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ROSIANE PEREIRA DA SILVA

Genomic selection and genome-wide association study with carcass composition indicator traits in Nellore cattle

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“Chega um momento em sua vida, que
você sabe: quem é imprescindível para
você, quem nunca foi, quem não é
mais, quem será sempre!”
(Charles Chaplin)

ABSTRACT

SILVA, R. P. **Genomic selection and genome-wide association study with carcass composition indicator traits in Nellore cattle**. 2021. Dr. Thesis – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

The growing global demand for safe and sustainable food production has motived a restructuring in the beef production sector aiming the production of better quality products without increasing the productive cost. Thus, animal breeding aims to improve economic productivity of future generations of domestic species through selection. Most of the economic important traits in livestock has a complex and quantitative expression, that is, they are influenced by a large number of genes and affected by environmental factors. However, there is no consensus among researches about the best methodology to obtain genomic prediction for each trait. There are different methods and pseudo-phenotypes used in genomic predictions, being necessary to determine the ideal for each trait of interest. In Chapter 2, the aim of this study was to estimate genetic parameters and identify genomic regions associated with carcass traits obtained by ultrasound and visual scores in Nellore cattle. Data from ~66,000 animals from the National Association of Breeders and Researchers (ANCP) were used. The variance components for back fat thickness (BF), rump fat thickness (RF) and *Longissimus* muscle area (LMA) were estimated considering a linear model whereas a threshold model were fitted for body structure (BS), finishing precocity (FP) and musculature (MS) traits were used. The SNP solutions were estimated using the ssGBLUP approach by considering windows of 10 consecutive SNPs. Regions that explained for more than 1.0% of the additive genetic variance were used. Gene enrichment analysis revealed GO biological processes that might be directly influenced the organism growth and development. In Chapter 3, the aim of this study was to compare the genomic prediction ability for carcass composition indicator traits in Nellore cattle using the Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP), single-step GBLUP (ssGBLUP), Bayesian methods (BayesA, BayesB, BayesC and BayesianLASSO) and an approach combining the pedigree matrix of genotyped

animals and the genomic matrix using a Bayesian analysis. Phenotypic and genotypic information on about 66, and 21,000 animals, respectively, evaluated by ANCP were available for BF, RF, LMA, BS, FP and MS. To obtain the prediction ability, the dataset was split into training (genotyped sires and dams with progenies) and validation (genotyped young animals without progeny records and without phenotypes) subsets. In terms of prediction ability and bias, Bayesian approaches were superior for visual scores traits and the ssGBLUP for carcass traits obtained by ultrasonography, however, more biased results were obtained for BF and RF using the ssGBLUP. The ssGBLUP model showed less biased prediction for low heritability traits, such as LMA, and also it has lower computational demand and it is a straightforward method for implementing genomic selection in beef cattle. In Chapter 4, the aim of this study was to estimate genetic parameters and to identify genomic regions associated with the calving ease (CE) in precocious Nellore heifers. A total of 1,277 CE phenotypes were collected and scored into two categories: i- non assisted calving, categorized as success (1) and ii- assisted calving where heifers required any form of assistance or intervention to give birth, categorized as failure (2). The direct and maternal heritability estimates for CE were low (0.18) and moderate (0.39) respectively, indicating that genetic progress for this trait is feasible, and so, it would respond favorably to direct selection. Gene enrichment analysis revealed processes that might directly influence fetal processes involved in female pregnancy and stress response.

Keywords: Bayesian models. Beef cattle. Calving ease. Carcass traits. GWAS. ssGBLUP.

RESUMO

SILVA, R. P. **Seleção genômica e estudo de associação genômica ampla de características indicadoras de composição de carcaça em bovinos da raça Nelore**. 2021. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

A crescente busca por produção alimentar sustentável tem incentivado uma reestruturação no setor de produção de carne visando obter produtos de melhor qualidade sem aumentar os custos de produção. Deste modo, o melhoramento genético animal tende a melhorar a produtividade econômica das futuras gerações de espécies de interesse econômico por meio da seleção. A maioria das características de interesse econômico na pecuária é de expressão quantitativa e complexa, ou seja, são influenciadas por vários genes e afetadas por fatores ambientais. No entanto, ainda não há consenso entre os pesquisadores sobre a melhor metodologia para a obtenção da predição genômica para cada característica. Existem diferentes métodos e pseudo-fenótipos utilizados em predições genômicas, sendo necessário determinar o ideal para a característica de interesse. No Capítulo 2, o objetivo deste estudo foi estimar parâmetros genéticos e identificar regiões genômicas associadas a características de carcaça obtidas por ultrassonografia e escores visuais em bovinos Nelore. Foram utilizados dados de aproximadamente 66.000 animais provenientes da Associação Nacional de Criadores e Pesquisadores (ANCP). Os componentes de variância para espessura de gordura subcutânea (EG), espessura de gordura subcutânea da garupa (EGP8) e área de olho de lombo (AOL) foram estimados considerando um modelo linear, e um modelo de limiar para as características, como estrutura corporal (E), precocidade (P) e musculosidade (M). As soluções dos SNPs foram estimadas por meio da metodologia ssGBLUP considerando janelas de 10 SNPs adjacentes. Regiões que representaram mais de 1.0% da variância genética aditiva foram utilizadas. A análise de enriquecimento funcional revelou processos biológicos que podem influenciar diretamente o crescimento e desenvolvimento animal. No Capítulo 3, objetivou-se com este estudo comparar a habilidade de predição genômica de características indicadoras de composição de carcaça em bovinos Nelore

usando os métodos BLUP, GBLUP, ssGBLUP, métodos Bayesianos (BayesA, BayesB, BayesC e BayesianLASSO) e uma metodologia combinando a matriz de parentesco dos animais genotipados com a matriz genômica e métodos Bayesianos. Foram obtidas informações fenotípicas e genotípicas de aproximadamente 66.000 e 21.000 animais, respectivamente, avaliados pela ANCP para BF, RF, LMA, BS, FP e MS. Para obter a habilidade de predição, o conjunto de dados foi dividido em subconjuntos de treinamento (touros genotipados e mães com progênies) e validação (animais jovens genotipados sem registros de progênies e sem fenótipos). Em relação a habilidade de predição e o viés, os métodos bayesianos foram superiores para as características de escores visuais e o ssGBLUP para as características de carcaça obtidas por ultrassonografia, entretanto, resultados mais tendenciosos foram obtidos para BF e RF quando utilizado o ssGBLUP. O modelo ssGBLUP apresentou predição menos viesada para as características de baixa herdabilidade, como LMA, e também apresentou menor demanda computacional, caracterizado por ser um método que não necesita de cálculo de pseudofenótipos para a implementação da seleção genômica em bovinos de corte. No Capítulo 4, o objetivo deste estudo foi estimar parâmetros genéticos e identificar regiões genômicas associadas à facilidade de parto (CE) em novilhas Nelore precoces. Um total de 1.277 fenótipos para CE foram coletados e classificados em duas categorias: i- parto não assistido, categorizado como sucesso (1) e ii- parto assistido, no qual as novilhas necessitaram de qualquer forma de assistência ou intervenção para parir, categorizado como falha (2). As estimativas de herdabilidade direta e materna para CE foram baixas (0,18) e moderadas (0,39), respectivamente, indicando que o progresso genético para essa característica é viável e, portanto, responderia favoravelmente à seleção direta. A análise de enriquecimento funcional revelou processos que podem influenciar diretamente os processos fetais envolvidos na gestação e na resposta ao estresse.

Palavras-chave: Bovinos de corte. Características de carcaça. Facilidade de Parto. GWAS. Modelos Bayesianos. ssGBLUP.

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LIST OF ABBREVIATIONS, ACRONYMS and INITIALS

ANCP	National Association of Breeders and Researchers
BF	Back Fat Thickness
BLUP	Best Linear Unbiased Prediction
BOA	Bayesian Output Analysis
BS	Body Structure
BTA	*Bos taurus* autosomes
CA	California
CE	Calving Ease
CG	Contemporary Group
cm^2	centimeter
DAVID	Database for Annotation, Visualization, and Integrated Discovery
dEPD	deregressed Expected Progeny Differences
DNA	Deoxyribonucleic Acid
DGV	Direct Genomic Values
EBV	Estimated Breeding Values
FDR	False Discovery Rate
FP	Finishing Precocity
GBLUP	Genomic Best Linear Unbiased Prediction
GEBV	Genomic Estimated Breeding Values
GO	Gene Ontology
GS	Genomic Selection
GWAS	Genome-Wide Association Study
HD	High-Density
HPD	Highest Posterior Density
HWE	Hardy-Weinberg Equilibrium
h^2	heritability
Inc.	Incorporated
KEEG	Kyoto Encyclopedia of Genes and Genomes
LD	Linkage Disequilibrium
LL	Low Limit
LMA	*Longissimus* Muscle Area

LOC	uncharacterized genes
MAF	Minor Allele Frequency
Mb	Megabase
MCMC	Monte Carlo Markov Chain
MHz	Mega-Hertz
mm	millimeter
MS	Musculature
MSE	Mean Squared Error
N	Number of records
NCBI	National Center for Biotechnology Information
PA	Prediction Ability
QTL	Quantitative Trait Loci
RC	Bias
REML	Restricted Maximum Likelihood
RF	Rump Fat Thickness
SD	Standard Deviations
SNP	Single Nucleotide Polymorphism
SP	São Paulo
ssGBLUP	single-step Genomic Best Linear Unbiased Prediction
ssGWAS	sinlge-step Genome-Wide Association Study
S-MGS	sire-maternal grandsire model
UL	Upper Limit
USA	United States of America

SUMMARY

CHAPTER 1 - General Considerations

1 INTRODUCTION

The demand for sustainable food production has grown and encouraged a restructuring in beef production sector in order to obtain better quality products without increasing production costs. In this way, animal breeding searches to improve the economic productivity of future generations of domestic species through selection. The most of economic interest traits in livestock has complex and quantitative expression, being influenced by several genes and affected by environmental factors. However, there is still no consensus among researchers on the best methodology for obtaining genomic prediction for each trait. There are different methods and pseudo-phenotypes used in genomic predictions, being necessary to determine the ideal for each interest trait.

The current market trend is to have animals with an appropriate carcass finishing, which remain for less time in pastures or feedlot, shortening the production cycle, allowing a higher economic return (GORDO et al., 2012). Therefore, the selection for reproductive and carcass traits obtained by ultrasound and visual scores is of fundamental importance, since its adoption will promote economic benefits to the production system. There is evidence that these traits should respond to selection, since moderate to high heritability estimates have been reported for carcass traits obtained by ultrasound and visual scores (HWANG et al., 2008; GORDO et al., 2012; GORDO et al., 2016; DO et al., 2016; MAGNABOSCO et al., 2017), indicating that it is possible to promote the carcass traits improvement in zebu breeds by selection. However, Gordo et al. 2012 estimated genetic correlations between them concluding that selection using visual scores to improve body structure, finishing precocity and musculature should lead to the same desired changes in carcass traits obtained by using ultrasonography in Nellore cattle. However, the correlated response is expected to be slower than direct selection using ultrasound records, suggesting that different set of genes influenced these traits. Concerning, the Calving Ease (CE) is determined by the calf and the dam through the direct and maternal effects, respectively. From the standpoint of the offspring, the maternal effect is purely environmental. However, it has a genetic component that could

contribute to selection purposes (DICKERSON, 1947). In this sense, estimates for direct and maternal heritabilities of CE varies from 0.072 to 0.13 and from 0.007 to 0.07 respectively (DJEMALI et al., 1987; HICKEY et al., 2007; LUO et al., 2002; MEIJERING, 1984; WELLER and GIANOLA, 1989; WELLER and RON, 1992). Additionally, there is no consensus about the genetic association between direct and genetic maternal effects for CE (BAR-ANAN et al., 1976; CARNIER et al., 1998; CUE and HAYES, 1985; DWYER et al., 1986; LUO et al., 2002; MANFREDI et al., 1991; MEIJERING, 1985; THOMPSON et al., 1981; TRUS and WILTON, 1988; WELLER et al., 1988).

Among the difficulties for inclusion and evaluation of reproductive and carcass traits in beef cattle breeding programs, it can be mentioned that they are late expression traits and difficult to measure. Considering the aforementioned, with advances in molecular biology techniques, the genomic selection (GS) has been proposed, which is performed based on Genomic Estimated Breeding Values (GEBV) predicted from a set of genetic markers, of the Single Nucleotide Polymorphism (SNP) type, covering the entire genome. This technology enables to predict the genetic merit of the animals without necessity to measure their own phenotypes or the closest relatives (MEUWISSEN et al., 2001).

In the literature, several methods to estimate breeding values were proposed, such as Bayesian methods by Meuwissen, Hayes and Goddard (2001) showing, through simulations, that these parametric methods are able to estimate genomic breeding values with remarkably high accuracy, even for animals without phenotypic records. Thus, many analytical methods have been proposed for genome-based prediction of genetic values, differing with respect to assumptions about the marker effects (MEUWISSEN; HAYES; GODDARD, 2001; HABIER et al. 2011), such as Genomic Best Linear Unbiased Predictor (GBLUP), Single-Step Genomic Best Linear Unbiased Predictor (ssGBLUP) and Bayesian models.

The apply of the most appropriate method for genomic evaluation could provide accurate predictions for reproductive and carcass finishing traits and selection for these traits could reduce the environmental impact and the beef production cost. The genome-wide association study of reproductive and carcass traits may help to better understand the main genes that affect these

traits, in addition to the functionality mechanism. Therefore, whether with Genome-Wide Association Study (GWAS) applications or genomic selection, the trend is that, more and more, marker-assisted selection will take effect in animal breeding programs, so with the availability of genomic data, this information can be incorporated into the genetic evaluations in order to increase the animals selection accuracy for reproductive and carcass traits.

2 OBJECTIVES

2.1 General Objective

The objective of this study was to compare methodologies to predict genomic breeding values and to detect SNP associated to reproductive and carcass traits obtained by ultrasound and visual scores in Nellore cattle.

2.2 Specific Objectives

The specific objectives were:

- To compare the prediction ability for carcass traits obtained by ultrasound and visual scores using different methodologies (GBLUP, ssGBLUP, BayesA, BayesB, BayesC and BayesianLASSO).
- To perform GWAS between SNP markers with body structure (BS), finishing precocity (FP), musculature (MS), back fat thickness (BF), rump fat thickness (RF), *Longissimus* muscle area (LMA) and calving ease (CE).
- To identify genomic regions, potential candidate genes, biological processes and molecular function.

3 LITERATURE REVIEW

3.1 Carcass traits obtained by ultrasound

Genetically superior animals' selection for economic interest traits has been one of the most important tools applied by breeding programs to improve

meat production aiming to increase producers' profit. In the beginning of beef cattle breeding programs, the mainly aim was to improve weight development traits, thus giving little emphasis to carcass composition indicator traits. Through the years, the meat industry observed the need to produce less-demanded muscles in the carcass into restructured products that were uniform in appearance and eating qualities (GRUNERT et al., 2004). However, from an economic viewpoint, the carcass composition indicator traits are considerable important, since the slaugter time antecipation is linked directly to efficiency and profitability of beef production. In this way, the genetic improvement of carcass composition indicator traits is relevant to maintain Brazil's position as the largest beef exporter.

The animal carcass has different muscles and fat depots, which according to their characteristic and localization may determine the price of different commercial cuts. There are several ways to evaluate the carcass quality to improve meat organoleptic characteristics. Some methods have the disadvantage to need to slaughter the animal, besides the long time and high price for evaluation. The ultrasound procedure is considered as a low cost technology and easy for application (STOUFFER, 2004), that is non-invasive procedure and allows evaluation without leaving residue in the meat. This technology has been used to evaluate some carcass traits, such as *Longissimus* muscle area and subcutaneous fat thickness (WILSON, 1992; FIGUEIREDO, 2001; YOKOO, 2009; BARBOSA et al., 2010).

The most important trait to carcass composition measurement is the *Longissimus* muscle area (LMA), which indicates the muscle amount, composition and noble cuts (GESUALDI JÚNIOR, et al., 2006). Other traits applied as carcass quality indicator are back fat thickness (BF) and rump fat thickness (RF), which are related to products quality, playing a key role in reducing the cold shortening, during carcass cooling processes, directly influencing the production system progress (CAETANO et al., 2013).

In Nellore breed, heritabilities estimates for LMA, BF and RF ranged from moderately to highly heritable (YOKOO et al., 2007; MAGNABOSCO et al., 2009; YOKOO et al., 2010), indicating that these carcass traits presented high genetic variability and may respond to individual selection immediately.

The meat production from Nellore (*Bos taurus indicus*) has been focused on quantitative level without standardization, which is not properly approved in most demanding markets. In addition, the restructure in beef production sector has been motived by the growing global demand for safe and sustainable food production (EUCLIDES FILHO et al., 2003; LOPES et al., 2012). Thus, to supply the international consumer demand, the genetic evaluations have been also focused on quality carcass traits aiming to improve the meat quality production without increasing the costs.

3.2 Carcass traits evaluated by visual scores

The knowledge of the productive genetic potential that influences the most relevant economic importance traits in beef cattle production is fundamental in the selection programs design. The visual evaluation scores have been used in some breeding programs aiming carcass useful results (DAL-FARRA; ROSO and SCHENKEL, 2002). In this sense, Koury Filho (2005), Yokoo et al. (2009) and Koury Filho et al. (2009) found moderate to high magnitude heritabilities for body structure (BS), finishing precocity (FP), musculature (MS), which may be explained by the morphological variability of Nellore herds.

The visual evaluation methodology has two practical applications in the selection process. First, may recognize the most negative and positive points that exist simultaneously in the animal. Second, may analyse morphological defects and qualities more constant in a simple and direct way, through the animal images obtained by scores, in the evaluation within the herd (KOURY FILHO et al., 2009). Studies realized in Nellore cattle verified that these traits presented accelerated response to direct selection (KOURY FILHO, 2005; FARIA et al., 2009).

The visual scores are evaluated by trained technicians, who first observed the entire management group, and then evaluated the average profile for each trait by sex, which served as a baseline. Then scores from 1, that represents lowest expression, to 6, highest expression, adapted from the method proposed by Koury Filho et al. (2010), were given to animals. The BS description visually predicts the body area as seen from the side, so body

length and rib depth. Small animals in the management group receive score 1, which gradually increases to 6 to large animals. For FP, animal strategical points are evaluated, in which the visual assessment scores of the rib depth in relation to the limbs length are carried out. Animals presenting shorter rib depth and longer limbs are frequently less precocious than those with longer rib depth and shorter limbs. Precocious phenotype requires less time for fat deposition, being desired in order to identify possibly earlier animals. Score 1 is related to animals with lower precocity and score 6 to early animals. MS iss assessed by the muscle mass distribution in the body and the muscles convexity in the carcass. Scores 1 to 6 are assigned to animals with muscle mass volume varying from thinner to thicker, respectively.

Visual scores selection brings economic benefits in medium and long period in beef cattle, with uniformity possibility of appropriate morphological types to different production systems (KOURY FILHO et al., 2009; FARIA et al., 2009). In this way, visual scores have been used to identify animals with different body frames with more adequate performance under feasible breeding conditions (GORDO et al., 2012).

3.3 Reproductive trait

Reproductive traits are of major economic to the sustainable breeding of beef cattle worldwide. Of these traits, calving ease (CE) is the essential maternal ability that must be considered among the breeding objectives of any beef cattle breed (PHOCAS et al., 1988; ROUGHSEDGE et al., 2005; ENNS et al., 2008; MCHUGH et al., 2014). Calving difficulty should be limited because it markedly affects the welfare of both the cow and calf and the profitability of herds, because of increased labour and veterinary costs, calf mortality rates and the time before a cow can breed again.

In Nellore beef cattle, birth weight plays an important role in a calf's ability to express its genetic potential, but should be supervisioned to prevent dystocia, since calving ease in this breed may be associated with moderately low birth weights (ARAÚJO et al., 2014; KAMEI et al., 2017). Hence, although a better herd management, such as heifer rearing and feeding during gestation, could improve calving difficulty, the direct selection and a proper breeding

approach targeting better CE might be a better choice in the long term (DEKKERS, 1994).

The CE is determined by the calf as well as by its dam, respectively, through effects termed direct and maternal. From the standpoint of the offspring, the maternal effect is purely environmental. However, it has a genetic component and thus could contribute to selection response (DICKERSON, 1947). In this sense, literature estimates of heritabilities on CE in cattle ranging from 0.072 to 0.13 for direct heritability and from 0.007 to 0.07 for maternal heritability (MEIJERING, 1984; DJEMALI et al., 1987; WELLER and GIANOLA., 1989; WELLER and RON, 1992; LUO et al., 2002; HICKEY et al., 2007). Moreover, some studies shown a negative genetic correlation between direct and maternal effects (CARNIER et al., 1998; CUE and HAYES, 1985; DWYER et al., 1986; MEIJERING, 1985; THOMPSON et al., 1981; TRUS and WILTON, 1988; WELLER et al., 1988), while others have reported a positive correlation (BAR-ANAN et al., 1976; MANFREDI et al., 1991b; LUO et al., 2002).

In Zebu cattle, the inclusion of reproductive traits in selection indices composition, such as age at first calving and early heifer pregnancy, is leading to earlier conception, gestation and calve of primiparous heifers. The early heifer pregnancy trait has moderate to high heritability, so the proportion of calves less than 30 months has been increasing in breeding programs as a result of selection for this trait, but has also been increasing problems of calving difficulty, which is something rare in the Nellore breed. Calving too early may be associated with an increased in calving difficulty or even dystocia (EASTHAM et al., 2018). Therefore, there is a growing concern about the reproductive performance of the females, in particular to calving events leading to economic losses in the farm (ALAM et al., 2017) and better explore the genetic structure harboring calving ease.

3.4 Genomic selection

Genomic selection is a form of marker-assisted selection on a genome-wide scale (MEUWISSEN et al., 2013). The effect of thousands of DNA (deoxyribonucleic acid) markers is simultaneously estimated combining phenotypic information, which can be used for the breeding value estimation

and selection candidates. Several methods to obtain the marker effects have been strongly studied and compared in order to find the most suitable for predicting genomic values for different traits.

Marker effects can be obtained assuming that all makers contributed equally to genetic variation (no major gene effect), or assuming that the prior distribution of marker or QTL (Quantitative Trait Loci) effects is not normal. The genomic BLUP estimates all marker effects at the same time and assumes the same variance for all SNP (MEUWISSEN et al., 2013). A disadvantage of this method is the overestimation of the markers variance with no effect and underestimation of the high effect markers variance, which can harm the accuracy of prediction. Therefore, accuracy of prediction is strongly dependent on many factors such as linkage disequilibrium (LD) (MEUWISSEN; HAYES; GODDARD, 2001), effective population size (GODDARD, 2009), marker density (MOSER et al., 2009), allele frequency distribution (LETTRE, 2011), number of genotyped animals (VAN RADEN et al., 2009; DAETWYLER et al., 2010; CALUS, 2011), the traits heritability and the method used to estimate marker effects (LOURENCO et al., 2014).

Genomic predictions can also be obtained considering different presuppositions in the model. The assumption that all SNP effects are normally distributed with a constant variance may be unrealistic (MEUWISSEN et al., 2013). In different circumstances, if phenotypes, pedigrees, and genotypes are available, a simple way to incorporate genomic information into evaluations it is by the ssGBLUP (MISZTAL et al., 2009). This approach consists of incorporating phenotypes, pedigrees and genomic information into only one step of evaluation. With this procedure, the relationship matrix based on pedigree (A) is combined with a genomic relationship matrix (G) based on information from SNP markers, into a single matrix of realized relationships (H).

Aguilar et al. (2010), comparing GBLUP and ssGBLUP methodologies, concluded that genomic evaluations using ssGBLUP were as accurate as those using multistep procedure. According to Lourenco et al. (2014), ssGBLUP has an advantage over multistep methods mainly because it uses phenotypes rather than pseudo-phenotypes and accounts for the entire population structure to estimate GEBV. Onogi et al. (2014) also concluded that genomic selection

implementation by ssGBLUP provided more accurate predictions than traditional BLUP even using only genotyped sires.

Another methodology used is the Bayesian method, which assumes different genetic variance across SNP with major effect. The dairy cattle genetic evaluation in the United States of America is currently performed by multistep methods if genomic information is available (VAN RADEN, 2008; VAN RADEN et al., 2009), this approach consists of predicting the GEBV (Genomic Estimated Breeding Values) by an index combining EBV (Estimated Breeding Values) and DGV (Direct Genomic Values).

The Bayesian approach may or not assume different variances on all chromosomes segments, considering that few SNPs have high effect and the majority small or null effect (VAN RADEN, 2008). Frequently, the animals number is smaller than the marker effects number to estimate, so the final marker effect estimates are strongly influenced by the prior information (MEUWISSEN et al., 2013). Pryce et al. (2012) found an advantage in the accuracy of genomic predictions for RF using Bayesian models over GBLUP, in Australian heifers. Also, Neves et al. (2014) reported that Bayesian regression models were more accurate than GBLUP in a Nellore population. In addition, Fernandes Júnior et al. (2016) worked with data from, approximately, 1,500 Nellore males for *Longissimus* muscle area, back fat thickness and hot carcass weight, they estimated empirical prediction accuracies considering three models (Bayesian Ridge Regression, BayesC and BayesianLASSO). The authors concluded that all models presented similar predictive performance, although BayesianLASSO, BayesC and Bayesian Ridge Regression showed the highest accuracies for *Longissimus* muscle area, back fat thickness and hot carcass weight, respectively. All these accuracies were calculated using the phenotype adjusted for fixed effects.

3.5 Genome-Wide Associaiton Study (GWAS)

The GEBV obtained can be used in association studies of economic important traits. The definition of Genome-Wide Associaton Study (GWAS) is finding associations between variants of SNPs with a specific trait by assuming that SNP is in LD with the causative mutation causing variation in the trait

(HAYES and GODDARD, 2010). The first successful GWAS was published by Klein et al. (2005), which worked with human data. In livestock, GWAS has gained popularity in mapping QTL to the economic important traits. Usually, due to different genetic architecture of breeds and polygenic nature of complex traits, different regions and genes are found to be associated with the same trait in different breeds of the same species (SHARMA et al., 2015).

The challenges of GWAS consist to choose carefully and strategically a homogeneous population for the study and to account for population stratification. If prudently chosen, the statistical models may be useful to minimize the chances of false positive associations. Consequently, the integration of all genotypes, pedigree, and phenotypic information available (from genotyped and ungenotyped animals) in one-step procedure (single-step GWAS) which consists in an alternative to simplify the traditional GWAS, allowing the use of any model, and all relationships simultaneously (WANG et al., 2012; WANG et al., 2014; FRAGOMENI at al., 2014). Most of the genomic studies for carcass indicator traits have been applied for taurine breeds in temperate regions (LU et al., 2013; ROBERTS, 2018), some researches have also been carried out in indicine breeds (ESPIGOLAN et al., 2015; ZHOU et al., 2016; SILVA et al., 2017). However, it is necessary to perform studies in different indicine cattle populations due to differences in environmental and management conditions, and also differences in allele frequency of genetic markers and QTL that would greatly influence the traits.

The GWAS has proved to be an ideal method to identify genes associated with various phenotypes and to elucidate the complex traits mechanisms. Therefore, the GWAS findings could be incorporated in a genetic evaluation model to increase accuracy of genetic prediction (BOICHARD et al., 2012) in domestic animals.

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CHAPTER 2 - Genomic regions and enrichment analyses associated with carcass composition indicator traits in Nellore cattle

ABSTRACT

SILVA, R. P. **Genomic regions and enrichment analyses associated with carcass composition indicator traits in Nellore cattle.** 2021. Dr. Thesis – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

The aim of this study was to estimate genetic parameters and identify genomic regions associated with carcass traits obtained by ultrasound and visual scores in Nellore cattle. Data from approximately 66,000 animals from the National Association of Breeders and Researchers (ANCP) were used. The variance components for back fat thickness (BF), rump fat thickness (RF) and *Longissimus* muscle area (LMA) were estimated considering a linear model whereas a threshold model for body structure (BS), finishing precocity (FP) and musculature (MS) traits. The SNP solutions were estimated using the ssGBLUP approach by considering windows of 10 consecutive SNPs. Regions that accounted for more than 1.0% of the additive genetic variance were used. Genes identified within the significant windows, such as *FOXA3*, *AP2S1*, *FKRP*, *NPASI* and *ATP6V1G1* were found to be related with MS, while *OMA1* and *FFGY* with BS and FP traits. The *PLTP*, *TNNC2* and *GPAT2* genes were found in the regions associated with LMA, as well as *TKT*, *FNDC5* and *CHRND* can strongly be related with fat deposition. Gene enrichment analysis revealed processes that might be directly influenced the organism growth and development. These results should help to better understand the genetic and physiological mechanisms regulating growth and body composition, muscle tissue development and subcutaneous fat expression, and this information might be useful for future genomic studies in Nellore cattle.

Keywords: Beef cattle. *Bos taurus indicus*. GWAS. Ultrasonography measurement. Visual score traits.

1 INTRODUCTION

Although the Nellore breed (*Bos taurus indicus*) has shown an outstanding contribution to the cow-calf industry in tropical and subtropical countries, research has reported that beef cuts from Nellore cattle often displays lower quality grades and more variable tenderness than those from *Bos taurus taurus* breeds (CUNDIFF, 2004; PEREIRA et al., 2014). The genetic evaluation for carcass traits is expensive and time-consuming since the information used to predict carcass merit is collected through structured carcass sire progeny tests (GORDO et al., 2012). However, faster and less expensive ways have been innovated to assess live animal carcass traits, such as carcass ultrasonography and visual scoring for precocity, muscling and conformation.

The carcass traits obtained by ultrasound and visual scores have moderate to high heritability, allowing reliable genetic merit prediction in several beef cattle breeds (RILEY et al., 2002; REVERTER et al., 2003; GORDO et al., 2016). Gordo et al. (2012) reported positive and favorable genetic correlations between ultrasound records and visual scores, concluding that both traits could be used to improve body structure, finishing precocity, muscling and carcass traits. Besides, the feasibility of visual scores to evaluate *postmortem* carcass traits in Nellore cattle was also confirmed by Gordo et al. (2016). Despite the selection advantages of carcass indicator traits, i.e. moderate to high heritability estimates and its measurement can be done directly in the selection candidates, it is still a common practice in beef cattle breeding programs a progeny-testing for unproven sires. Thus, reliable genetic evaluations earlier in animal's lifetime are necessary and appealing together with the application of technologies to increase the genetic evaluation reliability's and decrease the generation interval. In this sense, the use of genomic information can lower costs associated with progeny test and accelerate the genetic progress (MILLER, 2010).

Carcass traits obtained by ultrasound and visual scores are complex quantitative traits, which are believed to be influenced by many genomic polymorphisms of individually small effect (BOLORMAA et al., 2013). Genomic prediction reliability is influenced by the genetic architecture of the trait, which is particularly characterized by the number of loci that affect such trait and the

distribution size of the effects (HAYES et al., 2010). Genomic predictions rely on the presence of common genomic variants with a common substitution effect within each cattle breed. Consequently, it is relevant to determine the number of variants and the size of their effects on carcass indicator traits to increase the accuracy of genomic predictions.

Most of the significant genomic regions described in Genome-Wide Association Studies (GWAS) for carcass indicator traits in cattle were performed mainly in taurine breeds (LU et al., 2013; ROBERTS, 2018), suggesting that there is a deficit of studies with indicine cattle in tropical regions. In this sense, Silva et al. (2017) and Zhou et al. (2016) study in Nellore cattle applying the single-step genomic BLUP and copy number variation approach, respectively, have reported several genes associated with carcass traits, i.e. conformation, precocity and muscling, obtained by ultrasound and visual scores. In order to identify genomic regions associated with carcass indicator traits as well as to elucidate the genetic basis of them, it is necessary to encourage genomic studies since the indicine cattle is prevalent in herds under tropical and subtropical conditions.

2 OBJECTIVES

The aim of this study was to estimate genetic parameters and identify genomic regions associated with carcass indicator traits obtained by ultrasound and visual scores in Nellore cattle.

3 MATERIAL AND METHODS

3.1 Phenotypic Data

The phenotypic and genotypic information was provided by the National Association of Breeders and Researchers (ANCP, Ribeirão Preto-SP, Brazil). The dataset contained information from 18 Nellore herds distributed in the Southeast and Midwest regions of Brazil. The animals were pasture-reared in low throughput production systems, with or without the use of creep feeding and supplementation.

Visual scores of body structure (BS), finishing precocity (FP), and muscling (MS) were obtained (*in situ*) at yearling with approximately 550 days of age. Visual evaluation was performed by ten trained technicians, however, one technician performed almost 90% of the visual evaluations. Technicians underwent annual training to maintain visual evaluation standards and minimize the errors. The effect of the technician was nested within the contemporary group (CG) since the same person evaluated the entire CG, and therefore, it was not necessary to address this effect in the model. Visual evaluations were performed as follows: first, the trained technician observed the entire management group, separated for males and females, and then evaluated the average profile for each trait by sex, which served as a baseline. Then scores from 1 (lowest expression) to 6 (highest expression), adapted from the method proposed by Koury Filho et al. (2010), were given to the animals. According to Koury Filho (2005), BS is a visual estimate of the area of the animal when seen from the side, basically evaluating body length and rib depth. Greater areas correspond to higher scores. In the case of FP, higher scores are attributed to animals with greater rib depth in relation to the length of their limbs. Muscling is evaluated by visual evidence of muscle mass. Higher scores are attributed to “thicker” animals with more convex muscles and lower scores to “thinner” animals with a less convex rectilinear musculature and even concavities in the body.

Scanning and image analyses were carried out by technicians, equipment, and software accredited by ANCP following the Ultrasound Guidelines Council criteria. Animals were scanned for *Longissimus* muscle area (LMA) between the 12^{th} and 13^{th} ribs, back fat thickness (BF) over the *Longissimus* muscle at a point three-fourths the length ventrally of the LMA, and rump fat thickness (RF) at the junction of the *Biceps femoris* and *Gluteus medius* between the ischium and ilium and parallel to the vertebrae (GORDO et al., 2012). The carcass traits evaluated by ultrasonography were obtained using an ALOKA 500 V device, with a 3.5 MHz linear probe, measured in millimeters (mm). The CG were defined as follows: i) sex, farm, year, season of birth, and management group at 450 days of age for LMA, BF and RF; and ii) sex, farm, year, season of birth, and management group at 550 days for BS, FP and MS. Records with ±3.5 standard deviations from the CG mean and CG that had at

least 5 animals were considered. The overall structure of the data set analyzed is shown in Table 2.1.

Table 2.1 - Descriptive statistic for carcass traits obtained by ultrasound records and visual scores

Traits[1]	N	Mean	Mode	SD	Minimum	Maximum
BS	6,494	3.80	4	1.30	1	6
FP	6,494	3.90	4	1.31	1	6
MS	6,494	3.72	4	1.36	1	6
BF (mm)	12,831	2.77	---	1.71	0.45	18.54
RF (mm)	12,814	3.91	---	2.09	0.51	23.88
LMA (cm²)	12,827	55.73	---	12.30	19.32	104.64

[1]BS= body structure; FP= finishing precocity; MS= musculature; BF= back fat thickness; RF= rump fat; LMA= *Longissmus* muscle area.
Source: Elaborated by the authors.

3.2 Genomic data

A total of 963 animals were genotyped with the Illumina Bovine HD BeadChip (Illumina Inc., San Diego, CA, USA), which contains 777,962 markers. These animals were used as a reference population to impute genotypes from 8,652 animals genotyped with a low-density panel (Clarifide Nellore 2.0) containing roughly 20,000 markers. Imputation was implemented using FImpute 2.2 software (SARGOLZAEI; CHESNAIS; SCHENKEL, 2014). Prior to imputation, markers were edited for call rate in both populations and those with a value lower than 90% were excluded. After imputation, markers with a minor allele frequency (MAF) lower than 0.05, call rate lower than 90%, and failing Hardy-Weinberg Equilibrium (HWE) at a threshold of > 0.01 were not considered in the subsequent analyses. Markers with redundant position and those located in non-autosomal chromosomes were also excluded. After editing, a total of 8,545 genotyped animals and 460,838 markers were retained for the analyses.

3.3 Genetic parameter estimation

The variance components for LMA, BF and RF were estimated considering a linear model whereas a threshold model was applied for BS, FP

and MS. For all traits, a single step genomic BLUP (ssGBLUP) procedure was used (AGUILAR et al., 2010). The ssGBLUP is a modified version of the animal model (BLUP) with the additive relationship matrix (A^{-1}) replaced by H^{-1} (LEGARRA et al., 2014), as follows:

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

where, **H** is the relationship coefficients matrix between the animals, $\mathbf{A^{-1}}$ is the inverse of relationship matrix based on pedigree information, $\mathbf{G^{-1}}$ is the inverse of the genomic relationship matrix, which was constructed and scaled as described by VanRaden (2008), and $\mathbf{A_{22}^{-1}}$ is the inverse of the pedigree-based relationship matrix for genotyped animals.

The random additive genetic and residual effects and the fixed effects of CG were included in the model. The general animal model used was:

$$\mathbf{Y = X\beta + Za + e}$$

where, **y** is the observations vector; **β** is the vector of fixed effects; **a** is the vector of additive genetic effects; **X** is the incidence matrix for fixed effects; **Z** is the incidence matrix for additive genetic effects; **e** is the residual effect vector. For the linear animal model was assumed that **E[y]=Xβ**; the direct additive genetic and residual effects were normally distributed with means of zero and $\mathbf{Var(g)= H\otimes S_a}$; $\mathbf{Var(e)= I\otimes S_e}$; in which $\mathbf{S_a}$ is the additive genetic variance matrix; $\mathbf{S_e}$ is the residual variance matrix and **I** is the identity matrix.

For BS, FPS and MS analyses a threshold model was adopted, assuming that the underlying distribution (**U**) is determined by:

$$\mathbf{U\sim MVN\ (X\beta + Za + I\sigma_e^2)}$$

Priori distributions for additive genetic effect and residual followed the multivariate normal distributions, as follows:

$$P(a|\sigma_a^2) \sim MVN(0,\sigma_a^2)$$

$$P(e|\sigma_e^2) \sim MVN(0,\sigma_e^2)$$

After defining the model parameters, the link between categorical and continuous scales could be established based on the contribution of the probability of an observation that fit the first category, which is proportional to:

$$P(y_r=0|t,\theta)=P(U_r<t|t,\theta)=\emptyset\left((t-W_r'\theta)/\sigma_e\right)$$

where, $\mathbf{y_r}$ is the response variable for the r-th observation, with scores values ranging from 1 to 6, with 6 corresponding to the highest expression of the trait and 1 to the lowest expression; **t** is the threshold value arbitrarily assigned as the true value is unobservable; $\mathbf{U_r}$ is the value of the underlying variable for the r-th observation; **ø** is the cumulative distribution function of a standard normal variable; $\mathbf{W'_r}$ is the scale of the incidence matrix that linked ∅ to the r-th observation ; ∅ = (b', a') is the vector of the parameters of s with b (systematic effects) and a (random effects).

The analyses for BF, RF and LMA were performed using the restricted maximum likelihood (REML) method with the AIREMLF90 software (MISZTAL et al., 2015). For BS, FP and MS, the Bayesian analyses were carried out using the THRGIBBSF90 software (MISZTAL et al., 2015). In Bayesian analyses, Gibbs chains of 500,000 iterations were generated with an initial burn-in of 100,000 and a sampling interval of 100. Heritability and variances were calculated with the samples generated in every interaction, and subsequently, the mean of the samples were estimated. The highest posterior density (HPD) interval was constructed for all variance components and genetic parameters estimated at a 90% level of credibility. The convergence was tested using the Bayesian Output Analysis (BOA) implemented in R (2010) program.

3.4. Genome-Wide Association Study (GWAS)

The GWAS analysis was performed using the single-step GWAS (ssGWAS) methodology (WANG et al., 2012). The same linear models described for variance component estimation for BF, RF and LMA, and threshold models for BS, FPS and MS were applied in the ssGWAS. The animal effect was decomposed in genotyped (a_g) and non-genotyped (a_n) animals, as describe by Wang et al. (2012), considering the effect of genotyped animals as:

$$\mathbf{a_g = Zu}$$

where, **Z** is a matrix that relates genotypes of each locus and **u** is a vector of marker effects, and the variance of animal effects was assumed as:

$$\mathbf{var(a_g) = var(Zu) = ZDZ'\sigma_u^2 = G^*\sigma_a^2}$$

where, **D** is a diagonal matrix of weights for variances of markers (**D=I** for GBLUP), $\boldsymbol{\sigma_u^2}$ is the genetic additive variance captured by each SNP marker when no weights are present, and **G*** is the weighted genomic relationship matrix. The ratio of covariance of genetic effects ($\mathbf{a_g}$) and SNPs (**u**) is:

$$\mathbf{var}\begin{bmatrix} \mathbf{a_g} \\ \mathbf{u} \end{bmatrix} = \begin{bmatrix} \mathbf{ZDZ'} & \mathbf{ZD'} \\ \mathbf{DZ'} & \mathbf{D} \end{bmatrix} \boldsymbol{\sigma_u^2}$$

Sequentially:

$$\mathbf{G^* = \frac{var(a_g)}{\sigma_a^2} = \frac{var\,(Z_u)}{\sigma_a^2} = ZDZ'\lambda}$$

where, **λ** is a variance ratio or a normalizing constant. According to VanRaden et al. (2009),

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

where, **M** is the number of SNP and $\mathbf{p_i}$ is the allele frequency of the second allele of the i^{th} SNP. According to Strandén and Garrick (2009), the markers effect can be described by:

$$\hat{\mathbf{u}}= \frac{\sigma_u^2}{\sigma_a^2} \mathbf{DZ'G^{*-1}\hat{a}_g=DZ'[ZDZ']^{-1}\hat{a}_g}$$

The estimated SNP effects can be used to estimate the variance of each individual SNP effect (ZHANG et al., 2010) and apply a different weighting for each marker, such as:

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

The following iterative process described by Wang et al. (2012) was used considering **D** to estimate the SNP effects:

1. **D=I**;
2. To calculate the matrix **G=ZDZ´q**;
3. To calculate GEBVs for all animals in data set using ssGBLUP;
4. To calculate the SNP effect: $\hat{\mathbf{u}}= \lambda \mathbf{DZ'G^{*-1}\hat{a}_a}$;
5. To calculate the variance of each SNP: $\mathbf{d_i}= \hat{\mathbf{u}}_i^2 2\mathbf{p_i}(1-\mathbf{p_i})$, where **I** is the *i-th* marker;
6. To normalize the values of SNPs to keep constant the additive genetic variance;
7. Exit, or loop to step 2.

The markers effects were obtained by three iterations from step 2 to 7. The percentage of genetic variance explained by *i-th* region was calculated as described by Wang et al. (2014):

$$\frac{\mathbf{Var}(\mathbf{a_i})}{\sigma_a^2}\mathbf{x100} = \frac{\mathbf{Var}\left(\sum_{j=1}^{10} \mathbf{Z_j}\,\hat{\mathbf{u}}_j\right)}{\sigma_a^2}\mathbf{x100}$$

where, $\mathbf{a_i}$ is genetic value of the i-th region that consists of 10 consecutive SNPs, σ_a^2 is the total genetic variance, $\mathbf{Z_j}$ is vector of gene content of the j-th

SNP for all individual, and $\hat{\mathbf{u}}_j$ is marker effect of the *j-th* within the *i-th* region. The results were presented as the proportion of variance explained by each window of 10 SNPs.

3.5 Enrichment analysis

The chromosomal regions explaining more than 1.0% of the additive genetic variance were selected to explore and determine possible QTL. The windows were defined by 10 continuous adjacent SNPs. The bovine genome Map Viewer UMD3.1 available at "National Center for Biotechnology Information" (NCBI - http://www.ncbi.nlm.nih.gov) and the Ensembl Genome Browser (*Bos taurus* genes UMD3.1) (http://www.ensemble.org/index.html) were used for gene identification. GeneCards (http://www.genecards.org/) were used to describe annotated gene functions. Database for Annotation, Visualization, and Integrated Discovery (DAVID) v. 6.8 tool (HUANG; SHERMAN; LEMPICKI, 2009) was used to identify significant Gene Ontology (GO) ($p \leq 0.01$) terms and KEEG ($p \leq 0.05$) (Kyoto Encyclopedia of Genes and Genomes) pathways using the list of genes from the windows of 10 adjacent SNPs and the *Bos taurus taurus* annotation file as background.

4 RESULTS AND DISCUSSION

4.1 Genetic parameter estimates

Moderate heritability estimates for RF and BF, and high for BS, FP, MS and LMA were obtained (Table 2.2). The heritabilities estimated for carcass composition indicator traits indicated that genetic progress for these traits is feasible, and so, they would respond favorably to direct selection. Several studies in Nellore and Brahman cattle reported heritability estimates for BS, MS, FP, BF, RF and LMA ranging from 0.24 to 0.63, 0.29 to 0.48, 0.38 to 0.44, 0.10 to 0.36, 0.28 to 0.40 and 0.33 to 0.50, respectively (KOURY FILHO et al., 2009; GORDO et al., 2012; SMITH et al., 2007; MAGNABOSCO et al., 2017; KLUSKA et al., 2018). In temperate and tropically adapted beef breeds, the heritability estimates for BF, RF and LMA ranged from 0.30 to 0.38, 0.30 to 0.36

and 0.30 to 0.39, respectively (REVERTER et al., 2003). In an extensive review, Burrow et al. (2001) stated that the carcass composition indicator traits were moderately to highly heritable, indicating that these traits should respond adequately to selection.

Table 2.2 **–** Variance components and heritability estimates for carcass traits obtained by ultrasound records and visual scores

Traits[1]	σ_a^2	σ_e^2	h²	SD	HPD (LL)[2]	HPD (UL)[2]
BS	---	---	0.31	0.12	0.06	0.52
FP	---	---	0.44	0.16	0.11	0.74
MS	---	---	0.46	0.17	0.14	0.75
BF	0.14	0.69	0.17	0.06	---	---
RF	0.39	1.06	0.27	0.07	---	---
LMA	12.36	25.36	0.32	0.02	---	---

Additive genetic variance (σ_a^2), residual variance (σ_e^2), mean heritability (h²), Standard Deviation (SD), Low Limit (LL) and Upper Limit (UL).
[1]BS= body structure; FP= finishing precocity; MS= musculature; BF= back fat thickness; RF= rump fat thickness; LMA= *Longissimus* muscle area.
[2]95% highest posterior density of the estimates.
Source: Elaborated by the authors.

4.2 Associated Genomic Regions

A total of 10, 15, 11, 5, 5 and 7 genomic regions were identified for BS, FP, MS, BF, RF and LMA, respectively. The results displayed several genes with described biological functions within these regions (Tables 2.3, 2.4, 2.5, 2.6, 2.7 and 2.8).

4.2.1 Body structure (BS)

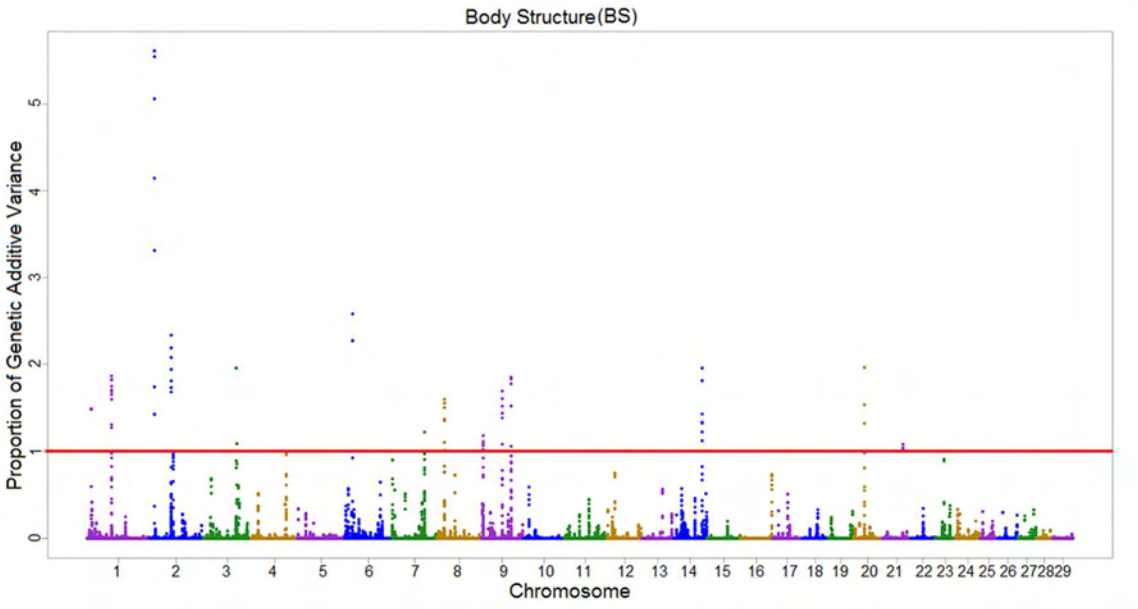
A total of 95 genes were identified within the genomic regions that explained more than 1.0% of the additive genetic variance for BS (Table 2.3), in which 48 of them had described biological functions and 47 were uncharacterized genes (Figure 1).

Table 2.3 - Genomic regions associated with body structure (BS)

BTA	Position (pb)	Candidate Genes	% variance explained by SNPs windows
1	9497908 - 9517678	CYYR1, LOC101903497, APP, TRNAR-CCU	1.49
1	63283014 - 63294181	TRNAR-GCG, LOC104970860, LOC104970861	1.87
2	12721239 - 12748110	LOC101907972, LOC787311, LOC100848878	5.61
2	56442434 - 56467655	TRNAC-GCA, LRP1B	2.34
3	86681730 - 86692101	C3H1orF87, LOC530929, LOC530875, LOC521656, LOC511936, LOC104971726, CYP2J2, HOOK1, LOC101903193, LOC104971742, LOC104971741, FGGY, LOC104971740	1.96
3	88010321 - 88040365	LOC104971748, LOC101903356, TRNAG-CCC, LOC100294994, JUN, MYSM1, TACSTD2, OMA1, LOC101903696, DAB1	1.09
6	21820803 - 21840435	LOC101905424, LOC101905590, LOC539947, LOC101905713, LOC539947	2.58
7	82527083 - 82553887	TENM2, MIR2462, WWC1, LOC101906156, RARS, LOC104968969, LOC529551, ZFYVE16, FAM151B, ANKRD34B, DHFR, MSH3, FBLL1	1.22
8	18415840 - 18441489	LOC101905259, TUSC1, LOC104969284	1.60
9	5526248 - 5586598	_____	1.18
9	55571261 - 55585039	MANEA, LOC101907134, LOC104969573, LOC104969572	1.69
9	78155378 - 78200348	ECT2L, REPS1, ABRACL, HECA, TXLNB, CITED2, LOC104969607, LOC100296379, TRNAC-GCA, LOC101902392	1.85
14	73018946 - 73043341	CDH17, LOC104974128, PDP1, LOC104974129, TRNAC-GCA, TMEM67, RBM12B, FAM92A, LOC101908004, BCL-XLP1	1.96
20	26014190 - 26028311	NDUFS4, LOC104975250, FST, LOC782165, LOC104975251, MOCS2, ITGA2, LOC104975252, ITGA1, PELO, LOC104975253	1.97
21	52490155 – 52521231	LRFN5, LOC104975419, LOC104972837, LOC104975421, LOC785161, LOC104975420	1.08

Source: Elaborated by the authors.

Figure 1 - Manhattan plot of the additive genetic variance explained by windows of 10 adjacent SNPs for body structure (BS) trait in Nellore cattle (the dots above the red line indicates the regions explaining more than 1.0% of the additive genetic variance)



Source: Elaborated by the authors.

Several genes were identified in *Bos taurus* autosomes (BTA) with functions that could better elucidate the mechanisms underlying this trait. The *CITED2* gene (Cbp/p300 Interacting Transactivator with Glu/Asp Rich Carboxy-Terminal Domain 2) located on BTA9 (78.15-78.20 Mb) express a protein in the skeletal muscle as well as other muscle cellular elements (DE GASPERI et al., 2016), and has been shown to play critical roles in mesenchymal origin tissues (LEONG et al., 2011). Despite having more two windows on BTA9 explaining 1.18% (5.53-5.59 Mb) and 1.69% (55.57-55.59 Mb) of the additive genetic variance of BS, no candidate gene was identified within these regions.

The *FST* gene (Follistatin) located on BTA20 (26.01-26.03 Mb) is highly expressed in ovary, skin and skeletal muscle in mammalians (TORTORIELLO et al., 2001), and it is known to have a prominent importance on muscle growth and development (AMTHOR et al., 2002). It is worth to highlight that the *FST* gene is known for its contribution to the myostatin pathway, which has been considered as a novel and unique negative regulator of muscle growth, and it appears to be involved in muscle homeostasis in adults as its expression is regulated during muscle atrophy (DOMINIQUE and GÉRARD, 2006).

4.2.2 Finishing precocity (FP)

A total of 208 genes were identified within the genomic regions explaining more than 1.0% of the additive genetic variance for FP (Table 2.4), in which 111 of them had described biological functions and 97 were uncharacterized genes (Figure 2).

Table 2.4 - Genomic regions associated with finishing precocity (FP)

BTA	Position (pb)	Candidate Genes	% variance explained by SNPs windows
1	63994643 - 64013367	LOC104970861, LOC104970862, LOC104970863, LOC104970864, IGSF11, LOC101901963	2.75
1	85677014 - 85698704	LOC104970936, LOC784318, TRNAR-UCU, LOC104970937, LOC104970938, LOC104970939, LOC104970941, LOC100849009, SOX2, LOC104970940, TRNAG-GCC	1.83
3	87539902 - 87583593	LOC104971740, FGGY, LOC104971743, LOC104971744, LOC104971745, LOC104971746, LOC104971747, LOC101903276, LOC104971748, LOC101903356, TRNAG-CCC, LOC100294994, JUN, MYSM1, TACSTD2, OMA1	2.34
6	57420282 - 57464144	ARAP2, LOC101906389, DTHD1, LOC104972734, LOC104972735, LOC100295559	1.01
11	13060084 - 13093451	LOC104973296, LOC104973297, LOC104973298, DYSF, LOC101903215, ZNF638, LOC100847214, PAIP2B, NAGK, TEX261, ANKRD53, ATP6V1B1, VAX2, LOC101905773, CD207, CLEC4F	2.02
12	18521528 - 18537665	LOC104973550, LOC783657, ITM2B, LOC101903713, TRNAE-UUC, LOC506251, LOC101903855, RB1, LPAR6, RCBTB2, CYSLTR2, LOC101903993, LOC101903925, TRNAW-CCA, FNDC3A, MLNR, LOC101904090, CDADC1, LOC104970162, CAB39L	2.32

Continue

Table 2.4 - Genomic regions associated with finishing precocity (FP)

13	16132850 - 16154301	LOC104973714, LOC101903221, GATA3, LOC104973715, TAF3, ATP5F1C, KIN, ITIH2, LOC100138864, ITIH5, LOC104973716, LOC101903501, SFMBT2	2.15
14	19653606 - 19687616	LOC100139328, LOC522769, HAS2, LOC515601, TRNAM-CAU, LOC104974009, LOC104974010	1.21
16	80055459 - 80070780	LOC101905566, PTPRC, LOC104974540, LOC104974541, MIR181B-1, MIR181A-1, LOC104974543, LOC104974542, LOC104974544, LOC104974545, LOC104974547, LOC104974546, LOC101904123, LOC104969997	2.24
17	23439209 - 23456842	LOC104974591, LOC783956	1.05
20	28432844 - 28472244	LOC101903570, LOC104975256, PARP8, LOC104975257, EMB, LOC104975258, LOC785429	1.09
22	21783249 - 21808384	EDEM1, ARL8B, LOC104975525, LOC101907920, BHLHE40, LOC104975524, ITPR1, SUMF1, LOC101907965, SETMAR, LOC104975546, LOC101908044, SEM1	1.10
23	29182707 - 29204327	RNF39, PPP1R11, ZNRD1, ZFP57, MOG, GABBR1, LOC786987, LOC504548, OR2H1, UBD, OR10C1, LOC523389, LOC786846, LOC785910, LOC785811, LOC789690, LOC785712, OR12D2, LOC514434, OR12D3, OR5V1, OR14J1, LOC516273, OR2J3, LOC784787, LOC789367, LOC789358, OR2J2, OR2W1	1.44
24	11012027 - 11031501	LOC101905214, CDH7, LOC104975735, CDH19	1.80
27	30454846 - 30491396	LOC104976101, LOC104976102, LOC783570, LOC104976105, UNC5D, LOC104976104	1.85

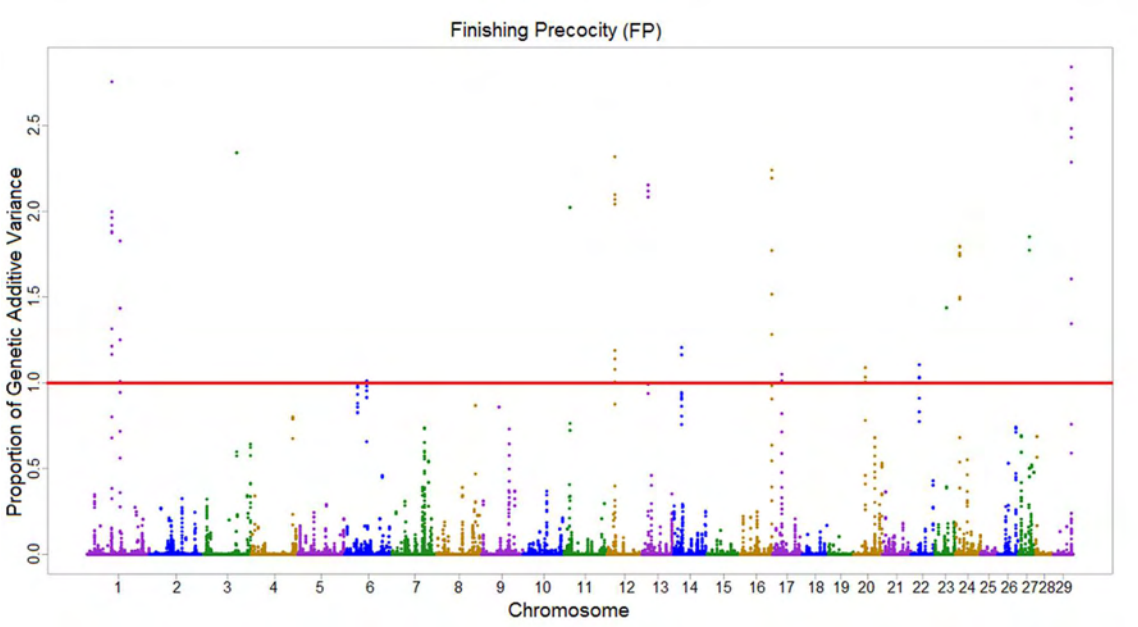
Continuation

Table 2.4 - Genomic regions associated with finishing precocity (FP)

29	46103755 - 46127317	PC, SYT12, RHOD, KDM2A, GRK2, SSH3, POLD4, CLCF1, RAD9A, PPP1CA, CARNS1, PTPRCAP, CORO1B, GPR152, CABP4, PITPNM1, CDK2AP2, CABP2, GSTP1, NDUFV1, DOC2G, NUDT8, TBX10, ALDH3B1, NDUFS8, TCIRG1, CHKA, KMT5B, LRP5L, TMEM134, TBC1D10C, RPS6KB2, UNC93B1, AIP, C29H11orf86, C29H11orf24, ANKRD13D, NDUFS8	2.84

Conclusion
Source: Elaborated by the authors.

Figure 2 - Manhattan plot of the additive genetic variance explained by windows of 10 adjacent SNPs for finishing precocity (FP) trait in Nellore cattle (the dots above the red line indicates the regions explaining more than 1.0% of the additive genetic variance)



Source: Elaborated by the authors.

The *SOX2* gene (SRY-box 2) located on BTA1 (85.68-85.70 Mb) acts during osteoblast differentiation, response to growth factor, and can be highlighted due to its manifestation in adult stem cells of several tissues (ARNOLD et al., 2011). On BTA22 (21.78-21.81 Mb), two genes were identified within the window explaining the highest genetic variance for FP (1.10%). The *ITPR1* gene (Inositol 1,4,5-Trisphosphate Receptor Type 1) regulates the

release of calcium from the endoplasmic reticulum, a fundamental process for ionic transport (HUANG et al., 2012). Additionally, this gene has been associated with feed efficiency in beef cattle (SANTANA et al., 2014a). The second gene, the *BHLHE40* (Basic Helix-Loop-Helix Family, Member E40) gene, participates during adipogenesis and myogenesis regulation (IIZUKA and HORIKAWA, 2008; LECOMTE et al., 2010), being such a candidate gene for fat deposition and growth-related traits (LAI et al., 2013).

4.2.3 Musculature (MS)

A total of 209 genes were identified within the genomic regions explaining more than 1.0% of the additive genetic variance for MS (Table 2.5), in which 135 of them had described biological functions and 74 were uncharacterized genes (Figure 3). Several genes related to energy homeostasis and metabolisms were found in a window explaining 1.03% of the additive variance (54.11-54.12 Mb on BTA18), such as the *SIX5* (Sine oculis homeobox, drosophila, homolog of, 5), *FOXA3* (Forkhead Box A3), and *AP2S1* (Adaptor-Related Protein Complex 2, Sigma-1 Subunit) genes.

Table 2.5 - Genomic regions associated with musculature (MS)

BTA	Position (pb)	Candidate Genes	% variance explained by SNPs windows
6	59281264 - 59352519	PGM2, LOC783708, LOC781379, LOC101906872, TBC1D1, LOC104972736, LOC104972737, LOC104972738, LOC101907152, LOC104972739, LOC101907197, TRNAI-AAU, KLF3, LOC104972740, LOC101907244, TLR10, TLR6, FAM114A1, LOC104972741, TMEM156	1.93
7	96320591 - 96341232	FAM172A, KIAA0825, SLF1, LOC104968998, MCTP1	1.01

Continue

Table 2.5 - Genomic regions associated with musculature (MS)

8	105893318 - 105927055	WHRN, ATP6V1G1, TMEM268, LOC104969457, TEX48, LOC101907680, TNFSF15, TNFSF8, LOC101908167, LOC104969458, LOC104972946, TNC, LOC101908213	2.97
10	22888748 - 22912474	LOC539243, LOC783489, LOC100295645, BVD1.23, LOC100299557, LOC506959, LOC407201, LOC407205, LOC100296565, LOC100298610, LOC785752, LOC100298572, LOC100298870, LOC101906952, LOC407203, LOC528363, LOC527826, LOC787726, LOC618561, LOC512980, LOC789048, LOC100296677, LOC100336256, LOC100297214, LOC100296317, LOC786608, LOC100296102, LOC786717, LOC786881, TRAV29DV5, TRNAW-CCA	6.59
12	18521528 - 18537665	LOC104973550, LOC783657, ITM2B, LOC101903713, TRNAE-UUC, LOC506251, LOC101903855, RB1, LPAR6, RCBTB2, CYSLTR2, LOC101903993, LOC101903925, TRNAW-CCA, FNDC3A, MLNR, LOC101904090, CDADC1, LOC104970162, CAB39L	2.43
13	24247404 - 24266248	TRNASTOP-UCA, SPAG6, LOC104973735, LOC101905701, MIR228K-2, LOC101905823, PIP4K2A, LOC104973737, ARMC3, MSRB2, LOC104973736, PTF1A, LOC100295196, OTUD1, LOC530173	1.11
13	71492185 - 71504029	LOC104973879, LOC101908081, LOC614378, LOC104973882, LOC101908176, LOC104973881, PTPRT	1.80

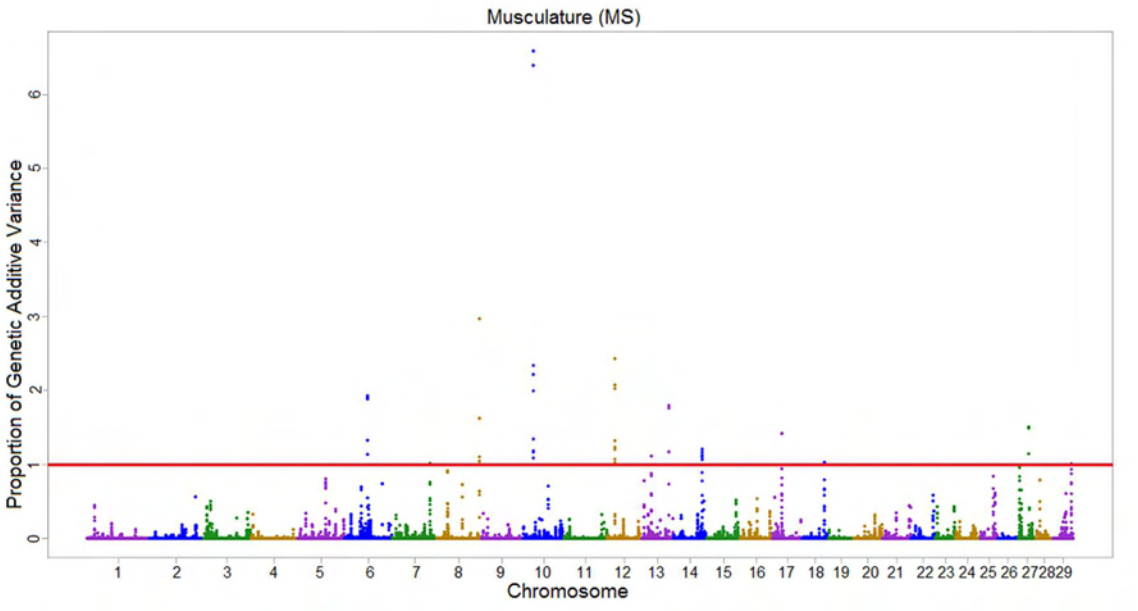
Continuation

Table 2.5 - Genomic regions associated with musculature (MS)

14	73018946 - 73043341	CDH17, LOC104974128, PDP1, LOC104974128, TRNAC-GCA, TMEM67, RBM12B, FAM92A, BCL-XLP1	1.21
17	23694684 - 23753069	LOC783956, LOC104974592	1.42
18	54107276 - 54119194	OPA3, GIPR, SNRPD2, QPCTL, FBX046, MYPOP, BHMG1, SIX5, DMWD, SYMPK, FOXA3, NOVA2, CCDC61, PGLYRP1, HIF3A, PPP5C, PNMA8A, CCDC8, PNMA8B, CALM3, PTGIR, GNG8, DACT3, PRKD2, FKRP, SLC1A5, AP2S1, ARHGAP35, NPAS1, ZC3H4, GPR4, NANOS2, DMPK, IGFL1, TMEM160, RSPH6A, STRN4	1.03
27	28142814 - 28188545	NRG1, LOC104976094, LOC104976095, LOC786349, TRNAG-CCC, LOC783293, LOC101903637, FUT10, MAK16, TTI2, LOC104976096, RNF122	1.50
29	46106562 - 46129111	PC, SYT12, RHOD, KDM2A, GRK2, SSH3, POLD4, CLCF1, RAD9A, PPP1CA, CARNS1, PTPRCAP, CORO1B, GPR152, CABP4, PITPNM1, CDK2AP2, CABP2, GSTP1, NDUFV1, DOC2G, NUDT8, TBX10, ALDH3B1, NDUFS8, TCIRG1, CHKA, KMT5B, LRP5L, AIP, GPR152, RPS6KB2, ANKRD13D, UNC93B1, TMEM134, TBC1D10C, C29H11orf24, C29H11orf86	1.01

Conclusion
Source: Elaborated by the authors.

Figure 3 - Manhattan plot of the additive genetic variance explained by windows of 10 adjacent SNPs for musculature (MS) trait in Nellore cattle (the dots above the red line indicates the regions explaining more than 1.0% of the additive genetic variance)



Source: Elaborated by the authors.

The *SIX5* gene belongs to the SIX Family associated with DNA binding specificity, it also appears to be critical for mediating protein-protein interactions, and has been implicated in developmental processes and in the maintenance of differentiated tissue states (BOUCHER et al., 2000). Some studies reported that members of the SIX family genes influenced the pubertal development and have a similar function of leptin and adiponectin, providing a susceptible link between energy homeostasis and GnRH release (AMSTALDEN et al., 2014; CARDOSO et al., 2015). The *FOXA3* gene was found to play an important role during early development and metabolism in mice (FRIEDMAN and KAESTNER, 2006), and the AP2S1 gene has been associated with beef quality traits in Simmental cattle (XIA et al., 2016) and intramuscular and fat thickness in Brangus cattle (WENG et al., 2016). Moreover, this window also harbored the *FKRP* (Fukutin Related Protein), *NPAS1* (Neuronal PAS Domain Protein 1) and *DMPK* (Distrophia Myotonica Protein Kinase) genes, which functions have been linked to body growth and developmental processes. The *FKRP* gene was associated with protein processing and was expressed in a wide range of tissues with the highest levels in skeletal muscle, placenta, and heart of mammals (BROCKINGTON, 2001). The *NPAS1* gene is a potential

candidate gene for bovine body growth and developmental processes, a biological process whose specific outcome is the organism or anatomical structure development (PAREEK et al., 2013). The *DMPK* gene plays an important role in muscle, heart and brain cells, it also regulates the production and function of important structures inside muscle cells interacting with other proteins (CARANGO et al., 1993).

The genomic region located on BTA8 (10.58-10.59 Mb) harbored the *TNC* (Troponin) and *ATP6V1G1* (ATPase V1 subunit G1) genes. These genes have functions associated with energy metabolism and growth. The *TNC* gene was related with growth and feed efficiency in Angus, Hereford and SimAngus cattle (SEABURY et al., 2017). The *ATP6V1G1* gene is responsible for the hydrolysis of ATP and has been associated with nucleic acid metabolism and energy metabolism, which encodes one of the G subunit proteins of the V1 domain complex (LI et al., 2011).

4.2.4 Back fat thickness (BF)

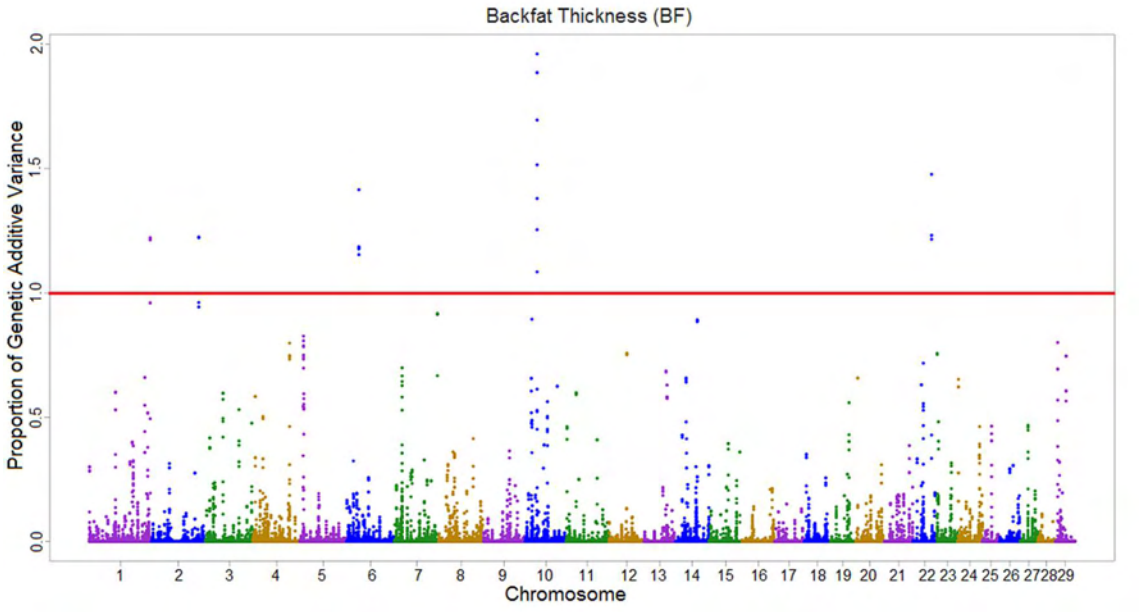
A total of 80 genes were identified within the genomic explaining more than 1.0% of the additive genetic variance for BF (Table 2.6), in which 56 of them had described biological functions and 24 were uncharacterized genes (Figure 4).

Table 2.6 - Genomic regions associated with back fat thickness (BF)

BTA	Position (pb)	Candidate Genes	% variance explained by SNPs windows
1	155379341 - 155410000	LOC104971081, GALNT15, LOC104971082, DPH3, OXNAD1, RFTN1, LOC101907685, DAZL, LOC101907852, LOC104971083, PLCL2, TBC1D5	1.22
2	121306996 - 121359593	LOC516378, LOC528262, ALPI, LOC100125266, ECEL1, PRSS56, CHRND, CHRNG, EIF4E2, LOC104971330, PHC2, A3GALT2, ZNF362, TRIM62, AZIN2, AK2, LOC104971331, MIR2357, RNF19B, TMEM54, HPCA, LOC104971332, FNDC5, S100PBP, YARS, KIAA1522, SYNC, RBBP4, ZBTB8OS, ZBTB8A	1.22
6	32605838 - 32657188	LOC104969897, ATOH1, LOC104970309, TRNAE-UUC, LOC536367	1.42
10	28988991 - 28997286	KATNBL1, EMC7, CHRM5, AVEN, LOC101901058, RYR3, LOC104973108, LOC104973109, TMCO5B, FMN1, C10H15orf24	1.96
22	48186729 - 48210434	CHDH, LOC101903574, CACNA1D, LOC783185, LOC101903766, DCP1A, TKT, LOC104975561, LOC100141064, PRKCD, TRNAG-UCC, RFT1, SFMBT1, LOC104975562, TMEM110, MUSTN1, ITIH4, ITIH3, LOC104975563, ITIH1, NEK4, CHCHD5	1.48

Source: Elaborated by the authors.

Figure 4 - Manhattan plot of the additive genetic variance explained by windows of 10 adjacent SNPs for back fat thickness (BF) trait in Nellore cattle (the dots above the red line indicates the regions explaining more than 1.0% of the additive genetic variance)



Source: Elaborated by the authors.

The *TKT* gene (Transketolase) on BTA22 (48.19-48.21 Mb) was associated with intramuscular fat in cattle (YANG et al., 2017) and sheep (GUO et al., 2014). The *FNDC5* gene (Fibronectin Type III Domain Containing 5) identified on BTA2 (121.31-121.36 Mb), was related with lipid and carbohydrate metabolism, body mass index, lean body mass, body circumference, accumulation of subcutaneous adipose tissue, circulating triglycerides, as well as fat cell size and intramyocellular lipid content (HUH et al., 2012; KURDIOVA et al., 2014; GAGGINI et al., 2017). Komolka et al. (2014) demonstrated a strong expression of total *FNDC5* mRNA in *Longissimus* dorsi muscle in adult cattle, emphasizing that this gene increases the list of proteins termed adipo-myokines and mediates the cross-talk between skeletal muscle and adipose tissue. The *CHRND* gene (Cholinergic receptor, nicotinic, delta polypeptide), located in the same genomic region as the *FNDC5* gene, was associated with muscle mass reduction during aging, influencing the final percentage of fat and muscle in beef cattle (SILVA-VIGNATO et al., 2016).

It should be noted that the region identified in the present study located on BTA1 (155.38-155.41 Mb) associated with BF partially overlapped with a region reported by Magalhães (2015), who performed a GWAS for beef

tenderness and marbling also in Nellore cattle. Besides, the *PLCL2* gene (Phospholipase C-Like 2) has been described in both studies, however, it has been associated with tenderness by Magalhães (2015). In addition, Otsuki et al., (1999) reported that this gene displayed a high gene expression in skeletal muscle and has an enzymatic function (Phospholipase C- PLC) related to lipid metabolism. These results suggest that the *PLCL2* gene is highly linked to fat accretion and body composition.

4.2.5 Rump fat tchickness (RF)

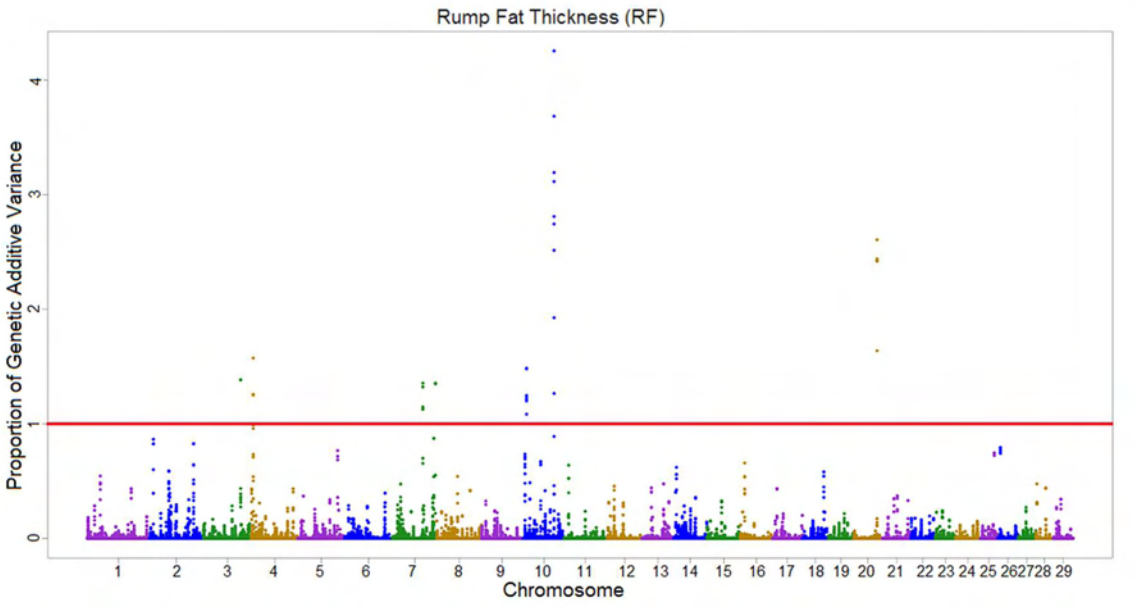
A total of 73 genes were identified within the genomic regions explaining more than 1.0% of the additive genetic variance for RF (Table 2.7), in which 34 of them had described biological functions and 39 were uncharacterized genes (Figure 5).

Table 2.7 - Genomic regions associated with rump fat thickness (RF)

BTA	Position (pb)	Candidate Genes	% variance explained by SNPs windows
3	97673293 - 97693026	LOC101905986, AGBL4, LOC101905744, BEND5, LOC104971807	1.38
4	6250490 - 6266978	ZPBP, VWC2, LOC101904266	1.57
7	78038681 - 78084430	TRNAE-UUC, LOC104968962	1.35
7	109549816 - 109566500	GIN1, LOC101905525, PPIP5K2, LOC101905593, C7H5orf30, PDZPH1P, NUDT12, LOC101905838, LOC104969011, LOC104969010, LOC104969009, LOC101906097, LOC787122, LOC104969008, LOC524356, LOC104969006, LOC513186, TRNAC-ACA, LOC104969017, EFNA5, LOC104969018, LOC104969019, FBXL17, LOC104969020, LOC523504, LOC104969021	1.35
10	7619876 - 7631950	SV2C, LOC101904784, LOC101904831, IQGAP2, LOC101905173, F2RL2, F2R, LOC100296562, F2RL1, LOC104973026, S100Z, LOC101907313, CRHBP, AGGF1	1.48
10	81454469 - 81461491	ZFP36L1, LOC101906193, ACTN1, LOC104973226, LOC104973227, DCAF5, LOC104973228, EXD2, LOC101906478, GALNT16, ERH, SLC39A9, PLEKHD1, CCDC177, SUSD6, LOC614945, SRSF5, SLC10A1, LOC104973229, LOC101907082, SMOC1	4.25
20	60404729 - 60436026	LOC101902130, LOC781723	2.61

Source: Elaborated by the authors.

Figure 5 - Manhattan plot of the additive genetic variance explained by windows of 10 adjacent SNPs for rump fat thickness (RF) trait in Nellore cattle (the dots above the red line indicates the regions explaining more than 1.0% of the additive genetic variance)



Source: Elaborated by the authors.

The window located on BTA7 (109.55-109.57 Mb) partially overlapped with a significant region described by Silva et al. (2017), who performed a GWAS for carcass traits (BF and RF) in Nellore cattle. These authors identified five genes in common with the present study, such as *GIN1* (Gypsy Retrotransposon Integrase 1), *LOC101905525* (Nuclease-Sensitive Element-Binding Protein 1 Pseudogene), *PPIP5K2* (Diphosphoinositol Pentakishosphate Kinase 2), *LOC101905593* (uncharacterized LOC101905593) and *C7H5orf30* (Chromosome 7 Open Reading Frame, Human C7H5orf30) genes.

4.2.6 *Longissimus* muscle area (LMA)

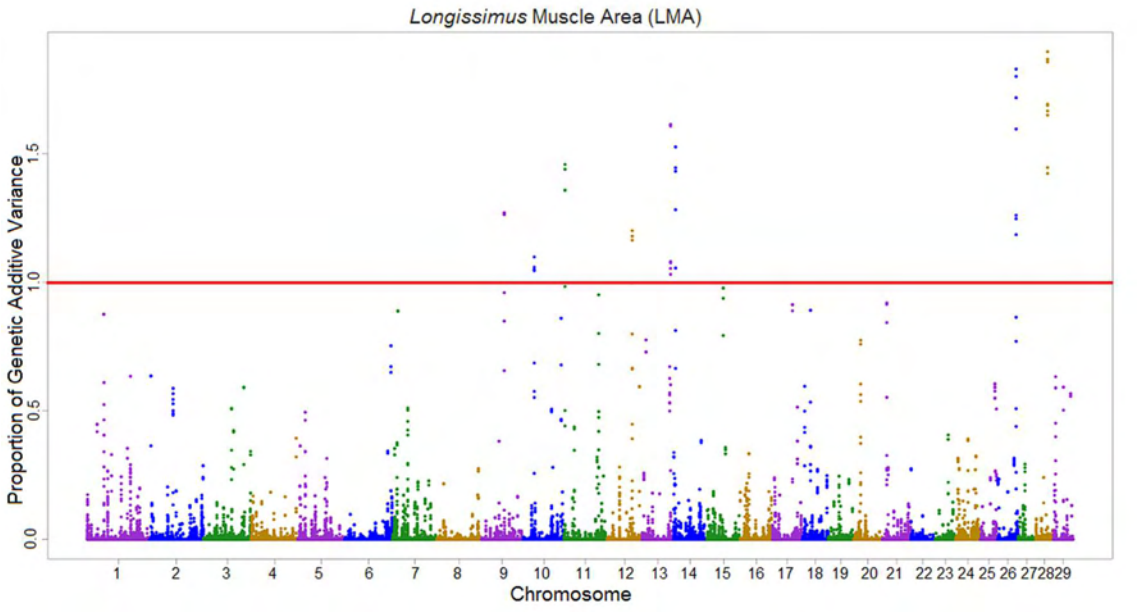
A total of 114 genes were identified in the genomic regions explaining more than 1.0% of the additive genetic variance for LMA (Table 2.8), in which 88 of them had described biological functions and 26 were uncharacterized genes (Figure 6).

Table 2.8 - Genomic regions associated with *Longissimus* muscle area (LMA)

BTA	Position (pb)	Candidate Genes	% variance explained by SNPs windows
9	60621625 - 60656837	LOC104969580, LOC782675, LOC101902479, MAP3K7, LOC104969579, BACH2	1.27
10	27128057 - 27141650	RNASE10, PNP, LOC790312, PIP4P1, APEX1, OSGEP, KLHL33, TEP1, PARP2, CCNB1IP1, TTC5, OR11H4, OR11H6, LOC783845, OR11G2, LOC783998, LOC526667, LOC617084, OR4M1, OR4N4, OR4N2, OR4K2, OR4K5, OR4K14, LOC515473, OR4K17, LOC787134, OR4L1, OR4N5, OR4K13, RPS29, OR11H7, OR4Q2, OR4Q3, OR4K1, OR4K15	1.10
11	1706353 - 1734496	LOC101906105, ACOXL, BUB1, TPC3, SMIM37, NPHP1, MALL, LOC101906286, MAL, LOC617833, MRPS5, ZNF514, ZNF2, PROM2, KCNIP3, FAHD2A, GPAT2, ADRA2B, ASTL	1.46
12	64443063 - 64462558	_____	1.20
13	75266762 - 75291860	TKDP1, TKDP4, TKDP2, LOC404103, PTI, LOC616039, WFDC2, SPINLW1, WFDC8, WFDC11, WFDC13, LOC104973895, WFDC3, DNTTIP1, UBE2C, TNNC2, ACOT8, SNX21, ZSWIM3, ZSWIM1, SPATA25, NEUTL2, CTSA, PLTP, PCIF1, ZNF335, MMP9, SLC12A5, CD40, CDH22, SPINT4, WFDC10A, NEURL2	1.61
14	5632525 - 5645865	LOC104973977, COL22A1, LOC104973976, LOC104973975, FAM135B, LOC1049973978	1.53
26	46941546 - 46952726	C26H10orf90, FAM196A, DOCK1, MIR2285C, NPS	1.83
28	33756807 - 33772666	KCNMA1, LOC104969723, LOC101907374, DLG5, POLR3A, RPS24, LOC104976196, LOC104969726, LOC104969725	1.90

Source: Elaborated by the authors.

Figure 6 - Manhattan plot of the additive genetic variance explained by windows of 10 adjacent SNPs for *Longissimus* muscle area (LMA) trait in Nellore cattle (the dots above the red line indicates the regions explaining more than 1.0% of the additive genetic variance)



Source: Elaborated by the authors.

Genes related to intramuscular fat, skeletal muscle, muscle hypertrophy and striated muscle were identified on BTA13 (75.27-75.29 Mb) in a window explaining 1.61% of the additive genetic variance for LMA, such as the *PLTP* (Phospholipid Transfer Protein), *TNNC2* (Troponin C, Fast), *DNTTIP1* (Deoxynucleotidyltransferase Terminal Interacting Protein 1) and *NEURL2* (Neuralized E3 Ubiquitin Protein Ligase 2) genes. The *PLTP* gene regulates the high-density lipoprotein metabolism (HUUSKONEN et al., 2001). Previous studies have shown that the *PLTP* gene was associated with fat metabolism, intramuscular fat and marbling, evidencing that this gene is a strong candidate gene for carcass traits in Hanwoo cattle (SEONG, et al., 2013; LIM et al., 2015). The *TNNC2* gene is expressed during the myoblast differentiation and skeletal muscle development, affecting beef quality traits in pigs and sheep (LI et al., 2008; XU et al., 2008). The *DNTTIP1* gene is up-regulated in the callipyge muscle and regulates several genes, leading to muscle hypertrophy (LUTZ, 2014). Fewer studies on *DNTTIP1* gene are available in the literature, and one of them revealed that this gene displayed a significant effect on the hypertrophy of *Longissimus* dorsi muscle in lambs (FLEMING-WADDELL et al., 2007). The

NEURL2 gene was related with the myofibril organization and was detected in skeletal and striated muscles (NASTASI et al., 2004; ROPKA-MOLIK et al., 2015).

The *GPAT2* gene (Glycerol-3-Phospahate Acyltransferase 2, Mitochondrial) on BTA11 (17.06-17.34 Mb) has been related with fat composition by acting on the triglycerides and glycerophospholipds biosynthesis (DIRCKS and SUL, 1997). This gene was showed to play a role in several growth-related traits and fatty acid composition in intramuscular fat and BF (REVILLA et al., 2017). Genes such as the *OMA1* (OMA1 Zinc Metallopeptidase) and *FGGY* (FGGY Carbohidrate Kinase Domain-Containing Protein) on BTA3 (88.01-88.04 Mb and 87.54-87.58 Mb, respectively) were associated with BS and FP, showing a pleiotropic effect. The *OMA1* gene was related with energy homeostasis and fat metabolism in humans (HEAD et al., 2009). The *FGGY* gene was also identified by Santana et al. (2014b) in a GWAS for average live weight gain in Nellore cattle. This gene encodes a carbohydrate kinase enzyme, which is able to use different sugars substrates (trioses and heptoses) to regulate the energetic balance (ZHANG et al., 2011).

The *RB1* gene (Retinoblastoma) located on BTA12 (18.52-18.54 Mb) was associated with FP and MS. Lim et al. (2013) reported that this gene was linked with marbling deposition in Hanwoo cattle. The authors showed direct association and biological functions related to fat and muscle, such as adipogenesis and muscle growth. The *CARNS1* gene (Carnosine Synthase 1) located on BTA29 (46.10-46.13 Mb) belongs to the ATP-grasp family of ligases and modulates carnosine concentrations in muscle tissue in pigs (YANG et al., 2014; D'AUSTOU-PAGÉ et al., 2017). This associated window partially overlapped with a window (46.11-46.38 Mb) described by Silva et al. (2017) in a GWAS investigating carcass traits in Nellore cattle. These authors identified six genes in common with the present study associated with BF, such as *NUDT8* (Nudix Hydrolase 8), *TBX10* (T-Box 10), *UNC93B1* (Unc-93 Homolog B1, C. Elegans), *ALDH3B1* (Aldehyde Dehydrogenase 3 Family member B1), *TCIRG1* (T-Cell Immune Regulator 1, ATPase H+ Transporting V0 Subunit a3) and *CHKA* (Choline Kinase Alpha) genes.

4.3 Functional Analyses

The functional analyses revealed several significant GO terms ($p < 0.01$) and KEGG pathways ($p \leq 0.05$) from the set of genes previously identified within the significant windows (Table 2.9).

Table 2.9 - DAVID Functional Annotation for gene category and pathway enrichment analysis

Term	Count	PValue	FDR	Genes
GO				
GO:0007606~sensory perception of chemical stimulus	21	0.0009	1.6E0	LOC784787, LOC785712, LOC785811, LOC785910, LOC789690, LOC516273, LOC514434, OR10C1, OR12D2, OR12D3, OR2H1, OR2J3, OR2W1, OR4K14, OR4K5, OR4L1, OR4M1, OR4N2, OR4N4, OR4N5, OR5V1
GO:0051482~positive regulation of cytosolic calcium ion concentration involved in phospholipase C-activating G-protein coupled signaling pathway	4	0.002	3.3E0	F2RL2, F2RL1, GPR4, F2R
GO:0004930~G-protein coupled receptor activity	34	0.002	2.9E0	F2RL2, OR2J3, F2RL1, LOC789690, OR4L1, OR11G2, LOC514434, GPR4, PTGIR, OR10C1, OR14J1, LOC785910, LOC783845, OR4K5, LOC523389, LOC785712, LOC516273, LOC785811, OR4M1, LOC783998, LOC784787, OR2W1, OR12D2, OR12D3, OR2H1, LPAR6, OR5V1, OR11H6, OR4K14, OR4N5, OR4N4, OR4N2, LOC786846, F2R
GO:0015057~thrombin receptor activity	3	0.002	3.4E0	F2RL2, F2RL1, F2R
GO:0035025~positive regulation of Rho protein signal transduction	4	0.002	4.1E0	F2RL2, F2RL1, GPR4, F2R

Continue

Table 2.9 - DAVID Functional Annotation for gene category and pathway enrichment analysis

GO:0004984~olfactory receptor activity	29	0.003	5.2E0	OR2J3, LOC789690, OR4L1, OR11G2, LOC514434, OR10C1, OR14J1, LOC785910, LOC783845, OR4K5, LOC523389, LOC785712, LOC516273, LOC785811, OR11H4, OR4M1, LOC783998, LOC784787, OR2W1, OR12D2, OR12D3, OR2H1, OR5V1, OR11H6, OR4K14, OR4N5, OR4N4, OR4N2, LOC786846
GO:0016712~oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen	4	0.005	7.7E0	CYP2J2, LOC521656, LOC530929, LOC511936
GO:0005576~extracellular region	19	0.014	1.7E1	WFDC10A, FST, TKDP1, WFDC13, WFDC8, RNASE10, NPS, FNDC5, ZPBP, AGGF1, ITIH1, SPINLW1, PTI, ITIH4, ITIH5, ITIH2, LOC404103, WFDC2, WFDC3
GO:0016471~vacuolar proton-transporting V-type ATPase complex	3	0.016	1.9E1	TCIRG1, ATP6V1G1, ATP6V1B1
GO:0005509~calcium ion binding	21	0.017	2.1E1	PDP1, TNNC2, CABP2, REPS1, SYT12, ACTN1, CABP4, EDEM1, S100Z, ITPR1, KCNIP3, CDH22, CDH7, DYSF, CDH17, SMOC1, RYR3, CDH19, HPCA, DOC2G, CALM3
GO:0045211~postsynaptic membrane	6	0.05	5.1E1	KCNMA1, CHRM5, GABBR1, CHRND, F2R, CHRNG
GO:0007271~synaptic transmission, cholinergic	3	0.1	8.1E1	CHRM5, CHRND, CHRNG
GO:0003713~transcription coactivator activity	6	0.12	8.2E1	GATA3, JUN, WWC1, APEX1, MYSM1, CITED2
GO:0031338~regulation of vesicle fusion	3	0.14	9.2E1	TBC1D10C, TBC1D5, TBC1D1
GO:0008137~NADH dehydrogenase (ubiquinone) activity	3	0.15	8.9E1	NDUFS4, NDUFV1, NDUFS8

Continuation

Table 2.9 - DAVID Functional Annotation for gene category and pathway enrichment analysis

GO:0022627~cytosolic small ribosomal subunit	3	0.19	9.4E1	RPS29, MRPS5, RPS24
KEGG Pathway				
bta04750:Inflammatory mediator regulation of TRP channels	9	0.002	5.7E0	PPP1CA, CYP2J2, LOC521656, F2RL1, CALM3, PRKCD, LOC530929, ITPR1, LOC511936
bta04740:Olfactory transduction	37	0.004	1.7E-1	OR2J3, LOC789690, OR4K17, LOC617084, OR2J2, OR4L1, OR11G2, LOC514434, LOC789367, LOC515473, LOC787134, OR10C1, OR14J1, LOC785910, LOC783845, OR4K5, LOC523389, OR4K2, LOC785712, LOC785811, LOC789358, OR11H4, OR4M1, LOC783998, LOC784787, OR2W1, OR12D2, OR12D3, OR2H1, OR4K14, OR5V1, OR11H6, OR4K13, OR4N5, OR4N4, CALM3, LOC786846
bta04726:Serotonergic synapse	8	0.01	2.5E0	GNG8, APP, CYP2J2, LOC521656, CACNA1D, LOC530929, ITPR1, LOC511936
bta00591:Linoleic acid metabolism	4	0.04	1.6E1	CYP2J2, LOC521656, LOC530929, LOC511936
bta04270:Vascular smooth muscle contraction	7	0.04	4.5E1	KCNMA1, PTGIR, PPP1CA, CALM3, PRKCD, CACNA1D, ITPR1
bta04913:Ovarian steroidogenesis	4	0.1	4.7E1	CYP2J2, LOC521656, LOC530929, LOC511936
bta05010:Alzheimer's disease	8	0.1	7.2E1	APP, NDUFS4, RYR3, NDUFV1, NDUFS8, CALM3, CACNA1D, ITPR1
bta04966:Collecting duct acid secretion	3	0.1	7.2E1	TCIRG1, ATP6V1G1, ATP6V1B1
bta05323:Rheumatoid arthritis	5	0.1	7.9E1	TCIRG1, JUN, ATP6V1G1, LOC786881, ATP6V1B1
bta04721:Synaptic vesicle cycle	4	0.1	8.8E1	TCIRG1, AP2S1, ATP6V1G1, ATP6V1B1
bta04022:cGMP-PKG signaling pathway	7	0.1	8.9E1	KCNMA1, PPP1CA, LOC787122, CALM3, ADRA2B, CACNA1D, ITPR1
bta04713:Circadian entrainment	5	0.1	8.9E1	GNG8, RYR3, CALM3, CACNA1D, ITPR1

Continuation

Table 2.9 - DAVID Functional Annotation for gene category and pathway enrichment analysis

bta04510:Focal adhesion	8	0.1	9.0E1	PPP1CA, DOCK1, TNC, JUN, ITGA1, ITGA2, ACTN1, ARHGAP35
bta04924:Renin secretion	4	0.1	9.1E1	KCNMA1, CALM3, CACNA1D, ITPR1
bta05031:Amphetamine addiction	4	0.1	9.1E1	PPP1CA, JUN, CALM3, CACNA1D
bta05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	4	0.1	9.2E1	ITGA1, ITGA2, ACTN1, CACNA1D
bta00190:Oxidative phosphorylation	6	0.1	9.3E1	TCIRG1, NDUFS4, NDUFV1, NDUFS8, ATP6V1G1, ATP6V1B1

Conclusion
Source: Elaborated by the authors.

The Cytosolic Small Ribosomal Subunit (GO:0022627) cellular component is defined as the small subunit of a ribosome located in the cytosol. Ribosomes are found in the endoplasmic reticulum and in the cytoplasm, and are formed by two ribonucleoprotein subunits (large and small) which have as a principal function to bind mRNA. Their function is to translate all mRNAs produced from nuclear genes and perform most of the cellular protein synthesis (MARYGOLD et al., 2007), influencing directly the growth and development of the organism.

Aerobic organisms need oxygen to accomplish several process for their survival, such as oxidation of substrates (sugars and fats), energy generation and the major source of ATP in mitochondria through the cellular respiration (HEINRICH and SCHUSTER, 2012). Therefore, the Oxidative Phosphorylation (bta00190) pathway is involved in the process in which ATP is the culmination of a series of energy transformations that are called cellular respiration, formed as a result of the transfer of electrons from NADH or FADH2 to O2 (BERG; TYMOCZKO; STRYER; 2002). Another highlighted GO term, related to energy production is the NADH Dehydrogenase (ubiquinone) Activity (GO:0008137) molecular function, that acts in the catalysis of the reaction (NADH + H+ + ubiquinone = NAD+ + ubiquinol), and has been important for the animal development since its function is related to energy production and antioxidative protection (MELLORS and TAPPEL, 1966).

The Sensory Perception of Chemical Stimulus (GO:0007606) biological process is a neurological process involved in a series of events required for an organism to receive a sensory chemical stimulus, convert it to a molecular signal, and recognize and characterize the signal. This biological process together with the Olfactory Transduction (bta04740) influence the odor perception through olfactory receptors and biochemical signaling events, affecting food preference and intake (DO et al., 2014; STAFUZZA et al., 2017). Olfactory receptors in the gut might serve as a sensor of chemical or nutritional status and have a role in nutrient absorption or digestive function (PALOUZIER-PAULIGNAN et al., 2012). Some studies have also found the potential relationship of the olfactory pathway with feed intake and beef quality traits in cattle, such as body condition score, live-weight, stature, and body size (VEERKAMP et al., 2012; LINDHOLM-PERRY et al., 2015; TAYE et al., 2018). Do et al. (2014) performed a pathway analysis for feed efficiency in pigs and showed metabolic and olfactory transduction pathways significantly associated with residual feed intake. In addition, Lemos et al. (2018) executed a GWAS between copy number variation regions and beef fatty acid profile in Nellore cattle and reported that the Olfactory Receptor Activity (GO:0004984) was significantly enriched.

5 CONCLUSIONS

The results obtained in the present study should help to elucidate the genetic and physiologic mechanism regulating body growth and composition, muscle and fat tissue development in Nellore cattle. Several genomic regions associated with body growth, homeostasis, energy, and protein metabolism were identified. Some genes, such as *FOXA3*, *AP2S1*, *FKRP*, *NPASI* and *ATP6V1G1* were more likely to be linked with musculature, meanwhile, the *OMA1* and *FFGY* genes were associated with body structure and finishing precocity. The *PLTP*, *TNNC2* and *GPAT2* genes were more likely to be related to LMA development, as well as the *TKT*, *FNDC5* and *CHRND* genes, which were possibly associated with fat deposition. The gene enrichment functional analysis revealed biological processes and molecular function influencing

directly the growth and development of the organism and the energy homeostasis.

The results showed that the main genomic regions explaining more than 1.0% of the additive genetic variance differed between ultrasound carcass and visual scores traits, suggesting that different groups of genes control these traits. Despite being controlled by different groups of genes, common metabolic pathways ($p < 0.05$) between these groups of traits were identified, i.e. G-protein coupled receptor activity (GO:0004930), olfactory receptor activity (GO:0004984) and calcium ion binding (GO:0005509), showing that the carcass and visual scores traits have similar metabolic and physiological basis. Several studies with Nellore cattle reported moderate to low genetic correlation estimates between ultrasound carcass and visual scores traits, reinforcing the concept that both groups of traits were partially controlled by the same groups of genes (GORDO et al., 2012; GORDO et al., 2016). The results of the present study showed that some of the genes influencing ultrasound carcass and visual scores traits participated in the same metabolic pathways, arguing the moderate to low genetic correlations reported between the group of traits.

The results described in here suggest that the in vivo carcass composition indicator traits have polygenic inheritance, controlled by several genetic variants of small effects. Hence, strategies such as genomic selection using the variability among all markers at the same time would be more appropriate to improve carcass indicator traits in candidates to selection (unproven sires) without phenotypic information for these traits. The identification and description of genome regions, genes and biological process that affect productive traits are important since this can be used in future fine mapping studies, which primary function is to search for informative causative mutations. The results also showed different genomic regions with known genes, but several uncharacterized genes (LOC) were identified, and this information would be useful for future genomic and genome assembly studies in indicine cattle.

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CHAPTER 3 - Genomic prediction ability for carcass composition indicator traits in Nellore cattle

ABSTRACT

SILVA, R. P. **Genomic prediction ability for carcass composition indicator traits in Nellore cattle.** 2021. Dr. Thesis – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

The aim of this study was to compare the genomic prediction ability for carcass composition indicator traits in Nellore cattle using the Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP), single-step GBLUP (ssGBLUP), Bayesian methods (BayesA, BayesB, BayesC and BayesianLASSO) and an approach combining the pedigree matrix of genotyped animals with both the genomic matrix and Bayesian methods. Phenotypic and genotypic information on about 66,000 and 21,000 animals, respectively, evaluated by National Association of Breeders and Researchers (ANCP) were available for body structure (BS), finishing precocity (FP), musculature (MS), *Longissimus* muscle area (LMA), back fat thickness (BF) and rump fat thickness (RF). The genotypes were obtained based on the low-density panel Zoetis CLARIFIDE® Nellore version 3.1 containing 30.754 markers. To obtain the prediction ability, the dataset was split into training (genotyped sires and dams with progenies) and validation (genotyped young animals without progeny records and without phenotypes) subsets. For genomic models, the predictive ability was assessed through the correlation between the deregressed expected progeny differences and DGVs. For BLUP model, the prediction ability was evaluated through the correlation between estimated breeding value (EBV) and deregressed expected progeny differences (dEPD). To evaluate the extent of prediction bias the linear regression coefficients between the response variable (dEPD) and DGVs (or EBVs for BLUP model) considering only the animals in the validation set, were calculated. In terms of prediction ability and bias, Bayesian approaches were superior for visual scores traits and the ssGBLUP for carcass traits obtained by ultrasonography, however, more biased results were obtained for BF and RF using the ssGBLUP. The ssGBLUP model showed less biased prediction for

low heritability traits, such as LMA, and also it has lower computational demand and it is a straightforward method for implementing genomic selection in beef cattle. Therefore, earlier reliable genetic evaluation of unproven sires trough genomic selection is appealing in order to increase the genetic response for carcass traits in the Nellore (*Bos taurus indicus*) beef cattle.

Keywords: Beef cattle. *Bos taurus indicus*. Genomic selection. Ultrasonography measurement. Visual score traits.

1 INTRODUCTION

In the last years, the carcass and beef traits in zebu breeds have been largely studied due to their economic relevance for the beef industry and consumers, and there is a growing concern and interest in improving the herd carcass genetic merit through selection (PEREIRA et al., 2015; GORDO et al., 2016). However, the genetic evaluation for these traits is expensive and difficult to measure since it requires the slaughter of the animals (GORDO et al., 2012). In this sense, faster and less expensive evaluation techniques, such as in vivo carcass ultrasonography and visual scoring, have been used as an alternative for the evaluation of carcass traits in animals. Gordo et al. (2016) confirmed the feasibility of visual scores to evaluate *postmortem* carcass traits in Nellore cattle. Carcass traits obtained by ultrasound and visual scores have moderate to high heritability, allowing reliable genetic merit prediction in several beef cattle breeds (RILEY et al., 2002; HWANG et al., 2008; DO et al., 2016). Moreover, Gordo et al. (2012) reported positive and favorable genetic correlation estimates between carcass traits obtained by ultrasound with visual scores, concluding that both traits could be used to improve body structure, finishing precocity, musculature and carcass traits.

Despite the advantages of visual scores and ultrasound records to evaluate carcass traits, only a part of the animals is phenotypically evaluated after weaning for these traits and it is necessary to wait until yearling to assess the phenotypic measures. These aspects undoubtedly preclude the genetic progress for beef and carcass traits in zebu breeds. In beef cattle, genomic selection was extensively implemented for the improvement of quantitative traits, and may be especially helpful for traits that are hard or expensive to measure, like carcass and beef traits (GORDO et al., 2012; 2016). Several methods have been proposed to predict the genomic estimated breeding values (GEBV) such as GBLUP, ssGBLUP and Bayesian methods. However, there is no consensus about the most suitable method to predict genomic values for beef and carcass traits. Saatchi et al. (2013), working with Hereford cattle performed genomic prediction for fat thickness, marbling and rib eye muscle area traits, and concluded that genomic selection using Bayesian models is feasible. In addition, Onogi et al. (2015) evaluated the predictive ability for

marbling score, carcass weight and ribeye area traits in Japanese Black cattle, using single-step genomic best linear unbiased method. Genomic selection improves the prediction ability for carcass composition indicator traits (STONE et al., 2005; ISHII et al., 2013; ELZO et al., 2013; FERNANDES JÚNIOR et al., 2016), enabling a significant enhancement of genetic gain even for traits with moderate to high heritability estimates.

To obtain reliable estimates of genetic trend and also perform accurate comparisons between animals of different generations, unbiased prediction is essential (HENDERSON et al., 1959). The majority of genomic models developed to date assume that all animals have been genotyped. However, in most of the situation in beef cattle the number of genotyped individuals is extremely small compared with the total number of individuals. Despite the models for genomic predictions assume an unselected genotyped population, in practice, genotyped individuals are highly selected, and genomic prediction models do not take this selection into account (HAYES et al., 2009). This scenario increased the bias of genomic predictions (VANRADEN et al., 2009a; 2009b; PATRY and DUCROCQ, 2011). Vitezica et al. (2011), in a simulated study reported that the predictions by a single-step method were less biased and more accurate than multi-step models, but under strong selection were less accurate. However, inflation in genomic predictions for young genotyped bulls in dairy cattle using the single-step method has been reported when those bulls have no progenies with phenotypes (AGUILAR et al., 2010; TSURUTA et al., 2011; MASUDA et al., 2018). According to Tsuruta et al. (2019), one reason for the bias could be incompatibility between the pedigree-based relationship matrix (A) and the genomic relationship matrix (G), and several strategies were developed to reduce the bias such as unknown parent groups.

Another important point is that differences in model prediction ability were reported mainly in small populations with limited number of genotyped and phenotyped animals in the reference population, however, no major differences between models were reported for large training populations (VANRADEN et al., 2009; BOLORMAA et al., 2013). There are several implications related to accuracy of pseudophenotypes used to estimate marker effects, impacting on bias and accuracy of genomic predictions. Thus, it is necessary to conduct genomic studies in order to evaluate the most adequate and suitable method for

genomic prediction for carcass indicator traits, since the number of genotyped and phenotyped animals for carcass traits is still limited in Nellore cattle.

2 OBJECTIVES

The aim of this study was to evaluate the genomic prediction ability for carcass composition indicator traits in Nellore cattle using the Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP), single-step GBLUP (ssGBLUP), Bayesian methods (BayesA, BayesB, BayesC and BayesianLASSO) and an approach combining the pedigree matrix of genotyped animals with both the genomic matrix and Bayesian methods.

3 MATERIAL AND METHODS

3.1 Phenotypic Data

The phenotypic and genotypic information belong to the Nellore Brazil Breeding Program, coordinated by the National Association of Breeders and Researchers (ANCP), Riberão Preto, Brazil. The dataset contained information on about 66,000 phenotyped animals from 18 Nellore herds distributed in the Southeast and Midwest regions of Brazil. The animals were pasture-reared in low throughput production systems, with or without the use of creep feeding and supplementation.

Approximately 57,000 phenotypic records were available for visual scores, such as body structure (BS), finishing precocity (FP) and musculature (MS), which were obtained (*in situ*) at yearling with approximately 550 days of age. Ten trained technicians performed visual evaluation, however, one technician performed almost 90% of these measures. Technicians underwent annual training to maintain visual evaluation standards and minimize the errors. The effect of the technician was nested within the contemporary group (CG) since the same person evaluated the entire CG, and therefore, it was not necessary to address this effect in the model. Visual evaluations were performed as follows: first, the trained technician observed the entire management group, separated for males and females, and then evaluated the

average profile for each trait by sex, which served as a baseline. Then scores from 1 (lowest expression) to 6 (highest expression), adapted from the method proposed by Koury Filho et al. (2010), were given to the animals. According to Koury Filho (2010), BS is a visual estimate of the area of the animal when seen from the side, basically evaluating body length and rib depth and establishing that greater areas correspond to higher scores. In the case of FP, higher scores are attributed to animals with greater rib depth in relation to the length of their limbs. Musculature is evaluated by visual evidence of muscle mass. Higher scores are attributed to “thicker” animals with more convex muscles and lower scores to “thinner” animals with a less convex rectilinear musculature and even concavities in the body.

The data set contained approximately 142,000 phenotypic records for carcass traits obtained by ultrasound, such as *Longissimus* muscle area (LMA), back fat thickness (BF) and rump fat thickness (RF). Scanning and image analyses were carried out by technicians, equipment, and software accredited by ANCP following the Ultrasound Guidelines Council criteria. Animals were scanned for *Longissimus* muscle area (LMA) between the 12th and 13th ribs, back fat thickness (BF) over the *Longissimus* muscle at a point three-fourths the length ventrally of the LMA, and rump fat thickness (RF) at the junction of the *Biceps femoris* and *Gluteus medius* between the ischium and ilium and parallel to the vertebrae (GORDO et al., 2012). The carcass traits evaluated by ultrasonography were obtained using an ALOKA 500 V device, with a 3.5 MHz linear probe, measured in millimeters (mm) for BF and RF, and in square centimeters (cm^2) for LMA. The CG were defined as follows: i) sex, farm, year, birth season, and management group at 450 days of age for LMA, BF and RF; and ii) sex, farm, year, season of birth, and management group at 550 days of age for BS, FP and MS.

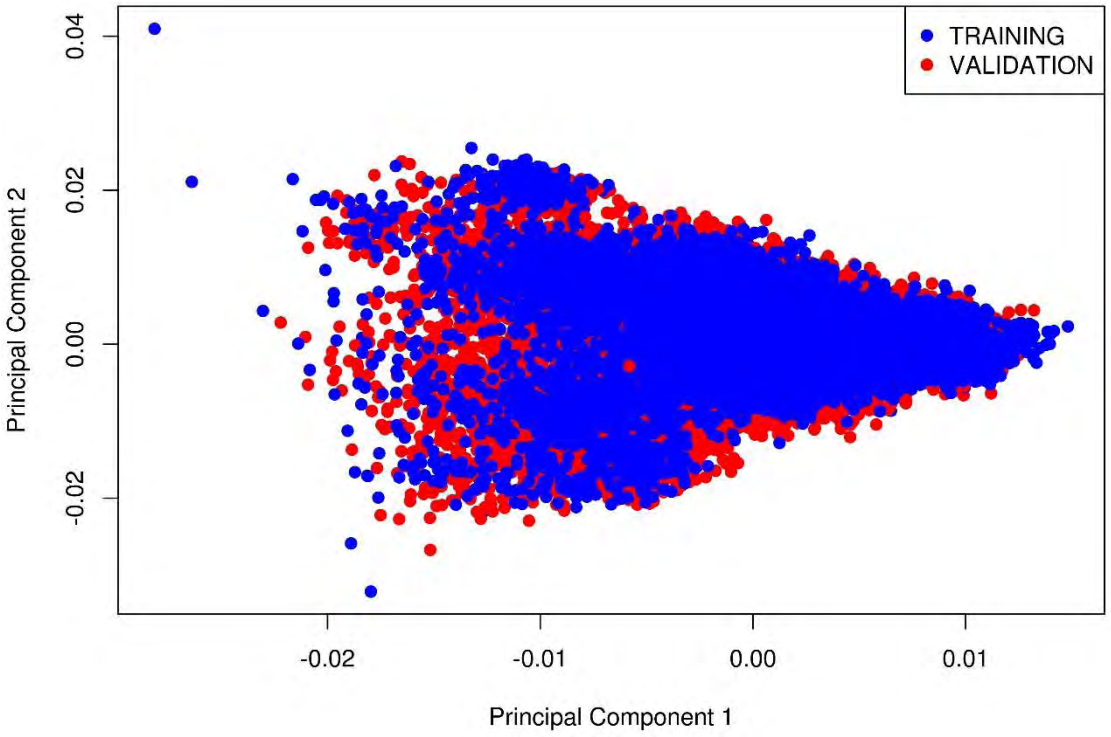
3.2 Genomic Data

A total of 20,827 animals were genotyped using a low-density panel (Zoetis CLARIFIDE® Nellore version 3.1) containing 30.754 SNP markers. Samples with call rate lower than 90%, markers with a minor allele frequency (MAF) lower than 0.05, call rate for SNPs lower than 90%, and failing Hardy-

Weinberg Equilibrium (HWE) at a threshold of > 0.01 were not considered in the subsequent analyses. Markers with redundant position and those located in non-autosomal chromosomes were also excluded, resulting in a final genotype file with 20,251 SNPs and 20,827 animals.

The principal component analysis was performed using information provided by SNPs after quality control criteria and employing the plot function inside R program (Figure 1). The results indicated the absence of subgroups among the evaluated animals either in the training or validation population, since no formation of major components demonstrating the presence of subpopulations was observed.

Figure 1 - First and second principal component of genomic relationship matrix (blue points: training population; red point: validation population)



Source: Elaborated by the authors.

3.3 Variance component estimation

Phenotypes, pedigree, and genotypes were used for variance component estimation under single-step genomic BLUP, through the restricted maximum likelihood method, using REMLF90 version 1.82 (Expectation Maximization

algorithm) and AIREMLF90 version 1.142 (Average Information algorithm) software belonged to the BLUPF90 family programs (MISZTAL, 2020). Thus, in the animal model, the inverse of the numerator relationship matrix ($\mathbf{A^{-1}}$) was replaced by ($\mathbf{H^{-1}}$), which was combine pedigree and genomic information. Matrix ($\mathbf{H^{-1}}$) can be obtained as follows (AGUILAR et al., 2010):

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

where, $\mathbf{G^{-1}}$ is the inverse of genomic relationship matrix and $\mathbf{A_{22}^{-1}}$ is the inverse of pedigree-based numerator relationship matrix for genotyped animals.

The general model can be represented as follows:

$$\mathbf{Y=Xb+Za+e},$$

where, **Y** is the vector of phenotypic observations, **X** is an incidence matrix that relates phenotypes and fixed effects, **b** is the vector of fixed effects, **Z** is an incidence matrix that relates animals to phenotypes, **a** is the vector of direct additive genetic effect, and **e** is a vector of residual effects. Assumptions were:

$$\mathbf{E}[\mathbf{Y}] = \mathbf{Xb}, \mathbf{var}[\mathbf{Y}] = \mathbf{Z}\Sigma\mathbf{Z}' + \mathbf{R},$$

with, $\Sigma = \mathbf{var}(\mathbf{a}) = \mathbf{H}\boldsymbol{\sigma}_{\mathbf{a}}^{\mathbf{2}}$ and $\mathbf{R} = \mathbf{I}\boldsymbol{\sigma}_{\mathbf{r}}^{\mathbf{2}}$ in the single-trait model, where $\mathbf{H}\boldsymbol{\sigma}_{\mathbf{a}}^{\mathbf{2}}$ is the additive genetic variance and $\mathbf{I}\boldsymbol{\sigma}_{\mathbf{r}}^{\mathbf{2}}$ the residual variance, **H** is the numerator relationship matrix among animals and **I** is the appropriate identity matrix. An inverted qui-square distribution was used for the prior values of the direct and residual genetic variances.

3.4 Prediction Models

The estimated breeding value (EBV) obtained through a traditional genetic evaluation performed without genomic information were deregressed

using the method proposed by Garrick and Taylor (2009), with the support of the DPR package implemented in R program, and the results were expressed in terms of deregressed expected progeny differences (dEPD), which was used as pseudo-phenotype in the genomic prediction analyses. The analyses were performed using the BLUPF90 family of programs (MISZTAL et al., 2002) available at http://nce.ads.uga.edu/wiki/doku.php, the Bayesian methods package (PÉREZ and DE LOS CAMPOS, 2014) and R software (R DEVELOPMENT CORE TEAM, 2017). The studied methods for genomic analysis were BLUP, GBLUP, ssGBLUP, BayesA, BayesB, BayesC, BayesianLASSO and an approach combining the pedigree-based numerator relationship matrix for genotyped animals ($\mathbf{A_{22}}$) with both the genomic matrix and Bayesian methods, hereafter referred as A22_BA, A22_BB, A22_BC, A22_BL and A22_G, as described in the next following topics.

3.4.1 Genomic Best Linear Unbiased Predictor (GBLUP)

The GBLUP model is similar to BLUP using a genomic relationship matrix (**G**) instead **A**. Solutions from GBLUP can be obtained with the model showed below:

$$\mathbf{y=1\mu+Zg+e,}$$

where **y** is the vector of pseudo-phenotypes (dEPD), **μ** is the overall mean, **1** is a vector of ones, **Z** is an incidence matrix of marker effects, **g** is a vector of marker effects, and **e** is a vector of residual effects. In the case of A22_G approach, the random effect of the $\mathbf{A_{22}}$ matrix was added to the equation described above for GBLUP. It was assumed $\mathbf{g} \sim \mathbf{N}(\mathbf{0}, \mathbf{G}\boldsymbol{\sigma}_{\mathbf{g}}^{\mathbf{2}})$, where $\boldsymbol{\sigma}_{\mathbf{g}}^{\mathbf{2}}$ is the variance of markers and **G** is the genomic relationship matrix. Random residuals were assumed $\mathbf{e} \sim \mathbf{N}(\mathbf{0}, \mathbf{I}\boldsymbol{\sigma}_{\mathbf{e}}^{\mathbf{2}})$, where I is an identity matrix and $\boldsymbol{\sigma}_{\mathbf{e}}^{\mathbf{2}}$ is the residual variance.

3.4.2 Single-Step Genomic Best Linear Unbiased Predictor (ssGBLUP)

The model used in ssGBLUP consists in combining **A** and **G** into a single matrix (**H**). Thus, the inverse of the numerator relationship matrix ($\mathbf{A^{-1}}$) was replaced by $\mathbf{H^{-1}}$, which combines pedigree and genomic information.

$$\mathbf{y=Zu+e,}$$

where, **y** is the vector of pseudo-phenotype (dEPD), **u** is the vector of direct additive genetic effects, and **Z** is an incidence matrix. Considering an infinitesimal model, $\mathbf{var(u) = H\sigma^2_u}$, where **H** is a combined relationship matrix that integrates the genomic derived relationships (**G** matrix) with population-based pedigree relationships (**A** matrix) and $\boldsymbol{\sigma^2_u}$ is the additive genetic variance, and $\mathbf{var(e) = I\sigma^2_e}$ where $\boldsymbol{\sigma^2_e}$ is the residual variance. The $\mathbf{H^{-1}}$ matrix was obtained as previously described in the topic "*Variance component estimation*".

3.4.3 Genomic prediction using the Bayesian methods

Genomic prediction models were fit using four Bayesian specifications: BayesA, BayesB, BayesC and BayesianLASSO. For these methods, the general statistical model was:

$$\mathbf{y} = \mathbf{1}\mu + \sum_{j=1}^{K} \mathbf{z}_j a_j + \mathbf{e},$$

where, **y** is a n × 1 vector of pseudo-phenotype (dEPD); **μ** is an intercept; **K** is the number of markers fitted; $\mathbf{z_j}$ is an n × 1 vector denoting the genotypes of the animals for marker **j**; $\mathbf{a_j}$ is the effect of marker **j**; and **e** is a vector of residual effects. The SNP genotypes were coded as 0, 1 and 2 for AA, AB and BB, respectively. The vector of residuals e was assumed to be distributed as $\mathbf{e} \sim N\left(\mathbf{0}, \mathbf{I}\sigma_e^2\right)$, where σ_e^2 is the residual variance. When models A22_BA, A22_BB, A22_BC and A22_BL were applied, the random effect of the pedigree-based numerator relationship matrix for genotyped animals ($\mathbf{A_{22}}$) was added to the general statistical equation described above for the Bayesian models.

3.4.3.1 BayesA

The BayesA method (MEUWISSEN et al., 2001) assumes that the conditional prior distribution of a marker effect $\mathbf{a_j}$ is Gaussian with null mean and marker-specific dispersion $\sigma^2_{a_j}$, independent from each other. The variance associated with the effect of each marker is assigned an independent and identically distributed scaled inverse chi-square prior distribution, $p\left(\sigma^2_{a_j}\right) = \chi^{-2}\left(\sigma^2_{a_j} \mid \nu, S^2\right)$, where **v** and $\mathbf{S^2}$ are known degrees of freedom and scale parameters, respectively. With these specifications, the marginal prior distribution of each marker effect, $p\left(a_j \mid \nu, S^2\right) = \int N\left(a_j \mid 0, \sigma^2_{a_j}\right)\chi^{-2}\left(\sigma^2_{a_j} \mid \nu, S^2\right)\partial\sigma^2_{a_j}$, is a t-distribution, i.e. $p\left(a_j \mid v, S^2\right) = t\left(0, v, S^2\right)$ (ROSA et al., 2003).

3.4.3.2 BayesB

In the BayesB method is assumed that most of the genetic markers have zero effect, and that only a few loci contribute with some genetic variance (MEUWISSEN et al., 2001). Conditional on the marker-specific variances $\sigma^2_{a_j}$, non-null marker effects are assumed Gaussian $N\left(a_j \mid 0, \sigma^2_{a_j}\right)$, such that the distribution of marker effects can be described with the following mixture model:

$$p\left(a_j \mid \sigma^2_{a_j}, \pi\right) = \begin{cases} 0 & \textit{with probability } \pi \\ N\left(0, \sigma^2_{aj}\right) & \textit{with probability } (1-\pi) \end{cases}$$

where **π** is the proportion of markers with null genetic effects.

Similarly as in BayesA approach, scaled inverse chi-square prior distributions are assumed for marker variances, i.e. $p\left(\sigma^2_{a_j}\right) = \chi^{-2}\left(\sigma^2_{a_j} \mid \nu, S^2\right)$, so that marginally, after integrating $\sigma^2_{a_j}$ out, the *prior* of marker effects takes the following form:

$$p(a_j \mid \pi) = \begin{cases} 0 & \text{with probability } \pi \\ t(0, v, S^2) & \text{with probability } (1-\pi) \end{cases}$$

Thus, BayesB can be reduced to BayesA by taking **π = 0**. $\mathbf{S^2}$ is given by $S^2 = \frac{\sigma_a^2(\nu-2)}{\nu}$ where $\sigma_a^2 = \frac{\sigma_S^2}{(1-\pi)\sum_{j=1}^{K} 2p_j(1-p_j)}$ and $\mathbf{p_j}$ is the allele frequency of the *j*th SNP, σ_S^2 is the additive-genetic variance explained by SNPs, and **π** is the *prior* probability that *j*th SNP has zero effect (HABIER et al., 2011).

3.4.3.3 BayesC

BayesC assumes the marker effects to be a mixture, with most marker effects to be zero, and a (usually) smaller part of markers to be nonzero. There is a common marker effect variance for all markers with nonzero effect. The prior probabilities of the SNP effects consist of a mixture of a probability mass point at zero (p = 1 − π) and a Gaussian distribution (p = π). BC considers a parameter π, which expresses the proportion of SNPs with non-null effects. This parameter is treated as unknown and has a beta density function a priori, i.e. π ~ beta (p_0, π_0), with parameter spaces $p_0 > 0$ and π_0 ε [0, 1]. It was assumed that π_0 was equal to 0.5 and p_0 was equal to 10 (PÉREZ and DE LOS CAMPOS, 2014).

3.4.3.4 BayesianLASSO

The general model for genomic prediction using BayesianLASSO model, expressed in matrix notation is:

$$\mathbf{y = 1\mu + Wg + e,}$$

where, **y** is the vector of pseudo-phenotype (dEPD), **μ** is the overall mean, **1** is a vector of ones, **g** is the vector of marker effects, **W** contains the genotype (coded 0 = AA, 1 = AB and 2 = BB) for each individual and each marker, and **e** is the vector of residual effects.

For Bayesian analyses, samples from posterior distributions were obtained by the Gibbs sampler based on 500,000 Monte Carlo Markov Chain (MCMC) samples with the first 100,000 discarded as burn-in. After burn-in, samples were thinned at a rate of 100. Convergence diagnostics, statistical and graphical analysis of Gibbs sampling were checked by visual inspection of trace plots of variance components using the Coda (RAFTERY and LEWIS, 1992) package.

3.5 Cross-validation and methodologies comparison

To obtain the prediction ability (PA), the dataset was split into training (8,703 animals) and validation (12,124 animals) subsets. The validation subset consisted of genotyped young animals without progeny records and without phenotypes, and genotyped sires and dams with progenies composed the training subset. Phenotypic and genotypic information from validation animals were omitted in the training set. Except for BLUP, the predictive ability of the methodologies was compared through the correlation between dEPD and DGVs. The correlation between EBV and dEPD was adopted as a measure of PA for BLUP model.

To evaluate the extent of prediction bias the linear regression between the response variable (dEPD) and DGVs (or EBVs for BLUP model) considering only the animals in the validation set, were calculated. Posteriorly, the regression coefficients were obtained to study the inflation/deflation level in relation to an applied response variable, so that the values closer to 1 (one) were considered the most desirable.

4 RESULTS AND DISCUSSION

The number of records, descriptive statistics and heritability estimates for studied traits are shown in Table 3.1. The heritability estimated for LMA (0.17) was similar to those reported by Miar et al. (2014) in crossbred beef cattle and Bolormaa et al. (2013) in *Bos taurus*, *Bos indicus* and composite beef cattle. The heritability estimated for BF was close to those reported by Gordo et al. (2012) in Nellore cattle and Miar et al. (2014) in crossbred beef cattle (0.24 and

0.31, respectively). For Nellore animals, Kluska et al. (2018) and Gordo et al. (2012) obtained heritability estimates similar for RF, 0.33 and 0.28, respectively. The estimated heritabilities for BS and PS agreed with those obtained by Yooko et al. (2009; 0.42 and 0.49, respectively), but the authors reported higher heritability estimates for MS (0.49). The heritability estimates for visual scores obtained in this study were similar to those reported in the literature, ranging from 0.24 to 0.45, 0.38 to 0.63 and 0.29 to 0.48 for BS, MS and FP, respectively (KOURY FILHO et al., 2010; FARIA et al., 2010; GORDO et al., 2012). The heritability estimates for carcass composition indicator traits pointed out that selection for these traits is feasible when phenotypic records are available.

Table 3.1 - Number of records (N), descriptive statistics and heritability estimates ($h^2 \pm SD$) for carcass traits obtained by ultrasound records and visual scores

Traits	N	Mean	Mode	Minimum	Maximum	$h^2 \pm SD$
BS	65,764	3.79	4	1	6	0.41 ± 0.12
FP	65,763	3.85	4	1	6	0.42 ± 0.15
MS	65,764	3.60	4	1	6	0.36 ± 0.13
LMA (cm^2)	141,030	54.31	--	18.59	117.42	0.17 ± 0.06
BF (mm)	140,227	2.89	--	0.09	26.25	0.27 ± 0.07
RF (mm)	140,750	3.96	--	0.09	27.18	0.32 ± 0.02

BS: body structure; FP: finishing precocity; MS: musculature; LMA: *Longissimus* muscle area; BF: backfat thickness and RF: rump fat.
Source: Elaborated by the authors.

The prediction ability and bias of genomic prediction for visual scores using BLUP, GBLUP, ssGBLUP and Bayesian models are presented in Table 3.2. The incorporation of genomic information improves the prediction ability for visual scores at yearling. Similar results were reported by Neves et al. (2014), Campos et al. (2018), Carreño et al. (2019) and Vargas et al. (2020) for visual scores in beef cattle. Despite the visual scores displayed moderate to high heritability estimates and the phenotypes can be assessed directly in the selection candidates, is necessary to maintain all the animals until yearling before selection decision. The use of genomic selection would allow await the discard and mating decisions, optimizing the management and natural resources.

Table 3.2 - Prediction ability (PA), bias (RC) and mean squared error (MSE) for visual scores using BLUP, genomic BLUP, single-step genomic BLUP and Bayesian models. Descriptive statistics of the results and the correlations among the predictions obtained with different methods can be found in Supplemental Appendices

Models	PA	RC	MSE
		BS	
BLUP	0.175	0.762	--
GBLUP	0.563	1.027	--
ssGBLUP	0.513	1.223	--
BA	0.569	1.021	0.033
BB	0.569	1.017	0.033
BC	0.554	1.018	0.033
BL	0.571	1.023	0.033
A22_BA	0.658	1.060	0.016
A22_BB	0.657	1.064	0.016
A22_BC	0.660	1.065	0.016
A22_BL	0.659	1.069	0.016
A22_G	0.652	1.057	0.016
		PS	
BLUP	0.276	0.612	--
GBLUP	0.623	1.026	--
ssGBLUP	0.656	1.053	--
BA	0.629	1.038	0.043
BB	0.635	1.033	0.043
BC	0.635	1.031	0.042
BL	0.642	1.046	0.043
A22_BA	0.697	1.101	0.019
A22_BB	0.696	1.110	0.019
A22_BC	0.698	1.109	0.019
A22_BL	0.700	1.117	0.019
A22_G	0.696	1.107	0.020
		MS	
BLUP	0.238	0.648	--
GBLUP	0.612	1.017	--
ssGBLUP	0.638	1.062	--
BA	0.623	1.025	0.035
BB	0.617	1.018	0.035
BC	0.618	1.021	0.035
BL	0.623	1.032	0.035
A22_BA	0.681	1.082	0.015
A22_BB	0.681	1.089	0.015
A22_BC	0.682	1.089	0.015
A22_BL	0.684	1.095	0.015
A22_G	0.679	1.085	0.016

Body Structure (BS); Finishing Precocity (FP) and Musculature (MS); Prediction ability was obtained through the correlation between deregressed expected progeny differences and direct genomic values (or estimated breeding values for BLUP model) (PA); Prediction bias was obtained using the linear regression coefficients between the deregressed expected progeny differences and direct genomic values (or estimated breeding values for BLUP model) considering only the animals in the validation set (RC).
Methods= Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP) and single-step Genomic BLUP (ssGBLUP).
Bayesian methods= BayesA (BA), BayesB (BB), BayesC (BC), BayesianLASSO (BL), BayesA considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BA), BayesB considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BB), BayesC considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BC), BayesianLASSO considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BL) and genomic relationship matrix considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_G).
Source: Elaborated by the authors.

For visual scores, the Bayesian models performed almost similar to ssGBLUP model, however, more biased predictions were obtained with the ssGBLUP model. Similar prediction accuracies between ssGBLUP and Bayesian methods for beef and dairy related traits were obtained in previous studies (HAYES et al., 2009; PRYCE et al., 2012; ERBE et al., 2012; CHEN et al., 2013; BOLORMAA et al., 2013). The GBLUP model displayed less biased prediction for MS and PS and lower or equal prediction ability compared to ssGBLUP and Bayesian methods. Previous studies suggested the superiority of Bayesian approaches when the number of SNPs is larger than the number of genotyped animals, i.e. many previous studies using real data (ERBE et al., 2012; PRYCE et al., 2012; GUNIA et al., 2014; NEVES et al., 2014; FERNANDES JÚNIOR et al., 2016), but this advantage was not confirmed in this study. Although the Bayesian models may provide rather different inferences regarding individual SNP effects, they frequently result in similar predictive abilities (GIANOLA, 2013). The approximation of results in the models can also be attributed to complex nature of the traits studied, considering that different models tend to show similar predictive ability when the traits are affected by many small-effect loci (DE LOS CAMPOS et al., 2013). In addition, it is noticed that in the Bayesian models when A_22 matrix was added (A22_BA, A22_BB, A22_BC, A22_BL and A22_G) the prediction ability

improves, but the bias also increases, which may be common when considering a more parameterized model.

The prediction ability and bias of genomic prediction for carcass traits obtained by ultrasonography using BLUP, GBLUP, ssGBLUP and Bayesian models are presented in Table 3.3. Comparing with BLUP model, the prediction ability and bias for carcass traits obtained by ultrasonography substantially improves when genomic information was included in the model. These results were in agreement with those reported in the literature for carcass composition indicator traits, where genomic selection improves the prediction ability (BOLORMAA et al., 2013; ELZO et al., 2013; ROLF et al., 2015; FERNANDES JÚNIOR et al., 2016). The implementation of genomic selection would allow making management decisions before yearling, when the ultrasound records were commonly obtained. The ssGBLUP model displayed equal or higher PA than the GBLUP and Bayesians models without A22 matrix. The advantages of ssGBLUP against GBLUP and Bayesian models in respect of predictive ability were also reported in previous studies (LOURENCO et al., 2014; ONOGI et al., 2014; SILVA et al., 2017; YAN et al., 2018).

Table 3.3 - Prediction ability (PA), bias (RC) and mean squared error (MSE) for carcass traits obtained by ultrasonography using BLUP, genomic BLUP, single-step genomic BLUP and Bayesian models. For more information and details, please check the Supplemental Appendices

Models	PA	RC	MSE
		LMA	
BLUP	0.235	0.511	--
GBLUP	0.557	1.056	--
ssGBLUP	0.623	1.025	--
BA	0.562	1.080	1.551
BB	0.555	1.068	1.552
BC	0.555	1.065	1.551
BL	0.562	1.087	1.549
A22_BA	0.662	1.146	1.035
A22_BB	0.657	1.152	1.027
A22_BC	0.661	1.154	1.027
A22_BL	0.660	1.167	1.031
A22_G	0.659	1.143	1.048
		BF	
BLUP	0.125	0.186	--
GBLUP	0.572	0.942	--
ssGBLUP	0.604	1.064	--
BA	0.586	1.022	0.018
BB	0.579	1.010	0.018
BC	0.577	1.010	0.018
BL	0.586	1.035	0.018
A22_BA	0.641	1.131	0.009
A22_BB	0.622	1.138	0.009
A22_BC	0.643	1.144	0.009
A22_BL	0.644	1.156	0.009
A22_G	0.639	1.134	0.010
		RF	
BLUP	0.143	0.205	--
GBLUP	0.567	0.980	--
ssGBLUP	0.614	1.046	--
BA	0.551	1.025	0.054
BB	0.544	0.990	0.054
BC	0.542	0.991	0.054
BL	0.550	1.022	0.054
A22_BA	0.617	1.122	0.032
A22_BB	0.609	1.123	0.032
A22_BC	0.613	1.131	0.033
A22_BL	0.624	1.148	0.032
A22_G	0.622	1.124	0.033

Longissimus Muscle Area (LMA); Back Fat Thickness (BF) and Rump Fat Thickness (RF); Prediction ability was obtained through the correlation between deregressed expected progeny differences and direct genomic values (or estimated breeding values for BLUP model) (PA); Prediction bias was obtained using the linear regression coefficients between the deregressed expected progeny differences and direct genomic values (or estimated breeding values for BLUP model) considering only the animals in the validation set (RC).
Methods= Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP) and single-step Genomic BLUP (ssGBLUP).
Bayesian methods= BayesA (BA), BayesB (BB), BayesC (BC), BayesianLASSO (BL), BayesA considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BA), BayesB considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BB), BayesC considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BC), BayesianLASSO considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BL) and genomic relationship matrix considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_G).
Source: Elaborated by the authors.

The prediction for LMA was less biased with ssGBLUP model, however, for RF and BF the predictions were less biased with the Bayesians models without $\mathbf{A}_{22}$ matrix. Comparing with the fatness carcass traits, the lower heritability estimated for LMA probably explained the less biased predictions with the ssGBLUP. The ssGBLUP is able to partially account for pre-selection, through considering all available information in the evaluation (i.e., phenotypes, genotypes and pedigree), thus minimizing possible selection bias on the predictions (LEGARRA et al., 2014). Despite the Bayesian models with $\mathbf{A}_{22}$ matrix (A22_BA, A22_BB, A22_BC, A22_BL and A22_G) displayed higher prediction ability, they also shown more biased predictions, particularly for LMA. Silva et al. (2017) also reported more biased prediction for feed efficiency related traits obtained using Bayesian methodology, especially for low heritability traits. In general, the prediction ability was almost similar among the models, probably, these results may be attributed to the polygenic nature of the evaluated traits. The carcass composition indicator traits are controlled by several QTLs of small effect as corroborated by Silva et al. (2019) in a previous genome-wide study using the same dataset. Daetwyler et al. (2013) also observed similar prediction ability among the genomic models (Bayesian methods, BLUP and GBLUP) for traits affected by many small-effect loci (polygenic nature).

5 CONCLUSIONS

In terms of prediction ability and bias, Bayesian approaches were superior for visual scores traits and the ssGBLUP for carcass traits obtained by ultrasonography, however, more biased results were obtained for BF and RF using the ssGBLUP. The ssGBLUP model showed less biased prediction for low heritability traits, such as LMA, and also it accounts lower computational demand and it is a straightforward method for implementing genomic selection in beef cattle. Carcass traits are assessed in selection candidates at yearling and displayed moderate to high heritability estimates, however, the progeny-test is a common practice in beef cattle breeding programs to improve the expected breeding value (EBV's) reliability for carcass traits in unproven sires. Therefore, earlier reliable genetic evaluations of unproven sires trough genomic selection is appealing in order to increase the genetic response for carcass traits.

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[1] De acordo com a Associação Brasileira de Normas Técnicas (ABNT NBR 6023).

APPENDIX A - Descriptive statistics for EBV (BLUP model) and GEBV considering all studied models and carcass traits obtained by ultrasound records and visual scores

Models	Mean						Standard Deviation						Minimum						Maximum					
	BS	FP	MS	LMA	BF	RF	BS	FP	MS	LMA	BF	RF	BS	FP	MS	LMA	BF	RF	BS	FP	MS	LMA	BF	RF
BLUP	0.483	0.365	0.381	1.269	0.055	0.093	0.283	0.400	0.363	1.844	0.232	0.366	-0.788	-1.446	-1.147	-4.845	-0.860	-1.798	1.420	1.693	1.602	7.254	1.098	1.975
GBLUP	0.490	0.334	0.358	1.219	0.053	0.087	0.230	0.366	0.338	1.579	0.207	0.324	-0.409	-0.958	-0.793	-3.385	-0.572	-1.122	1.365	1.412	1.435	7.009	1.035	1.555
ssGBLUP	0.099	0.211	0.187	0.703	0.077	0.104	0.404	0.569	0.509	2.531	0.343	0.548	-1.432	-1.920	-1.847	-7.805	-1.172	-1.788	1.826	2.066	1.927	10.099	1.694	2.206
BA	0.491	0.332	0.357	1.206	0.051	0.084	0.222	0.363	0.336	1.541	0.201	0.318	-0.413	-0.943	-0.808	-3.159	-0.493	-0.998	1.285	1.366	1.424	6.671	1.012	1.493
BB	0.491	0.331	0.357	1.200	0.053	0.083	0.225	0.364	0.338	1.560	0.202	0.322	-0.456	-0.919	-0.748	-3.228	-0.521	-1.054	1.268	1.347	1.468	6.637	0.999	1.497
BC	0.489	0.332	0.355	1.202	0.051	0.084	0.226	0.365	0.339	1.565	0.204	0.322	-0.436	-0.951	-0.818	-3.338	-0.542	-1.098	1.301	1.367	1.439	6.969	1.034	1.508
BL	0.492	0.331	0.356	1.204	0.052	0.085	0.218	0.359	0.332	1.528	0.198	0.314	-0.379	-0.886	-0.765	-3.135	-0.487	-1.014	1.289	1.343	1.425	6.601	1.030	1.518
A22_BA	0.482	0.340	0.362	1.221	0.054	0.088	0.228	0.346	0.319	1.529	0.199	0.312	-0.561	-1.131	-0.963	-3.779	-0.673	-1.392	1.189	1.556	1.412	6.934	0.927	1.500
A22_BB	0.481	0.338	0.360	1.206	0.050	0.085	0.227	0.343	0.317	1.523	0.197	0.312	-0.596	-1.125	-0.982	-4.112	-0.656	-1.382	1.190	1.462	1.431	6.949	0.929	1.491
A22_BC	0.481	0.339	0.360	1.214	0.051	0.087	0.226	0.343	0.316	1.518	0.196	0.311	-0.593	-1.129	-0.955	-3.682	-0.655	-1.388	1.204	1.513	1.429	6.734	0.916	1.439
A22_BL	0.479	0.335	0.357	1.199	0.049	0.084	0.224	0.342	0.317	1.502	0.195	0.306	-0.555	-1.165	-0.988	-3.820	-0.657	-1.379	1.188	1.500	1.440	6.625	0.925	1.506
A22_G	0.484	0.338	0.361	1.223	0.052	0.088	0.228	0.344	0.318	1.528	0.197	0.311	-0.549	-1.161	-0.970	-3.881	-0.666	-1.369	1.239	1.523	1.443	6.875	0.940	1.466

Traits = BS: body structure; FP: finishing precocity; MS: musculature; LMA: *Longissimus* muscle area; BF: backfat thickness and RF: rump fat
Methods = Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP) and single-step Genomic BLUP (ssGBLUP)
Bayesian methods = BayesA (BA), BayesB (BB), BayesC (BC), BayesianLASSO (BL), BayesA considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BA), BayesB considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BB), BayesC considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BC), BayesianLASSO considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BL) and genomic relationship matrix considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_G)
Source: Elaborated by the authors.

APPENDIX B - Correlations for EBV (BLUP model) and GEBV obtained between all studied models and traits BS (below the diagonal) and FP (above the diagonal)

BS		BLUP	GBLUP	ssGBLUP	BA	BB	BC	BL	A22_BA	A22_BB	A22_BC	A22_BL	A22_G		FP
	BLUP		0.875	0.755	0.882	0.874	0.875	0.881	0.962	0.960	0.963	0.965	0.960	BLUP	
	GBLUP	0.814		0.871	0.973	0.965	0.966	0.973	0.933	0.926	0.924	0.910	0.929	GBLUP	
	ssGBLUP	0.559	0.752		0.881	0.873	0.874	0.882	0.812	0.801	0.795	0.768	0.796	ssGBLUP	
	BA	0.809	0.965	0.755		0.991	0.992	0.998	0.931	0.923	0.919	0.902	0.914	BA	
	BB	0.800	0.952	0.748	0.987		0.991	0.992	0.923	0.916	0.912	0.894	0.906	BB	
	BC	0.798	0.951	0.749	0.986	0.985		0.991	0.923	0.916	0.912	0.893	0.906	BC	
	BL	0.811	0.964	0.756	0.996	0.986	0.985		0.903	0.923	0.919	0.902	0.914	BL	
	A22_BA	0.932	0.893	0.656	0.887	0.876	0.875	0.888		0.992	0.993	0.989	0.991	A22_BA	
	A22_BB	0.931	0.882	0.644	0.876	0.866	0.864	0.876	0.986		0.991	0.988	0.989	A22_BB	
	A22_BC	0.934	0.879	0.635	0.870	0.860	0.859	0.872	0.985	0.984		0.992	0.994	A22_BC	
	A22_BL	0.934	0.863	0.604	0.851	0.840	0.839	0.853	0.983	0.982	0.986		0.993	A22_BL	
	A22_G	0.932	0.900	0.657	0.878	0.868	0.866	0.880	0.985	0.983	0.985	0.984		A22_G	
		BLUP	GBLUP	ssGBLUP	BA	BB	BC	BL	A22_BA	A22_BB	A22_BC	A22_BL	A22_G		

Traits = BS: body structure; FP: finishing precocity
Methods = Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP) and single-step Genomic BLUP (ssGBLUP)
Bayesian methods = BayesA (BA), BayesB (BB), BayesC (BC), BayesianLASSO (BL), BayesA considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BA), BayesB considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BB), BayesC considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BC), BayesianLASSO considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BL) and genomic relationship matrix considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_G)
Source: Elaborated by the authors.

APPENDIX C - Correlations for EBV (BLUP model) and GEBV obtained between all studied models and traits MS (below the diagonal) and LMA (above the diagonal)

MS		BLUP	GBLUP	ssGBLUP	BA	BB	BC	BL	A22_BA	A22_BB	A22_BC	A22_BL	A22_G		LMA
	BLUP		0.859	0.741	0.866	0.858	0.857	0.866	0.953	0.950	0.954	0.956	0.949	BLUP	
	GBLUP	0.876		0.888	0.977	0.969	0.970	0.977	0.919	0.911	0.907	0.888	0.925	GBLUP	
	ssGBLUP	0.752	0.864		0.886	0.878	0.880	0.885	0.803	0.790	0.782	0.748	0.806	ssGBLUP	
	BA	0.882	0.975	0.874		0.991	0.990	0.997	0.916	0.908	0.905	0.883	0.914	BA	
	BB	0.876	0.966	0.865	0.991		0.990	0.991	0.908	0.902	0.897	0.875	0.905	BB	
	BC	0.874	0.968	0.866	0.992	0.990		0.990	0.907	0.901	0.896	0.874	0.905	BC	
	BL	0.882	0.974	0.876	0.997	0.991	0.992		0.917	0.908	0.904	0.883	0.914	BL	
	A22_BA	0.961	0.932	0.801	0.931	0.924	0.924	0.931		0.985	0.988	0.984	0.988	A22_BA	
	A22_BB	0.962	0.923	0.794	0.921	0.915	0.915	0.921	0.992		0.985	0.982	0.984	A22_BB	
	A22_BC	0.963	0.924	0.791	0.919	0.913	0.913	0.919	0.993	0.992		0.986	0.984	A22_BC	
	A22_BL	0.964	0.910	0.765	0.902	0.896	0.896	0.903	0.990	0.991	0.992		0.983	A22_BL	
	A22_G	0.959	0.927	0.793	0.915	0.908	0.908	0.915	0.991	0.993	0.993	0.995		A22_G	
		BLUP	GBLUP	ssGBLUP	BA	BB	BC	BL	A22_BA	A22_BB	A22_BC	A22_BL	A22_G		

Traits = MS: musculature; LMA: Longissimus muscle area
Methods = Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP) and single-step Genomic BLUP (ssGBLUP)
Bayesian methods = BayesA (BA), BayesB (BB), BayesC (BC), BayesianLASSO (BL), BayesA considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BA), BayesB considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BB), BayesC considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BC), BayesianLASSO considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BL) and genomic relationship matrix considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_G)
Source: Elaborated by the authors.

APPENDIX D - Correlations for EBV (BLUP model) and GEBV obtained between all studied models and traits BF (below the diagonal) and RF (above the diagonal)

BF		BLUP	GBLUP	ssGBLUP	BA	BB	BC	BL	A22_BA	A22_BB	A22_BC	A22_BL	A22_G		RF
	BLUP		0.845	0.695	0.850	0.844	0.842	0.851	0.942	0.939	0.944	0.947	0.939	BLUP	
	GBLUP	0.856		0.862	0.975	0.967	0.966	0.976	0.923	0.915	0.910	0.886	0.925	GBLUP	
	ssGBLUP	0.692	0.848		0.856	0.851	0.848	0.856	0.785	0.771	0.759	0.712	0.783	ssGBLUP	
	BA	0.862	0.975	0.851		0.991	0.988	0.997	0.922	0.913	0.907	0.881	0.914	BA	
	BB	0.854	0.967	0.844	0.992		0.989	0.990	0.914	0.909	0.901	0.874	0.908	BB	
	BC	0.852	0.968	0.843	0.991	0.991		0.988	0.912	0.906	0.897	0.871	0.904	BC	
	BL	0.862	0.974	0.849	0.997	0.994	0.991		0.922	0.914	0.908	0.882	0.915	BL	
	A22_BA	0.950	0.926	0.776	0.922	0.915	0.915	0.923		0.986	0.988	0.982	0.989	A22_BA	
	A22_BB	0.951	0.917	0.760	0.914	0.907	0.907	0.915	0.988		0.986	0.980	0.985	A22_BB	
	A22_BC	0.953	0.914	0.751	0.909	0.902	0.901	0.909	0.989	0.987		0.986	0.987	A22_BC	
	A22_BL	0.955	0.894	0.709	0.885	0.878	0.877	0.885	0.984	0.983	0.987		0.982	A22_BL	
	A22_G	0.949	0.927	0.770	0.915	0.907	0.907	0.915	0.991	0.987	0.989	0.985		A22_G	
		BLUP	GBLUP	ssGBLUP	BA	BB	BC	BL	A22_BA	A22_BB	A22_BC	A22_BL	A22_G		

Traits = BF: backfat thickness; RF: rump fat
Methods = Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP) and single-step Genomic BLUP (ssGBLUP)
Bayesian methods = BayesA (BA), BayesB (BB), BayesC (BC), BayesianLASSO (BL), BayesA considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BA), BayesB considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BB), BayesC considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BC), BayesianLASSO considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_BL) and genomic relationship matrix considering the random effect of pedigree-based numerator relationship matrix for genotyped animals (A22_G)
Source: Elaborated by the authors.

CHAPTER 4 - Genetic parameters and genomic regions associated with calving ease in primiparous Nellore heifers

ABSTRACT

SILVA, R. P. **Genetic parameters and genomic regions associated with calving ease in primiparous Nellore heifers.** 2021. Dr. Thesis – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

The aim of this study was to estimate genetic parameters and to identify genomic regions associated with the calving ease (CE) in precocious Nellore heifers. A total of 1,277 CE phenotypes were collected and scored into two categories: i- non assisted calving, categorized as success (1) and ii- assisted calving where heifers required any form of assistance or intervention to give birth, categorized as failure (2). A pedigree structure containing the identification of the animal's sire and dam was used, with the relationship matrix comprising a total of 6,511 animals. Genotypic data from 1,201 animals were obtained using low-density panel (Clarifide Nelore 3.1) encompassing over 29,001 single nucleotide polymorphisms (SNP) markers. A threshold sire-maternal grandsire model (S-MGS) was used to estimate the genetic parameters, which included sire, maternal grandsire and residual effects as random effects and the fixed effects of contemporary groups (farm and year of calving, sex) and birth weight of the calf as covariable (linear effect). Genomic breeding values were estimated using an animal model with the direct and maternal genetic variances which were previously obtained by means of a S-MGS threshold model. The direct and maternal heritability estimates for CE were obtained considering the covariance of direct and maternal effects fixed as zero. Regions that accounted for more than 0.5% of the additive genetic variance were used. The direct and maternal heritability estimates for CE were low (0.18) and moderate (0.39) respectively, indicating that genetic progress for this trait is feasible, and so, it would respond favorably to direct selection. Genes identified within the significant windows, such as *CA8*, *FAM110B*, *TOX*, *ARID1A*, *RBM15*, *HSF1* and *PLAG1* were found to be related with maternal and direct effects on CE.

Gene enrichment analysis revealed processes that might directly influence fetal processes involved in female pregnancy and stress response. These results should help to better understand the genetic and physiological mechanisms regulating placenta development and fetal development, and this information might be useful for future genomic studies in Nellore cattle.

Keywords: Beef cattle. Candidate genes. Distocia. Functional analyses. Reproduction traits.

1 INTRODUCTION

Calving ease (CE) is an important reproductive trait in beef cattle because influences animal welfare and herd profitability (CARNIER et al., 2000). Dystocia or delayed births might affect the short-term farm profits through the loss of calves, death of dams, extra labors and veterinary fees, but also affects the long-term animal performances i.e. health issues, fertility problems, reduced production and involuntary culling (MEIJERING, 1984).

In Nellore cattle, birth weight plays an important role in a calf's ability to express its genetic potential for growth related traits, since birth weight is genetically correlated with other production traits, like weaning weight and adult cow weight (BOLIGON et al., 2010). However, birth weight should be supervised to prevent dystocia, mainly in young heifers, since the low frequency of distocia in Nellore breed was more associated with birth weight than with maternal facility to give birth (ARAÚJO et al., 2014; KAMEI et al., 2017). Hence, although a better herd management of the heifer, such as an adequate rearing and feeding regime during late gestation could improve calving facility, a direct selection and a proper breeding approach targeting better CE might be a better choice in the long term (DEKKERS, 1994).

The CE is determined by the calf and the dam through the effects termed direct and maternal, respectively. From the standpoint of the offspring, the maternal effect is purely environmental. However, it has a genetic component that could contribute to selection purposes (DICKERSON, 1947; WILLHAM,1963). In this sense, estimates for direct and maternal heritabilities of CE varies from 0.072 to 0.13 and from 0.007 to 0.07 respectively (DJEMALI et al., 1987; HICKEY et al., 2007; LUO et al., 2002; MEIJERING, 1984; WELLER and GIANOLA, 1989; WELLER and RON, 1992). Additionally, there is no consensus about the genetic association between direct and genetic maternal effects for CE (BAR-ANAN et al., 1976; CARNIER et al., 1998; CUE and HAYES, 1985; DWYER et al., 1986; LUO et al., 2002; MANFREDI et al., 1991; MEIJERING, 1985; THOMPSON et al., 1981; TRUS and WILTON, 1988; WELLER et al., 1988).

In Zebu cattle, the inclusion of reproductive traits in selection indices, such as the age at first calving and early pregnancy in heifer, is leading to

earlier conception, gestation and calving of primiparous heifers. The early pregnancy trait in heifers displayed moderate to high heritability and the selection for this trait is feasible (KLUSKA et al., 2018; BONAMY et al., 2019). In recent years, the proportion of calves being born to heifers with less than 30 months of age has increased in Nellore breeding programs as a result of selection for early heifer pregnancy, but problems associated with calving has also raised, been something uncommon in the Nellore breed. Therefore, there is a growing concern about the reproductive performance of sexual precocious heifers, in particular with calving events, and also explore the genetic architecture and physiological mechanism related with CE (ALAM et al., 2017).
Most of the genomic regions related with CE in cattle reported in genome-wide association studies (GWAS) were performed mainly in taurine breeds (ABO-ISMAIL et al., 2017; FRISCHKNECHT et al., 2017). The identification of genomic regions associated with CE would be of support to select for CE in indicine cattle, since this trait is not easy to measure in heifers under extensive conditions. It will also allow to understand the genetic background for the direct and maternal effects.

2 OBJECTIVES

The aim of this study was to estimate genetic parameters and identify genomic regions associated with calving ease in early sexual precocious Nellore heifers.

3 MATERIAL AND METHODS

3.1 Ethics approval

The collection of phenotypic information is not categorized as an experiment, since the interventions are related to farming practices (law Nº 11.794; October, 8, 2008; subsection VII of § 1o of clause 225 of Brazilian Federal Constitution). Thus, this study was not submitted to an ethics committee to evaluate the scientific use of animals, since the records were provided by commercial beef cattle farms.

3.2 Phenotypic and Genotypic Data

The phenotypic information was provided by two farms, Genética Aditiva (N= 392) and Nellore Naviraí (N= 885), which participates in the Brazilian Nellore breeding program belonging to the National Association of Breeders and Researchers (ANCP, Ribeirão Preto-SP, Brazil). This study included field records on parturition from primiparous Nellore heifers that calved between 2015 and 2019. A total of 1,277 field based CE phenotypes were collected, and all records were scored into two categories of birth events: i- non assisted calving, categorized as success (1) representing 86% of the records and ii- assisted calving where heifers required any form of assistance or intervention to give birth, categorized as failure (2), representing the remaining 14% records. A pedigree structure containing the identification of the animal's sire and dam was used, with the relationship matrix comprising a total of 6,511 animals.

Genotypic data from 1,201 animals were obtained using low-density panel (Clarifide Nelore 3.1) encompassing over 29,001 single nucleotide polymorphisms (SNP) markers, which were built specifically for *Bos Indicus* breeds. The quality control for genomic information was performed by the PREGSF90 program (MISZTAL et al., 2015), considering only autosomal chromosomes, and SNPs with allele frequency less than 0.05, Hardy-Weinberg equilibrium p-value less than 10-5 and SNPs with a call rate less than 0.95 were excluded. For samples, a call rate of at least p-value of 0.90 was required. After quality control, 1,199 genotyped animals and 20,516 SNPs remained for analyses.

3.3 Estimation of Genetic Parameters

Considering that CE was recorded as a binary trait, a threshold sire-maternal grandsire model (S-MGS) was used to estimate the genetic parameters, which included sire, maternal grandsire and residual effects as random effects and the fixed effects of contemporary groups (farm and year of calving, sex) and birth weight of the animal as a covariable (linear effect). The sire-maternal grandsire model is described in matrix form as:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{W}_S\mathbf{a}_S + \mathbf{W}_{MGS}\mathbf{a}_{MGS} + \mathbf{e},$$

where **y**, **b**, $\mathbf{a_s}$, $\mathbf{a_{mgs}}$ and **e** are vectors of phenotypes, fixed effect of contemporary group, random additive genetic effects of the sires, random additive genetic effects of the maternal grandsires and random residuals effects, respectively; **X**, $\mathbf{W_s}$ and $\mathbf{W_{MGS}}$ are the corresponding incidence matrices for **b**, $\mathbf{a_s}$ and $\mathbf{a_{mgs}}$. The covariance between direct and maternal effects was set to zero. It was assumed that the CE phenotypes (**y**) were the outcome of an unobserved underlying normally distributed continuous scale called liability (THOMPSON and BAKER, 1981; GIANOLA and FOULLEY, 1983), so that **y** is linked to an underling liability (θ). The observed binary response takes a value of **1** if θ was larger than a fixed threshold (τ), and **0** otherwise. Formally, this can be presented as follows:

$$\mathbf{y} = \begin{cases} 1, & \text{if } \theta > \tau \\ 0, & \text{if } \theta \leq \tau \end{cases}.$$

In threshold models for binary data, the threshold (τ) and mean on the liability scale are not identifiable, and usually the threshold is set to an arbitrary value: $\tau = 0$, such that **y** = 1 if $\tau > 0$ and 0 otherwise, and the mean on the liability scale models the average probability for **y** = 1.

The variance components and genetic parameters were estimated using a Bayesian approach via Gibbs sampling algorithm with THRGIBBS1F90 (MISZTAL et al., 2015) assigning the genetic correlation between direct and maternal effects to zero and a value of 1 to the residual variance. To consider genomic information in the S-MGS model, the single-step Genomic Best Linear Unbiased Predictor (ssGBLUP) approach was used. This methodology combines pedigree and genomic relationships in a single realized relationship matrix (**H**), and the inverse of **H** replaces the inverse of the pedigree-based

relationship matrix ($\mathbf{A^{-1}}$) in the BLUP mixed model equations. Matrix $\mathbf{H^{-1}}$ can be obtained as follows (AGUILAR et al., 2010):

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

where $\mathbf{G^{-1}}$ is the inverse of the genomic relationship matrix and $\mathbf{A_{22}^{-1}}$ is the inverse of pedigree-based numerator relationship matrix for genotyped animals. According to (VANRADEN, 2008), the **G** matrix can be calculated:

$$\mathbf{G} = \frac{(\mathbf{M} - \mathbf{P})(\mathbf{M} - \mathbf{P})'}{\mathbf{2} \sum_{\mathbf{j=i}}^{\mathbf{m}} \mathbf{p_j} \, (\mathbf{1} - \mathbf{p_j})},$$

where **M** is a matrix of marker alleles with n lines (n = number of genotyped animals) and m columns (m = number of markers), and **P** is a matrix containing: $2(\mathbf{p_j}-0.5)$, with $\mathbf{p_j}$ being the frequency of the second allele. Elements of **M** are set to 0 and 2 for both homozygous and to 1 for the heterozygous genotype.

Samples from posterior distributions for THRGIBBS1F90 were obtained by the Gibbs sampler based on 600,000 Monte Carlo Markov Chain samples with the first 100,000 discarded as burn-in. After burn-in, samples were thinned at a rate of 100, resulting in 5000 mildly correlated samples for posterior inference. Convergence diagnostics, statistical and graphical analysis of Gibbs sampling were checked by visual inspection of trace plots of variance components using the Coda R package (RAFTERY and LEWIS, 1992; R CORE TEAM, 2013).

After verifying the Gibbs chain convergence, a *posteriori* distribution estimates were computed for direct and maternal heritabilities. Following the derivations proposed by Willham (1972), the solutions for sire variance (σ_S^2), maternal grandsire variance (σ_{MGS}^2) and covariance between sire and maternal grandsire ($\sigma_{S,MGS}$) were converted to direct (D) and maternal (M) effects according to the equations:

$$\sigma_D^2 = 4\,\sigma_S^2$$

$$\sigma^2_M = 4\,\sigma^2_{MGS} - 4\,\sigma_{S,MGS} + \sigma^2_S$$

The $\sigma_{S,MGS}$ was set to zero and the phenotypic variance was defined by $\sigma^2_P = \sigma^2_S + \sigma^2_{MGS} + \sigma^2_E$, where σ^2_E is the residual variance. Based on this, the direct (h^2_D) and maternal (h^2_M) heritabilities were obtained:

$$h^2_D = \frac{\sigma^2_D}{\sigma^2_P} \quad \text{and} \quad h^2_M = \frac{\sigma^2_M}{\sigma^2_P}.$$

3.4 Genome-wide association study (GWAS)

Genomic breeding values were estimated using the THRGIBBS1F90 (MISZTAL et al., 2015) with an animal model using the direct and maternal genetic variances previously obtained using the S-MGS threshold model. The GWAS analysis for CE was performed applying the single-step GWAS (ssGWAS) methodology (WANG et al., 2012), which allows the use of the whole information available from genotyped and ungenotyped animals in one-step procedure. According to Wang et al. (2012), the estimation of animal effect for genotyped individuals was calculated as $a_g = \mathbf{Zu}$, where **Z** is the matrix that related genotypes of each locus, **u** is a vector of marker effects, and the variance for animal effects was assumed as:

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

where **D** is a diagonal matrix of weights, $\sigma^2_{\mathbf{u}}$ is the genetic additive variance captured by each SNP when no weights are present, and **G*** is the weighted genomic relationship matrix. In regular GBLUP-based methods, **D = I**, which gives a weight of 1 to all SNP. The ratio of covariance of genetic effects ($\mathbf{a_g}$) and the respective SNP effects is:

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

and weighted genomic relationship matrix is obtained:

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

where λ is a normalizing constant described by (**VanRaden** et al., 2009) as:

$$\lambda = \frac{\sigma^2_{\mathbf{u}}}{\sigma^2_{\mathbf{a}}} = \frac{1}{\sum_{\mathrm{i}=1}^{\mathrm{M}} 2\mathrm{p_i}(1-\mathrm{p_i})}$$

where M is the number of SNPs and $\mathbf{p_i}$ is the frequency of the second allele in the *i-th* SNP. Markers effects can be calculated following (STRANDÉN and GARRICK, 2009):

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

The estimated SNP effects can be used to obtain the variance of each individual SNP and apply a different weighting for each marker. The weights were calculated using the nonlinear method as described in (VANRADEN, 2008):

$$\mathbf{d_{ii}} \;=\; \hat{\mathbf{u}}_{\mathbf{i}}^{\mathbf{2}} \;/\mathbf{v}^{|\,\mathbf{s_i} - \mathbf{2}\,|}$$

where **v** is a scale parameter standing for the departure from normality and $\mathbf{s_i}$ is the number of standard deviations from the mean for each $\mathbf{2}\sum_{\mathbf{j}=\mathbf{i}}^{\mathbf{m}} \mathbf{p_j}\,(\mathbf{1}-\mathbf{p_j})$.

The estimation of SNP effects and variances were obtained using the software POSTGSF90 (MISZTAL et al., 2015) in three iterative processes, each one consisting of seven steps (WANG et al., 2012): 1. Before the first iteration, set **D = I**; 2. Construct the genomic relationship matrix (**G**); 3. Calculate the genomic estimated breeding vales (GEBV); 4. Calculate SNP effects; 5.

Calculate de weight of each SNP using the nonlinear A method; 6. Normalize **D**; and 7. Iterate from step 2, using the updated **D**.

Results were summarized using 1-Mb sliding SNP windows and reported as percentage of genetic variance due to direct and maternal effects explained by *i-th* window according to (WANG et al., 2014):

$$\frac{\mathrm{var}(\mathbf{a}_i)}{\sigma^2_{\mathbf{a}}} \text{ x } 100 \;=\; \frac{\mathrm{var}\left(\sum_{j=1}^{1Mb} \mathbf{Z}_j\, \hat{\mathbf{u}}_j\right)}{\sigma^2_{\mathbf{a}}} \text{ x } 100$$

where $\mathbf{a_i}$ is the additive genetic value for *i-th* window, $\sigma^2_{\mathbf{a}}$ is the total genetic variance, $\mathbf{Z_j}$ is a vector of SNP content of the *j-th* SNP for all individuals, and $\hat{\mathbf{u}}_j$ is the effect of the *j-th* SNP within the *i-th* region. The window size was defined after an analysis performed with the R software, in which the average and mode haplotype block was obtained from the studied population. In addition, the window size was based on the linkage disequilibrium of Zebu genome (ESPIGOLAN et al., 2013).

3.5 Enrichment analysis

The chromosomal regions that explained over 0.5% of the direct and maternal additive genetic variance were selected to explore and determine possible quantitative trait loci (QTL). The windows were defined by 1-Mb continuous adjacent SNPs. The bovine genome Map Viewer UMD3.1 available at "National Center for Biotechnology Information" (NCBI - http://www.ncbi.nlm.nih.gov) and the Ensemble Genome Browser (*Bos taurus* genes UMD3.1) (http://www.ensemble.org/index.html) were used for gene identification. GeneCards (http://www.genecards.org/) were used to describe annotated gene functions. Database for Annotation, Visualization, and Integrated Discovery (DAVID) v. 6.8 tool (HUANG et al., 2009) was used to identify significant Gene Ontology (GO) ($p \leq 0.01$) terms and KEEG ($p \leq 0.05$) (Kyoto Encyclopedia of Genes and Genomes) pathways using the list of genes from the windows of 10 adjacent SNPs and the *Bos taurus taurus* annotation file as background. The convergence for all parameter estimates was verified by

using Geweke's (GEWEKE, 1992). The burn-in period considered was sufficient to reach convergence in all parameter estimates.

4 RESULTS AND DISCUSSION

4.1 Genetic Parameter Estimates

The direct and maternal heritability estimates for CE (Table 4.1) were moderate (0.18) and high (0.39), respectively, indicating that genetic progress for this trait is feasible. Literature estimates of heritabilities on calving ease in cattle using sire model or sire-maternal grandsire model ranged from 0.072 to 0.13 for direct heritability and from 0.007 to 0.07 for maternal heritability (CUBAS et al., 1991; CUE and HAYES, 1985; DJEMALI et al., 1987; HICKEY et al., 2007; LUO et al., 2002; MANFREDI et al., 1991; VOSTRÝ et al., 2015; WELLER and GIANOLA, 1989; WELLER and RON, 1992; WIGGANS et al., 2003). For Dutch Holstein-Friesian and Canadian Holstein cattle displayed a direct heritability for CE varying from 0.08 to 0.14, and a maternal heritability of varying from 0.04 to 0.08, obtained by sire-maternal grandsire model (EAGLEN and BIJMA, 2009; LUO et al., 2002), being lower than those obtained in this study. It is important to highlight that the results obtained in this study are based on records from only two farms (i.e. less environmental variation) and applying a sire-maternal grandsire model, which could biased upwards the heritability estimates.

Table 4.1 - Direct (h^2_D) and maternal (h^2_M) heritability estimates for calving ease (CE)

Parameters	Posterior Means	SD	SE	95% HPD	
				Low Limit	High Limit
h^2_D	0.18	0.14	0.0020	$4.15e^{-4}$	0.47
h^2_M	0.39	0.20	0.0028	$9.20e^{-2}$	0.78

SE = standard error; SD= standard deviation; HPD= highest posterior density
Source: Elaborated by the authors.

4.2 Maternal effect for calving ease

A total of 17 genomic regions and 436 genes were identified that explained more than 0.5% of the maternal genetic variance (Table 4.2), in which 402 of them have described biological functions and 34 are uncharacterized genes (Figure 1).

Table 4.2 - Genomic regions associated with calving ease (Maternal Effect)

BTA	Position (pb)	Candidate Genes	% variance explained by SNPs windows
1	99410715-100300830	MECOM, GOLIM4, LRRC77, SERPINI1, PDCD10, ENSBTAG00000011427, WDR49, SERPINI2	0.94
2	126677480-127673633	FAM76A, IFI6, FGR, AHDC1, WASF2, GPR3, CD164L2, MAP3K6, SYTL1, TMEM222, SLC9A1, TENT5B, KDF1, NUDC, NR0B2, GPATCH3, GPN2, SFN, ZDHHC18, PIGV, ARID1A, RPS6KA1, HMGN2, DHDDS, LIN28A, ZNF683, CRYBG2, CD52, UBXN11, SH3BGRL3, CEP85, ENSBTAG00000039276, CATSPER4, ENSBTAG00000047148, CNKSR1, ZNF593, FAM110D, PDIK1L, TRIM63, SLC30A2, EXTL1, PAFAH2, STMN1, PAQR7, AUNIP, MTFR1L, SELENON, MAN1C1, LDLRAP1, MACO1	0.52
3	15158180-16053896	SEMA4A, LMNA, MEX3A, RAB25, LAMTOR2, UBQLN4, SSR2, ARHGEF2, RXFP4, KHDC4, RIT1, SYT11, ENSBTAG00000045596, GON4L, MSTO1, DAP3, ASH1L, FDPS, PKLR, HCN3, CLK2, SCAMP3, FAM189B, GBA, MTX1, THBS3, MUC1, TRIM46, KRTCAP2, DPM3, SLC50A1, EFNA1, EFNA3, EFNA4, ADAM15, DCST1, DCST2, ZBTB7B, LENEP, FLAD1, CKS1B, SHC1, PYGO2, PBXIP1, PMVK, KCNN3, ADAR, CHRNB2, UBE2Q1, TDRD10, ENSBTAG00000045672, SHE, IL6R, ATP8B2, AQP10, HAX1, UBAP2L, C3H1orf43, C3H1orf189, TPM3, NUP210L, RPS27, ENSBTAG00000018987, RAB13, JTB, CREB3L4, SLC39A1, CRTC2, DENND4B	0.88

Continue

Table 4.2 - Genomic regions associated with calving ease (Maternal Effect)

3	32440899-33388282	TMIGD3, ADORA3, ATP5PB, WDR77, OVGP1, ENSBTAG00000034841, ENSBTAG00000023535, CHIA, CHI3L2, DENND2D, CEPT1, DRAM2, LRIF1, CD53, KCNA3, KCNA2, KCNA10, CYM, PROK1, LAMTOR5, SLC16A4, RBM15, KCNC4, SLC6A17, UBL4B, ALX3, STRIP1, AHCYL1, CSF1, ENSBTAG00000001845, GSTM3, ENSBTAG00000031788, GSTM1, ENSBTAG00000016472, ENSBTAG00000012692, GSTM1	8.73
11	12786137-13757086	EXOC6B, CYP26B1, DYSF, ZNF638, PAIP2B, NAGK, TEX261, ANKRD53, ATP6V1B1, VAX2, CD207, CLEC4F, FIGLA, ADD2, TGFA, FAM136A, XDH	1.52
11	53132515-53778535	EXOC6B, CYP26B1, DYSF, ZNF638, PAIP2B, NAGK, TEX261, ANKRD53, ATP6V1B1, VAX2, CD207, CLEC4F, FIGLA, ADD2, TGFA, FAM136A, XDH	0.55
12	33535830-34395464	GPR12, WASF3, CDK8, RNF6, ENSBTAG00000025428, SHISA2, ATP8A2, NUP58, MTMR6, ENSBTAG00000019545, C1QTNF9, MIPEP, TNFRSF19, SACS	0.68
12	78600379-79466865	MBNL2, RAP2A, IPO5, FARP1, STK24, SLC15A1, DOCK9	0.58
14	1868636-2857000	ZNF16, C14H8orf33, ZNF34, RPL8, ZNF7, COMMD5, ARHGAP39, C14H8orf82, LRRC24, LRRC14, RECQL4, MFSD3, GPT, PPP1R16A, FOXH1, KIFC2, CYHR1, TONSL, VPS28, SLC39A4, CPSF1, ADCK5, SLC52A2, FBXL6, TMEM249, SCRT1, DGAT1, HSF1, BOP1, SCX, MROH1, ENSBTAG00000039978, HGH1, WDR97, MAF1, SHARPIN, CYC1, GPAA1, EXOSC4, OPLAH, ENSBTAG00000015040, SPATC1, GRINA, PARP10, PLEC, NRBP2, PUF60, SCRIB, FAM83H, MAPK15, CCDC166, ZNF623, ENSBTAG00000046866, TSTA3, PYCR3, TIGD5, EEF1D, NAPRT, MROH6 GSDMD, ZC3H3, MAFA, RHPN1, ENSBTAG00000003606, LY6H, LY6E, ENSBTAG00000004596, GML, LY6K, ENSBTAG00000037824, LY6D, LYNX1, LYPD2, SLURP1, THEM6, PSCA, ARC, ENSBTAG00000026340, ADGRB1, TSNARE1	0.61
14	26223437-27155254	FAM110B, ENSBTAG00000047136, UBXN2B CYP7A1, SDCBP, NSMAF, TOX, CA8	0.91
15	24038481-24954026	NCAM1, TTC12, ANKK1, DRD2, TMPRSS5, ZW10, CLDN25, USP28, HTR3B, HTR3A, ZBTB16, ENSBTAG00000046254, ENSBTAG00000008564, C15H11orf71, RBM7, REXO2, NXPE4	1.50

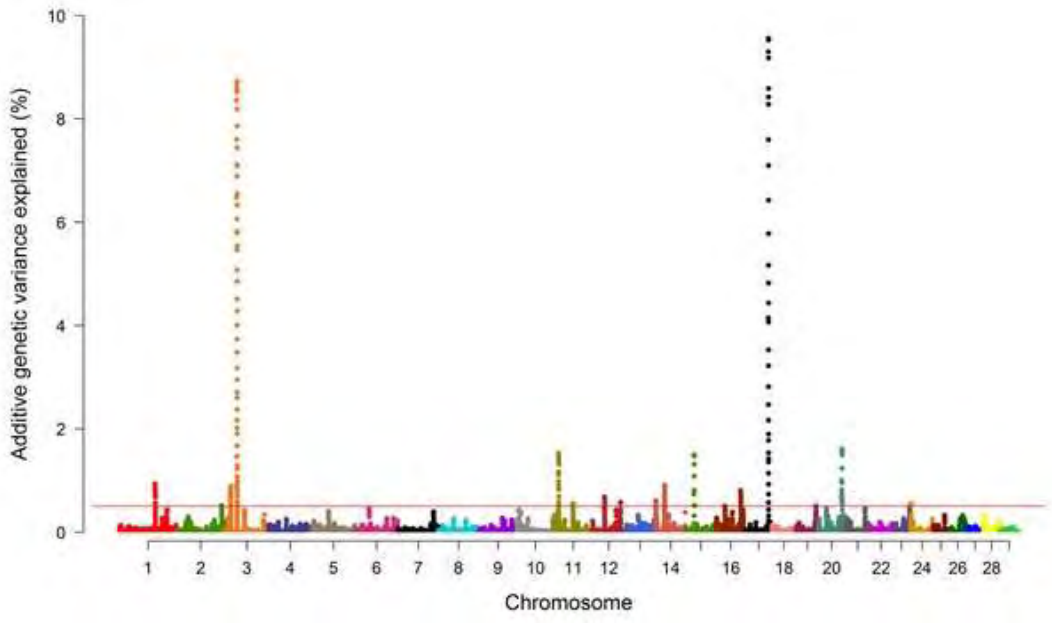
Continuation

Table 4.2 - Genomic regions associated with calving ease (Maternal Effect)

16	25000153-25953743	MARK1, C16H1orf115, MARC2, MARC1, HLX, DUSP10, HMCN1, PRG4, TPR, ENSBTAG00000047559, ODR4, PDC, PTGS2, PLA2G4A, KCNK2	0.51
16	68714302-69671934	MARK1, C16H1orf115, MARC2, MARC1, HLX, DUSP10, HMCN1, PRG4, TPR, ENSBTAG00000047559, ODR4, PDC, PTGS2, PLA2G4A, KCNK2	0.80
17	64938681-65923565	TMEM116, ADAM1B, MAPKAPK5, ALDH2, BICDL1, RAB35, GCN1, RPLP0, PXN, SIRT4, ENSBTAG00000026732, ENSBTAG00000047084, MSI1, COX6A1, TRIAP1, GATC, SRSF9, DYNLL1, COQ5, RNF10, POP5, CABP1, MLEC, UNC119B, ACADS, SPPL3, HNF1A, C17H12orf43, OASL, ENSBTAG00000046887, ENSBTAG00000032047, ANKRD13A, GIT2, TCHP, GLTP, TRPV4, FAM222A, MVK, MMAB, UBE3B, KCTD10, MYO1H, FOXN4, ACACB, UNG, ALKBH2, USP30, SVOP, DAO, SSH1	9.56
20	64911501-65773057	MTRR, FASTKD3, ADCY2	1.60
23	49437644-50388461	FARS2, LYRM4, PPP1R3G, RPP40, CDYL, ECI2, ENSBTAG00000015176, FAM217A, PRPF4B, PXDC1, SLC22A23, PSMG4, TUBB2B, TUBB2A, BPHL, ENSBTAG00000045888, RIPK1, NQO2, ENSBTAG00000017483, ENSBTAG00000017949, SERPINB6, SERPINB9, SERPINB1, WRNIP1, MYLK4	0.51
24	1704623-2620915	ATP9B, GALR1, MBP, ZNF236, ZNF516	0.56

Conclusion
Source: Elaborated by the authors.

Figure 1 - Manhattan plot of the maternal genetic variance explained by 1-Mb continuous adjacent SNPs for calving ease in Nellore cattle. The dots above the red line indicates the regions explaining more than 0.5% of the maternal genetic variance



Source: Elaborated by the authors.

The window located on BTA14 (26.22-27.15 Mb) overlapped with a significant region (21.95-28.4 Mb) described by Fortes et al., (2012), whom performed a GWAS for sexual puberty related traits in Brahman cattle. These authors identified four genes in common with the present study, such as *CA8* (P-type ATPase), *FAM110B* (family with sequence similarity 110 member B), *NSMAF* (neutral sphingomyelinase (N-SMase) activation associated factor) and *TOX* (thymocyte selection associated high mobility group box) genes. A locus on BTA14 has been associated with calving ease in German Fleckvieh beef breed (PAUSCH et al., 2011).

The genes *ARID1A* (AT-rich interaction domain 1A), *RBM15* (RNA Binding Motif Protein 15) and *HSF1* (Heat Shock Transcription Factor 1), found on BTA2 (126.67-127.67 MB), BTA3 (32.44-33.38 Mb) and BTA14 (1.86-2.85 Mb), respectively, have functions associated with placenta development and fetal development especially in female pregnancy and stress response. These genes could be of great importance for CE, since mal function of placenta can lead to significant maternal morbidities, such as preeclampsia and fetal complications, restricting fetal growth which might end with fetal demise (WANG

et al., 2016). In a study with mice, Xiao et al., (1999) reported defects on the placenta development of *HSF1* gene-deficient animals, indicating that the cells and tissues requirements were specific for *HSF1* gene. In addition, the females also presented infertility in adulthood. In addition, Wang et al., (2016) working with multiparous female mice reported that placentas from *ARID1A* gene deficient samples manifested lower levels of Prostaglandin, an important hormone during the placenta development. These authors concluded that *ARID1A* gene is essential for normal placental morphology and fertility in parous female mice. Moreover, female mice with conditional deletion of *ARID1A* gene showed progressive decline in fertility with consecutives parturitions, with a loss of pups in the post implantation phase beginning on the third pregnancy. Raffel et al., (2009) studied the *RMB15* gene demonstrating placental defects in the spongiotrophoblast and syncytial trophoblast layers, resulting in an arrest of vascular branching morphogenesis in gene deficient female mice and a growth retardation and incomplete closure of the notochord on their embryos. Similar to the results obtained in the present study, Purfield et al., (2015) working with Limousin and Charolais beef breeds, reported several genomic regions associated with calving ease at the BTA2, suggesting that these regions harbor QTL for calving easy in beef breeds.

Saatchi et al. (2014), in a study with large-effect pleiotropic or closely linked QTL segregate within and across ten US cattle breeds, reported a genomic region at BTA6 associated with direct calving ease and weaning weight. This QTL explained more than 10% of the direct additive genetic variance of birth weight and calving ease in Hereford cattle breed. The largest effect found for this QTL was for calving ease in Hereford where it explained 32% of additive genetic variance. Like in the present study, many previous studies have reported the presence of QTL at BTA6 associated with direct calving ease (BONGIORNI et al., 2012; HÖGLUND et al., 2012).

4.3 Direct effect for calving ease

A total of 18 genomic regions and 341 genes were identified that explained more than 0.5% of the additive direct genetic variance (Table 4.3), in

which 284 of them had described biological functions and 57 are uncharacterized genes (Figure 2).

Table 4.3 - Genomic regions associated with calving ease (Direct Effect)

BTA	Position (pb)	Candidate Genes	% variance explained by SNPs windows
3	32440899-33388282	TMIGD3, ADORA3, ATP5PB, WDR77, OVGP1, ENSBTAG00000034841, ENSBTAG00000023535, CHIA, CHI3L2, DENND2D, CEPT1, DRAM2, LRIF1, CD53, KCNA3, KCNA2, KCNA10, CYM, PROK1 LAMTOR5, SLC16A4, RBM15, KCNC4, SLC6A17, UBL4B, ALX3, STRIP1, AHCYL1, CSF1, ENSBTAG00000001845,GSTM3, ENSBTAG00000031788, GSTM1, ENSBTAG00000016472, ENSBTAG00000012692, GSTM1	3.89
3	9797974-10746542	VANGL2, NHLH1, NCSTN, COPA, PEX19, DCAF8, PEA15, CASQ1, ATP1A4, ATP1A2, IGSF8, KCNJ9, KCNJ10, PIGM, SLAMF9, IGSF9, TAGLN2, CFAP45, VSIG8, SLAMF8, FCRL6, DUSP23, CRP, ENSBTAG00000046656, APCS, OR10J5, OR10J1, OR10J4, ENSBTAG00000020765, OR10J3, FCER1A, ENSBTAG00000022528, MPTX, ACKR1, CADM3, ENSBTAG00000011511, ENSBTAG00000020838, ENSBTAG00000048292, OR6N2, OR6N1, OR6K6, ENSBTAG00000038961, OR6K3, OR6K2, SPTA1, OR10Z1, ENSBTAG00000039653	0.66
3	15158180-16053896	SEMA4A, LMNA, MEX3A, RAB25, AMTOR2, UBQLN4, SSR2, ARHGEF2, RXFP4, KHDC4, RIT1, SYT11, ENSBTAG00000045596, GON4L, MSTO1, DAP3, ASH1L, FDPS, PKLR, HCN3, CLK2, SCAMP3, FAM189B, GBA, MTX1, THBS3, MUC1, TRIM46, KRTCAP2, DPM3, SLC50A1, EFNA1, EFNA3, EFNA4, ADAM15, DCST1, DCST2, ZBTB7B, LENEP, FLAD1, CKS1B, SHC1, PYGO2, PBXIP1, PMVK, KCNN3, ADAR, CHRNB2, UBE2Q1, TDRD10, ENSBTAG00000045672, SHE, IL6R, ATP8B2, AQP10, HAX1, UBAP2L, C3H1orf43, C3H1orf189, TPM3, NUP210L, RPS27, ENSBTAG00000018987, RAB13, JTB, CREB3L4, SLC39A1, CRTC2, DENND4B	3.60
4	80804444-81715533	SUGCT, MPLKIP, CDK13, RALA, YAE1, POU6F2	0.97
6	88350890-89288321	RUFY3, GRSF1, MOB1B, DCK, SLC4A4, GC, NPFFR2, ADAMTS3	0.87
9	52430564-53156338	ENSBTAG00000017170, MMS22L, KLHL32	1.45
11	53132515-53778535	-	0.71

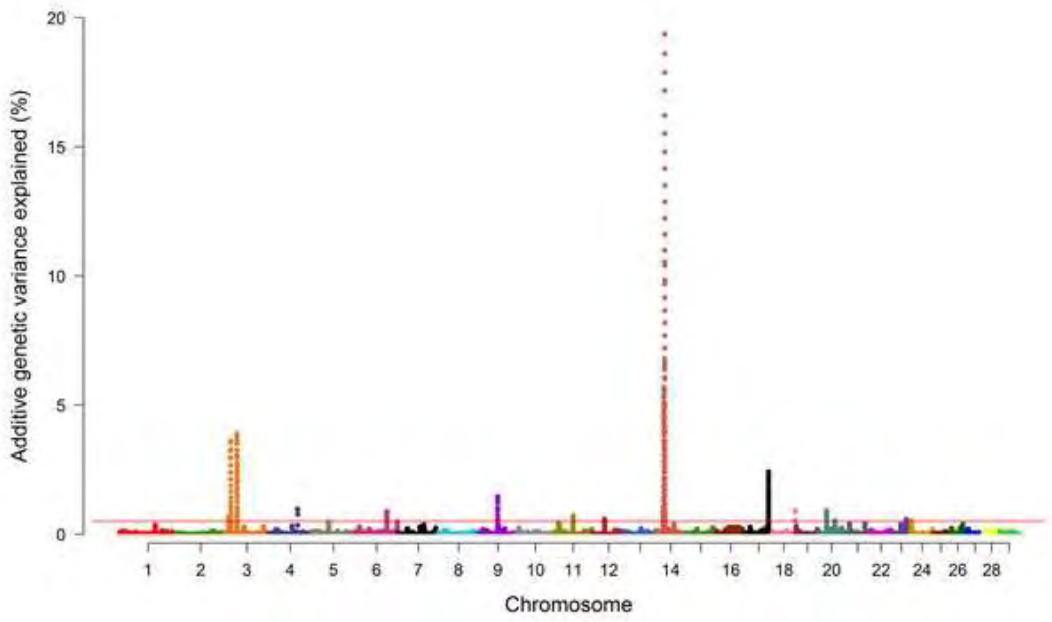
Continue

Table 4.3 - Genomic regions associated with calving ease (Direct Effect)

12	33978213-34960812	ENSBTAG00000025428, SHISA2, ATP8A2, NUP58, MTMR6, ENSBTAG00000019545, C1QTNF9, MIPEP, TNFRSF19, SACS, SGCG	0.58
14	21619549-22587081	EFCAB1, SNAI2, PPDPFL, SNTG1, PCMTD1, ST18, ENSBTAG00000026306	1.06
14	24049812-25015640	ATP6V1H, RGS20, TCEA1, LYPLA1, MRPL15, ENSBTAG00000033627, POLR2K, SOX17, RP1, ENSBTAG00000047303, XKR4, TMEM68, TGS1, LYN, RPS20, MOS, PLAG1	5.69
14	25058053-25869266	CHCHD7, ENSBTAG00000039031, SDR16C5, SDR16C6, PENK, IMPAD1	0.73
14	26223437-27155254	FAM110B, ENSBTAG00000047136, UBXN2B, CYP7A1, SDCBP, NSMAF, TOX, CA8	19.36
17	64784269-65771136	ENSBTAG00000008762, TRAFD1, NAA25, ERP29, TMEM116, ADAM1B, MAPKAPK5, ALDH2, BICDL1, RAB35, GCN1, RPLP0, PXN, SIRT4, ENSBTAG00000026732, ENSBTAG00000047084, MSI1, COX6A1, TRIAP1, GATC, SRSF9, DYNLL1, COQ5, RNF10, POP5, CABP1, MLEC, UNC119B, ACADS, SPPL3, HNF1A, C17H12orf43, OASL, ENSBTAG00000046887, ENSBTAG00000032047, ANKRD13A, GIT2, TCHP, GLTP, TRPV4, FAM222A, MVK, MMAB, UBE3B, KCTD10, MYO1H, FOXN4, ACACB, UNG, ALKBH2, USP30	2.41
18	64724506-65722095	MGC157368, ENSBTAG00000023338, AURKC, ENSBTAG00000038926, ZNF304, ENSBTAG00000037721, ENSBTAG00000039341, ENSBTAG00000037375, ENSBTAG00000011262, ENSBTAG00000000195, ZNF772, ZNF419, ENSBTAG00000009460, ENSBTAG00000037981, ENSBTAG00000048261, ENSBTAG00000038240, ENSBTAG00000047568, ENSBTAG00000038635, ENSBTAG00000040169, ENSBTAG00000015866, ZSCAN4, ENSBTAG00000003447, ENSBTAG00000038088, ENSBTAG00000038715, ENSBTAG00000040442, ZNF814, ENSBTAG00000027787, ZNF606, ZNF135, ZNF329, ZNF274, ZNF8, ENSBTAG00000047405, A1BG, RPS5, ZNF584, ZNF132, ENSBTAG00000039453, ZNF446, SLC27A5, TRIM28, CHMP2A, UBE2M, MZF1, ENSBTAG00000046987, ENSBTAG00000037595	0.90
20	21539446-22441853	ENSBTAG00000026505, GPBP1, ENSBTAG00000047548, MIER3, SETD9, MAP3K1	0.90
20	45354483-46296840	CDH9	0.52
23	39285499-40258674	RNF144B, DEK, KDM1B, TPMT, NHLRC1, KIF13A, NUP153, FAM8A1, CAP2, RBM24, STMND1, ATXN1, ENSBTAG00000019672, GMPR	0.56
24	1654864-2620915	ATP9B, GALR1, MBP, ZNF236, ZNF516	0.52

Conclusion
Source: Elaborated by the authors.

Figure 2 - Manhattan plot of the additive direct genetic variance explained by 1-Mb continuous adjacent SNPs for calving ease in Nellore cattle. The dots above the red line indicates the regions explaining more than 0.5% of the additive direct genetic variance



Source: Elaborated by the authors.

The windows found on chromosome 14 are located between the 21 and 27 Mb regions. Some studies have identified SNPs in these regions that were associated with various traits in different cattle breeds, such as age at puberty in males and females, postpartum anestrus interval, calving ease, rump height, height, body size, stillbirth rate, growth hormone levels (*IGF-1*), weight, fat deposition and indicator traits of sexual precocity (BOLORMAA et al., 2011, 2010; FORTES et al., 2012; HAWKEN et al., 2011; IRANO et al., 2016; KARIM et al., 2011; PAUSCH et al., 2011; SAATCHI et al., 2014).

Utsunomiya et al. (2013) reported a QTL on BTA14 in the vicinity of *PLAG1* (pleomorphic adenoma gene 1) associated with variation in birth weight in Nellore cattle suggesting that *PLAG1* may also be responsible for variation in body weights in Bos indicus cattle. The *PLAG1* gene encodes a transcription factor that is broadly expressed during fetal development and which is downregulated at birth (KARIM et al., 2011). This gene regulates several growth factors, including *IGF2*, a key regulator of body size (VAN DYCK et al., 2007; VOZ et al., 2004). The functional candidacy of *PLAG1* is well supported by its

regulation of the expression of insulin-like growth factors (IGF) (VOZ et al., 2000). These factors are major mediators of the growth pathway and the hypothalamic-pituitary-gonadal axis (VELAZQUEZ et al., 2008), and serum concentrations of IGF1 in cattle have been shown to be highly heritable, negatively correlated with weight and carcass traits (DAVIS et al., 2003; DAVIS and SIMMEN, 2006) and primarily determined by variants in the *PLAG1* chromosomal region (FORTES et al., 2013). Similar to the present study, Höglund et al. (2012) and Fang and Pausch (2019) also reported several QTL at the BT18 harboring possible candidate genes for calving easy and gestation length in dairy cattle.

4.4 Functional analyses

The functional analysis revealed three GO biological processes, six GO molecular functions, and four KEGG pathways (Table 4.4) shared between maternal and direct effects. Among them, the Ras protein signal transduction (GO:0007265), ephrin receptor binding (GO:0046875), delayed rectifier potassium channel activity (GO:0005251), glutathione transferase activity (GO:0004364) and glutathione metabolism (bta00480), were of interest for our study.

Table 4.4 - DAVID Functional Annotation for gene category and pathway enrichment analysis

Biological Process	PValue	Genes
GO:0008152~metabolic process	0.002420	ECI2, ENSBTAG00000016472, CDYL, GSTM3, ENSBTAG00000031788, GSTM1, ENSBTAG00000037673, ENSBTAG00000012692
GO:0010839~negative regulation of keratinocyte proliferation	0.022972	SLURP1, KDF1, SFN
GO:0007265~Ras protein signal transduction	0.023433	RIT1, MAPKAPK5, USP28, SDCBP
GO:0061436~establishment of skin barrier	0.030856	CYP26B1, KDF1, SFN
GO:0030336~negative regulation of cell migration	0.036977	RAP2A, SLURP1, ADAM15, STK24, DRD2
GO:0045188~regulation of circadian sleep/wake cycle, non-REM sleep	0.039533	KCNA2, CHRNB2
GO:0042126~nitrate metabolic process	0.039533	MARC1, MARC2
GO:0051260~protein homooligomerization	0.046114	KCNA3, RIPK1, HSF1, KCNA2, KCNA10, KCNC4, KCTD10
Cellular Component		
GO:0005741~mitochondrial outer membrane	0.019179	MTX1, USP30, MSTO1, MARC2, HAX1, DAO
GO:0016023~cytoplasmic, membrane-bounded vesicle	0.023273	ADD2, SLC39A4, RAB25, SLC30A2, HAX1, PLA2G4A
GO:0005874~microtubule	0.032670	PBXIP1, DYNLL1, NUDC, ARHGEF2, TUBB2B, TUBB2A, STMN1, KIFC2
GO:0005794~Golgi apparatus	0.033866	ASH1L, GRINA, ATP8B2, CREB3L4, PARP10, GOLIM4, NAPRT, ZDHHC18, PDCD10, IPO5, MECOM, DUSP10, DENND4B, CEP85, SLC50A1, WDR77, DRAM2, PLA2G4A
GO:1904602~serotonin-activated cation-selective channel complex	0.039193	HTR3B, HTR3A
GO:0008076~voltage-gated potassium channel complex	0.039728	KCNA3, KCNA2, KCNA10, KCNK2, KCNC4

Continue

Table 4.4 - DAVID Functional Annotation for gene category and pathway enrichment analysis

Molecular Function		
GO:0004364~glutathione transferase activity	3.61E-04	ENSBTAG00000016472, GSTM3, ENSBTAG00000031788, GSTM1, ENSBTAG00000037673, ENSBTAG00000012692
GO:0043546~molybdopterin cofactor binding	0.002382	XDH, MARC1, MARC2
GO:0008061~chitin binding	0.005799	CHIA, CHI3L2, OVGP1
GO:0046875~ephrin receptor binding	0.016493	EFNA4, EFNA1, EFNA3
GO:0005251~delayed rectifier potassium channel activity	0.024259	KCNA3, KCNA2, KCNA10, KCNC4
GO:0004867~serine-type endopeptidase inhibitor activity	0.028925	SERPINI2, ENSBTAG00000017483, ENSBTAG00000017949, SERPINI1, SERPINB9, SERPINB1, SERPINB6
GO:0004012~phospholipid-translocating ATPase activity	0.031629	ATP8B2, ENSBTAG00000025428, ATP9B
GO:0008940~nitrate reductase activity	0.040065	MARC1, MARC2
KEGG pathway		
bta00480:Glutathione metabolism	3.88E-04	ENSBTAG00000016472, GSTM3, ENSBTAG00000031788, GSTM1, OPLAH, ENSBTAG00000037673, ENSBTAG00000012692
bta01100:Metabolic pathways	4.15E-04	COX6A1, AHCYL1, ENSBTAG00000008564, ATP5PB, FDPS, TSTA3, MMAB, MTMR6, ENSBTAG00000004596, CEPT1, GPAA1, CYC1, ATP6V1B1, DAO, XDH, ENSBTAG00000047084, DGAT1, ACACB, ENSBTAG00000007835, CYP26B1, ACADS, COQ5, DPM3, PKLR, GBA, PYCR3, CYP7A1, MVK, ALDH2, EXTL1, PMVK, NAPRT, PIGV, FLAD1, MAN1C1, PAFAH2, PTGS2, PLA2G4A, GATC
bta00982:Drug metabolism - cytochrome P450	0.003756	ENSBTAG00000016472, GSTM3, ENSBTAG00000031788, GSTM1, ENSBTAG00000037673, ENSBTAG00000012692
bta00980:Metabolism of xenobiotics by cytochrome P450	0.004045	ENSBTAG00000016472, GSTM3, ENSBTAG00000031788, GSTM1, ENSBTAG00000037673, ENSBTAG00000012692
bta00900:Terpenoid backbone biosynthesis	0.006852	MVK, FDPS, DHDDS, PMVK

Conclusion
Source: Elaborated by the authors.

The Ras protein signal transduction (GO:0007265) encompass several intracellular molecular signals that are mediated by a member of the Ras superfamily of proteins. Streyl et al. (2012), in a transcriptome study of bovine placentomes (12 days before the estimated day of calving), identified the genes of Ras protein signal transduction upregulated, which could be involved in the detachment of the fetal membranes. The ephrin receptor binding (GO:0046875) includes interactions selectively and non-covalently with an ephrin receptor. Purfield et al. (2015) performed a GWAS for calving difficulty in beef and dairy bulls and identified genes involved in ephrin signaling pathway as candidate genes for maternal calving difficulty, regulating of a host of processes critical to fetal development including axon guidance (CAI et al., 2002; 2001). The delayed rectifier potassium channel activity (GO:0005251) molecular function is related to the transmembrane transfer of potassium ions by a delayed rectifying voltage-gated channel. Potassium channels comprise one group of proteins that are crucial to maintain the uterus in a state of quiescence during pregnancy and alterations of K+ channels contribute to pathophysiological conditions such as dystocia, pre-term labor and post-term labor in humans (BRAINARD et al., 2007). The glutathione transferase activity (GO:0004364) molecular function and the glutathione metabolism (bta00480) pathway are related to glutathione, an antioxidant that acts in nutrient metabolism, antioxidant defense and regulation of several pathways crucial for whole body homeostasis and cellular events such as cell proliferation, apoptosis, signal transduction, cytokine production and immune response (WU et al., 2004).

Regarding to direct effect, a total of eight GO biological processes, one GO molecular function and three KEGG pathways were identified as being overrepresented. Among them, we highlighted the ephrin receptor signaling pathway (GO:0048013) and the potassium ion import (GO:0010107), in which the role of ephrin and the potassium channels on dystocia were previously discussed, in addition to the activation of MAPK activity (GO:0000187), isopentenyl diphosphate biosynthetic process, mevalonate pathway (GO:0019287) and regulation of adenylate cyclase activity (GO:0045761).

The activation of MAPK activity (GO:0000187) biological process is related to the initiation of the activity of the mitogen-activated protein kinase (MAPK), which transduces several extracellular signals to the transcriptional

machinery through a cascade of protein phosphorylation, including its important role at the late stage of gestation contributing to parturition (TAKANAMI-OHNISHI et al., 2001; WANG and STJERNHOLM, 2007). The isopentenyl diphosphate biosynthetic process, mevalonate pathway (GO:0019287) encompass chemical reactions and pathways resulting in the formation of isopentenyl diphosphate, via the intermediate mevalonate. The mevalonate pathway regulates innate immunity in the bovine endometrium (HEALEY et al., 2016).

The regulation of adenylate cyclase activity (GO:0045761) biological process is related to all processes that modulates the extent rate or frequency of adenylate cyclase activity, an enzyme that converts ATP to cAMP. Studies in humans have been reported that the cAMP promotes the myometrial relaxation via activation of cAMP-dependent protein kinase and this uterine quiescence is vital to permit the fetus to grow and mature (CARVAJAL, 2014; PRICE and BERNAL, 2001).

Regarding to maternal effects, a total of five GO biological processes, three GO molecular functions and one KEGG pathways were identified as being overrepresented. The negative regulation of cell migration (GO:0030336) biological process encompass all processes that reduces, prevents or stops the extent, rate or frequency of cell migration. Boro et al. (2015) showed the importance of migration of inflammatory cells to the site of feto-maternal junction during parturition, which is a critical step for fetal membrane separation. Retention of fetal membranes in cows is associated with low level of pro-inflammatory mediators and alterations in inflammatory process (BEAGLEY et al., 2010; STREYL et al., 2012).

The results showed that the main genomic regions explaining more than 0.5% of the additive genetic variance for CE differed between maternal and direct effects, suggesting that different groups of genes control direct and maternal effects. Despite being controlled by different groups of genes, common metabolic pathways ($p < 0.05$) between these groups were identified, which are: Ras protein signal transduction (GO:0007265), ephrin receptor binding (GO:0046875), delayed rectifier potassium channel activity (GO:0005251), glutathione transferase activity (GO:0004364) and glutathione

metabolism (bta00480), showing that the maternal and direct effects on CE have similar metabolic and physiological basis.

5 CONCLUSION

In conclusion, direct and maternal heritability estimates for CE were moderate to high, indicating that genetic progress for this trait is feasible. The results of the present study showed that some of the genes influencing maternal and direct effects on CE participated in the same metabolic pathways. Several genes and GO related to placental development, infertility, maternal calving difficult, dystocia in cows, emphasizing that placental abnormalities could increase maternal morbidities. In addition, genes that affect placenta and fetal development and fetal processes involved in female pregnancy were found.

The results described in this study suggest that the CE trait has polygenic inheritance, controlled by several genetic variants of small effects. Hence, strategies such as genomic selection using the variability among all markers at the same time would be more appropriate to identify the candidates for CE selection. The identification and description of genome regions, genes and biological process that affect calving easy in indicine cattle are important since this can be used in future fine mapping studies, to search for informative causative mutations. The results also showed different genomic regions with known genes, but several uncharacterized genes were identified, and this information would be useful for future genomic and genome assembly studies in indicine cattle.

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