and 4; **M1LT**: comparison among linear model 1 and threshold model 1; **M2LT**: comparison among linear model 2 and threshold model 2.
STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length.
-: not a categorical trait; +: equal model in the comparison; NS: non-significant.
**: Difference between accuracies for bulls with more than 20 phenotyped daughters were statistically significative (p<0.05).

In this study, linear models performed better than threshold models. Accuracies averages for almost all comparisons was higher for linear models than threshold models, and differences in the sires' accuracy were statistically significant. Furthermore, linear M1 and M2 showed smaller AIC and BIC values than threshold M1 and M2. The spearman's rank correlation showed that regardless of which model was used, the animal's ranking will not be impacted.

Meijering (1985) found no advantage of using threshold models over linear models, although being theoretically a better option. Vanderick et al. (2014) reported that the threshold model had better goodness of fit over linear models. However, according to the authors, no clear advantage was found in terms of predictive ability and the linear model would be more suitable and practical to apply in the genetic evaluation of the studied trait. Weller et al. (1988) concluded that there were few advantages of using threshold over linear models. However, they showed that even though threshold models estimate larger variance components, the rank correlation estimated between the two models were higher than 0.90.

Theoretically, threshold models fit categorical data better than linear models (Gianola, 1982), although they require more computational capacity than linear models (Jamrozik et al., 1991). The main advantage of using threshold models is that fewer interactions are expected in the underlaying scale than the observed scale, leading to the estimation of fewer parameters in the threshold model. The use of a threshold model is justified whether heritability estimates are higher for the threshold model than the linear model, and the accuracy is similar in both models (Kadarmideen et al., 2000).

When linear models, including only statistically significant fixed effects were compared against models, including all fixed effects, the first model fitted the data better due to the smallest AIC and BIC values. For almost all traits, differences were imperceptible on heritability estimates, animal’s ranking, and accuracy. However, for traits where CG was not statistically significant to be included in the model (body length, rear legs – side view and rear view, and teat diameter), the exclusion of CG had a large impact on heritability estimates, animal’s ranking and sires’ accuracies.

Models where CG was included as random effect performed better than models where CG was included as fixed effect, based on the AIC and BIC values. Genetic additive variance was very similar between models and heritability estimates were smaller for models with CG as random effect for all traits. Animal’s ranking did not change largely between models for most of the traits in both comparisons. However, between M1 and M3, ρ had a huge impact for body length, rear legs – side and rear view, and teat diameter. Average accuracies for models with CG as random were higher (except for body length, rear legs – side view, fore udder attachment, and teat diameter between M1 and M3 and for rear legs – side view, fore udder attachment, rear udder width, temperament, and navel length between M2 and M4).

4. Conclusion

When threshold and linear models were compared no advantages were observed in the use of threshold M1 and M2 over linear M1 and M2, respectively. When all linear models were compared, models with only statistically significant effects performed better over the model with all effects based on the AIC and BIC parameters. Models where CG was included as random effect performed better than models where CG was included as fixed effect. Linear models should consider CG as random effect and only statistically significant fixed effects.

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CHAPTER 3 – Genetic parameters estimation for linear conformation traits, milking ease, temperament, and 305-day cumulative milk yield of Dairy Gir cattle

ABSTRACT – Milk yield is the most important trait in the Dairy Gir breeding program and the selection for this single trait can affect the body structure, health, and lifespan which will lead to premature culling. Conformation traits can be associated with milk yield and can help in the indirect selection of this trait. Besides, they are easy to be assess and measurements are obtained relatively early in the animal’s life. Thus, the objective of this study was estimated genetic and phenotypic parameters for conformation traits, milking ease, temperament, and 305-day cumulative milk yield to contribute to the breeding process for the Dairy Gir cattle. The data set used in this study was provided by Embrapa Dairy Cattle and belong to the Brazilian Dairy Gir National Breeding Program (Programa Nacional de Melhoramento do Gir Leiteiro – PNMGL). Records were collected between 1992 and 2018, in herds located in the southeast of Brazil. The data set ranged from 4,441 to 9,738 records from first to seventh lactation. The traits evaluated in this study belong to the linear evaluation and were separated in four categories: corporal dimension, feet and legs, mammary system, and handling. Two-trait analyses were performed to estimate the genetic and phenotypic parameters among all conformation traits and 305-day cumulative milk yield. All analyses were performed using the average Information Restricted Maximum Likelihood (AIREML) method under a repeatability model. Heritability estimates for all traits ranged from 0.07 to 0.50, except for rear-legs – rear view (0.01) which was a very small and poorly reliable estimate. Among all traits, stature, teat length, and navel length presented the higher heritability estimates. Repeatability estimates ranged from 0.22 to 0.79. Heritability and repeatability estimates for traits measured in a continuous scale were higher than for traits measured in a discrete scale. Genetic correlation between rear udder width and udder depth (0.69), fore udder attachment and udder depth (-0.68), udder depth and milk yield (0.56), and rear udder width and milk yield (0.59) showing that a wide rear udder and deep udder are genetically correlated with milk yield and selection for deep udder will lead to a weak fore udder attachment. Milking ease and temperament had genetic correlation estimate of 0.40 which will contribute in the selection process whereas the aim is calmer and easier to milk cows. Genetic correlation between rump length, rear udder width, udder depth, and teat diameter and milk yield was 0.35, 0.59, 0.56, and 0.42, respectively. Therefore, it is possible change conformation, handling traits, and 305-day cumulative milk yield through selection, except rear legs – rear view. The selection for rump length, rear udder width, udder depth, and teat diameter can contribute to increase the mean of milk yield due to their genetic correlation estimates. Selection for udder depth, fore udder attachment, and rear udder width need to be done carefully due to low heritability estimates and unfavorable genetic correlation between them and milk yield. The results obtained in this study can be helpful in the Dairy Gir breeding program.

Keywords: Dairy cattle, handling traits, genetic parameters, Zebu cattle.

1. Introduction

The evaluation of conformation traits started in 1925, in Canada, in pure breed Holstein. In the begin, the linear evaluation was a subjectively way to identify more productive animals in the herd through body conformation and measures which was later replaced by dairy control. Over the years, the linear evaluation has been improved and this information were included in the genetic evaluation (Short and Lawlor, 1992; McManus and Saueressig, 1998). The linear evaluation system in the Brazilian Dairy Gir started in 1994, and currently sixteen conformation traits, milking ease, and temperament are evaluated in the national breeding program (McManus and Saueressig, 1998; Panetto et al., 2019).

Conformation traits are easy to be assess and measurements are obtained relatively early in the animal’s life, besides they have been found related with health, welfare and longevity for European cattle (Sewalem et al., 2004; Kern et al., 2014). For instance, traits that describe the cow’s rump were have a linear association with calving ease and uterus and vaginal prolapse (Nogalski and Mordas, 2012; Sawa et al., 2013). Feet and legs traits were genetically associated with hoof lesions and had a linear association with lifespan (Sewalem et al., 2004; Chapinal et al., 2013). Mammary system traits were genetically correlated with mastitis incidence and somatic cell counts (Bharti et al., 2015; Dube et al., 2008). And handling traits are related with milking process where calmer animals and easier to be milked can maximize the process and reduce residual milk (Costa et al., 2015).

Despite the importance of the conformation and handling traits, milk yield continues been the most important trait in the Dairy Gir cattle. However, the preferential selection for milk yield can affect body structure as well as health and lifespan which can lead to premature culling (Onyiro and Brotherstone, 2008). Thereby, understand how conformation, handling traits, and milk yield relate between themselves and how they respond to selection it is important in a breeding program.

Several studies were done to estimate genetic parameters for conformation and handling traits and milk yield. However, most of them were with European cattle in temperate countries (Mrode et al., 1999; Berry et al., 2004; Wiggans et al., 2004; De Hass et al., 2007; Kramer et al., 2013) and usually, these results vary a lot between population even in the same breed which make important individual studies for each population (Nemcova et al., 2011). Few studies are available for Brazilian Dairy Gir aiming estimate genetic parameters for conformation traits and the relationship between them and milk yield but not all traits in the linear evaluation system were included in these reports (Wenceslau et al., 2000; Lagrotta et al., 2010). Therefore, the objective of this study was estimated genetic and phenotypic parameters for conformation traits, milking ease, temperament, and 305-day cumulative milk yield to contribute for the Dairy Gir cattle breeding program.

2. Material and Methods

2.1. Data description

The data set used in this study was provided by Embrapa Dairy Cattle (Embrapa Gado de Leite) and it is part of the Brazilian Dairy Gir National Breeding Program (Programa Nacional de Melhoramento do Gir Leiteiro – PNMGL). Records were collected by the Brazilian Association of Dairy Gir Breeders (Associação Brasileira de Criadores dos Gir Leiteiro – ABCGIL) and Brazilian Association of Zebu Breeders (Associação Brasileira dos Criadores de Zebu), between 1992 and 2018, in herds located in southeast region of Brazil.

In the group of traits measured and recorded in centimeters: stature is the length of the animal from the top of the rump to the ground. Heart girth is the thoracic perimeter. Body length is the distance between the end of the shoulder blade to the ilium. Navel length is the distance from the abdominal wall to the end of the navel. The rump length is the distance between the tip of the ilium and the tip of the ischium in the same animal side. The pin width is the distance between the tip of the ischium on the left to the right side. The hook width is the distance between the tip of the ilium on the left side until the right side of the animal. Teat length is the teat size and teat diameter is the teat thickness.

In the group of traits measured using a visual score (9-point scale): foot angle is the angle formed between two imaginary line, 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 is the angulation formed between the tibia and metatarsus bones (1 – straight and 9 – curved). Rear legs – rear view evaluate the proximity of the hocks (1 – hocked-in and 9 – bow legged). Fore udder attachment measures the fixation of the anterior udder to the abdominal wall (1 – weak and 9 – strong). Rear udder width is 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 is the distance from the hock to the floor of the udder (1 – shallow and 9 – deep). Milking ease measures the time and effort spent at the cows' milking measured by the farmer (1 – faster and 9 – slow) and temperament is the behavior of the animal during management measured by the farmer (1 – tame and 9 – aggressive). To a better understand on how these traits are measured, see Panetto et al. (2019).

Rump angle is recorded in angle degrees, using a slope inclinometer protractor or, in part of the cases, approximated from a visual linear score. It is the difference between the height of the pin and the hip bones. 305-day cumulative milk yield was record in kilogram.

The traits were separated in four categories for a better understanding: 1) corporal dimension: stature, heart girth, body length, rump length, pin width, hook width, rump angle, and navel angle; 2) feet and legs: foot angle, rear legs – rear view, and rear legs – side view; 3) mammary system: fore udder attachment, rear attachment width, udder depth, teat length, and teat diameter; 4) handling: milking ease and temperament.

The raw data set contained 10,956 linear type evaluations, from first to seventh lactation, of 7,913 Gir cows and 7,234 records of 305-day cumulative milk yield from 6,888 Gir cows. In the first data edition, data with possible annotation errors and with values outside the biological range for traits evaluated in cm and angles degrees were removed from the data file. Lactations that was not finished naturally for reasons like mastitis, culling, waiver by the farmer in the continuous record of milk production were removed from posterior analyses. The pedigree file used in the analysis for all traits was constituted by 18,402 animals, which considered up to 15 generations from phenotyped animals.

2.2. Data distribution and transformation

Skewness and Kurtosis tests were performed using the R-package "moments" (Komsta and Novomestky, 2015), in order to verify whether the data were normally distributed. Thereafter, several data transformations were tested, including log transformation, square-root transformation, cube root transformation, Yeo-Johnson transformation, and Ordered Quantile normalizing transformation. All analyses were performed in the R software (R Core Team, 2008). The Ordered Quantile normalizing method (Peterson and Cavanaugh, 2019) was chosen to transform records measured by score because it presented the best Skewness and Kurtosis values after the transformation which indicated a high approximation of the normally distribution.

2.3. Data analyses

Before the analyses, contemporary group (herd and year of evaluation) with less than 5 records within group were removed. The number of contemporary group (CG) removed varied from 548 to 573 for all traits analyzed. Disconnected CG were also removed from the data set which led to the removal of 17 to 67 animals from 3 to 9 CG within all traits analyzed. Connectedness between CG was checked using the AMC program (Roso and Schenkel, 2006), in which the degree of connectedness is measured through the total number of direct genetic links due to a common ancestor. The program used ten or more genetic links to consider a CG connected.

One-trait analyses were performed to estimate the variance components which were used to estimate heritability and repeatability posteriorly. Two-trait analyses were performed among all conformation traits and between them and 305-day cumulative milk yield to obtain phenotypic, genetic, permanent environmental, and residual correlation. All analyses were performed using the average Information Restricted Maximum Likelihood (AIREML) method (Gilmour et al., 1995) with the ASREML software (Gilmour et al., 2009) under a repeatability model.

The general statistic model used in the analyses was:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{Wpe} + \boldsymbol{\varepsilon}$$

where: y is the observation vector for the linear conformation traits, milking ease or temperament; $\mathbf{X}$ is the incidence matrix of fixed effects; $\mathbf{Z}$ is the incidence matrix for the animal additive genetic effect; $\mathbf{W}$ is the incidence matrix for the permanent environment effect; $\boldsymbol{\beta}$ is the vector of fixed effects; $\mathbf{a}$ is the vector of animal random effects; $\mathbf{pe}$ is the vector related to permanent environment random effects; $\boldsymbol{\varepsilon}$ is the vector of random errors or residual effects associated with each observation.

For the model, it was assumed that $\mathrm{E}[\mathbf{y}] = \mathbf{X\beta}; \mathrm{Var}(\mathbf{a}) = \mathbf{A} \otimes \mathbf{G}$, $\mathrm{Var}(\mathbf{pe}) = \mathbf{I} \otimes \mathbf{W}$, and $\mathrm{Var}(\mathbf{e}) = \mathbf{I} \otimes \mathbf{R}$, where $\mathbf{G}$ is the additive genetic covariance matrix; $\mathbf{A}$ is the numerator matrix of additive genetic relationships; $\mathbf{W}$ is the permanent environmental covariance matrix; $\mathbf{R}$ is the residual covariance matrix; $\mathbf{I}$ is the identity matrix; and $\otimes$ is the direct product or the Kronecker product between two matrices. Genetic and residual effects were assumed to be independent.

The general variance and covariance matrix structure of the random effects in the model were:

$$\begin{bmatrix} \boldsymbol{a} \\ \boldsymbol{e} \end{bmatrix} \sim N(0, V); V = \begin{bmatrix} \boldsymbol{A} \otimes \boldsymbol{G} & 0 & 0 \\ 0 & \boldsymbol{I} \otimes \boldsymbol{W} & 0 \\ 0 & 0 & \boldsymbol{I} \otimes \boldsymbol{R} \end{bmatrix}$$

where: $\boldsymbol{G}$ is the direct additive genetic effects covariance matrix; $\boldsymbol{A}$ is the parentage matrix; $\boldsymbol{W}$ is the permanent environmental matrix; $\boldsymbol{R}$ is the residual covariance matrix; $\boldsymbol{I}$ is the identity matrix and $\otimes$ is the Kronecker product between two matrices.

The fixed effects considered in the model for conformation and handling traits were CG composed by herd and year of evaluation, season of evaluation, evaluator, diet, and age of the dam in the evaluation moment as covariate (with linear and quadratic effects). For 305-day cumulative milk yield, the fixed effects considered in the model were CG composed by herd and year of parturition, season of parturition, number of milking, diet, and age of the dam in the delivery as covariate (with linear and quadratic effects).

A two-tailed t-test was performed to verify whether the correlations were significantly ($p<0.05$) different from zero. The DISTT function (version in Portuguese, and TDIST in English) on Microsoft Excel® were applied.

3. Results and Discussion

The number of records varied from 9,738 to 5,579 for all conformation and handling traits. The number of records for 305-day cumulative milk yield was 4,441 (Table 1). Mean, median, and mode for almost all traits are around the ideal pattern for the breed as proposed by Panetto et al. (2019) for the Brazilian Dairy Gir National Breeding Program. Nevertheless, rumple angle and navel length need more attention from the breeding program since ideal values for both traits are less than 23 degrees and close to 1 cm as possible, respectively (Panetto et al., 2019). Median and mode values were 25 and 30 degrees, respectively for rump angle and 10 cm for navel length for both mode and median.

Table 1. Descriptive statistic for conformation and handling traits, and 305-day cumulative milk yield (Milk yield)

Trait	N	Min	Max	Mean	SD	Median	Mode
Stature (cm)	9,698	119	155	136.90	4.92	137	136
Hearth girth (cm)	9,576	141	210	176.00	8.91	176	180
Body length (cm)	9,432	86	127	103.50	5.97	103	102
Rump length (cm)	9,677	31	59	42.77	4.30	43	45
Pin width (cm)	9,536	10	29	18.27	2.62	18	18
Hook width (cm)	9,546	33	62	46.26	4.36	47	48
Rump angle (degree)	5,987	1	50	24.42	7.74	25	30
Foot angle (score)	8,778	1	9	-	-	5	5
Rear legs – side view (score)	6,073	1	9	-	-	5	5
Rear legs – rear view (score)	6,056	1	9	-	-	5	5
Fore udder attachment (score)	5,655	1	9	-	-	5	5
Rear udder width (score)	5,853	1	9	-	-	5	5
Udder depth (score)	6,034	1	9	-	-	5	5
Teat length (cm)	9,738	3	14	7.50	1.92	7	7
Teat diameter (cm)	9,692	1	8	3.87	0.87	4	4
Milking ease (score)	9,520	1	9	-	-	3	5
Temperament (score)	9,702	1	9	-	-	4	5
Navel length (cm)	5,579	1	20	10.20	3.16	10	10
Milk yield (kg)	4,441	103	12,275	3,858.00	1,883.53	3,513	1,506

N: number of records; **Min**: minimum value for the evaluated trait; **Max**: maximum value for the evaluated trait; **Mean**: mean by trait; **SD**: mean standard deviation by trait; **Median**: median by trait; **Mode**: mode by trait;

Genetic additive, permanent environmental, and residual variance and their respective standard error are presented in Table 2. Repeatability estimates for conformation traits (Table 3) tend to be high because is expected that a trait repeat the same score or measurement over different evaluations through the years. On the other hand, heritability estimates normally range from low to high (Table 3) and traits on feet and legs category has the lowest estimates and corporal dimension category has the highest estimates, with stature been the trait with the highest estimate between all traits (Berry et al., 2004; De Haas et al., 2007).

Heritability estimates for traits in the corporal dimension category ranged from 0.12 to 0.50 (Table 3). Stature was the trait with the highest estimate, so a higher response to selection can be expect from this trait. Heritability estimates in this study agree with some results in the literature of 0.54 for Dairy Gir cattle in Brazil and 0.53 for Holstein cattle in Turkey (Lagrotta et al., 2010; Duru et al., 2012). However, it is possible found higher (0.74) and smaller (0.39) estimates for stature in the literature (Rennó et al., 2003; De Hass et al., 2007). Heritability estimates for heart girth in this study was close to reports in the literature (0.23 to 0.38) (Wenceslau et al., 2000; Daliri et al., 2008).

Table 2. Genetic additive (σ_a^2), permanent environmental (σ_{pe}^2), and residual variance (σ_e^2) and their respective standard error (se) for all traits evaluated

Trait	σ_a^2	sea	σ_{pe}^2	sepe	σ_e^2	see
STA	8.56	0.72	4.98	0.49	3.54	0.10
HG	12.62	1.66	18.50	1.36	17.38	0.47
BL	4.48	0.68	4.15	0.60	14.15	0.36
RL	1.18	0.17	1.53	0.14	2.54	0.07
PW	0.65	0.10	0.65	0.09	1.78	0.05
HW	1.38	0.22	1.93	0.20	4.06	0.11
RA	3.49	0.93	8.82	1.13	17.57	0.84
FA	0.05	0.01	0.10	0.02	0.53	0.01
LSV	0.07	0.02	0.20	0.03	0.45	0.02
LRV	0.004	0.01	0.26	0.02	0.42	0.02
FUA	0.05	0.02	0.31	0.02	0.28	0.02
RUW	0.04	0.01	0.13	0.02	0.29	0.01
UD	0.08	0.02	0.15	0.02	0.44	0.02
TL	0.93	0.09	0.56	0.06	0.76	0.02
TD	0.09	0.01	0.12	0.01	0.24	0.01
ME	0.09	0.02	0.13	0.01	0.34	0.01
TEM	0.07	0.01	0.19	0.02	0.35	0.01
NL	3.17	0.42	2.86	0.32	1.94	0.11
MY	189,596.00	41,669.40	449,329.00	85,586.50	559,333.00	77,685.10

STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length; **MY**: 305-day cumulative milk yield. **sea**: standard error of additive genetic variance estimates; **sepe**: standard error of permanent environmental variance estimates; **see**: standard error of residual variance estimates.

Table 3. Heritability (h^2) and repeatability (r) estimates for all traits

Trait	h^2	seh²	r	ser
Stature (cm)	0.50	0.03	0.79	0.01
Hearth girth (cm)	0.26	0.03	0.64	0.01
Body length (cm)	0.20	0.03	0.38	0.02
Rump length (cm)	0.22	0.03	0.52	0.01
Pin width (cm)	0.21	0.03	0.42	0.02
Hook width (cm)	0.19	0.03	0.45	0.02
Rump angle (degree)	0.12	0.03	0.41	0.03
Foot angle (score)	0.07	0.01	0.22	0.02
Rear legs – side view (score)	0.10	0.03	0.37	0.03
Rear legs – rear view (score)	0.01	0.01	0.38	0.03
Fore udder attachment (score)	0.07	0.02	0.56	0.03
Rear udder width (score)	0.08	0.03	0.37	0.03
Udder depth (score)	0.12	0.03	0.34	0.03
Teat length (cm)	0.41	0.03	0.66	0.01
Teat diameter (cm)	0.20	0.03	0.47	0.02
Milking ease (score)	0.15	0.03	0.39	0.02
Temperament (score)	0.11	0.02	0.43	0.02
Navel length (cm)	0.40	0.05	0.76	0.01
Milk yield (kg)	0.16	0.03	0.53	0.07

seh²: standard error of heritability estimates; **ser**: standard error of repeatability estimates;

Cows with sloping or raised rump needed more human intervention at calving increasing costs with veterinary, as well as these animals had more incidence of uterus and vaginal prolapse (Sawa et al., 2013). Besides the rump angle, smaller rump can contribute to difficult the calving (Dadati et al., 1985; Nogalski and Mordas, 2012). Heritability estimates for pin width and rump angle (Table 3) are close to some reports in the literature that ranged from 0.16 to 0.49 and from 0.09 to 0.41, respectively (Daliri et al., 2008; Duru et al., 2012).

In this study, heritability estimate for rump angle (Table 3) is close to the reports in the literature of 0.23 and 0.26 by Daliri et al. (2008) and Lagrotta et al. (2010), respectively. Differently, hook width had heritability estimate a little smaller than the result found in the literature of 0.26 by Lagrotta et al. (2010) for the Gir breed in Brazil. This divergence may be due to the differences in the models or number of animals used in the studies.

The last two traits in the corporal dimension category are body length and navel length. The heritability estimate for body length (Table 3) is close to values of 0.11 and 0.18 reported by Wenceslau et al. (2000) and Lagrotta et al. (2010), respectively. It is important notice that both studies were performed in the Gir cattle in Brazil and differences in the results may be due to population size or statistic model applied. Heritability estimate for navel length in this study was moderate which indicate a bigger response from the selection process for this trait. However, this value is smaller than estimates in the literature of 0.93 for Sahiwal cows (Khan and Khan, 2016). However, Khan and Khan (2016) justified that the high heritability estimates may be due to a small dataset used and the use of only one classifier for all linear classifications. Longer navel disturbs the animals in the copulation moment and is more susceptible to pathologies, such as foreskin prolapse, due to constant contact with grass which explain the importance of this trait.

Heritability estimate for foot angle (Table 3) agrees with the literature where mostly of the estimates ranged from 0.09 to 0.18 (Daliri et al., 2008; Onyiro and Brotherstone, 2008; Duru et al., 2012; Campos et al., 2015). Heritability estimate for rear legs – side view (Table 3) also agrees with mostly of the results in the literature which varied from 0.07 to 0.22 (Wiggans et al., 2004; Ptak et al., 2011; Campos et al., 2012). Heritability estimate for rear legs – rear view in this study (Table 3) is lower than reports in the literature. Nemcova et al. (2011), Ptak et al. (2011), and Duru et al. (2012) estimated heritability of 0.10, 0.09, and 0.11, respectively. However, standard error of the heritability estimated by Duru et al. (2012) was higher (0.18) than the estimate itself which happened in this study as well, making the estimate poorly reliable.

According to Berry et al. (2004) feet and legs category has the smaller heritability estimates among all categories. These traits are highly related with the environment where the animals are living. No slippery, level floor, and cleaned paving are associated with better score for feet and legs and less locomotive problems (Onyiro and Brotherstone, 2008). Sewalem et al. (2004) concluded that cows with extreme score classification for foot angle and rear legs – side view are more susceptible to be discarded from the herd, reducing their lifespan. Thus, based in the low heritability estimates for the feet and legs in this study, less locomotor problems and better scores for these traits can be expected if environmental improvement were implanted when compared with genetic selection.

The mammary system is the most important group of traits for the milk production system, because it impacts directly the cow's milk yield. Carlström et al. (2016) estimated a positive genetic correlation between udder health and udder depth and fore udder attachment suggesting that shallow udders with strong udder fore attachments are associated with lower risk of clinical mastitis. However, increase udder health by genetic selection can be a little slow, whereas in this study, fore udder attachment and udder depth had lower heritability estimates (Table 3). These estimates were slightly smaller than results in the literature that ranged from 0.10 to 0.36 for fore udder attachment (Rennó et al., 2003; Campos et al., 2012) and from 0.14 to 0.40 for udder depth (Wiggans et al., 2004; Dube et al., 2008).

Kramer et al. (2013) estimated heritability of 0.42 for udder depth measured in cm above hock and not in scores as usually is made. According the authors, this estimate is higher than results in literature which is around 0.30. This difference can be explained by the fact that the trait was measured in a continuous scale (cm). According to the authors, this result and others in the literature agree with Ilahi and Kadarmideen (2004) who stated that evaluation using a continuous scale lead to a higher heritabilities.

Heritability estimates for teat length in this study (Table 3) was higher than most of the results in the literature which ranged from 0.007 to 0.38 (Daliri et al., 2008; Campos et al., 2015). However, some authors estimated heritability for teat length of

0.44, 0.45, and 0.46 (Wenceslau et al., 2000; Lagrotta et al., 2010; Duru et al., 2012). Teat length also has a huge impact in the udder health where animals with long teats are associated with more probability of clinical mastitis (Sorensen et al., 2000). Another trait associated with risk of clinical mastitis is teat diameter (Carlström et al., 2016). Besides of few reports in the literature, heritability estimates for this trait in this study was closed to the results of Lagrotta et al. (2010) of 0.24. A higher response to selection can be expected from teat length when compared to teat diameter due to high heritability estimate for the first trait.

Reports in the literature did not associated rear udder width with udder health before, as happened with other traits in the mammary system category. For this trait, heritability estimate in this study (Table 3) was smaller than the results reported in the literature that range from 0.11 to 0.28 for several breeds such as Holstein, Brown Swiss, Jersey, Ayrshire, Guernsey, and Milking Shorthorns (Wiggans et al., 2004; Campos et al., 2012). Due to the lower heritability estimate is expected little gain for rear udder width from the selection process for the Dairy Gir cattle.

Compare heritability estimates for milking ease and temperament from this study with results in the literature can be complicated. Beyond the several differences between studies, the methodology used to evaluate the traits differ notably between studies. Heritability estimates for milking ease in this study (Table 3) was close to 0.11 reported by Berry et al. (2004) and 0.10 by Resing and Ruten (2005). For both studies the trait was evaluated with the same methodology of this study, where scores from 1 to 9 were attributed to the cow by the farmer. For temperament the heritability estimated in this study was close to the value estimated by Berry et al. (2004) of 0.12 where the animal general behavior was evaluated in score from 1 to 9 by the farmer as same as in this study. For both traits, low gain in the genetic selection can be expected for the population in this study.

Unfortunately, studies about milking ease and temperament are scarce in the literature. Milking ease can be measured subjectively in scores (from 1 to 9 or 1 to 5) by the farmer or more objectively in kilograms per minute during the hole milking or in the main milking phase (Kramer et al., 2013). Higher heritability estimates can be achieved when objectively measurements are performed. Ilahi and Kadarmideen (2004) estimated heritabilities for milking ease of 0.46 and 0.48 for Brown Swiss and

Simmental breeds, respectively and 0.25 for Holstein cattle. The authors affirmed that differences in the results were attributed to the use of milk in kg per minute as measurement for Brown Swiss and Simmental breeds while the Holstein cattle were measured in scores from 1 to 5. Genetic variance of continuous traits is usually higher when compared to discrete traits due to the loss of information when information is truncated in categories (Ilahi and Kadarmideen, 2004). Despite these differences, Resing and Ruten (2005) estimated genetic correlation between milking speed measured in score and in kg per minute (0.79) and affirmed that the owners score are useful data for genetic evaluation of milking speed.

Beside the difference for genetic parameters between traits evaluated in continuous and categorical scale, the use of a scale by the farmer to evaluate an animal can have positive and negative points (Kramer et al., 2013). Farmers know their animals very well because they deal with them daily; thus, they can evaluate the animal’s behavior and milking difficulty more accurate. However, they do not have the experience on evaluate a cow in a standardized scale as an independent person which can difficult the use of the full 9-point scale. But this problem can be bypassed with the use of a scale with less values (5-point scale) as suggested by Resing and Ruten (2005). However, the use of scale with odd numbers can lead to an instinctive preference of the mean value by the farmer as reported by Ilahi and Kadarmideen (2004). So, to avoid this problem the use of a scale with even number can be a solution (6-point scale) (Kramer et al., 2013).

Heritability estimate for 305-day cumulative milk yield in this study (Table 3) is smaller than results in the literature with values of 0.20, 0.21, 0.22, 0.24, and 0.25 for Holstein, Dairy Gir, and Brown Swiss (Rennó et al., 2003; Daliri et al., 2008; Lagrotta et al., 2010, Duru et al., 2012; Campos et al., 2015). The result obtained here is smaller than the heritability estimated in the national breeding program of 0.20, but it is important highlight that in the breeding program besides the Dairy Gir cows, crossbred Gir x Holstein are also included in the genetic parameter estimates (Panetto et al., 2019).

Among all conformation and handling traits those with the highest response to the selection process will be stature, teat length, and navel length due to their heritabilities estimates (0.50, 0.41, and 0.40, respectively), and traits with the smallest response to the selection process will be foot angle, fore udder attachment, and rear udder width with heritabilities estimates of 0.07, 0.07, and 0.08, respectively. Rear legs – rear view was disregarded from the rank with lowest estimates because the estimate is not reliable.

Repeatability estimates ranged from 0.22 to 0.79 for all studied traits (Table 3). High repeatability estimates for conformation traits can be expected, since a score or measurement for a trait tends to be the same, or very similar, over the evaluations. The traits with the high estimates were stature, navel length, and teat length (0.79, 0.76, and 0.66) which mean that there is a correlation among measurements over time and it is not necessary several measurements to evaluate these traits. On the other hand, foot angle, udder depth, rear legs – side view, and rear udder width had the smaller repeatability estimates (0.22, 0.34, 0.37, and 0.37, respectively). Most of the traits evaluated in a continuous scale had higher repeatability estimates and most of the traits evaluated in a categorical scale had smaller repeatability estimates. According to Kramer et al. (2013), repeatability estimate (0.71) for udder depth was high because the trait was evaluated in centimeters.

Significant ($p<0.05$) phenotypic and genetic correlation between all nineteen traits and their respective standard error obtained in the two traits analyses are presented in Table 4 and significant ($p<0.05$) permanent environmental and residual correlation estimates among all nineteen traits and their respective standard error obtained in the two traits analyses are presented in Table 5. Heritabilities and their standard error mean obtained in the two traits analyses were similar to the values obtained in one-trait analyses and it is presented in Table 4 and Table 5. All phenotypic, genetic, permanent environmental and residual correlation estimates are showed in Appendix C and Appendix D on appendix.

Genetic correlation estimates between stature and almost all traits in the corporal dimension category were positive (Table 4) meaning that genes involved in the expression of stature also affects heart girth, body length, rump length, pin width, hook width, and rump angle in the same direction. So, if selection is applied for higher animals, it is expected an increase in the hearth girth, body length, rump length, rump length, pin width, and hook width mean, as well as changes in the rump angle to a low rump. Cows with low rump angle needs more force to deliver the calf and more veterinary intervention at the calving. The stress during the birth leads to a higher placenta retention. In addition, sloping rump could lead to more incidence of uterus and vaginal prolapse (Nogalski, 2002; Nogalski and Mordas, 2012; Sawa et al., 2013). Thus, selection for stature need to be done with caution.

Despite few reports in the literature on genetic correlation between stature and hearth girth, body length, rump length, and hook width, the genetic correlation estimates in this study (Table 4) were close to the results reported by Lagrotta et al. (2010) of 0.69, 0.76, 0.81, and 0.42, respectively. On the other hand, several genetic correlation estimates between stature and pin width and rump angle can be found. Estimate between stature and pin width in this study was close to 0.41, 0.42, and 0.48 reported by Nemcova et al. (2011), Rennó et al. (2003), and Esteves et al. (2004), respectively. However, the same estimate in the literature also ranged from 0.35 to 0.77 (Campos et al., 2012; Wiggans et al., 2004). The genetic correlation estimate between stature and rump angle in this study was a little higher than the estimates in the literature that range from -0.09 to 0.29 (Wiggans et al., 2004).

Genetic correlation between hearth girth, body length, rump length, pin width, and hook width ranged from 0.29 to 0.65 in this study (Table 4) which demonstrate that selection for any of these traits will lead to a response in the same direction for the other. These results agree with Lagrotta et al. (2010) which estimated genetic correlation ranging from 0.15 to 0.72 for the same traits in the Dairy Gir cattle in Brazil.

A larger distance between the two pin bones, measured by pin width, had a favorable genetic correlation with ease calving (Dadati et al., 1985). However, selection for bigger rump width will lead to a steep foot angle due to the significant ($p<0.05$) positive genetic correlation estimated among pin width and foot angle (Table 4). Animals with extreme score for feet and legs (such as steep foot angle) are more likely to be discarded from the herd (Sewalem et al., 2004). Higher and negative genetic correlation (Table 4) was estimated between rump angle and rear legs – side view which is good whereas for the Dairy Gir cattle intermediary phenotype for both traits are the best choice. Thereby, if a balance were maintained in the selection for both traits, the animals will present a body conformation that will not compromise the rump and legs.

Table 4. Significative (p<0.05) phenotypic correlation (above diagonal), genetic correlation (below diagonal), heritability (diagonal), and standard error (parentheses) estimated in the two traits analyses among all nineteen traits

	STA	**HG**	**BL**	**RL**	**PW**	**HW**	**RA**	**FA**	**LSV**	**LRV**	**FUA**	**RUW**	**UD**	**TL**	**TD**	**ME**	**TEM**	**NL**	**MY**
STA	**0.50** **(0.03)**	0.45* (0.01)	0.37* (0.01)	0.44* (0.01)	0.25* (0.01)	0.40* (0.01)	0.18* (0.02)	0.04* (0.01)	-0.05* (0.02)	NS	NS	NS	0.05* (0.02)	0.11* (0.02)	0.13* (0.01)	NS	NS	0.07* (0.02)	0.11* (0.02)
HG	0.64* (0.05)	**0.26** **(0.03)**	0.30* (0.01)	0.45* (0.01)	0.33* (0.01)	0.50* (0.01)	NS	NS	-0.04* (0.02)	NS	NS	0.08* (0.02)	0.07* (0.02)	0.10* (0.01)	0.40* (0.09)	NS	NS	0.08* (0.02)	0.08* (0.02)
BL	0.73* (0.05)	0.52* (0.08)	**0.20** **(0.03)**	0.30* (0.01)	0.25* (0.01)	0.31* (0.01)	NS	NS	NS	NS	NS	0.06* (0.02)	0.06* (0.02)	0.08* (0.01)	0.09* (0.01)	NS	-0.04* (0.01)	0.08* (0.02)	0.10* (0.02)
RL	0.76* (0.04)	0.59* (0.07)	0.55* (0.07)	**0.23** **(0.03)**	0.24* (0.01)	0.45* (0.01)	-0.06* (0.02)	NS	NS	NS	NS	0.05* (0.01)	0.06* (0.01)	0.11* (0.01)	0.14* (0.01)	NS	-0.06* (0.01)	0.06* (0.02)	0.11* (0.02)
PW	0.45* (0.07)	0.33* (0.09)	0.45* (0.09)	0.29* (0.09)	**0.21** **(0.03)**	0.34* (0.01)	NS	NS	-0.03* (0.01)	NS	NS	0.06* (0.02)	0.06* (0.02)	0.08* (0.01)	0.08* (0.01)	NS	NS	0.07* (0.02)	0.05* (0.02)
HW	0.65* (0.06)	0.62* (0.06)	0.53* (0.08)	0.65* (0.06)	0.35* (0.09)	**0.19** **(0.03)**	-0.07* (0.02)	NS	-0.03* (0.02)	NS	NS	0.08* (0.02)	0.07* (0.02)	0.08* (0.01)	0.12* (0.01)	NS	-0.04* (0.01)	0.08* (0.02)	0.07* (0.02)
RA	0.34* (0.11)	NS	NS	NS	NS	NS	**0.12** **(0.03)**	-0.04* (0.01)	NS	NS	NS	NS	0.04* (0.01)	NS	NS	NS	NS	NS	NS
FA	NS	NS	NS	NS	0.32* (0.14)	NS	NS	**0.07** **(0.02)**	-0.12* (0.01)	0.07* (0.01)	0.07* (0.01)	NS	-0.04* (0.01)	NS	NS	NS	NS	NS	NS
LSV	NS	NS	NS	NS	NS	NS	-0.42* (0.16)	NS	**0.10** **(0.03)**	NS	NS	NS	NS	NS	NS	NS	0.04* (0.01)	NS	NS
LRV	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.01** **(0.01)**	0.06* (0.01)	0.06* (0.01)	NS	NS	NS	NS	NS	NS	NS
FUA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.07** **(0.02)**	0.06* (0.02)	-0.19* (0.01)	-0.05* (0.02)	NS	NS	NS	-0.07* (0.02)	NS
RUW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.08** **(0.03)**	0.16* (0.01)	0.04* (0.02)	0.07* (0.01)	NS	-0.04* (0.01)	NS	0.17* (0.02)
UD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.68* (0.14)	0.69* (0.15)	**0.11** **(0.03)**	0.11* (0.02)	0.14* (0.01)	NS	NS	0.05* (0.02)	0.13* (0.02)
TL	0.15* (0.06)	0.24* (0.08)	0.21* (0.08)	0.30* (0.08)	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.41** **(0.03)**	0.53* (0.01)	0.08* (0.01)	NS	NS	0.10* (0.02)
TD	0.31* (0.08)	0.40* (0.09)	0.33* (0.10)	0.48* (0.09)	0.24* (0.10)	0.33* (0.10)	NS	NS	NS	NS	-0.40* (0.15)	NS	0.45* (0.13)	0.82* (0.04)	**0.20** **(0.03)**	0.05* (0.01)	NS	NS	0.15* (0.02)
ME	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.27* (0.09)	NS	**0.15** **(0.03)**	0.20* (0.01)	NS	-0.05* (0.02)
TEM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.40* (0.12)	**0.11** **(0.02)**	NS	-0.07* (0.02)
NL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.40** **(0.05)**	NS
MY	NS	NS	NS	0.35* (0.12)	NS	NS	NS	NS	NS	NS	NS	0.59* (0.15)	0.56* (0.15)	NS	0.42* (0.12)	NS	NS	NS	**0.16** **(0.03)**

STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length; **MY**: 305-day cumulative milk yield.
*: Significantly (p<0.05) different from zero using the two-tailed t-test; **NS**: non-significant.

Table 5. Significative (p<0.05) residual correlation (above diagonal), permanent environmental correlation (below diagonal), heritability (diagonal), and standard error (parentheses) estimated in the two traits analyses among all nineteen traits

	STA	**HG**	**BL**	**RL**	**PW**	**HW**	**RA**	**FA**	**LSV**	**LRV**	**FUA**	**RUW**	**UD**	**TL**	**TD**	**ME**	**TEM**	**NL**	**MY**
STA	**0.50** **(0.03)**	0.17* (0.02)	0.05* (0.02)	0.09* (0.02)	0.05* (0.02)	0.09* (0.02)	0.16* (0.03)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
HG	0.53* (0.05)	**0.26** **(0.03)**	0.08* (0.02)	0.19* (0.02)	0.15* (0.02)	0.21* (0.02)	-0.10* (0.03)	NS	NS	NS	NS	NS	NS	NS	0.08* (0.02)	NS	NS	NS	NS
BL	0.53* (0.06)	0.53* (0.06)	**0.20** **(0.03)**	0.10* (0.02)	0.09* (0.02)	0.14* (0.02)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
RL	0.54* (0.05)	0.68* (0.04)	0.52* (0.07)	**0.23** **(0.03)**	0.18* (0.02)	0.16* (0.02)	-0.13* (0.03)	NS	NS	NS	NS	NS	NS	NS	0.05* (0.02)	NS	NS	NS	NS
PW	0.34* (0.07)	0.62* (0.06)	0.52* (0.08)	0.33* (0.07)	**0.21** **(0.03)**	0.13* (0.02)	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.05* (0.02)	NS	NS	NS
HW	0.59* (0.05)	0.83* (0.04)	0.56* (0.07)	0.79* (0.04)	0.80* (0.06)	**0.19** **(0.03)**	NS	-0.05* (0.02)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
RA	NS	NS	0.28* (0.12)	NS	NS	NS	**0.12** **(0.03)**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FA	NS	NS	NS	NS	NS	NS	NS	**0.07** **(0.02)**	-0.10* (0.03)	0.09* (0.03)	NS	NS	NS	NS	NS	NS	NS	NS	NS
LSV	NS	NS	NS	NS	NS	NS	NS	NS	**0.10** **(0.03)**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LRV	NS	NS	NS	NS	NS	0.26* (0.08)	NS	NS	NS	**0.01** **(0.01)**	NS	NS	NS	NS	NS	NS	NS	NS	NS
FUA	NS	NS	NS	NS	NS	NS	NS	0.34* (0.09)	NS	NS	**0.07** **(0.02)**	0.11* (0.04)	-0.14* (0.04)	NS	NS	NS	NS	NS	NS
RUW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.08** **(0.03)**	0.08* (0.03)	0.08* (0.03)	NS	NS	NS	NS	NS
UD	NS	NS	0.38* (0.13)	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.11** **(0.03)**	NS	NS	NS	NS	NS	NS
TL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.41** **(0.03)**	0.34* (0.02)	NS	NS	NS	NS
TD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.57* (0.05)	**0.20** **(0.03)**	NS	NS	-0.13* (0.04)	NS
ME	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.15** **(0.03)**	0.10* (0.02)	NS	NS
TEM	NS	NS	NS	-0.18* (0.06)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.35* (0.06)	**0.11** **(0.02)**	NS	-0.11* (0.05)
NL	NS	0.16* (0.07)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.40** **(0.05)**	NS
MY	0.27* (0.08)	0.25* (0.08)	NS	0.19* (0.08)	NS	NS	NS	NS	NS	NS	NS	NS	0.35* (0.13)	0.21* (0.09)	NS	NS	NS	NS	**0.16** **(0.03)**

STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length; **MY**: 305-day cumulative milk yield.
*: Significantly (p<0.05) different from zero using the two-tailed t-test; **NS**: non-significant.

Genetic correlation estimates (Table 4) were significant ($p<0.05$) between teat length and stature, hearth girth, body length, and rump length. It is necessary attention when selection is applied to increase the mean of the traits in the corporal dimension category because is expected an increase in the mean for teat length. Longer teat is associated with more probability of clinical mastitis (Sorensen et al., 2000; Dube et al., 2008). Genetic correlation estimates in this study are closer to results estimated by Lagrotta et al. (2010) of 0.23 (stature), 0.18 (hearth girth), 0.23 (body length), and 0.15 (rump length).

Teat diameter is not a trait ordinarily evaluated in the breeding programs. So, genetic correlation estimates between this trait with other are rare. Positive and moderate to high genetic correlations were estimated here between teat diameter and stature, hearth girth, body length, rump length, pin width, and hook width (Table 4) which were close for most of the traits with the results estimated by Lagrotta et al. (2010) between teat diameter and stature (0.50), hearth girth (0.44), body length (0.52), rump length (0.43), pin width (0.16), and hook width (0.28). For stature and body length the estimates made by the authors were a little higher. If selection were applied for increase the traits average in the corporal dimension category it can be expected an increase in the average for teat diameter which is worrisome since cows with too thick teats are associated with high somatic cell count (Bharti et al., 2015); however, too thin teats can cause incompatibility with milking machine.

Negative and high genetic correlation was estimated among fore udder attachment and udder depth (Table 4). Both traits have a high correlation with udder health where deeper udder and udder with weak fore attachment are more likely to get dirty, increase somatic cell counts and develop clinical mastitis (Bharti et al., 2015; Carlström et al., 2016). So, if selection is applied to udder well attached to the abdominal wall (strong fore udder attachment – score 9) is expected a shallow udder (bigger distance from the hock to the udder floor – score 1) as a secondary response which is excellent for healthier udder. The estimate in this study was smaller than values reported in the literature that ranged from 0.75 to 0.92 (Berry et al., 2004; Dube et al., 2008; Nemcova et al., 2011). Difference in the signal can be attributed to the inverse score scale used to evaluate the animals (score 1 for the studies in the literature and 9 in this study).

Significant ($p<0.05$) negative genetic correlation was estimated between fore udder attachment and teat diameter (Table 4). If selection was applied for a well attached fore udder it can be expected a reduce in the teat diameter mean. To thinner teat can cause milking machine incompatibility. Genetic correlation estimates between fore udder attachment and teat diameter are rare in the literature. However, Lagrotta et al. (2010) reported a value of -0.19 in the Brazilian Dairy Gir population. Although the population in both studies are from the same country and breed, divergence in results can be assigned to differences in the number of animals used, statistic model used, and other factors.

Selection for a wide rear udder can increase milk production due to a significant positive and high genetic correlation between both traits. As well as, selection for wide rear udder can lead to a deeper udder due to the high and positive genetic correlation between both traits (Table 4). However, deeper udder is highly associated with a high somatic cell counts and increased risk of clinical mastitis (Lund et al., 1994; Sorensen et al., 2000; Bharti et al., 2015). This estimate in the literature varies from negative to positive and comparison between results can be difficult since in some studies is not detailed the phenotype associated to the score scale.

Udder depth is also genetically correlated with teat diameter (Table 4) where selection for deeper udder will result in an increase of the teat diameter mean. This genetic correlation may not be a problem in the breeding program since deep udder and too thick teat is not the objective and intermediate values for both traits will result in a more functional and healthier udder.

The higher and positive genetic correlation estimate in this study among teat length and teat diameter (Table 4) mean that the same genes control the expression of both traits. Lagrotta et al. (2010) reported genetic correlation of 0.80 among these traits in the Brazilian Dairy Gir cattle and the authors also highlighted that due to the high estimate, teat diameter could be eliminated from the breeding program. Teat length is also genetically correlated with milking ease and the estimate in this study (Table 4) is closer to the result reported by Resing and Ruten (2005) of -0.23. The negative signal is due to the use of an inverse scale, where cows easier (faster) to milk received a score 9 in the report and in this study a score 1. However, both results mean that selection for longer teats will deteriorate milking ease.

Genetic correlation estimates between milking ease and temperament (Table 4) was close to the values reported by Berry et al. (2004) of 0.56 meaning that the same genes control both traits and calmer cows will be easier to milk. According to Berry et al. (2004) a positive relationship between both traits is expected due to the effect of fear in the milk letdown. Animals more aggressive will release high quantities of cortisol by the adrenal in the blood flow. The cortisol will inhibit the central nervous system release oxytocin. The oxytocin is responsible for the continuous milk ejection and the lack or reduced quantity of this hormone will cease or hinder the milking flow (Bruckmaier and Blum, 1998).

Significant ($p<0.05$) genetic correlations between 305-cumulative milk yield and rump length, rear udder width, udder depth, and teat diameter were estimated in this study (Table 4). Genetic correlation estimates among milk yield and rump length and teat diameter are scarce in the literature because rump length and teat diameter are not normally evaluated. Estimate among milk yield and rump length in this study differ from the result reported by Lagrotta et al. (2010) of 0.02 in the Dairy Gir cattle. The results in this study, showed that both traits are controlled by the same genes and selection for a long rump will result in the increase of milk yield average.

Genetic correlation between milk yield and teat diameter in this study (Table 4) is higher than results reported by Wenceslau et al. (2000) and Lagrotta et al. (2010) of -0.12 and 0.26, respectively. These differences may be due to several reasons such as number of animals evaluated, statistical model used, and other. Although, increase milk production is an objective, thicker teat can cause incompatibility with milking machines which lead to liner slips and consequently increase somatic cell count and the probability of develop clinical mastitis (Bharti et al., 2015).

Between milk yield and rear udder width, the genetic correlation estimated in this study (Table 4) is similar to results in the literature of 0.40, 0.60, and 0.61 (Esteves et al., 2004; Alphonsus et al., 2010; Khan and Khan, 2016a). Thereby, if selection is applied to increase rear udder width is expected an increased in milk production mean. In the same way, if selection is applied to increase udder deep is expected increase milk production mean due to the genetic correlation estimated in this study which is higher than results in the literature that ranged from -0.13 to -0.46 (Duru et al., 2012; Campos et al., 2015). However, selection for deeper udder can increase probability of develop clinical mastitis which it is not desirable (Juozaitiene et al., 2006; Dube et al., 2008).

The number of significant ($p<0.05$) phenotypic correlation estimates (Table 4) was bigger that permanent environmental, genetic, and residual. However, mostly of these correlations were smaller than 0.15. Phenotypic correlation among stature and all traits in the corporal dimension, were positive and ranged from 0.18 to 0.45. Hence, taller cows will have a bigger hearth girth, body length, rump length, pin width, hook width, and a low rump. In the same direction, phenotypic correlation among hearth girth, body length, rump length, pin width, and hook width were positive and varied from 0.24 to 0.45. Therefore, cows with a bigger perimeter thoracic will present bigger body and rump length and a larger pin and hook width.

Phenotypic correlation (Table 4) between teat length and stature, hearth girth, body length, and rump length and between teat diameter and stature, body length, rump length, pin width, and hook width were significant ($p<0.05$) and positive; however, with low magnitude and smaller than genetic correlation estimates. A moderate phenotypic correlation was estimated between hearth girth and teat diameter meaning that cows with bigger hearth girth will present thicker teat.

Negative phenotypic correlation estimates between udder depth and fore udder attachment and positive estimate between udder depth and rear udder width (Table 4) showed that cows with deeper udder will present a weaker fore udder attachment and a wider posterior udder. The same relationship among these traits were found in the genetic correlation but with higher estimates. Moderate and positive phenotypic between among teat length and teat diameter estimated in this study demonstrate that cows with longer teat will also present a thicker teat.

Two pair of traits that present significant ($p<0.05$) phenotypic correlation estimate (Table 4) were milking ease and temperament and 305-day cumulative milk yield and rear udder width; however, the phenotypic correlation estimates were smaller than genetic correlation. Phenotypic correlation estimated between milking ease and temperament showed that calmer cows tend to be easier (faster) to be milked. Phenotypic correlation estimated between milk yield and rear udder width indicate that cows with a wider posterior udder will produce more milk.

Significant ($p<0.05$) permanent environmental correlation estimates (Table 5) were positive and ranged from 0.16 to 0.83, except for the correlation between rump length and temperament that was negative. Permanent environmental correlations between stature, hearth girth, body length, rump length, pin width, and hook width for pair of traits ranged from 0.33 to 0.83. Significant ($p<0.05$) permanent environmental correlation was estimated between hearth girth and navel length, body length and rump angle, body length and udder depth, and hook width and rear legs – rear view. These positive correlations indicate that the same permanent environment that affects one trait, affects the other in the same direction. The negative permanent environment between rump length and temperament indicate that the same permanent environment affects both traits; however, in opposite directions.

Positive permanent environmental correlation (Table 5) were estimated between fore udder attachment and foot angle, teat length and teat diameter, milking ease and temperament. Thus, the same permanent environment that affects one trait affects the other in the same direction. Positive permanent environmental correlation was estimated among 305-day cumulative milk yield and stature, hearth girth, body length, rear udder width, and teat length. Thus, effect of permanent environmental that acts in the milk yield expression also contributes in the expression of the conformation traits.

Significant ($p<0.05$) residual correlation (Table 5) among conformation and handling traits ranged from -0.14 to 0.34. A residual correlation that highlighted among other results was the estimate between temperament and 305-day cumulative milk yield of -0.11. This result was import not of its magnitude, but because of the non-additive effects of genes and temporary environmental that affects in opposite direction both traits. More reactive cows tend to release more cortisol in the blood flow which will cease or reduce the milk flow (Bruckmaier and Blum, 1998). This effect is more accentuated in the first lactation (due to the new environmental, management or milking machine noise), especially if they are Zebu cattle without the calf (Fordyce et al., 1988; Costa et al., 2015). Both effects can explain this low residual correlation among milk yield and temperament where more reactive cows will produce less milk.

Comparison between reports can be difficult since estimates of genetic parameters for these traits normally vary a lot between population even in the same breed. These differences can be explained by selection strategies for type and production, level of production by different populations or breeds, different scales used for score the traits, statistical model definitions, breeds analyzed, number of records, data editing, accuracy and consistency of classifiers, and traits definitions (Samoré et al., 2010; Nemcova et al., 2011).

Conformation traits can be very helpful in a breeding program; however, it is important record information about mastitis, somatic cell counts, calving problems, foot disease, locomotion problems, the floor where the animals are living (such as grass, concrete), among other. All these records together can provide more information about which direction the breeding program should pursue. For example, if the animals present more clinical mastitis, than more importance should be given to traits related to udder health.

Among the traits in the corporal dimension category, navel length is probably the most important trait for the Dairy Gir cattle. The navel can disturb the animals in the reproduction process as well as bigger navel are more likely to get hurt and infected. Thus, selection for this trait must be applied with the aiming to reduce navel length. Due to its moderate heritability and no genetic correlation with other studied trait, reduce navel length mean can be easy and fast done. Traits in the feet and legs category can be very important for dairy cattle, mainly if these animals are raised in stables with hard floor with no access to a soft pavement or grass. However, due to small heritability estimates a higher gain can be obtained with environment management than with genetic selection.

Selection for traits in the mammary system and handling category can be a little complicated because of the genetic correlation between these traits. Milking ease and temperament are positive genetically correlated, selection for fast cows to be milked will result in calmer cows which is an aiming. However, milking ease and teat length have a positive moderate genetic correlation, where selection for fast cows to be milk can reduce the teat length and to small teat can be a problem in the attachment of the milking machine; thus, selection for faster cows to be milk need to be done carefully. Teat length and teat diameter have a high positive genetic correlation which can allow the selection for only one trait. Whereas, teat length has a higher heritability estimate than teat diameter, this trait must be chosen in the selection process. However, it is important the continuous record of teat diameter as a way of follow the trait changes.

A negative and moderate genetic correlation between teat diameter and fore udder attachment estimated in this study need a little attention. A well attached anterior udder is the expected because is related to a healthier udder. However, if selection is applied to this trait it can be expect an increase in the teat diameter which is not favorable, so selection for these traits needs to be done with attention. Teat diameter and milk yield are also positive genetically related, so selection for thicker teats can increase the mean of milk yield; however, to thicker teat can be incompatible with the milking machine.

A positive genetic correlation was estimated between udder depth and rear udder width, udder depth and milk yield, rear udder width and milk yield. Thus, selection for cows with deeper udder and wider rear udder will increase the mean for milk yield. However, deeper udder is more likely to develop clinical mastitis which make the deeper udder phenotype undesirable. In addition, udder depth and fore udder attachment have negative genetic correlation, so if selection is applied for deeper udder the cows will present a weak fore udder attachment. It is recommended the selection for well attached fore udder and a wider rear udder.

With the results in this study, a restructuring in the Dairy Gir linear evaluation can be made. If possible, the evaluation and measurement of traits should be done using a continuous scale instead of a categorical scale. Milking ease and temperament when recorded by the farmer should be done using a scale with less levels and using even number (4- or 6-point scales). Records about somatic cell count, clinical mastitis, intervention by a human at calving, cesarean sections, uterus or vaginal prolapses, locomotor problems, foot diseases, pavement where the animals are maintained, and problems with the milking machine attachment in automatic systems should be done or should be continue done if they have already been taken. If these records are available, studies about the relationship between these factors and conformation and handling traits should be performed.

4. Conclusion

It is possible change conformation and handling traits, and 305-day cumulative milk yield through selection. However, for rear legs – rear view this may not be possible due to heritability estimate which was very small and poorly reliable. Among all traits, stature, teat length, and navel length will present the higher response to selection.

The selection to increase rump length, rear udder width, udder depth, and teat diameter can contribute to the selection to increase milk yield due to their genetic association. Among all conformation and handling traits, udder depth, fore udder attachment, and rear udder width can be the most difficult to select due to low heritability estimates and unfavorable genetic correlation between them and between them and milk yield. The results in this study can be very helpful in the Dairy Gir breeding program.

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CHAPTER 4 – Weighted single-step genome-wide association study for udder conformation traits, milking ease, and temperament of Dairy Gir cattle

ABSTRACT – Udder conformation traits have the hugest impact on the functional herd life. Cows with shorter teats and shallow udders are less susceptible to mastitis and surfer traumas. Similarly, handling traits are also important in a dairy farmer since reactive animals are more likely to cause accidents and animals slower to be milked delay the milking process. Genome-wide association studies (GWAS) have been a helpful tool to identify genetic variants associated with important economic traits. This study aimed to identify genes and quantitative trait loci (QTL) related to udder conformation traits, milking ease and temperament in the Dairy Gir cattle. Mammary system conformation traits used in this association study were fore udder attachment (FUA), rear udder width (RUW), udder depth (UD), teat length (TL), and teat diameter (TD) and the handling traits were milking ease (ME) and temperament (TEM). The genotype and phenotype records were provided by Embrapa Dairy Cattle. After genotype quality control, 409,488 SNPs and 2,729 genotyped animals remained in the analysis. Weighted single-step genome-wide association methodology was performed. Genomic moving windows were defined based on 20 adjacent SNPs. Windows that explained more than 1% of the total genetic variance were selected for further analyses. Genes and biological pathways were annotated using the ASR-UCD1.2 bovine genome assembly, and QTL annotation was performed using the UMD3.1 bovine genome assembly. Gene searching within each moving window and QTL annotations were performed using the GALLO R package. Candidate gene list was used to identify functional information and biological pathways from Gene Ontology (GO), Medical Subject Headings (Mesh), Kyoto Encyclopedia of Genes and Genomes (KEGG), and WikiPathways. Fore udder attachment was associated with three windows located on BTA1, BTA21, and BTA28 that explained more than 1% of the genetic additive variance. A total of six candidate genes were identified in these genomic regions (*URB1* – BTA1; *KLC1*, *XRCC3*, *ZFYVE21*, and *PPP1R13B* – BTA21; *PGBD5* – BTA28) and six QTL. The *KLC1* and *XRCC3* genes were showed by some authors to be expressed in mammary gland tissue which make them candidates genes related to the studied trait. For rear udder width, one genetic window located on BTA1 explained more than 1% of the genetic additive variance. Inside this window, the gene *URB1* and four QTL were identified. The genomic windows in BTA1 found for fore udder attachment and rear udder width, overlap and the *URB1* gene was found associated with both traits. Five windows explaining more than 1% of the genetic additive variance was located on BTA5, BTA6, BTA9, BTA14, and BTA16 for udder depth. Three candidate genes were identified located on BTA5 (*U6*, *FAM227A*, and the gene with Ensembl identification ENSBTAG00000048693), one on BTA6 (*RNA5S1*), and two on BTA14 (*CYP7B1* and *BHLHE22*). Besides, eleven QTL were identified on BTA6, BTA14, and BTA16. The *CYP7B1* gene was related to onset the puberty, estrogenic functions, and reproductive organs and mammary gland development. The *BHLHE22* gene was found up-regulated in women breast tissue that had a full-term pregnancy. One window explaining more than 1% of the genetic additive variance was associated with teat length (BTA17) and two windows for teat diameter (BTA23 and BTA27). Inside the window for teat length one gene (*KLHL2*) was identified; however, with no apparent relationship with the phenotype. For milking ease and temperament, two (BTA5 and BTA19) and three (CRO 4, 18, and 29) windows was associated with the traits, respectively. One gene (*CA10*) was identified for milking ease within BTA19 which was associated with synapses function in the cerebrum and plays a role in secretory pathways. Two genes were identified for temperament located on BTA4 and BTA18 (*TFEC* and *CDH11*) and were related to behavior and bipolar disorder in human and with autism in mice. A total of three QTL for milking ease located on BTA5 and five QTL for temperament on BTA4, BTA18, and BTA29 were identified. Strong candidate genes were associated with the studied traits. It is possible highlight the *KLC1* and *CYP7B1* which were found associated with mammary gland tissue and the *CDH11* gene associated with behavior in mice and human. These results might be very helpful to understand the genetic structure of the studied traits, can be applied in the genomic selection of the breed, and be a start point to other studies related to udder conformation and handling traits in Zebu cattle.

Keywords: *Bos taurus indicus*, dairy cattle, moving windows, SNP, WssGWAS

1. Introduction

Conformation traits are important in dairy cattle breeding programs because they can be a good indicator of animal's longevity in the herd. Cows with better body conformation are less susceptible to diseases. As showed by Schneider et al. (2003), among all conformation traits, udder traits have the hugest impact on functional herd life. For instance, cows with udder well-attached and stronger ligaments are kept longer in the herd. In addition, cows with shorter teats and shallow udders are less susceptible to mastitis and to surfer traumas in the teats and udders (Lund et al., 1994; Juozaitiene et al., 2006; Dube et al., 2008).

Another important trait for the dairy industry is the animal's behavior. It is well known that *Bos indicus* animals are more reactive and aggressive than *Bos taurus* animals. In the daily management of a dairy farm, reactive animals are more likely to hurt the farmer, injury other animals, and increase susceptibly to mastitis by dropping the teat cup in an automatic milking system (Fordyce et al., 1988; Costa et al., 2015). Lund et al. (1994) estimated genetic correlation between temperament and disease (-0.96), and temperament and milking speed (0.33), showing that more docile cows tend to be healthier and faster to milk. The time expended to milk a cow is very important, since slow animals delay the milking process which are undesirable (Luttinen and

Juga, 1997) and fast animals have large somatic cell score average (Rupp and Boichard, 1999).

Genome-wide association studies (GWAS) have been a helpful tool to identify genetic variants associated with phenotypes of interest (Fragomeni et al., 2014; Oliveira et al., 2019). GWAS relies on the linkage disequilibrium (LD) between a genetic variant and a nearby SNP or group of SNPs. The LD strongly depends on the population history, mainly to the effective population size, which can change over time (Goddard and Hayes, 2009; Kruglyak, 1999). The use of HD SNP panels can increase the power to identify regions with genes of interest (Abo-Ismail et al., 2017).

The LD patterns among different populations from the same species can differ (O’Brien et al., 2014), as well as the QTL effects among breeds (Raven et al., 2014). These differences can affect the results and repeatability of GWAS, being necessary to investigate the LD before each genomic study and perform the association studies between genotypes and phenotypes for each breed. The decay of LD decreases the power of GWAS, since the lower the LD, less is the chance of finding markers associated with a QTL (O’Brien et al., 2014; Qanbari et al., 2010).

A modified version of the single-step GWAS (ssGWAS) (Misztal et al., 2009; Christensen and Lund, 2010) was proposed by Wang et al. (2012). Weighted single-step GWAS (Wang et al., 2012) converts the genomic estimated breeding value (GEBV) obtained in the ssGWAS into marker effects and their weights in an iterative process. In this approach, pedigree, phenotype, and genotype information from all animals are used in a single-step with any model that best fits the data (Wang et al., 2012; Wang et al., 2014). The ssGWAS can be performed using single markers, several adjacent markers, or base-pair building windows distributed over the genome. Usually, each window explains the genetic variability of a chromosome region. Use several adjacent markers to measure the genetic phenomenon that occur in a genome region instead of single marker can considerably decrease the sampling error, while maintaining the true signal (Beissinger et al., 2015).

In Holstein, several association studies have been reported for different traits, including milk yield and conformation traits (Abo-Ismail et al., 2017; Cole et al., 2011; Oliveira et al., 2019; Oliveira et al., 2019a). However, different from Holstein, few studies have been performed with the objective of finding genomic regions associated with different traits in dairy Gir and no studies have been found for conformation traits, milking ease, and temperament for this breed. Therefore, the objective of this study was to identify QTL and candidate genes related to udder conformation, temperament and milking ease in the Dairy Gir cattle.

2. Material and Methods

2.1. Phenotypic data and pedigree

The data set used for the association study was provided by Embrapa Dairy Cattle (Embrapa Gado de Leite) and it is part of the Brazilian Dairy Gir National Breeding Program (Programa Nacional de Melhoramento do Gir Leiteiro – PNMGL). Records were collected by the Brazilian Association of Dairy Gir Breeders (Associação Brasileira dos Criadores de Gir Leiteiro – ABCGIL) and Brazilian Association of Zebu Breeders (Associação Brasileira dos Criadores de Zebu - ABCZ), between 1992 and 2018, in herds located in southeast region of Brazil.

In this association study were used five conformation traits, fore udder attachment (FUA), rear udder width (RUW), udder depth (UD), teats length (TL), and teats diameter (TD) which belong to the mammary system category and two handling traits, milking ease (ME) and temperament (TEM). All traits were recorded in score (9-point scale), except TL and TD which were measured in centimeters. Description on how traits were recorded can be found in Panetto et al. (2019).

For the data edition, records with possible annotation errors and data recorded in centimeter or degrees outside the possible biological range were removed from the data file. The number of phenotyped animals varied between traits and the data were record from first to seventh lactation. All descriptive statistical are presented in Table 1. The pedigree file used in the analysis for all traits was constituted by 18,402 animals, which considered up to 15 generations.

Traits evaluated in score were transformed to achieve the normal distribution. The Ordered Quantile normalizing method was chosen to transform the values because it presented the best values of Skewness and Kurtosis after data transformation. Analyses were performed using the package “bestNormalize” (Peterson, 2019) available in the R software (R Core Team, 2008).

Table 1. Descriptive statistics for traits which association study was performed

Trait	N	Min - Max	Mean	SD	Median	Mode
Conformation						
Fore udder attachment (score)	5617	1 - 9	-		5	5
Rear udder width (score)	5822	1 - 9	-		5	5
Udder depth (score)	6003	1 - 9	-		5	5
Teat length (cm)	9690	3 - 14	7.51	1.92	7	7
Teat diameter (cm)	9666	1 - 8	3.87	0.87	4	4
Handling						
Milking ease (score)	9486	1 - 9	-		3	5
Temperament (score)	9672	1 - 9	-		4	5

N: number of records; **Min**: minimum value for the evaluated trait; **Max**: maximum value for the evaluated trait; **Mean**: mean; **SD:** standard deviation; **Median**: median by trait; **Mode**: mode by trait.

2.2. Description and edition of genotypic data

Animals were genotyped using four different SNP panels: BovineHD BeadChip (Illumina, Inc., San Diego, CA) with 777,962 markers, BovineSNP50 BeadChip v2 (Illumina, Inc., San Diego, CA) with 53,714 markers, GGP Indicus (Neogen Genomics Corporation, Lincoln, OR) with 35,090 markers, and Z-Chip (Neogen Genomics Corporation, Lincoln, OR) with 27,533 markers distributed across the genome. Animals genotyped with low-density panels (i.e., BovineSNP50, GGP Indicus, and Z-Chip) were imputed to the BovineHD panel.

After the imputation, only autosomal chromosomes were kept in the analysis, and a quality control was performed. SNP with minor allele frequency (MAF) below 0.02, deviating from Hardy-Weinberg equilibrium ($p<10^{-6}$), GenCall score below 0.70, call-rate below 0.98, and SNP with correlation between each other above 90% were removed. Samples with call rate below 0.90, with parent progeny conflict, and possible duplicated samples were also excluded. After quality control, 409,488 SNPs and 2,729 genotyped animals remained in the analysis.

2.3. Statistical analysis

The general statistic model used was:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Za} + \mathbf{Wpe} + \mathbf{Sd} + \boldsymbol{\varepsilon}$$

where: $\mathbf{y}$ is the observation vector for the linear conformation traits, milking ease or temperament; $\mathbf{X}$ is the incidence matrix of fixed effects; $\mathbf{Z}$ is the incidence matrix for the animal additive genetic effect; $\mathbf{W}$ is the incidence matrix for the permanent environment effect; $\boldsymbol{\beta}$ is the vector of fixed effects; $\mathbf{a}$ is the vector of animal random effects; $\mathbf{pe}$ is the vector related to permanent environment random effects; $\mathbf{S}$ is the incidence matrix for the CG effect; $\mathbf{d}$ is the vector of CG and $\boldsymbol{\varepsilon}$ is the vector of random errors or residual effects associated with each observation.

Only fixed effects that were statistically significant ($p<0.05$) were included in the models. To defined which fixed effects would be included in the analyses a general linear model (GLM) was performed considering a $p<0.05$. The effects tested were season of evaluation (SE), evaluator (E), diet (D), age of the dam as covariate (with linear, I; and quadratic, Q effects). The CG was composed by H and YE and was considered as a random effect among with animal, permanent environment, and residual effects. The statistical model used for each trait is described in Table 2.

Table 2. Linear model used for each trait

Trait	Model
Conformation	
Fore udder attachment (score)	SE + D + I
Rear udder width (score)	SE + D + I
Udder depth (score)	E + D + I + Q
Teat length (cm)	SE + E + D + I + Q
Teat diameter (cm)	E + I + Q
Handling	
Milking ease (score)	E + I + Q
Temperament (score)	SE + E + I + Q

SE: season of evaluation; **E**: evaluator; **D**: diet; **I**: linear effect of age of the dam; **Q**: quadratic effect of age of the dam.

The genomic association was performed using the BLUPF90 family of programs (Misztal et al., 2002), using the weighted ssGWAS (WssGWAS) methodology proposed by Wang et al. (2012), in which the animal effect was decomposed into genotyped (a_g) and ungenotyped (a_n) animals. The animal effects are a function of SNP effects:

$$(\mathbf{a_g}) = \mathbf{Zu},$$

where: $\mathbf{Z}$ is a matrix relating genotypes of each locus, and $\mathbf{u}$ is a vector of SNP marker effects. The variance for the animal effect was assumed as:

$$\mathrm{var}\,(\mathbf{a_g}) = \mathrm{var}\,(\mathbf{Zu}) = \mathbf{ZDZ}'\sigma^2_{\mathrm{u}} = \mathbf{G}^*\sigma^2_{\mathrm{a}},$$

where: $\mathbf{D}$ is a diagonal matrix of weights for variances of SNP ($\mathbf{D} = \mathbf{I}$ for GBLUP); σ^2_u is the genetic additive variance captured by each SNP marker when no weights are present and $\boldsymbol{G}^*$ is the weighted genomic relationship matrix. The joint (co)variance of animal effects $(\mathbf{a_g})$ and SNP effects (u) was:

$$\mathrm{var}\begin{bmatrix}\mathbf{a_g}\\ \mathbf{u}\end{bmatrix} = \begin{bmatrix}\mathbf{ZDZ'} & \mathbf{ZD'}\\ \mathbf{DZ'} & \mathbf{D}\end{bmatrix}\sigma^2_u,$$

subsequently,

$$\mathbf{G}^* = \frac{\mathrm{var}\left(\mathbf{a_g}\right)}{\sigma^2_a} = \frac{\mathrm{var}\left(\mathbf{Zu}\right)}{\sigma^2_a} = \mathbf{ZDZ'}\frac{\sigma^2_u}{\sigma^2_a} = \mathbf{ZDZ'}\lambda\,,$$

where: λ is the variance ratio or a normalizing constant. According to VanRaden et al. (2008),

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

where: M is the number of SNPs and p_i is the allele frequency of the second allele for the ith marker. The final equation to predict SNP effects, which uses the weighted genomic relationship matrix $\mathbf{G}^*$, is defined as:

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

The SNP effect estimates were used to estimate the variance for the SNP effects as (Zhang et al., 2010):

$$\hat{\sigma}^2_{u,i} = \hat{\sigma}^2_i\,2p_i(1-\,p_i).$$

The GEBV were converted to SNP effects. Thereafter, weights for the SNP effects were adjusted iteratively using 2 iterations for all traits. Moving windows of 20 adjacent SNP were used in all analysis. The windows' variance was calculated through the sum of 20 adjacent markers, and only the windows' combination that explained the highest variance were maintained in order to avoid overlapping windows. This criterion was chosen to avoid counting the same SNP effect more than once, as suggested by Fragomeni et al. (2014) and sustained by Oliveira et al. (2019a). Windows that explained more than 1% of the total genetic variance were selected for further analyses.

To decide the number of SNP to be considered in the moving window, linkage disequilibrium was calculated by chromosome using the preGSf90 program (Aguilar et al., 2014). After that, the mean linkage disequilibrium was calculated for different distances in mega base pairs (Mb). The windows size was compared against the mean linkage disequilibrium for the different size of windows and the window with 20 SNP was chosen due to the more appropriated linkage disequilibrium.

2.4. Candidate genes, biological pathways search, and QTL annotation

Genes and biological pathways were annotated using the ARS-UCD1.2 bovine genome assembly, and QTL annotation were performed using the UMD3.1 bovine genome assembly. Gene searching within each moving window and QTL annotations were performed using the GALLO package (Fonseca et al., 2020) in the software R (R Core Team, 2008).

The list of candidate genes was used to identify functional information and biological pathways, considering the biological evidence in the relationship between terms and gene. It is important to highlight that the results were not filtered based on a p-value threshold. In this step, we aimed to create a functional profile of all annotated genes within the candidate windows and not perform enrichment analysis. Functional information was recovered from Gene Ontology (GO) using the terms “Biological Process”, “Cellular Component”, and “Molecular Function” and from Medical Subject Headings (MeSH) using the terms “Anatomy”, “Diseases”, “Biological Sciences”, and “Chemical and Drugs”. Metabolic pathways were searched in the Kyoto Encyclopedia of Genes and Genomes (KEGG) and WikiPathways. Functional information recovered and metabolic pathways searching were carried out using the WebGestaltR package in the software R (Wang e Liao, 2020).

The QTL enrichment was performed for QTL classes and for the trait per chromosome using the qtl_enrich function from the GALLO package, using a p<0.05.

3. Results and Discussion

The windows explaining more than 1% of the variance, Manhattan plots and results from gene and QTL search, as well as functional information obtained in the analyses will be discussed by each trait below.

As suggested by Ardlie et al. (2002), levels of linkage disequilibrium (r^2) above 0.3 among markers give to the GWAS enough effectiveness for the association. In this study, to achieve this level of r^2 (0.30) was necessary use windows smaller than 0.135 mega base pair (mb), since the mean r^2 in this distance was 0.30, as shown in Figure 1. The window of 20 SNPs presented a size ranging from 0.115 to 0.116 mb for all traits, which provided LD of 0.317. Higher r^2 levels increase the possibility of find QTL associated with markers, as well as smaller distance between marker and QTL reduce the possibility of recombination between them (O'Brien et al., 2014).

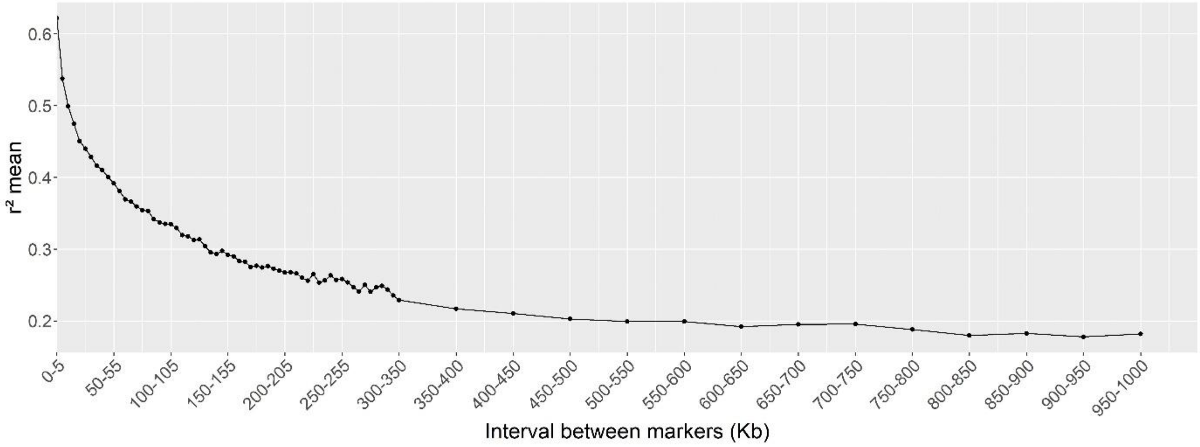

Figure 1. Linkage disequilibrium decay calculated as the r^2 mean within interval of 5 Kb between markers from 0 to 300 Kb and within a 50Kb of interval between markers from 300 to 1000 Kb

Fore udder attachment

Three genomic windows that explained more than 1% each of the total genetic additive variance were found on chromosomes 1, 21, and 28 for fore udder attachment. The additive genetic variance explained, chromosome number, start and end base pair for each window, and the windows' size (in base pair) for these windows are presented in Table 3 and the Manhattan plot is presented in Figure 2.

Six genes were annotated within the three identified candidate windows. All genes identified are protein coding genes named URB1 Ribosome Biogenesis Homolog (*URB1*) on chromosome 1, Kinesin Light Chain 1 (*KLC1*), X-ray Repair Cross Complementing 3 (*XRCC3*), Zing Finger FYVE-Type Containing 21 (*ZFYVE21*), Protein Phosphatase 1 Regulatory Subunit 13B (*PPP1R13B*) on chromosome 21, and PiggyBac Transposable Element Derived 5 (*PGBD5*) on chromosome 28.

No function for the *URB1* (chromosome 1) gene was found related to the mammary gland phenotype. However, this gene plays a role in the biogenesis of the 60S ribosomal subunit (Giaever et al., 2002). Ribosomes are the machinery responsible for the protein synthesis which is important in the mammary gland tissue cellular process (He et al., 2017). This gene has been found responsible in the development of Zebrafish's digestive organs where animals with a unfunctional copy of this gene lost the ribosome biogenesis protein *URB1* leading to a hypoplastic digestive organs (He et al., 2017). As well as, a mutation in this gene lead to embryonic lethality in Landrace pigs (Derks et al., 2019).

Table 3. Windows that explained more than 1% of the variance for fore udder attachment

Chr	**IP**	**FP**	**Size (bp)**	**VE (%)**
1	3128547	3153221	24,674	1.41588
21	68254999	68351453	96,454	1.13090
28	2325557	2353694	28,137	1.08296

Chr: chromosome number where the window is located; **IP**: window initial position in base pair; **FP**: window final position in base pair; **Size (bp)**: window size in base pair; **VE (%)**: percentage of variance explained by each window.

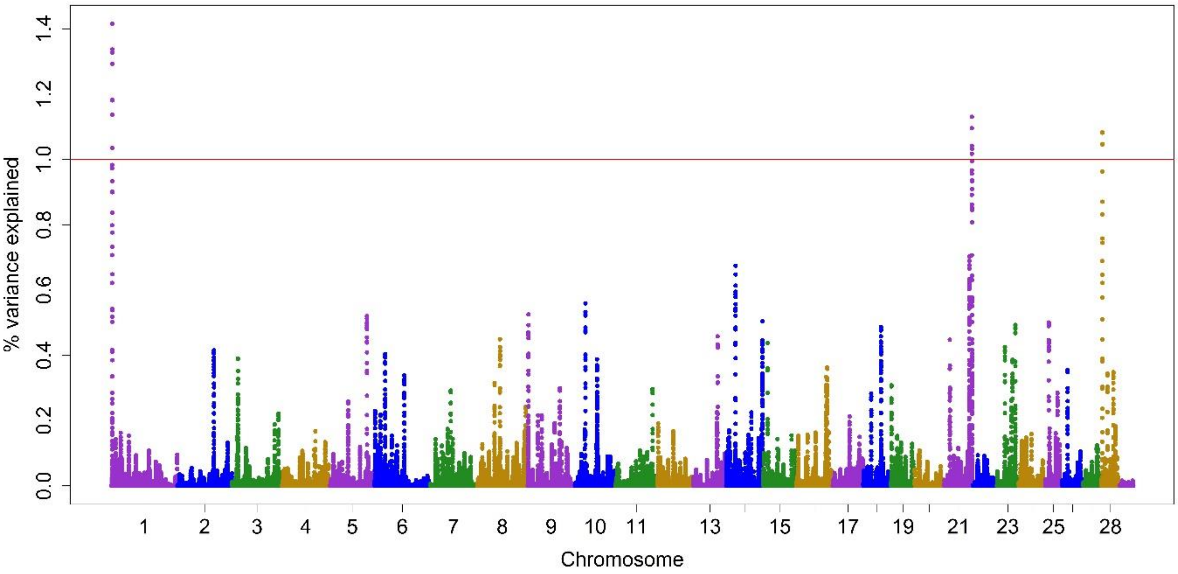

Figure 2. Manhattan plot of percentage of genetic additive variance explained for SNP-phenotype association for fore udder attachment. The horizontal line represents the 1% threshold imposed to select windows for further analyses

The first gene on chromosome 21, the *KLC1* gene, is responsible for the encoding of kinesin light chain 1 protein and is related to the activity of cell differentiation. Two different molecules can control its expression, the estrogen (E_2) and the Transforming Growth Factor β (TGF-β). The E_2 is essential in several process in the body, especially the regulation of growth and differentiation of the mammary gland tissue due the high dependency of E_2 by this organ (Aboghe et al., 2009). The E_2 was responsible for the *KLC1* downregulation in mammary gland tissue of mice with a knockout in the nuclear hormone receptors β. While TGF-β, is essential in some cellular process such as cell growth, differentiation, motility, and apoptosis. In addition, for some specific cells this molecule induces epithelial to mesenchymal transition

(EMT) (Xie et al., 2003). Xie et al. (2003) studied the effect of TGF- β in gene expression of the mammary epithelial cell of mice and reported that the *KLC1* gene was expressed because of the TGF-β effect in the cell cycle control and EMT, which are highly related to the phenotype of anterior udder.

The second gene on chromosome 21, the *XRCC3*, produces a protein that is part of a machinery responsible for repairing of double-stranded DNA breaks by using homologous recombination. Polymorphisms in this gene can be associated with an increased risk of breast cancer development despite some disagreements among different authors (The Breast Cancer Association Consortium, 2006). However, the possibility of this gene be associated with breast cancer shows that it is related to the mammary tissue. Due to lactation, the cells in the mammary tissue change a lot along time, which can explain why the *XRCC3* gene was found associated with fore udder attachment.

The third gene presented in chromosome 21 window was the *ZFYVE21* gene. This gene was found related to tissue inflammation process (Fang et al., 2019), and was also found differently expressed among follicular thyroid cancer and follicular thyroid adenoma (Wojtas et al., 2017). However, it is important to highlight that according to Bhat-Nakshatri et al. (2008) this gene is estrogen inducible and repressible, which is an important hormone in the development of the breast tissue. Besides, it was found expressed in breast cancer cells (Bhat-Nakshatri et al., 2008). Due to its response to the estrogen, this gene could be related to fore udder attachment trait playing a role alone or in interaction with the other genes on the same window.

The *PPP1R13B* gene, the fourth gene on chromosome 21, is responsible for encoding a protein from the apoptosis-stimulating protein of p53 family, which are proteins responsible by cells apoptosis. This gene was found expressed in human prostate tissue (Felgueiras and Fardilha, 2014) and might not be directly relate to the trait. The fact that it was used sliding windows in the analyses, these gene could be close to other genes more likely to be related to fore udder attachment which could make it show up in the functional analyses. However, the mammary tissue has a mechanism of proliferation and apoptosis, which could explain its importance.

In humans, the *PGBD5* gene, found on chromosome 28, is responsible for encoding the *PGBD5* transposase, which is responsible for the movement of transposons of the piggyBac family. Transposons rearrange and increase variety of DNA sequences; however, knowledge about its function and where it is expressed is limited (Henssen et al., 2015). Thus, it is difficult to understand the relation between this gene and the studied trait. Xiang et al. (2017) found a SNP within the *PGBD5* gene with pleiotropic effects among traits in dairy cattle and in beef cattle. However, similarly as in this study, the authors could not explain the relationship between the *PGBD5* and the studied traits.

The complete functional information recovered from GO are presented in Appendix E on the appendix. As well as all the metabolic pathways (Appendix G on appendix) recovered from KEGG and WikiPathways. All terms found associated with the genes in the windows that explain more than 1% of the additive genetic variance are related in somehow to cell replication, maintenance of the cell structure, cellular death, and metabolic control. All those terms might be related to fore udder attachment. The phenotype describes the udder attachment to the abdominal wall, and differences in the phenotype are basically related to more mammary tissue (which includes the mammary gland support tissue) in the junction among the anterior udder and the abdominal wall.

Among the pathways found related to the genes, two could be related to the studied trait. The first, “homologous recombination”, is the process to correct both stands break in the DNA, which is a basic function of the *XRCC3* gene. This biological function is expected in the mammary gland tissue since cell replication is recurrent, mainly in the lactation phase, which is when the cows are evaluated. The second pathway related to fore udder attachment is the “integrated breast cancer pathway”, which includes the most important proteins related to breast cancer.

The QTL search within the windows revealed four QTL located on chromosome 1 and two on chromosome 2, details can be seen in Appendix H on appendix. The QTLs found are related to body weight (3 QTL) and to reproduction traits (3 QTL). However, neither of them can be related to udder attachment. No terms were found in the QTL enrichment for trait within chromosome, on the other hand, the QTL enrichment ($p<0.05$) for class within chromosome result in one term, “Production”. Besides the result means that more “production” terms were found than the expected, this term do not explain much about the studied trait.

Rear udder width

One genomic window was found explaining more than 1% of the total genetic variance for rear udder width which was located on chromosome 1. The additive genetic variance explained, chromosome number, start and end base pair for the window, and the window's size (in base pair) for rear udder width are presented in Table 4. Manhattan plot is presented in Figure 3.

Table 4. Window that explained more than 1% of the variance for rear udder width

Chr	IP	FP	Size (bp)	VE (%)
1	3131867	3160224	28,357	1.37642

Chr: chromosome number where the window is located; **IP**: window initial position in base pair; **FP**: window final position in base pair; **Size (bp)**: window size in base pair; **VE (%)**: percentage of variance explained by each window.

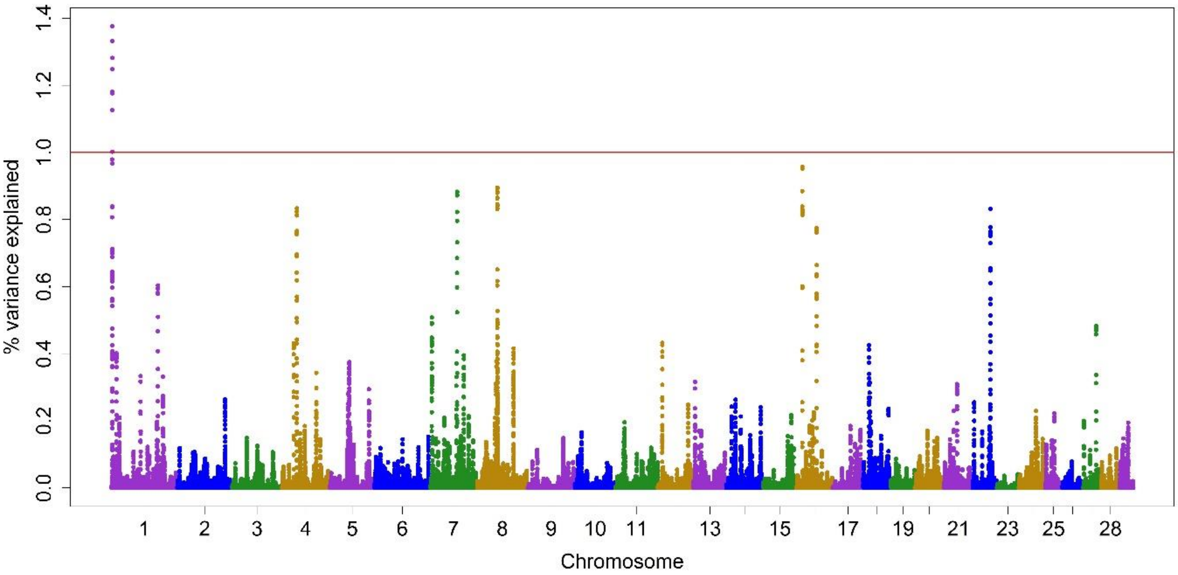

Figure 3. Manhattan plot of percentage of variance explained for SNP-phenotype association for rear udder width. The horizontal line represents the 1% threshold imposed to selected windows for further analyses

The *URB1* gene, previous described for fore udder attachment, also was annotated within the window for rear udder width. The fact that both traits represent the development of the mammary gland and the same gene was found for both traits, in regions that overlap on chromosome 1, it seeming that this gene have a relevant importance in the mammary tissue development and further studies should be developed with the aim of understand the role played by the *URB1* gene in the mammary gland in dairy Gir cattle.

We did not find GO and MeSH terms, and metabolic pathways associated with the gene. A total of four QTL were found in the window on chromosome 1 (Appendix H on appendix). Three of QTLs is related to body weight trait and one is related to calving ease trait. It is not clear the association between body weight and rear udder width; however, with calving ease it might have some association. Cows with a large rear udder probably have a bigger rump width which can explain a QTL for calving ease in a region that explain the rear udder width. The QTL enrichment for trait inside chromosome returned zero results and the enrichment for class inside chromosome result in 130 terms, being 128 times “Reproduction” and 2 times “Production”.

Udder Depth

Five genomic windows that explained more than 1% each of the total genetic additive variance were found on chromosomes 5, 6, 9, 14 and 16 for udder depth. The additive genetic variance explained, chromosome number, start and end base pair for each window, and the windows’ size (in base pair) for udder depth are presented in Table 5 and the Manhattan plot is presented in Figure 4.

Six genes were annotated within the five windows. On chromosome 5 were identified three genes, the *U6*, the Family with Sequence Similarity 227 Member A (*FAM227A*), and the gene with Ensembl’s identification ENSBTAG00000048693. The RNA, 5S Ribosomal 1 (*RNA5S1*) gene was annotated on chromosome 6 and the genes Cytochrome P450 Family 7 Subfamily B Member 1 (*CYP7B1*) and Basic Helix-Loop-Helix Family Member E22 (*BHLHE22*) were found on the chromosome 14.

Table 5. Windows that explained more than 1% of the variance for udder depth

Chr	IP	FP	Size (bp)	VE (%)
5	110258876	110287236	28,360	1.04534
6	115216126	115289243	73,117	2.36727
9	82611144	82672803	61,659	1.09437
14	29154723	29295803	141,080	1.23406
16	69850869	69896396	45,527	1.99485

Chr: chromosome number where the window is located; **IP**: window initial position in base pair; **FP**: window final position in base pair; **Size (bp)**: window size in base pair; **VE (%)**: percentage of variance explained by each window.

The gene ENSBTAG00000048693 (chromosome 5) did not have an official gene symbol related to it. The search for the orthologues for this gene revealed seven results in which the Domestic Yank (*Bos grunniens*) had 98.66% of similarity; however, in the same way, the gene did not have a gene symbol related to it. The second gene, *FAM227A* (chromosome 5), has not been well described in the literature. The last gene on chromosome 5, U6, is a gene responsible for the coding of small nuclear RNA (snRNA), which is responsible for the splicing of precursor messenger RNA (pre-mRNA) (Das et al., 1988). There is a lack of the information about how snRNA behaves, mainly its function in the expression of the udder depth phenotype.

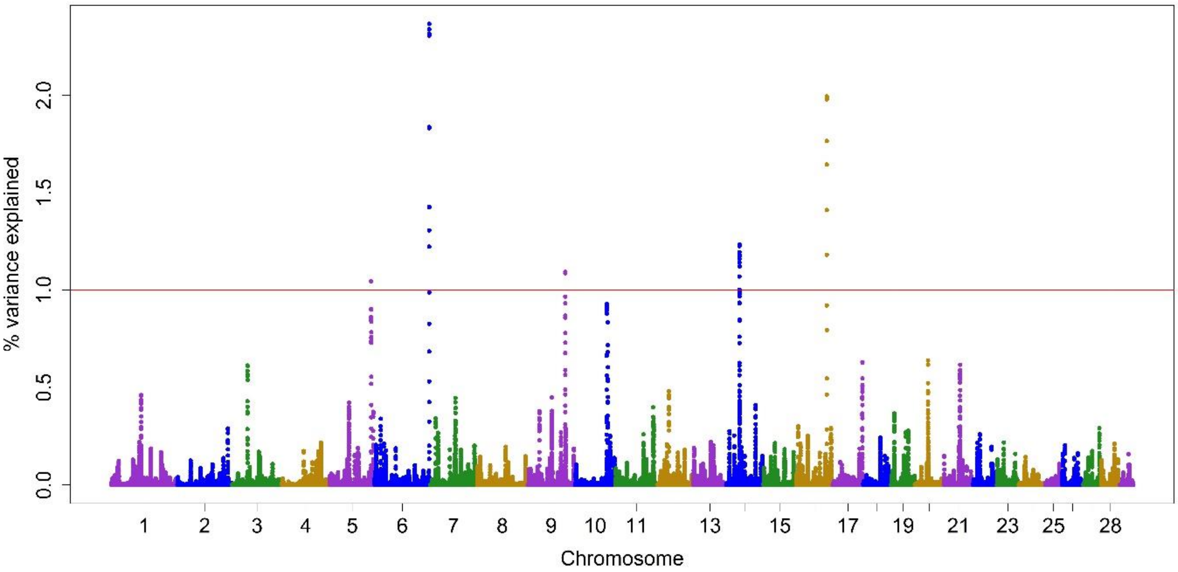

Figure 4. Manhattan plot of percentage of variance explained for SNP-phenotype association for udder depth. The horizontal line represents the 1% threshold imposed to selected windows for further analyses

The only gene within chromosome 6 window was the *RNA5S1* gene that encode the 5S ribosomal RNA (rRNA) which is found in almost all ribosomes (Szymanski et al., 2002). The ribosomes are responsible for the protein synthesis. Although this gene does not present a strong and apparently relationship with the phenotype, it is important remember that the mammary gland during lactation need the protein synthesis for its incorporation in the milk as well as for the tissue function.

The *CYP7B1* gene (chromosome 14) is responsible for the synthesis of the CYP7B1 enzyme. This enzyme plays a role in the catabolism of 27-hydroxycholesterol (27HC), and animals with a knockout in this gene had increased the 27HC levels circulating, which resulted in the promotion of tumor growth in mice models (Nelson et al., 2013; Cedó et al., 2016). Another interesting result about the function of the *CYP7B1* gene was reported by Omoto et al. (2005), where the CYP7B1 enzyme seems to regulate the tissue selective for 17βadiol (3βAdiol). The 3βAdiol has estrogenic functions in the female reproductive organs (mammary gland and uterus), mostly in the onset of puberty.

According to Omoto et al. (2005), knockout mice (KM) in the *CYP7B1* gene submitted to the administration of 3βAdiol had more ductal elongation and ramifying in the mammary gland than the wild mice (WM), with two to four weeks of age. KM had precocious puberty, but they also presented early suspension of ovarian function when compared to WM. At six months, KM had more extensive ductal formation in the mammary glands, and the lumina of the uterus were larger than WM group. At 15 months, both uterus and mammary gland of KM showed signs of atrophy. The early and faster development of mammary gland and uterus in knockout mice means that the CYP7B1 enzyme plays an important role in the inactivation and excretion of 3βAdiol (since it was responsible by the development of organs).

Another probable reason for the more prominent development of the mammary gland is the higher expression levels of estrogen receptor α (ERα) in the mammary epithelium when compared to estrogen receptor β (ERβ) in KM. When both receptors are expressed in equilibrium, there is no mammary gland proliferation due to estrogen because probably, they nullify each other, which appears to have occurred in the wild type mice. The high expression of ERα in the knockout mice seems enough for these receptors to dominate the signalization and lead to proliferation of the mammary gland (Omoto et al., 2005).

Santucci-Pereira et al. (2014) compared the gene expression of breast tissue from nulliparous and parous premenopausal women between 30 and 47 years old with no records of breast pathology. The women in the parous group were divided among women in which the last full-term pregnancy was less than five years and more than five years. The *BHLHE22* gene, found in the window on chromosome 14, was found up-regulated in both parous groups, meaning that the gene started to be up-regulated probably in pregnancy and continued up-expressed regardless of the amount of time that has passed since the last gestation. The authors classified this gene involved in the developmental process, together with *FZD8* and *KRT5*, also long-term up-regulated.

The GO and MeSH term search did not return any term. The pathway search resulted in five terms (Appendix G on appendix). The “Steroid hormone biosynthesis” pathway might be related to udder depth. The estrogen is a very important steroid hormone in the mammary gland tissue and other reproductive organs development. Udder depth describe the quantity of mammary tissue is developed in determined animal, thus is expect found a pathway related to the synthesis of hormones which help the development of this tissue.

Eleven QTL were identified within the windows on chromosome 6, 14, and 16 (Appendix H on appendix). However, among associated traits with the QTL, none of them seems to be related to udder depth. The QTL enrichment did not result in trait inside chromosome but return one term in the enrichment for class inside chromosome. The term “Production” on chromosome 14 which does not relate to the phenotype.

Teat Length

One genomic window that explained more than 1% of the total genetic additive variance was found in chromosome 17 for teat length. The additive genetic variance explained, chromosome number, start and end base pair for the window, and the window’s size (in base pair) for teat length are presented in Table 6 and the Manhattan plot is presented in Figure 5.

Table 6. Windows that explained more than 1% of the variance for teat length

Chr	IP	FP	Size (bp)	VE (%)
17	433330	508226	74,896	1.99158

Chr: chromosome number where the window is located; **IP**: window initial position in base pair; **FP**: window final position in base pair; **Size (bp)**: window size in base pair; **VE (%)**: percentage of variance explained by each window.

Only the Kelch-like family member 2 (*KLHL2*) gene was annotated in the genomic window on chromosome 17. Some functions were studied between *KLHL2* and with-no-lysine kinase (WNK) (Takahashi et al., 2013; Kasagi et al., 2017). However, nothing that could be related to the teat length in cattle. Two GO terms were found related to the *KLHL2* gene (Appendix E in appendix). However, the terms have not clear association with teat length.

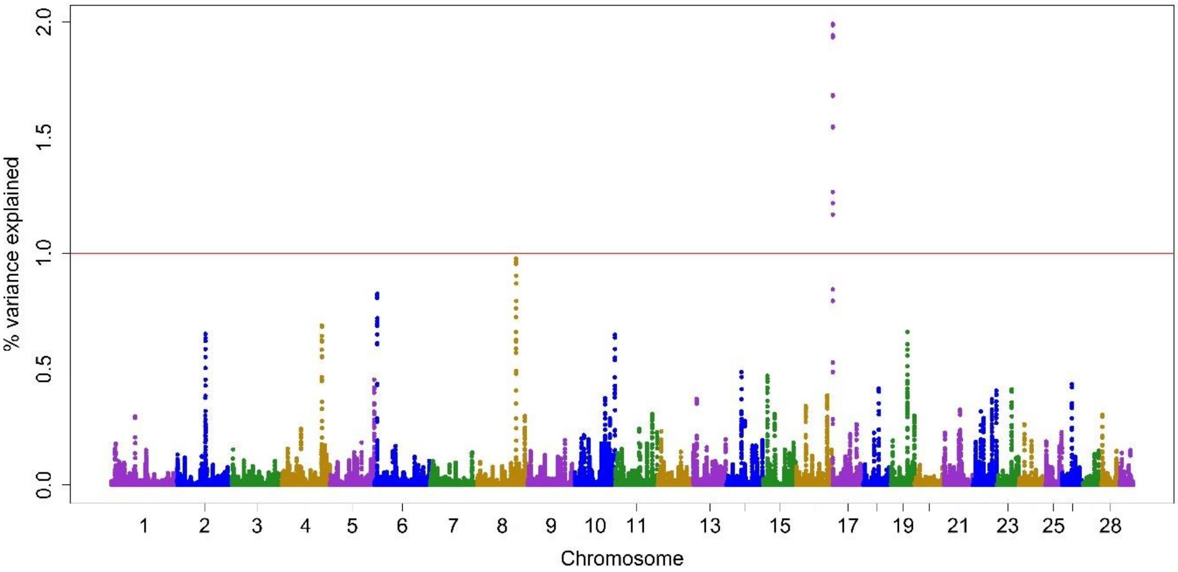

Figure 5. Manhattan plot of percentage of variance explained for SNP-phenotype association for teat length. The horizontal line represents the 1% threshold imposed to selected windows for further analyses

Teat Diameter

Two genomic windows that explained more than 1% each of the total genetic additive variance were found on chromosomes 23 and 27 for teat diameter. The additive genetic variance explained, chromosome number, start and end base pair for each window, and the windows' size (in base pair) for teat diameter are presented in Table 7 and the Manhattan plot is presented in Figure 6.

Table 7. Windows that explained more than 1% of the variance for teat diameter

Chr	**IP**	**FP**	**Size (bp)**	**VE (%)**
23	1396095	1500882	104,787	1.44808
27	8148149	8232468	84,319	1.01567

Chr: chromosome number where the window is located; **IP**: window initial position in base pair; **FP**: window final position in base pair; **Size (bp)**: window size in base pair; **VE (%)**: percentage of variance explained by each window.

No annotated genes within the two genomic windows on chromosome 23 and 27 was found. However, two QTL, one in each chromosome (Appendix H on appendix) were found. QTL were related to shear force and milk iron content, which does not relate to the teat diameter. The QTL enrichment for trait and class within chromosome did not return any trait or term.

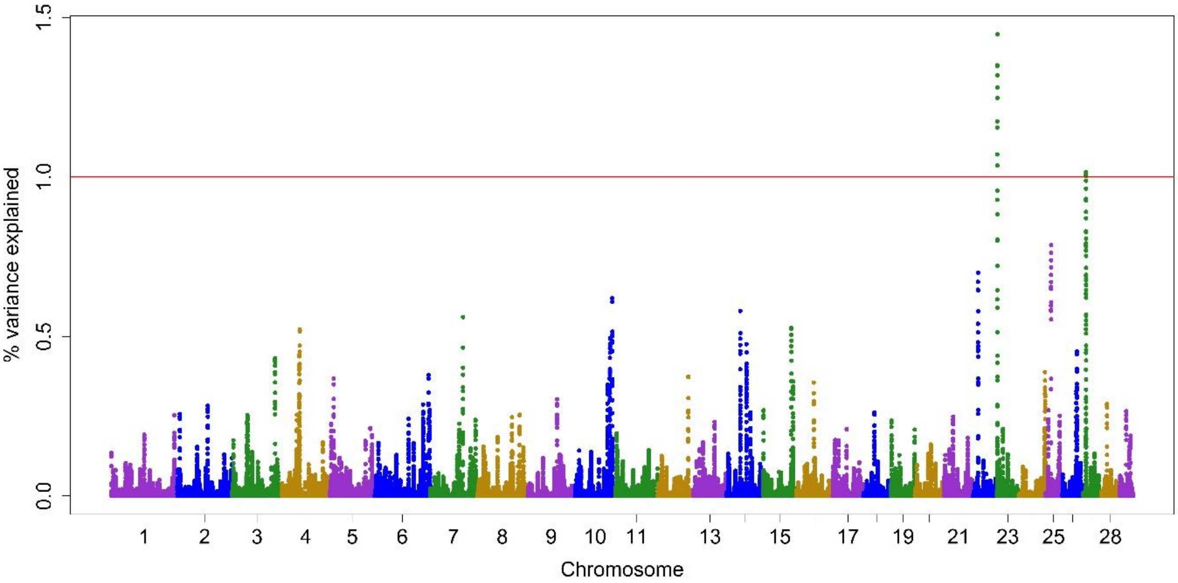

Figure 6. Manhattan plot of percentage of variance explained for SNP-phenotype association for teat diameter. The horizontal line represents the 1% threshold imposed to selected windows for further analyses

Milking Ease

Two genomic windows that explained more than 1% each of the total genetic additive variance were found on chromosomes 5 and 19 for milking ease. The additive genetic variance explained, chromosome number, start and end base pair for each window, and the windows' size (in base pair) for milking ease are presented in Table 8 and the Manhattan plot is presented in Figure 7.

Table 8. Windows that explained more than 1% of the variance for milking ease

Chr	IP	FP	Size (bp)	VE (%)
5	47043710	47109714	66,004	1.71275
19	1403909	1472676	68,767	6.23190

Chr: chromosome number where the window is located; **IP**: window initial position in base pair; **FP**: window final position in base pair; **Size (bp)**: window size in base pair; **VE (%)**: percentage of variance explained by each window.

The Carbonic Anhydrase 10 (*CA10*) is a protein-coding gene and was annotated within the genomic window on chromosome 19. Carbonic anhydrases enzyme coded by the Carbonic Anhydrase family genes participate in the acid-base homeostasis. However, this protein can be inactivated due to the loss of some histidine which demonstrates that this protein can be related to other functions (Sterky et al., 2016). This inactive protein binds to neurexin and this complex acts in the synaptic function of neurons cells, mostly in the secretory pathway. This gene was found highly expressed in the cerebellum, spinal cord, and cerebral cortex (Sterky et al., 2016). The milking ease trait can be impacted by the amount of oxytocin circulating in the blood flow. If the *CA10* gene play a role in secretory pathways it can be related to the studied trait even with the oxytocin being produced and released by the hypothalamus and posterior pituitary, respectively.

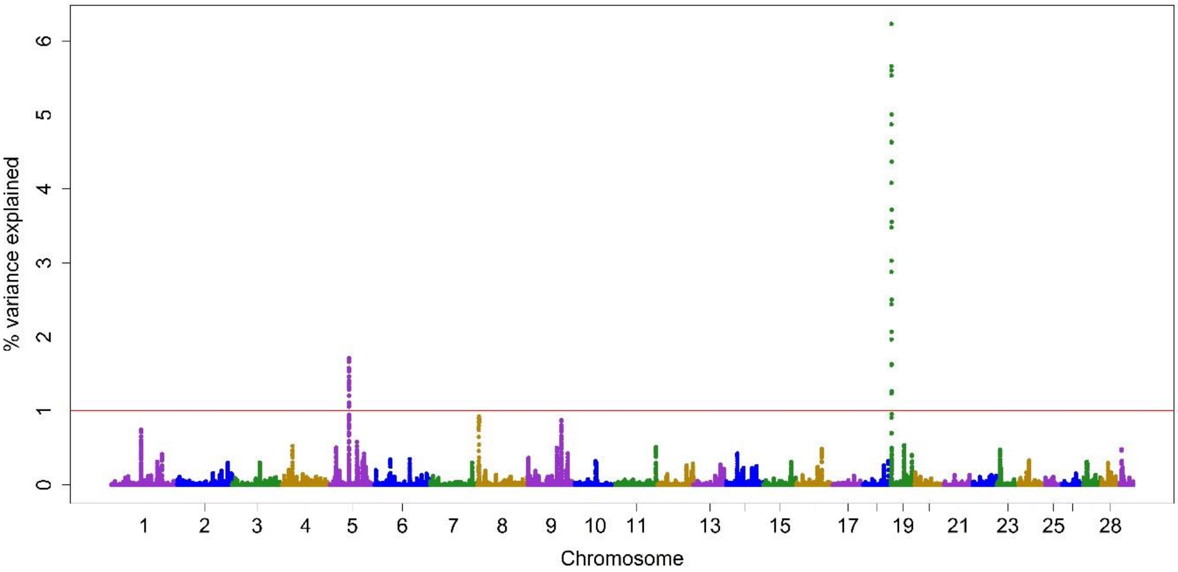

Figure 7. Manhattan plot of percentage of variance explained for SNP-phenotype association for milking ease. The horizontal line represents the 1% threshold imposed to selected windows for further analyses

Two terms were identified associated with *CA10* gene, the “Lyase activity” and "Zinc ion binding” terms. A total of three QTL (Appendix H in appendix) within the genomic window on chromosome 5 were identified; however, “Intramuscular fat”, “Body weight (birth)”, and “Intramuscular Fat” QTL are related to production traits in beef cattle. The QTL enrichment for trait within chromosome return zero results, and the QTL enrichment for class within chromosome returned 25 results. All 25 results were the term “Production” on chromosome 5.

Temperament

Three genomic windows that explained more than 1% each of the total genetic additive variance were found on chromosomes 4, 18, and 29 for temperament. The additive genetic variance explained, chromosome number, start and end base pair for each window, and the windows’ size (in base pair) for temperament are presented in Table 9 and the Manhattan plot is presented in Figure 8.

Table 9. Windows that explained more than 1% of the variance for temperament

Chr	IP	FP	Size (bp)	VE (%)
4	52310668	52358788	48,120	1.86311
18	32817160	32874467	57,307	1.61359
29	13270456	13197492	72,964	2.40851

Chr: chromosome number where the window is located; **IP**: window initial position in base pair; **FP**: window final position in base pair; **Size (bp)**: window size in base pair; **VE (%)**: percentage of variance explained by each window.

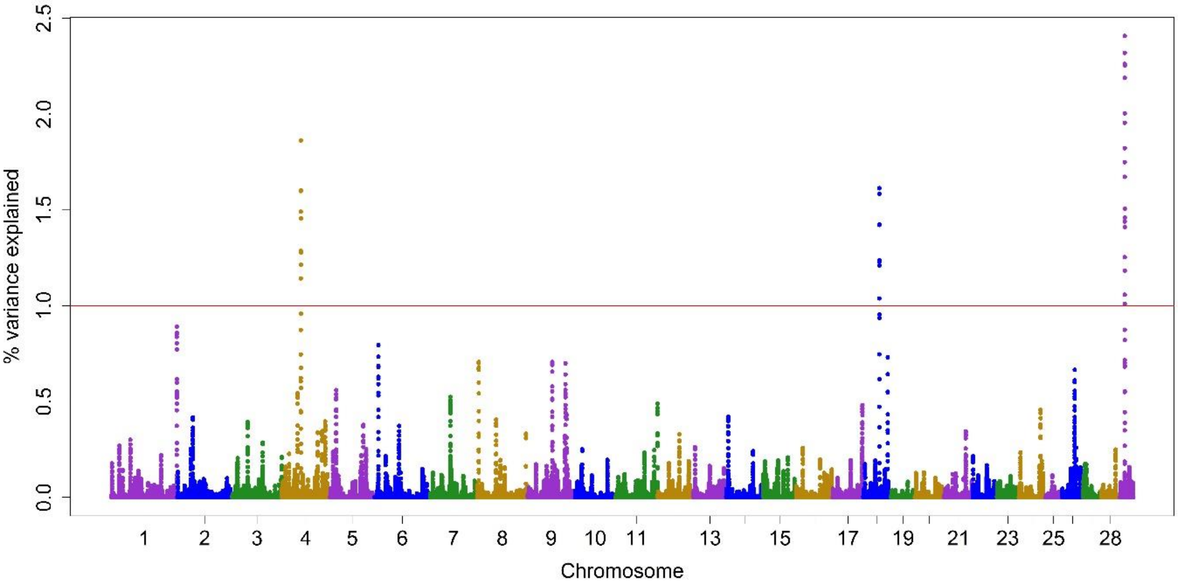

Figure 8. Manhattan plot of percentage of variance explained for SNP-phenotype association for temperament. The horizontal line represents the 1% threshold imposed to selected windows for further analyses

One candidate gene was annotated within the genomic window on chromosome 4, and one gene was annotated within the genomic window on chromosome 18. The first gene, Transcription Factor EC (*TFEC*), reported on chromosome 4, a regulatory gene, plays a role as a transcriptional activator for the non-muscle myosin II heavy chain A (NMHC-A) that are expressed in the spleen, thymus, and epithelium (Chung et al., 2001). The myosin participates in the cellular contractile, cytokinesis, cell migration, and cell attachment (Chung et al., 2001). This gene was also found down-regulated in female pigs submitted to heat stress and it was associated with four common risky behaviors (automobile speeding, alcohol drinking, smoking, and multiple sexual partners) (Ye et al., 2020; Srikanth et al., 2020). Risky behavior can be a socially unacceptable violation behavior or a socially accepted behavior (Ye et al., 2020).

The second gene, Cadherin 11 (*CDH11*), reported on chromosome 18 might be related to bovine temperament since it has been associated with autism spectrum disorder (ASD) in mice and with human diagnosed with both bipolar disorder and comorbid alcoholism. Besides both associations being about diseases, it is important to notice that both diseases are related to the individual behavioral (Lydall et al., 2011; Wu et al., 2020). Wu et al. (2020) studied mice's behavior with a knockout in the *CDH11* gene and showed that knockout mice presented hyperactivity, increased repetitive behavior, and reduced sociability. According to the authors, it seems that the *CDH11* gene plays an important role in mediating synapses formation during the wiring of circuits in the cortex, cerebellum, and striatum, which are regions responsible for repetitive behaviors.

Temperament is a complex trait to describe and evaluate and is composed of several traces combined, such as fear, stubbornness, aggressiveness, shyness, curiosity, and nervousness (Costa et al., 2015). Both genes in this study have been related to different behavioral responses in humans and mice. The *TFEC* is related to not health or inconsequential choices in humans and the knockout in the *CDH11* gene in mice leads to hyperactivity, increased repetitive behavior, and reduced sociability. Thus, both genes can be highly associated with the temperament in Dairy Gir cattle.

A total of 17 GO terms (Appendix E in appendix) and six MeSH terms (Appendix F in appendix) were identified. No pathway was found associated with the two genes. The MeSH terms found do not seem to be related to the phenotype. On the other hand, some Go terms, “Central Nervous system development”, “Neuron development”, “Response to abiotic stimulus”, “Schaffer collateral – CA1 synapse”, seems to be related to the phenotype. “Response to an abiotic stimulus”, is basically how the temperament trait is evaluated where the animal's response to new stimulus is observed, such as in the milking. The “Schaffer collateral – CA1 synapse” is related to the memory formation and emotional network.

Five QTL were found within (Appendix H in appendix) the three genomic windows. Two of than “Milk kappa-casein percentage” and “Milk Glycosylated kappa-casein percentage” may be related to the phenotype. The QTL enrichment for trait inside chromosome result in “Milk kappa-casein percentage” and “Milk Glycosylated kappa-casein percentage” traits which means that the association among the genomic regions and milk protein content is not random.

The total protein contents have been reported altered (increased or reduced) in cows treated with adrenocorticotrophic hormone (ACTH) which is responsible for increasing the production and release of cortisol by the adrenal gland (Flux et al., 1954) and in cows submitted to different levels of heat stress (Bernabucci et al., 2002; Smith et al., 2013; Hammami et al., 2015). It is important to notice that cows with a reactive temperament displays higher levels of cortisol in the blood flow than calmer cows which can explain why protein percentage traits were found related to the temperament trait (Hemsworth et al., 1989).

The lack in the literature of association studies for udder conformation and handling traits in Dairy Zebu cattle can make the analogy among our results and the results in the literature difficult. Ashwell et al. (2005) identified a suggestive and significant QTL for fore udder attachment located on chromosome 28 for Holstein cattle, which was found in this studied as well. However, the position of the two regions differ.

Several studies associated udder depth with genomic regions located on the same chromosomes identified in this studied (chromosomes 5, 6, 9, 14, and 16) despite the regions' location being different. Schrooten et al. (2000) associated the phenotype with a region on chromosome 5 in Dutch Holstein cattle. Abo-Ismail et al. (2017) found SNP and Pausch et al. (2016) found QTL on chromosome 6 associated with udder depth in Holstein cattle and Fleckvieh cattle, respectively. Cole et al. (2011) found a SNP on chromosome 9 related to udder depth in Holstein cattle. Pausch et al. (2016) also found QTL on chromosome 14 associated with udder depth for Fleckvieh cattle. Ashwell et al. (2005) identified a suggestive and significant QTL for udder depth on chromosome 16 for Holstein cattle.

Ashwell et al. (2005) identified a suggestive and significant QTL, and Cole et al. (2011) found four SNP on chromosome 17 associated with teat length, both studies were performed in Holstein cattle. Fang and Pausch (2019) found one significant SNP associated with milking speed on chromosome 19 for Brown Swiss cattle. Abo-Ismail et al. (2014) found two significant SNP on chromosome 5 and one on chromosome 19, Jardim et al. (2018) found one significant SNP on chromosome 5 and two on chromosome 19, and Marete et al. (2018) found one significant SNP on each chromosomes 5 and 19 associated with milking speed in Holstein cattle. Milking ease and milking speed is the description of the same trait. It can be measured subjectively by scores (from 1 to 9 or 1 to 5) by the farmer and it is called milking ease or in kilograms per minute during the whole milking or in the main milking phase, which is called milking speed.

4. Conclusion

The association study provided several QTL and genes related to the phenotypes. Genes associated to the mammary gland tissue, behavior, and secretory pathways were identified and might be very helpful to understand the genetic structure of the studied traits and can be applied in the genomic selection of the breed as well as being a start point to other studies related to udder conformation and handling traits in Zebu cattle.

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APPENDIX

Appendix A. Standard errors of the estimated additive genetic, permanent environmental, and residual variances for all models evaluated

Trait	**M1L**			**M2L**			**M3L**			**M4L**			**M1T**		**M2T**	
	seA	**sePE**	**seE**	**seA**	**sePE**	**seE**	**seA**	**sePE**	**seE**	**seA**	**sePE**	**seE**	**seA**	**sePE**	**seA**	**sePE**
STA	0.72	0.49	0.10	0.72	0.49	0.10	0.73	0.49	0.10	0.72	0.49	0.10	-	-	-	-
HG	1.66	1.36	0.47	1.66	1.36	0.47	1.74	1.38	0.47	1.74	1.38	0.47	-	-	-	-
BL	0.78	0.63	0.38	0.68	0.60	0.36	0.69	0.60	0.36	0.69	0.60	0.36	-	-	-	-
RL	0.17	0.14	0.07	0.17	0.14	0.07	0.17	0.14	0.07	0.17	0.14	0.07	-	-	-	-
PW	0.10	0.09	0.05	0.10	0.09	0.05	0.10	0.08	0.05	0.10	0.08	0.05	-	-	-	-
HW	0.21	0.20	0.13	0.21	1.20	0.11	0.21	0.20	0.13	0.23	0.20	0.11	-	-	-	-
RA	0.94	1.14	0.85	0.94	1.14	0.85	0.91	1.12	0.85	0.91	1.12	0.85	-	-	-	-
FA	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.09	0.10	0.09	0.11
LSV	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.13	0.13	0.20	0.20
LRV	0.00	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.00	0.09	0.06	0.14
FAU	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.22	0.27	0.24	0.28
RUW	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.17	0.19	0.19	0.21
UD	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.21	0.20	0.21	0.20
TL	0.09	0.06	0.02	0.09	0.06	0.02	0.09	0.06	0.02	0.09	0.06	0.02	-	-	-	-
TD	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	-	-	-	-
ME	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.16	0.14	0.16	0.14
TEM	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.14	0.14	0.14	0.14
NL	0.42	0.32	0.11	0.42	0.32	0.11	0.42	0.31	0.10	0.42	0.31	0.10	-	-	-	-

M1L: model 1 using linear methodology; **M2L**: model 2 using linear methodology; **M3L**: model 3 using linear methodology; **M4L**: model 4 using linear methodology; **M1T**: model 1 using threshold methodology; **M2T**: model 2 using threshold methodology.

seA: standard error of additive genetic variance; **sePE**: standard error of permanent environmental variance; **seE**: standard error of residual variance.

STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length.

Appendix B. Standard errors of the heritability and repeatability for all models evaluated

Trait	M1L		M2L		M3L		M4L		M1T		M2T	
	seh^2	se$r$	seh^2	se$r$	seh^2	se$r$	seh^2	se$r$	seh^2	se$r$	seh^2	se$r$
STA	0.03	0.01	0.03	0.01	0.03	0.01	0.03	0.01	-	-	-	-
HG	0.03	0.01	0.03	0.01	0.02	0.02	0.02	0.02	-	-	-	-
BL	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	-	-	-	-
RL	0.03	0.01	0.03	0.01	0.02	0.01	0.02	0.01	-	-	-	-
PW	0.03	0.02	0.03	0.02	0.02	0.01	0.02	0.01	-	-	-	-
HW	0.03	0.02	0.03	0.02	0.01	0.01	0.02	0.01	-	-	-	-
RA	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	-	-	-	-
FA	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.01
LSV	0.02	0.03	0.03	0.03	0.02	0.03	0.02	0.03	0.02	0.01	0.03	0.01
LRV	0.01	0.03	0.01	0.03	0.01	0.03	0.01	0.03	0.00	0.01	0.01	0.01
FAU	0.02	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01
RUW	0.02	0.03	0.03	0.03	0.01	0.02	0.02	0.02	0.02	0.01	0.03	0.01
UD	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.03	0.03	0.01	0.03	0.01
TL	0.03	0.01	0.03	0.01	0.03	0.01	0.03	0.01	-	-	-	-
TD	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	-	-	-	-
ME	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.03	0.01
TEM	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01
NL	0.05	0.01	0.05	0.01	0.04	0.02	0.04	0.02	-	-	-	-

M1L: model 1 using linear methodology; **M2L**: model 2 using linear methodology; **M3L**: model 3 using linear methodology; **M4L**: model 4 using linear methodology; **M1T**: model 1 using threshold methodology; **M2T**: model 2 using threshold methodology. **seh^2**: standard error of heritability; **se$r$**: standard error of repeatability.

STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length.

Appendix C. Phenotypic correlation (above diagonal), genetic correlation (below diagonal), heritability (diagonal), and standard error (parentheses) estimated in the two traits analyses among all nineteen traits

	STA	**HG**	**BL**	**RL**	**PW**	**HW**	**RA**	**FA**	**LSV**	**LRV**	**FUA**	**RUW**	**UD**	**TL**	**TD**	**ME**	**TEM**	**NL**	**MY**
STA	**0.50** **(0.03)**	0.45* (0.01)	0.37* (0.01)	0.44* (0.01)	0.25* (0.01)	0.40* (0.01)	0.18* (0.02)	0.04* (0.01)	-0.05* (0.02)	0.00 (0.01)	-0.03 (0.02)	0.03 (0.02)	0.05* (0.02)	0.11* (0.02)	0.13* (0.01)	0.01 (0.01)	-0.03 (0.01)	0.07* (0.02)	0.11* (0.02)
HG	0.64* (0.05)	**0.26** **(0.03)**	0.30* (0.01)	0.45* (0.01)	0.33* (0.01)	0.50* (0.01)	-0.03 (0.02)	0.03 (0.01)	-0.04* (0.02)	0.02 (0.01)	0.02 (0.02)	0.08* (0.02)	0.07* (0.02)	0.10* (0.01)	0.40* (0.09)	0.02 (0.01)	-0.02 (0.01)	0.08* (0.02)	0.08* (0.02)
BL	0.73* (0.05)	0.52* (0.08)	**0.20** **(0.03)**	0.30* (0.01)	0.25* (0.01)	0.31* (0.01)	0.02 (0.02)	0.02 (0.01)	-0.03 (0.02)	0.00 (0.01)	0.01 (0.02)	0.06* (0.02)	0.06* (0.02)	0.08* (0.01)	0.09* (0.01)	0.01 (0.01)	-0.04* (0.01)	0.08* (0.02)	0.10* (0.02)
RL	0.76* (0.04)	0.59* (0.07)	0.55* (0.07)	**0.23** **(0.03)**	0.24* (0.01)	0.45* (0.01)	-0.06* (0.02)	0.02 (0.01)	-0.01 (0.01)	0.02 (0.01)	-0.01 (0.02)	0.05* (0.01)	0.06* (0.01)	0.11* (0.01)	0.14* (0.01)	-0.01 (0.01)	-0.06* (0.01)	0.06* (0.02)	0.11* (0.02)
PW	0.45* (0.07)	0.33* (0.09)	0.45* (0.09)	0.29* (0.09)	**0.21** **(0.03)**	0.34* (0.01)	-0.03 (0.02)	0.02 (0.01)	-0.03* (0.01)	0.00 (0.01)	0.00 (0.02)	0.06* (0.02)	0.06* (0.02)	0.08* (0.01)	0.08* (0.01)	-0.01 (0.01)	-0.01 (0.01)	0.07* (0.02)	0.05* (0.02)
HW	0.65* (0.06)	0.62* (0.06)	0.53* (0.08)	0.65* (0.06)	0.35* (0.09)	**0.19** **(0.03)**	-0.07* (0.02)	0.00 (0.01)	-0.03* (0.02)	0.02 (0.01)	0.01 (0.02)	0.08* (0.02)	0.07* (0.02)	0.08* (0.01)	0.12* (0.01)	0.01 (0.01)	-0.04* (0.01)	0.08* (0.02)	0.07* (0.02)
RA	0.34* (0.11)	0.07 (0.14)	-0.13 (0.15)	0.03 (0.15)	-0.05 (0.15)	-0.16 (0.15)	**0.12** **(0.03)**	-0.04* (0.01)	-0.02 (0.01)	-0.02 (0.01)	0.00 (0.02)	-0.02 (0.01)	0.04* (0.01)	0.00 (0.02)	0.01 (0.01)	0.00 (0.01)	0.01 (0.01)	-0.01 (0.02)	0.00 (0.02)
FA	0.11 (0.12)	0.24 (0.13)	0.09 (0.15)	0.04 (0.14)	0.32* (0.14)	0.03 (0.15)	-0.03 (0.20)	**0.07** **(0.02)**	-0.12* (0.01)	0.07* (0.01)	0.07* (0.01)	0.00 (0.01)	-0.04* (0.01)	-0.03 (0.01)	-0.01 (0.01)	-0.01 (0.01)	0.01 (0.01)	-0.01 (0.02)	0.00 (0.02)
LSV	-0.24 (0.12)	0.01 (0.15)	-0.19 (0.16)	0.00 (0.15)	-0.33 (0.16)	0.09 (0.16)	-0.42* (0.16)	-0.41 (0.20)	**0.10** **(0.03)**	0.01 (0.01)	-0.02 (0.02)	0.02 (0.01)	0.03 (0.01)	0.02 (0.02)	0.02 (0.01)	0.03 (0.01)	0.04* (0.01)	0.03 (0.02)	0.00 (0.02)
LRV	-0.33 (0.37)	-0.30 (0.40)	-0.54 (0.37)	-0.52 (0.35)	-0.43 (0.38)	-0.63 (0.41)	-0.53 (0.45)	0.74 (0.62)	0.35 (0.44)	**0.01** **(0.01)**	0.06* (0.01)	0.06* (0.01)	0.01 (0.01)	0.01 (0.01)	-0.02 (0.01)	0.01 (0.01)	0.00 (0.01)	0.01 (0.02)	0.03 (0.02)
FUA	-0.21 (0.14)	0.06 (0.17)	-0.03 (0.19)	-0.11 (0.18)	-0.22 (0.18)	0.16 (0.18)	0.04 (0.21)	0.38 (0.21)	-0.14 (0.22)	-0.11 (0.55)	**0.07** **(0.02)**	0.06* (0.02)	-0.19* (0.01)	-0.05* (0.02)	-0.03 (0.02)	-0.01 (0.01)	0.00 (0.01)	-0.07* (0.02)	-0.02 (0.02)
RUW	0.10 (0.14)	0.12 (0.16)	-0.03 (0.18)	0.22 (0.16)	0.22 (0.16)	0.35 (0.17)	-0.13 (0.20)	-0.02 (0.22)	0.37 (0.22)	-0.61 (0.46)	-0.36 (0.24)	**0.08** **(0.03)**	0.16* (0.01)	0.04* (0.02)	0.07* (0.01)	-0.02 (0.01)	-0.04* (0.01)	0.03 (0.02)	0.17* (0.02)
UD	0.18 (0.11)	0.15 (0.13)	0.09 (0.15)	0.16 (0.14)	0.05 (0.15)	0.08 (0.15)	0.36 (0.16)	-0.38 (0.18)	0.13 (0.19)	-0.71 (0.74)	-0.68* (0.14)	0.69* (0.15)	**0.11** **(0.03)**	0.11* (0.02)	0.14* (0.01)	0.02 (0.01)	-0.01 (0.01)	0.05* (0.02)	0.13* (0.02)
TL	0.15* (0.06)	0.24* (0.08)	0.21* (0.08)	0.30* (0.08)	0.14 (0.08)	0.14 (0.09)	-0.22 (0.12)	-0.09 (0.12)	0.09 (0.13)	-0.26 (0.39)	-0.19 (0.15)	-0.18 (0.14)	0.21 (0.12)	**0.41** **(0.03)**	0.53* (0.01)	0.08* (0.01)	0.00 (0.01)	0.04 (0.02)	0.10* (0.02)
TD	0.31* (0.08)	0.40* (0.09)	0.33* (0.10)	0.48* (0.09)	0.24* (0.10)	0.33* (0.10)	-0.19 (0.14)	-0.08 (0.14)	0.11 (0.16)	-0.75 (0.44)	-0.40* (0.15)	0.11 (0.17)	0.45* (0.13)	0.82* (0.04)	**0.20** **(0.03)**	0.05* (0.01)	0.00 (0.01)	0.00 (0.02)	0.15* (0.02)
ME	0.07 (0.09)	0.19 (0.11)	0.04 (0.12)	-0.06 (0.11)	0.19 (0.11)	0.08 (0.12)	-0.11 (0.16)	-0.07 (0.16)	-0.01 (0.17)	0.20 (0.46)	-0.10 (0.19)	-0.25 (0.18)	0.18 (0.15)	0.27* (0.09)	0.20 (0.11)	**0.15** **(0.03)**	0.20* (0.01)	0.01 (0.02)	-0.05* (0.02)
TEM	0.02 (0.10)	0.00 (0.12)	-0.18 (0.13)	0.02 (0.12)	0.17 (0.13)	0.01 (0.01)	-0.02 (0.17)	0.22 (0.16)	0.05 (0.17)	0.63 (0.51)	0.35 (0.18)	-0.08 (0.19)	0.13 (0.16)	-0.06 (0.10)	0.01 (0.13)	0.40* (0.12)	**0.11** **(0.02)**	-0.02 (0.02)	-0.07* (0.02)
NL	0.06 (0.08)	0.04 (0.10)	0.19 (0.10)	0.02 (0.10)	0.03 (0.11)	0.01 (0.11)	-0.13 (0.14)	0.07 (0.15)	0.29 (0.14)	0.40 (0.71)	-0.33 (0.17)	0.19 (0.15)	0.19 (0.13)	0.02 (0.09)	-0.03 (0.11)	0.05 (0.12)	-0.22 (0.12)	**0.40** **(0.05)**	0.00 (0.02)
MY	0.04 (0.11)	-0.05 (0.13)	0.08 (0.13)	0.35* (0.12)	0.14 (0.13)	0.14 (0.13)	-0.07 (0.17)	0.06 (0.17)	-0.17 (0.17)	-0.26 (0.46)	-0.38 (0.20)	0.59* (0.15)	0.56* (0.15)	0.20 (0.11)	0.42* (0.12)	-0.15 (0.14)	-0.04 (0.15)	0.17 (0.13)	**0.16** **(0.03)**

STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length; **MY**: 305-day cumulative milk yield.

*: Significantly (p<0.05) different from zero using the two-tailed t-test.

Appendix D. Residual correlation (above diagonal), permanent environmental correlation (below diagonal), heritability (diagonal), and standard error (parentheses) estimated in the two traits analyses among all nineteen traits

	STA	**HG**	**BL**	**RL**	**PW**	**HW**	**RA**	**FA**	**LSV**	**LRV**	**FUA**	**RUW**	**UD**	**TL**	**TD**	**ME**	**TEM**	**NL**	**MY**
STA	**0.50**	0.17*	0.05*	0.09*	0.05*	0.09*	0.16*	0.00	-0.01	0.00	-0.01	0.01	-0.04	0.03	0.04	-0.02	-0.01	0.01	0.03
	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.02)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.05)
HG	0.53*	**0.26**	0.08*	0.19*	0.15*	0.21*	-0.10*	-0.03	-0.02	-0.03	0.06	0.02	0.01	0.03	0.08*	0.01	0.00	0.03	0.00
	(0.05)	**(0.03)**	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.02)	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.05)
BL	0.53*	0.53*	**0.20**	0.10*	0.09*	0.14*	-0.04	-0.01	0.02	-0.02	0.00	0.01	-0.05	0.01	0.00	0.04	-0.01	-0.07	0.09
	(0.06)	(0.06)	**(0.03)**	(0.02)	(0.02)	(0.02)	(0.04)	(0.02)	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.05)
RL	0.54*	0.68*	0.52*	**0.23**	0.18*	0.16*	-0.13*	0.01	-0.06	0.01	-0.01	0.00	0.07	0.03	0.05*	0.00	-0.01	0.05	-0.03
	(0.05)	(0.04)	(0.07)	**(0.03)**	(0.02)	(0.02)	(0.03)	(0.02)	(0.03)	(0.03)	(0.04)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.05)
PW	0.34*	0.62*	0.52*	0.33*	**0.21**	0.13*	-0.06	-0.03	0.01	0.02	-0.01	0.00	0.03	0.03	0.04	-0.05*	-0.03	0.02	-0.01
	(0.07)	(0.06)	(0.08)	(0.07)	**(0.03)**	(0.02)	(0.03)	(0.02)	(0.03)	(0.03)	(0.04)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.05)
HW	0.59*	0.83*	0.56*	0.79*	0.80*	**0.19**	-0.07	-0.05*	-0.03	-0.06	0.00	0.00	0.03	0.01	0.02	0.02	-0.03	0.05	0.09
	(0.05)	(0.04)	(0.07)	(0.04)	(0.06)	**(0.03)**	(0.04)	(0.02)	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.05)
RA	0.15	0.00	0.28*	0.00	0.04	-0.01	**0.12**	-0.07	-0.01	-0.01	0.07	0.02	-0.01	0.00	0.00	0.02	0.00	-0.05	-0.03
	(0.08)	(0.08)	(0.12)	(0.09)	(0.11)	(0.10)	**(0.03)**	(0.04)	(0.03)	(0.03)	(0.04)	(0.03)	(0.03)	(0.04)	(0.03)	(0.03)	(0.03)	(0.04)	(0.07)
FA	0.10	0.03	0.10	0.01	0.00	0.14	0.05	**0.07**	-0.10*	0.09*	-0.09	0.05	-0.02	0.01	0.01	-0.02	0.00	0.00	0.07
	(0.09)	(0.08)	(0.11)	(0.09)	(0.10)	(0.09)	(0.13)	**(0.02)**	(0.03)	(0.03)	(0.04)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.05)
LSV	0.02	-0.10	-0.07	0.09	0.03	-0.11	0.15	-0.06	**0.10**	0.01	-0.03	0.02	0.02	0.07	0.04	-0.01	0.00	0.05	-0.01
	(0.08)	(0.08)	(0.12)	(0.09)	(0.11)	(0.10)	(0.10)	(0.13)	**(0.03)**	(0.03)	(0.04)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.04)	(0.06)
LRV	0.06	0.13	0.12	0.11	0.03	0.26*	0.01	-0.01	-0.01	**0.01**	0.05	0.05	0.02	0.07	0.00	0.00	-0.05	0.00	0.10
	(0.07)	(0.06)	(0.10)	(0.07)	(0.09)	(0.08)	(0.07)	(0.10)	(0.07)	**(0.01)**	(0.04)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.04)	(0.06)
FUA	0.04	-0.02	0.06	0.03	0.09	-0.01	-0.10	0.34*	0.03	0.07	**0.07**	0.11*	-0.14*	-0.01	0.06	-0.06	-0.07	-0.06	0.07
	(0.06)	(0.06)	(0.09)	(0.07)	(0.08)	(0.07)	(0.07)	(0.09)	(0.07)	(0.05)	**(0.02)**	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.03)	(0.04)	(0.07)
RUW	0.02	0.16	0.24	0.07	0.11	0.13	-0.05	-0.13	-0.08	0.14	0.07	**0.08**	0.08*	0.08*	0.04	-0.03	-0.06	0.01	0.13
	(0.08)	(0.07)	(0.12)	(0.08)	(0.11)	(0.09)	(0.09)	(0.13)	(0.09)	(0.07)	(0.07)	**(0.03)**	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.04)	(0.06)
UD	0.06	0.12	0.38*	0.00	0.13	0.15	0.02	0.04	0.01	0.04	-0.14	0.16	**0.11**	0.05	0.07	0.04	0.01	0.02	-0.07
	(0.09)	(0.08)	(0.13)	(0.10)	(0.12)	(0.11)	(0.10)	(0.14)	(0.10)	(0.08)	(0.08)	(0.09)	**(0.03)**	(0.03)	(0.03)	(0.03)	(0.03)	(0.04)	(0.06)
TL	0.12	0.04	0.05	0.02	0.09	0.16	0.18	-0.09	-0.10	-0.03	-0.03	0.14	0.15	**0.41**	0.34*	-0.02	0.00	-0.08	-0.03
	(0.07)	(0.06)	(0.09)	(0.07)	(0.08)	(0.07)	(0.10)	(0.09)	(0.10)	(0.07)	(0.07)	(0.09)	(0.10)	**(0.03)**	(0.02)	(0.02)	(0.02)	(0.04)	(0.05)
TD	0.06	0.08	0.13	0.05	0.04	0.15	0.13	-0.01	-0.07	0.03	-0.03	0.13	0.13	0.57*	**0.20**	-0.02	0.01	-0.13*	0.04
	(0.07)	(0.06)	(0.08)	(0.07)	(0.08)	(0.07)	(0.09)	(0.09)	(0.09)	(0.07)	(0.07)	(0.09)	(0.10)	(0.05)	**(0.03)**	(0.02)	(0.02)	(0.04)	(0.05)
ME	-0.03	-0.09	-0.08	-0.01	-0.09	-0.05	0.00	0.04	0.15	0.00	0.10	0.08	-0.14	0.07	0.12	**0.15**	0.10*	0.08	0.06
	(0.07)	(0.06)	(0.09)	(0.07)	(0.09)	(0.08)	(0.10)	(0.10)	(0.10)	(0.08)	(0.08)	(0.10)	(0.11)	(0.08)	(0.07)	**(0.03)**	(0.02)	(0.04)	(0.05)
TEM	-0.10	-0.05	-0.03	-0.18*	-0.07	-0.09	0.02	-0.04	0.11	0.04	-0.01	0.03	-0.11	0.04	-0.02	0.35*	**0.11**	0.02	-0.11*
	(0.06)	(0.05)	(0.08)	(0.06)	(0.07)	(0.06)	(0.08)	(0.08)	(0.08)	(0.06)	(0.06)	(0.08)	(0.09)	(0.07)	(0.06)	(0.06)	**(0.02)**	(0.04)	(0.05)
NL	0.14	0.16*	0.22	0.11	0.18	0.19	0.10	-0.10	-0.17	-0.03	0.00	-0.03	0.01	0.18	0.18	-0.10	0.05	**0.40**	0.00
	(0.08)	(0.07)	(0.11)	(0.08)	(0.10)	(0.09)	(0.08)	(0.11)	(0.09)	(0.06)	(0.06)	(0.08)	(0.09)	(0.09)	(0.09)	(0.09)	(0.07)	**(0.05)**	(0.07)
MY	0.27*	0.25*	0.16	0.19*	0.11	0.00	0.07	-0.19	0.10	-0.04	-0.04	0.10	0.35*	0.21*	0.16	-0.21	-0.02	-0.12	**0.16**
	(0.08)	(0.08)	(0.11)	(0.08)	(0.11)	(0.09)	(0.12)	(0.13)	(0.11)	(0.09)	(0.08)	(0.11)	(0.13)	(0.09)	(0.09)	(0.10)	(0.09)	(0.09)	**(0.03)**

STA: stature; **HG**: heart girth; **BL**: body length; **RL**: rump length; **PW**: pin width; **HW**: hook width; **RA**: rump angle; **FA**: foot angle; **LSV**: rear legs – side view; **LRV**: rear legs – rear view; **FUA**: fore udder attachment; **RUW**: rear udder width; **UD**: udder depth; **TL**: teat length; **TD**: teat diameter; **ME**: milking ease; **TEM**: temperament; **NL**: navel length; **MY**: 305-day cumulative milk yield.

*: Significantly (p<0.05) different from zero using the two-tailed t-test.

Appendix E. Gene Ontology terms related to the genes found in the windows explaining more than 1% of the total genetic variance for all traits

ID	Gene	Description
	Fore udder attachment	
	Gene Ontology "Biological Process"	
GO:0032200	XRCC3	Telomere organization;
GO:0033044	XRCC3	Regulation of chromosome organization
GO:0060249	XRCC3	Anatomical structure homeostasis
GO:0007059	XRCC3	Chromosome segregation
GO:0045787	XRCC3	Positive regulation of cell cycle
GO:0044770	XRCC3	Cell cycle phase transition
GO:0048285	XRCC3	Organelle fission
GO:0045786	XRCC3	Negative regulation of cell cycle
GO:0051493	XRCC3	Regulation of cytoskeleton organization
GO:0010564	XRCC3	Regulation of cell cycle process
GO:0051129	XRCC3	Negative regulation of cellular component organization
GO:0007017	XRCC3	Microtubule-based process
GO:0006974	XRCC3	Cellular response to DNA damage stimulus
GO:0000278	XRCC3	Mitotic cell cycle
GO:0006259	XRCC3	DNA metabolic process
	Gene Ontology "Cellular Component"	
GO:0005875	KLC1	Microtubule associated complex
GO:0005657	XRCC3	Replication fork
GO:0005759	XRCC3	Mitochondrial matrix
GO:0048471	XRCC3	Perinuclear region of cytoplasm
GO:0005768	ZFYVE21	Endosome
GO:0030054	ZFYVE21	Cell junction
	Gene Ontology "Molecular Function"	
GO:0003774	KLC1	Motor activity
GO:0140097	XRCC3	Catalytic activity, acting on DNA
GO:0016887	XRCC3	ATPase activity
GO:0016788	XRCC3	Hydrolase activity, acting on ester bonds
	Teat Length	
	Gene Ontology "Cellular Component"	
GO:0015629	KLHL2	Actin cytoskeleton
GO:1990234	KLHL2	Transferase complex
	Milking Ease	
	Gene Ontology "Molecular Function"	
GO:0016829	CA10	Lyase activity
GO:0008270	CA10	Zinc ion binding
	Temperament	
	Gene Ontology "Biological Process"	
GO:0034330	CDH11	Cell junction organization
GO:0099536	CDH11	Synaptic signaling
GO:0098609	CDH11	Cell-cell adhesion
GO:0007417	CDH11	Central nervous system development
GO:0048666	CDH11	Neuron development
GO:0009628	TFEC	Response to abiotic stimulus
GO:0032989	CDH11	Cellular component morphogenesis

Appendix E. Continued

	Gene Ontology “Cellular Component”	
GO:0098685	CDH11	Schaffer collateral - CA1 synapse
GO:0098978	CDH11	Glutamatergic synapse
GO:0019898	CDH11	Extrinsic component of membrane
GO:0098797	CDH11	Plasma membrane protein complex
GO:0009986	CDH11	Cell surface
GO:0030054	CDH11	Cell junction
	Gene Ontology “Molecular Function”	
GO:0050839	CDH11	Cell adhesion molecule binding
GO:0005509	CDH11	Calcium ion binding
GO:0042803	CDH11	Protein homodimerization activity
GO:0008092	CDH11	Cytoskeletal protein binding

ID: Gene Ontology.

Appendix F. Medical Subject Headings (MeSH) terms related to the genes found in the windows explaining more than 1% of the total genetic variance for all traits

ID	**Gene**	**Description**
	Temperament	
	MeSH “Anatomy”	
D010006	CDH11	Osteoblasts
	MeSH “Biological Sciences”	
D002454	CDH11	Cell Differentiation
	MeSH “Chemical and Drugs”	
D015675	CDH11	Osteocalcin
D017886	CDH11	Durapatite
D015820	CDH11	Cadherins
D053495	CDH11	Osteopontin

ID: MeSH term information identification.

Appendix G. Metabolic pathways recovered from Kyoto Encyclopedia of Genes and Genomes (KEGG) and WikiPathways (WIKI) related to the genes found in the windows explaining more than 1% of the total genetic variance for all traits

ID	**Gene**	**Description**
	Fore Udder Attachment	
	KEGG	
BTA03440	XRCC3	Homologous recombination
BTA05132	KLC1	Salmonella infection
	WikiPathways	
WP3275	XRCC3	Fluoropyrimidine Activity
WP3260	XRCC3	Integrated Breast Cancer Pathway
	Udder Depth	
	KEGG	
BTA00120	CYP7B1	Primary bile acid biosynthesis
BTA00140	CYP7B1	Steroid hormone biosynthesis
	WikiPathways	
WP3248	CYP7B1	Tryptophan metabolism
WP1077	CYP7B1	Oxidation by Cytochrome P450
WP1006	CYP7B1	Metapathway biotransformation

ID: KEGG and WIKI metabolic pathway identification.

Appendix H. Relevant quantitative trait loci (QTL) found in the windows explaining more than 1% of the total genetic variance for all traits

ID	CHR	Class	Gene	Trait
Fore Udder Attachment				
24743	1	Production	-	Body weight (weaning)
24744	1	Production	-	Body weight (weaning)
24745	1	Production	-	Body weight (weaning)
106485	1	Reproduction	-	Calving Ease
124004	21	Reproduction	-	Sire conception rate
126885	21	Reproduction	-	Interval to first estrus after calving
Rear Udder Width				
24743	1	Production	-	Body weight (weaning)
24744	1	Production	-	Body weight (weaning)
24745	1	Production	-	Body weight (weaning)
106485	1	Reproduction	-	Calving Ease
Udder Depth				
106583	6	Exterior	-	Udder swelling score
139022	14	Reproduction	-	Scrotal circumference
138431	14	Reproduction	-	Scrotal circumference
138438	14	Reproduction	-	Scrotal circumference
20703	14	Meat and Carcass	-	Subcutaneous fat
56461	14	Production	-	Residual feed intake
63712	14	Reproduction	-	Reproductive efficiency
63753	14	Reproduction	-	Age at first calving
68659	14	Production	-	Body weight gain
22885	14	Meat and Carcass	-	Fat thickness at the 12th rib
56624	16	Milk	-	Milk palmitoleic acid content
Teat Diameter				
69601	23	Milk	-	Milk iron content
20826	27	Meat and Carcass	-	Shear force
Milking Ease				
22866	5	Meat and Carcass	-	Intramuscular fat
22757	5	Production	-	Body weight (birth)
22863	5	Meat and Carcass	-	Intramuscular fat
Temperament				
138508	4	Reproduction	-	Conception rate
106465	4	Meat and Carcass	-	Pelvic area
30537	18	Reproduction	-	Birth index
111026	29	Milk	-	Milk kappa-casein percentage
115139	29	Milk	-	Milk glycosylated kappa-casein percentage

ID: QTL identification in the QTL database named “Cattle QTLdb”; **CHR:** chromosome number; **Class:** class that the QTL is classified; **Gene:** Associated gene to the QTL; **Trait:** trait associated to the QTL.