UNIVERSIDADE ESTADUAL PAULISTA

FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS

CAMPUS JABOTICABAL

GENOMIC SELECTION IN NELLORE CATTLE: NEW APPROACHES FOR REPRODUCTIVE TRAITS AND POLLED CHARACTER

Larissa Bordin Temp

Animal Scientist

2025

DISS. / TEMP

L.B.

2025

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Student: Larissa Bordin Temp

Advisor: Prof. Dr. Fernando Sebastian Baldi Rey

Coadvisor: Drª. Ludmilla Costa Brunes

Master’s thesis presented to the Graduate program in Animal Science at the Faculty of Agricultural and Veterinary Science – UNESP, Jaboticabal campus, as part of the requirements for obtaining the title of Master’s in Animal Science.

2025

CATALOG CARD

T282g

Temp, Larissa Bordin

Genomic selection in Nellore cattle: new approaches for reproductive traits and polled character / Larissa Bordin Temp. -- Jaboticabal, 2025

84 p. : tabs., fotos

Dissertação (mestrado) - Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientador: Fernando Sebastian Baldi Rey

Coorientadora: Ludmilla Costa Brunes

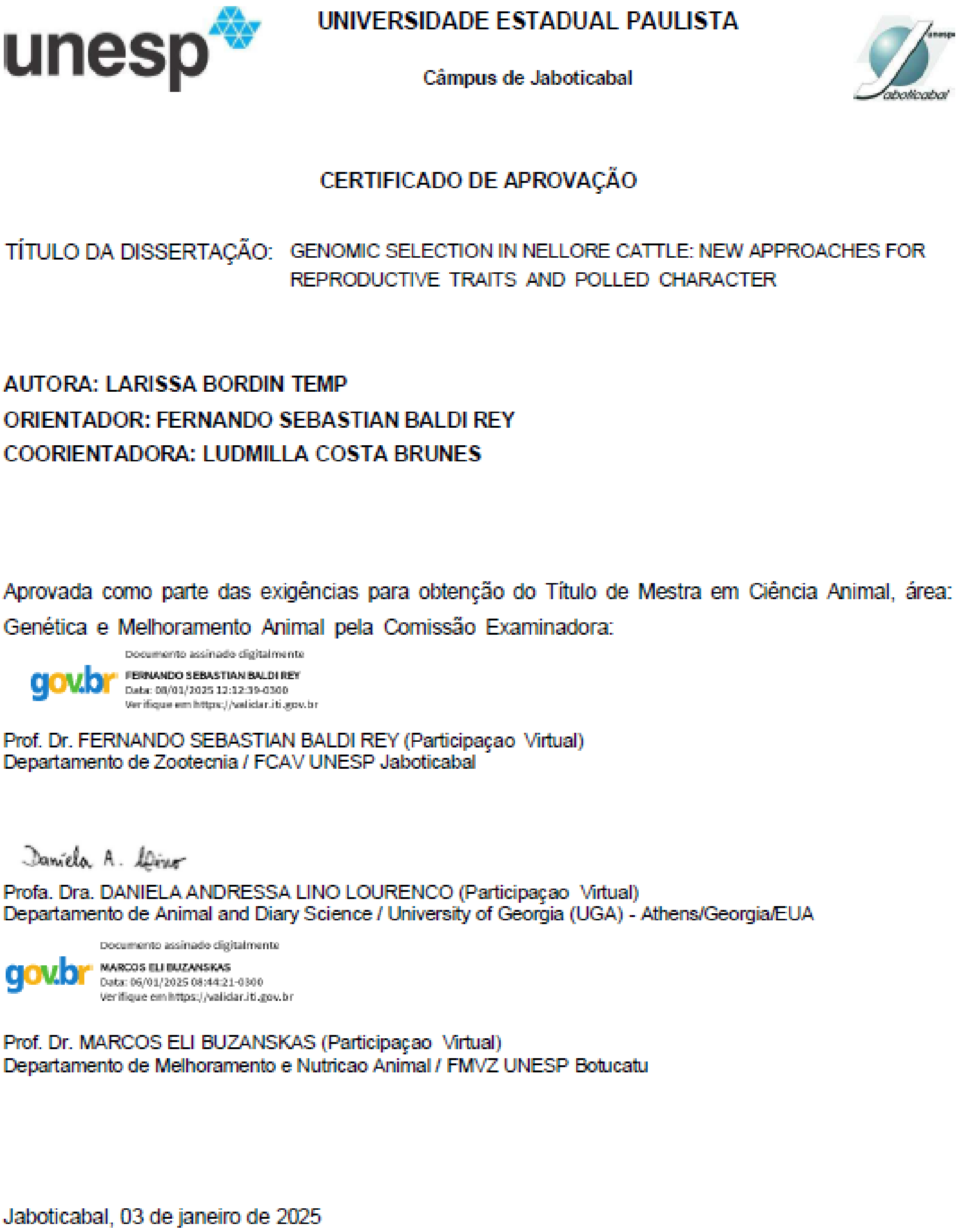
1. Bos indicus. 2. Pedigree. 3. Genetic groups. 4. Metafounders. 5. ssGBLUP. I. Título.

POTENCIAL IMPACT OF THIS RESEARCH

First study: In modern beef cattle farming, reducing horns is essential to prevent accidents and minimize economic losses from hide and muscle damage. Dehorning is a common practice but causes pain and stress to the animals. Selecting naturally polled animals offers a humane alternative by increasing the incidence of the polled gene. This research focuses on analyzing the impact of horn development classification, sex effects, and non-autosomal SNP markers on genetic parameters and genomic prediction in Nellore cattle. Twelve models were evaluated using two, three, and four phenotypic categories of horn development. Including non-autosomal SNPs in models with four categories improved prediction accuracy by 5.26%, bias and dispersion reduction by 37%, and 4.55%, compared to models with only autosomal SNPs. These findings suggest that the genetic architecture of horn development is more complex than binary coding allows. Genomic selection for polled animals is a viable strategy, and genetic dehorning could be adopted as a cost-effective, non-invasive solution to increase the frequency of hornless cattle.

Second study: The development of tools that enhance accuracy and reduce bias in genomic evaluations of young animals is essential, as is creating strategies to lower genotyping and phenotyping costs. The innovative aspect of this research lies in the analysis of the performance of genomic prediction models that incorporate metafounders, unknown parent groups, and allele frequency weighting based on the genomic matrix, using real data from many Nellore herds with pedigree, phenotypic, and genotypic information. The main focus was on reproductive and longevity traits, which are of high economic relevance to beef cattle production and enable the evaluation of populations with incomplete pedigree records. The results obtained will allow for the evaluation of the impact of the tested methodologies on predictive ability, providing guidelines for their use in future research or for implementation in genetic evaluations based on genomic data. Furthermore, the use of metafounders can clarify discrepancies between pedigree and genomic matrices, allowing for the proper definition of models used to estimate genetic values for economically important traits, even in complex populations with limited pedigree records.

CERTIFICATE OF APPROVAL



UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: GENOMIC SELECTION IN NELLORE CATTLE: NEW APPROACHES FOR REPRODUCTIVE TRAITS AND POLLED CHARACTER

AUTORA: LARISSA BORDIN TEMP
ORIENTADOR: FERNANDO SEBASTIAN BALDI REY
COORIENTADORA: LUDMILLA COSTA BRUNES

Aprovada como parte das exigências para obtenção do Título de Mestra em Ciência Animal, área: Genética e Melhoramento Animal pela Comissão Examinadora:

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FERNANDO SEBASTIAN BALDI REY
Data: 08/01/2025 12:12:39-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. FERNANDO SEBASTIAN BALDI REY (Participaçao Virtual)
Departamento de Zootecnia / FCAV UNESP Jaboticabal

Daniela A. Lino

Profa. Dra. DANIELA ANDRESSA LINO LOURENCO (Participaçao Virtual)
Departamento de Animal and Diary Science / University of Georgia (UGA) - Athens/Georgia/EUA

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Documento assinado digitalmente
MARCOS ELI BUZANSKAS
Data: 06/01/2025 08:44:21-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. MARCOS ELI BUZANSKAS (Participaçao Virtual)
Departamento de Melhoramento e Nutricao Animal / FMVZ UNESP Botucatu

Jaboticabal, 03 de janeiro de 2025

AUTHOR’S CURRICULAR DATA

Larissa Bordin Temp, daughter of Marcio Moacir Temp and Luci Bordin Temp, and sister of Gabriel Bordin Temp, was born in Agudo, Rio Grande do Sul, on October 12, 1997. On March 6, 2017, she began her studies in Animal Science at the Federal University of Santa Maria (UFSM), where she participated as a scholarship student in the Bromatology and Ruminant Nutrition Laboratory and as a scholarship student in the Animal Breeding Laboratory (LMA). She also collaborated in the Beef Cattle Laboratory (LBC) and in the Junior Company TechVet Jr. She was a member of the Group of Studies in Additives in Animal Production (GEAPA) and a member of the Animal Breeding Study Group (GMA). She also served as a teaching assistant in the Animal Genetic Improvement I and II courses. Larissa completed her mandatory internship at DOGMA – Doctors of Genetics and Animal Breeding. She graduated on July 20, 2022, earning the title of Animal Scientist. On August 1, 2022, she began the Master's Program in Genetics and Animal Breeding, now the Animal Science Graduate Program, at the School of Agricultural and Veterinary Sciences – UNESP/Campus Jaboticabal. During the master's, she actively participated in several research projects, with summaries and papers published in the field, as well as a period abroad at the University of Georgia under the supervision of Professor Dr. Daniela Lourenco.

EPIGRAPH

“Progress is not achieved by luck or chance, but through hard work and dedication.”

Marie Curie

DEDICATION

To my parents, Marcio Moacir Temp e Luci Bordin Temp, for being my safe harbor and my strength.

I dedicate and offer this work.

ACKNOWLEDGMENTS

To God for everything.

To my family, thank you for your support and encouragement throughout my master’s degree. You were essential in this journey.

To the Graduate Program in Animal Science at the School of Agricultural and Veterinary Sciences (FCAV/UNESP) for the opportunity to undertake this next stage of my life.

To my advisor, Prof. Dr. Fernando Baldi, I am deeply grateful for agreeing to guide me during my master’s degree. Your teachings in Animal Breeding and guidance in becoming a better person and professional have been invaluable. You have been more than an advisor, you have been a father figure who has always led me down the best paths.

To my co-advisor, Ludmilla Costa Brunes, who has been an excellent mentor and a great friend. I am deeply grateful for the knowledge you shared with me and your unwavering support in Animal Breeding. I thank God for bringing us together on this journey.

To my friends from Jaboticabal and UNESP, who have become my second family, I am thankful for the support and the countless joyful moments we have shared. Gabriel Gubiani, in particular, has become my best friend and post-graduation brother. Each of you holds a special place in my heart, and I am grateful for you every day.

To Patrícia Iana Schmidt, my friend and work partner at DOGMA – Doctors of Genetics and Animal Breeding, I am deeply grateful for our friendship, which began in 2021. May our journey continue strong for many years, full of collaboration, mutual support, and significant professional achievements together.

To professors Daniela Andressa Lino Lourenco, Jorge Hidalgo, and Ignacy Misztal, my sincere thanks for warmly welcoming me during my stay at the University of Georgia (UGA) and for all the valuable teachings. To the friends I made in the department, you remain a special part of my life, and I am eternally grateful for the memories and lessons.

To the Animal Genetics and Breeding Laboratory (labGEN), where I was fortunate to work with exceptional colleagues. Every day I learned more, both personally and professionally. Together, we have a promising future, and I know the

world is small compared to the greatness of our dreams and our desire to soar even higher.

Finally, I thank the São Paulo Research Foundation (FAPESP) for the research grants in Brazil (Process No. 2022/12134-0) and abroad (Process No. 2023/08817-8), which were essential for my academic development during my master’s degree. These opportunities expanded my network of contacts worldwide and significantly contributed to my growth as a better-qualified professional.

SUMMARY

Seleção genômica em bovinos nelore: novas abordagens para características reprodutivas e caráter mocho

RESUMO – Os programas de melhoramento genético focados na raça Nelore são essenciais para promover ganhos de produtividade e garantir a sustentabilidade do setor pecuário. Utilizando dados da Associação Nacional de Criadores e Pesquisadores (ANCP), o presente estudo teve dois objetivos principais. O primeiro foi estimar parâmetros genéticos e avaliar a influência da classificação fenotípica do desenvolvimento de cornos, do sexo dos animais e da inclusão de marcadores SNPs não autossômicos na predição genômica do caráter mocho em bovinos Nelore, utilizando o método de predição linear não viesada genômica de etapa única (ssGBLUP). Foram analisadas duas, três e quatro categorias fenotípicas para o desenvolvimento de cornos, em um total de 12 modelos estatísticos. As estimativas de herdabilidade variaram de 0,44 a 0,83. A inclusão de SNPs não autossômicos nos modelos com quatro categorias fenotípicas resultou em uma melhoria de 5,26% na acurácia da predição, redução de 37% no viés e aprimoramento de 4,55% na dispersão, em comparação com os modelos que consideraram apenas SNPs autossômicos. Esses resultados sugerem que a utilização de informações genômicas na seleção para a ausência de cornos é uma estratégia viável, representando uma abordagem não invasiva e de baixo custo para aumentar a frequência de animais mochos. O segundo objetivo do estudo foi avaliar o uso de metafundadores (MF) e grupos de pais desconhecidos (UPGs) na avaliação genômica de características relacionadas com precocidade sexual, fertilidade, longevidade e produtividade. Foram utilizados registros de circunferência escrotal aos 365 dias de idade (CE365), idade ao primeiro parto (IPP) e produtividade acumulada de matrizes (PAC). Quatro modelos foram implementados, levando em consideração UPGs e MF com base em diferentes critérios: rebanhos comerciais e registrados, paternidade incerta e patriarcas (animais nascidos no Brasil de pais importados). As estimativas de herdabilidade variaram de 0,07 a 0,40. A acurácia e o viés das predições foram avaliados utilizando o método de regressão linear. Embora os modelos com MF tenham apresentado pequenas melhorias na acurácia, os resultados indicam que o aumento da quantidade de genótipos e fenótipos disponíveis pode contribuir para aprimorar as estimativas de MF e, consequentemente, a acurácia preditiva.

Palavras-chave: *Bos indicus*, pedigree, metafundadores, grupos genéticos, ssGBLUP

GENOMIC SELECTION IN NELLORE CATTLE: NEW APPROACHES FOR REPRODUCTIVE TRAITS AND POLLED CHARACTER

ABSTRACT – Genetic improvement programs focused on the Nellore breed are essential for promoting productivity gains and ensuring the sustainability of the livestock sector. Using data from the National Association of Breeders and Researchers (ANCP), this study had two main objectives. The first was to estimate genetic parameters and assess the influence of the phenotypic classification of horn development, animal sex, and the inclusion of non-autosomal SNP markers on the genomic prediction of the polled trait in Nellore cattle, using the single-step genomic best linear unbiased prediction (ssGBLUP) method. Two, three, and four phenotypic categories for horn development were analyzed, in a total of 12 statistical models. Heritability estimates ranged from 0.44 to 0.83. The inclusion of non-autosomal SNPs in models with four phenotypic categories resulted in a 5.26% improvement in prediction accuracy, a 37% reduction in bias, and a 4.55% improvement in dispersion compared to models that only considered autosomal SNPs. These results suggest that the use of genomic information in selection for the polled trait is a feasible strategy, representing a non-invasive and low-cost approach to increasing the frequency of polled animals. The second objective of the study was to evaluate the use of metafounders (MF) and unknown parent groups (UPGs) in the genomic evaluation of traits related to sexual precocity, fertility, longevity, and productivity. Records for scrotal circumference at 365 days (SC365), age at first calving (AFC), and accumulated cow productivity (ACP) were used. Four models were implemented, taking into account UPGs and MF based on different criteria: commercial and registered herds, uncertain paternity, and patriarchs (animals born in Brazil from imported sires). Heritability estimates ranged from 0.07 to 0.40. The accuracy and bias of predictions were assessed using the linear regression method. Although the models with MF showed small improvements in accuracy, the results suggest that increasing the number of genotypes and phenotypes available may help improve MF estimates and, consequently, predictive accuracy.

Keyword: *Bos indicus*, pedigree, metafounders, genetic groups, ssGBLUP

LIST OF ABBREVIATIONS

ACP	Accumulated Productivity
AFC	Age at First Calving
ANCP	Nation Association of Breeders and Researchers
BLUP	Best Linear Unbiased Prediction
CEIP	Special Certificate of Identification and Production
CG	Contemporary Groups
EPD	Expected Progeny Difference
GEBVS	Genomic Estimated Breeding Values
GWAS	Genome-Wide Association
HIER	Hierarchical Animal Model
IBGE	Brazilian Institute of Geography and Statistics
LR	Linear Regression method
MAPA	Ministry of Agriculture, Livestock and Supply
MF	Metafounders
PO	Purebred
SC365	Scrotal Circumference
SNP	Single Nucleotide Polymorphism
ssGBLUP	Single Step Genomic BLUP
UPG	Unknown Parent Groups

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CHAPTER 1- GENERAL CONSIDERATIONS

1.1 INTRODUCTION

Beef cattle farming plays a crucial role in tropical countries' socioeconomic landscape, standing out as one of the Brazil country's leading sectors for beef production (ABIEC, 2024). This position has been achieved through significant investments and the adoption of technologies, resulting in a notable increase in productivity and improvement in the quality of the final product. Genetic improvement programs dedicated to the Nellore breed are pivotal in boosting beef productivity, offering important support for the livestock industry to realize its full potential. The Nellore breed has the highest number of both polled and horned animals among indicine cattle in Brazil (Peripolli et al., 2018) and are descendants of purebred imports from India (PROZEBU, 1978). The current Nellore population includes contributions from six predominant lineages: Kavardi Imp, Taj Mahal Imp, Kurupathy Imp, Golias Imp, Godhavari Imp, and Rastã Imp (Magnabosco et al., 1997) where those lineages are renowned for their superior productive and reproductive performance (Oliveira et al., 2002).

Therefore, it is crucial to share more effective selection strategies and methods in the field to enhance genetic gain and the ability to predict characteristics of economic importance for the sector. Some of these traits are related to sexual precocity, longevity, and production. In the production domain for Zebu breeds, there is the trait of presence or absence of horns in cattle. Since, there is a global trend towards reducing horn presence to prevent accidents during the handling of these animals and reduce the possibility of economic losses associated with carcass damage (Youngers et al., 2017; Stafuzza et al., 2018; Temp et al., 2024). Instead of proceeding with dehorning, which causes stress and pain to the animals and is considered an invasive procedure difficult to adopt on a large scale (Bates et al., 2016; Knierim et al., 2015), an alternative would be the production of hornless animals, thereby increasing the presence of alleles for the polled trait in the Nellore breed (Temp et al., 2024).

Moreover, sexual precocity, according to Sartori et al. (2010), is important for *Bos taurus indicus* animals, mainly due to their tendency for delayed development compared to *Bos taurus taurus* breeds. Nonetheless, the incorporation of specific reproductive traits as selection criteria in breeding programs proceeds slowly. This delay primarily stems from the low heritability of such traits, which typically emerge later and are constrained by gender (Sbardella et al., 2021). Researchers in the field of genetic improvement have placed considerable emphasis on reproductive traits associated with sexual precocity in females, such as Age at First Calving (AFC), and Scrotal Circumference (SC365) in males. They also focus on measures of longevity, such as Accumulated Productivity (ACP), aiming to enhance reproductive indices (Kluska et al., 2018; Brunes et al., 2020; Fernandes Júnior, 2018).

Among the traits related to the sex of the animals there are situations where the pedigree has gaps. For example, in beef cattle, there is often more information available about the parents of males than females (Misztal et al., 2013; Garcia-Baccino et al., 2017). Over the years, several methods have been developed to increase the reliability of genetic evaluations of animals with uncertain pedigrees and genetic differences between animal groups in genetic evaluations (Kluska et al., 2021). Quass (1988) proposed the Unknown parent groups (UPG) methodology to account for missing pedigree in a population, that can be formed based on different criteria of missing parents like a year of birth, breed composition, sex, among others (Legarra et al., 2007; Kluska et al., 2021). The redefinition of groups to get better connectivity and increase the number of animals and phenotypes can help to obtain a correct genetic trend and increase the reliability of GEBV estimates (Tsutura, et al., 2014; Kluska et al., 2021), benefiting traits assessed late or limited to sex.

Legarra et al. (2009), Misztal et al. (2009), and Aguilar et al. (2010) proposed the ssGBLUP methodology that integrates the pedigree relationship matrix (**A**) with the genomic relationship matrix (**G**) into a single matrix (**H**) to predict the genomic estimated breeding values of animals. This methodology requires genomic and pedigree relationships to refer to the same base (Legarra et al., 2014), where pedigree relationships can be projected for non-genotyped animals (Legarra, Aguilar, Misztal, 2009). UPGs can be considered either fixed or random effects in the model, and three different approaches are employed to incorporated UPGs in ssGBLUP: 1) through the additive relationship matrix based on pedigree where $\mathbf{A}^{-1}$ is replaced by $\mathbf{A}^*$ using Quass and Polak transformation; 2) contributions added to $\mathbf{A}^{-1}$ e $\mathbf{A}_{22}$ matrices; and 3) contributions are added to genomic and pedigree-based relationship matrices (**G,** $\mathbf{A}^{-1}$ and $\mathbf{A}_{22}$) (Misztal et al., 2013; Kluska et al., 2021). According Tsuruta et al. (2014), Braford et al (2019), Tsuruta et al (2019) and Kluska et al (2021) have demonstrated that incorporating UPGs into the model, provided these groups have enough number of records and animals, has the potential to mitigate the bias of GEBV.

However, in some situations, the **G** and **A** matrix can be incompatible resulting in biased estimates using ssBLUP (Misztal and Legarra, 2017) because not all animals of the pedigree have genotypes (Legarra et al., 2014; Legarra et al., 2015), and to solve this point the matrices need to refer to the same base population (Legarra et al., 2015). For solving this incompatibility between matrices **G** and **A**, Legarra et al. (2015) proposed a method in which is possible to include multiple base generation through metafounders (MF), that can be understood as pseudo individuals added into the pedigree in a similar way as UPGs, however, the relationship across MF are taken into account (Kluska et al., 2021). Lourenco et al. (2016) implemented another approach to enhance the compatibility between **G** and **A**, which involves utilizing breed-specific allele frequencies during the construction of **G**. Given that the genomic relationship matrix utilized in genomic evaluation considers allele frequencies for centering and scaling, it is crucial that the population being evaluated exhibits homogeneous allele frequencies (Lourenco et al., 2016).

The development of tools capable of increasing the accuracy and reducing the bias of genomic evaluations in young animals is of utmost importance to achieve more significant response to selection, as well as being effective strategies for reducing genotyping and phenotyping costs. Studies with UPG and MF for traits related to reproduction and longevity are still scarce in the literature, as well as the effect of sex and non-autosomal SNP markers on genomic prediction ability for polled trait in Nellore beef cattle. Furthermore, the evaluation of these methodologies may clarify discrepancies between pedigree and genomic matrices, allowing for a more precise definition of the models and his effects employed in estimating genetic values for economically important traits in beef cattle production, even in complex populations with pedigree data limitations.

1.2 OBJECTIVES

1.2.1 General objectives

The objectives of this study were: 1) estimate genetic parameters and evaluate the influence of phenotype classification of horn development, animal sex effect, and non-autosomal SNP markers on genomic prediction ability for horn development in Nellore cattle using the single-step genomic best linear unbiased prediction method; 2) evaluate the use of MF and UPG in the genomic evaluation of traits associated with sexual precocity, fertility, longevity, and production in Nellore cattle.

1.2.2 Specific objectives

- Estimate the (co)variance components using the traditional BLUP method for the traits SC365, AFC and ACP;
- Evaluate the effect of using one or multiple MF and UPG in predicting the estimated genomic breeding values (GEBVs) using the single-step genomic BLUP (ssGBLUP) method;
- Assess the impact of including MF and UPG in accuracy, bias, and dispersion estimates using the LR method (linear regression);
- Evaluate different strategies for obtaining the **G** matrix, implemented in the ssGBLUP method considering: a) the traditional approach of ssGBLUP; b) the **G** matrix centered and scaled for specific allele frequencies of the patriarchs;
- Verify the impact of using weighted genomic matrices on genomic predictions for SC365, AFC and ACP, bias and prediction ability in young genotyped animals using three genetic groups: commercial and registered, uncertain paternity, and patriarchs (offspring of the first individuals of the breed introduced in Brazil).

1.3 LITERATURE REVIEW

1.3.1 Beef cattle production: Nellore breed

Beef cattle farming plays a crucial role in Brazil's socioeconomic landscape, standing out as one of the country's leading sectors for beef production. This prominent position has been achieved through significant investments and the adoption of technologies, resulting in a notable increase in productivity and improvement in the quality of the final product.

In 2023, the Brazilian livestock industry recorded the world's largest commercial cattle herd, totaling 194.36 million heads (Table 1). From January to September 2023, Brazilian beef production reached 63.9 million tons, marking a 4.5% growth compared to the previous record set in 2019, according to data released by the Brazilian Institute of Geography and Statistics (IBGE) (IBGE, 2023).

Table 1. World bovine herd data, in millions of heads from 2021 to 2024.

Countries	**2021**	**2022**	**2023**	**Oct/ 2024**
India	305.500	306.700	307.400	307.635
Brazil	193.195	193.780	194.365	193.900
China	95.621	98.182	102.160	105.500
United States	93.790	92.077	89.274	87.600
European Union	76.551	75.705	74.808	74.200
Argentina	53.540	53.400	54.100	52.500
Australia	23.021	23.944	25.800	27.430
Others	38.039	38.314	37.948	37.473

Source: (Foreign Agricultural Service/USDA, 2023)

The national bovine herd of Brazil is predominantly composed of 80% zebu breeds, notably including Nellore (*Bos indicus indicus*), as mentioned in the study by Brunes et al. in 2018. Imports of zebu cattle began in 1870, initiated by the Rio de Janeiro and Bahia states. Nellore is a zebu cattle breed from India, specifically from the Ongole region in Andhra Pradesh. This breed was introduced to Brazil during the colonial period and has become one of the most popular breeds in the country's beef cattle industry (Vozzi et al., 2007; Maiorano et al., 2022).

Firstly, Nellore cattle are known for their remarkable adaptability to the tropical conditions of Brazil (Sousa et al., 2016). These animals have adapted well to the country's tropical conditions due to their hardiness, natural resistance to ecto and endoparasites, and heat, as well as good feed conversion even when fed with low-quality native pastures, which are common in many regions of the country (Sousa et al., 2016). Another relevant aspect is the quality of their meat, as they produce high-quality meat that is appreciated for its texture and flavor. This meat quality makes them attractive for domestic and international consumer markets. The breed's short and light-colored coat is an additional characteristic that contributes to their adaptation to Brazil's hot climate, helping them cope better with the heat (Vozzi et al., 2007; Maiorano et al., 2022).

The history of the Nellore breed in Brazil dates to the late 19th century and extends until 1930, when Pedro Marques Nunes imported the bulls Rajá, Sheik, and Marajá. The last two imports of Nellore breeding bulls influenced the breed's formation between 1960 and 1962. During this period, a group of notable sires arrived in Brazil at Fernando de Noronha, including Kavardi, Golias, Rastã, Checurupadu, Godhavari, Padu, and Akasamu. These animals underwent a quarantine period and became the essential foundation for the development of the main Nellore lineages in the country (ACNP, 2023).

Regarding genetic selection for the Nellore breed in Brazil, it is essential to mention the ongoing certification systems: Purebred (PO) and Special Certificate of Identification and Production (CEIP). In 1971, the Zebu Breeders Association (ABCZ), through the certification of animals proven to be PO via the closed pedigree book (animals should have purebred ancestors registered for up to two generations), aimed to preserve the integrity and uniformity of the breed. In herds with PO identification, the intensity of selection is lower compared to commercial herds, and animals that fall into this category are registered by ABCZ and evaluated by genetic improvement programs such as the one coordinated by the National Association of Breeders and Researchers (ANCP) (Rosa et al., 1997; Maiorano et al., 2022).

In 1989, the Ministry of Agriculture, Livestock, and Supply (MAPA) launched the CEIP to promote the use of genetically superior animals in livestock farming. To obtain this certification, animals must come from breeding programs that conduct controlled data recording and genetic evaluation, allowing only 20 to 30% of them to be considered eligible for such certification. CEIP bulls are predominantly used in commercial herds, aiming to improve beef production, while PO bulls are preferred by producers who value the tradition of genetic improvement in restricted pedigrees (Ferraz et al., 2012; Carvalheiro, 2014; Maiorano et al., 2022).

Commercial herds in tropical and subtropical regions typically operate in low-input production systems with limited technology adoption, resulting in a lack of reliable genetic evaluations for sires (Ferraz and Felício, 2010). In contrast, herds with registered animals, often associated with breeder organizations, generally have better access to technology and more comprehensive phenotypic, pedigree, and genotypic data. In many extensive commercial herds, pedigree information is either incomplete or unavailable due to the frequent use of multiple-sire mating systems (Cavani et al., 2021; Tonussi et al., 2021; Londoño-Gil et al., 2024). Although these bulls possess high genetic value for economically important traits and are evaluated by companies authorized by the MAPA, identifying paternity is a significant challenge. The literature indicates that approximately 40% of Brazilian commercial herds originate from multiple-sire matings, with no record of the sire. This occurs because, in many farms, bulls are used for only one breeding season and subsequently discarded (Tonussi et al., 2017). However, tools like unknown parent groups and single-step genomic BLUP software have been developed to provide more accurate relationship estimates between individuals and across herds, enhancing genetic predictions. These advancements are particularly beneficial for commercial beef cattle systems, where most animals, especially younger ones, lack genetic evaluations or have relatively low accuracy in existing estimates (Carvalheiro et al., 2014; Londoño-Gil et al., 2024).

Genetic breeding programs dedicated to the Nellore breed are pivotal in boosting beef productivity, offering crucial support for the livestock industry to realize its full potential. Therefore, it is crucial to share more effective selection strategies and methods in the field to enhance genetic gain, especially in herds that have unknown pedigree, and the ability to predict characteristics of economic importance for the sector.

1.3.2 Reproduction and longevity traits

Researchers in the field of genetic improvement have placed great emphasis on reproductive traits related to sexual precocity in females and efficiency indicators with the aim of improving reproductive indices (Kluska et al., 2018; Brunes et al., 2020; Fernandes Júnior, 2018). The use of early-maturing heifers aims to reduce the age at first calving and increase herd profitability by reducing the number of unproductive categories, increasing the ability to produce a greater number of offspring compared to later-maturing females. These traits are not just important, they are crucial for the success of beef cattle production. Additionally, they have more time to recover post-partum, wean heavier offspring throughout their productive lives, and exhibit longer retention in the herd with satisfactory reproductive performance (Schmidt et al., 2018; Fernandes Júnior et al., 2018).

Breeding programs have developed reproductive indices to evaluate the performance of females, such as the index of Accumulated Cow Productivity (ACP), which indicates the productivity of cows in kilograms of weaned calves per year (Lobo et al., 2000). ACP has a favorable correlation with growth traits and age at first calving because females who calve early and maintain calving intervals are more fertile and reproductively efficient (Schwenberg et al., 2001; Grossi et al., 2016). In the literature, estimates of low to moderate magnitude are found in the Nellore breed, as reported by Schwengber et al. (2001), Chud et al. (2014), Grossi et al. (2016), Negreiros et al. (2022), and Negreiros et al. (2024), ranging between 0.11 and 0.17.

Among reproductive traits, age at first calving (AFC) stands out as it allows the registration of the onset of reproductive life in heifers and reflects the herd's productivity, with its advancement directly related to the efficiency and profitability of beef production. AFC depends on the age at which females are incorporated into the breeding herd and the reproductive management adopted (Notter, 1995). Thus, the phenotypic information obtained can often be biased due to the late exposure of females capable of reproduction. Females challenged at a younger age have a higher chance of conceiving earlier. The heritability for this trait in the Nellore breed is low to moderate, ranging between 0.06 and 0.25 (Ambrosini et al., 2016; Claus et al., 2017; Kluska et al., 2018; Schmidt et al., 2018; Silva Neto et al., 2020), indicating that there is a strong environmental influence on the phenotypic expression of this trait.

In breeding programs, the main selection criteria associated with sexual precocity and fertility in males is scrotal circumference, evaluated at different ages due to its high repeatability, moderate to high heritability, and close relationship with other reproductive traits (Bonamy et al., 2018; Kluska et al., 2018). In the literature, estimates of moderate to high heritability magnitude are reported, ranging between 0.33 and 0.48 for this trait in the Nellore breed, as reported by Kluska et al. (2018), Carvalho Filho et al. (2020), and Negreiros et al. (2022).

1.3.3 Horn development

The morphology of horns in bovines varies significantly between species and breeds. There are three main phenotypes based on the presence or absence of horns: polled (lacking horns), horned, and an intermediate phenotype known as scurs—loose and mobile horn tissue (Duijvesteijn et al., 2018; Gehrke et al., 2020). Gehrke et al.

(2020) describe a wide range of horn phenotypes, ranging from smoothly polled animals to those with frontal bumps, scabs, scurs up to 10 cm long, and regular horns. Davis et al. (2011) defines horns as permanent, paired appendages consisting of an outer part of keratin and an inner part of living tissue. In contrast, scurs are shorter and do not grow at the same rate as horns (Capitan et al., 2011). Typically, horns are visible shortly after birth, while scurs begin to appear after four months of age (Lange et al., 1990).

In nature, horns serve several purposes: defense against predators, establishing dominance among males for mating privileges, and protecting offspring from aggression (Estes, 1991). Historically, farmers favored cattle with horns because they were used as draught animals and were practical for attaching harnesses (Rosenberger & Robeis, 2005). However, horned animals can pose risks to farmers and other livestock on farms, such as causing injuries during interactions or damaging carcasses. Studies by Sambraus (1978) and Goonewardene & Hand (1991) suggest that polled animals may exhibit calmer temperaments than horned animals.

Research indicates notable differences between horned and dehorned animals: dehorned dairy cows show reduced activity and displacement (Knierim et al., 2015) and horned herds demonstrate higher levels of physical aggression (Menke et al., 1999). Cows also use horns to establish dominance within the herd and for self-grooming (Bouissou, 1972; Beilharz & Zeeb, 1982). While horns are associated with thermoregulation and nasal heat exchange (Taylor, 1966; Hoefs, 2000; Knierim et al., 2015), the presence of horns increases the risk of injuries in herds. Costa & Silva (2007) showed that the presence of horned animals within a batch cand influence the degree and amount of bruising, as these animals tend to be dominant over hornless cattle, and they display their dominance using their horns to push and injure other cattle. In dairy farming, injuries from horns are often superficial but can escalate to severe injuries in the udder and vulva, impacting producers economically (Knierim et al., 2015).

Studies by Meischke et al. (1974) and Shaw et al. (1976) highlight increased bruising in horned cattle during slaughterhouse transport, requiring more extensive trimming of bruised tissue compared to hornless cattle. In the Australian industry, the incidence of bruising in horned cattle was found to be twice as high as in polled cattle (Collins & Huey, 2014). Similarly, Huertas et al. (2010) observed in Uruguay a positive correlation between the presence of bruised carcasses and batches of horned animals.

According to Vaz et al. (2012), these bruises negatively impact earnings and affect the quality and location of the best cuts of meat from the carcasses. When comparing taurine and zebu cattle breeds, a higher number of injuries were found on the carcasses of zebu cattle compared to taurine cattle. Specifically, groups of horned animals exhibited more bruising on their carcasses compared to polled animals (Mendonça et al., 2016). The same author concluded that zebu and horned animals are more susceptible to carcass bruising, leading to greater economic losses for producers and industries.

For farmers, raising hornless cattle can be economically beneficial, as it reduces the risk of injuries to both livestock and handlers (Knierim et al., 2015). Additionally, dehorning or disbudding processes can cause considerable stress and pain to the animals, with dehorning generally being more harmful to their welfare than disbudding (Knierim et al., 2015). To encourage more humane practices, there is increasing interest in breeding polled cattle to eliminate the need for dehorning (Bates et al., 2016; Knierim et al., 2015). The use of genetic selection to produce polled animals brings benefits such as promoting animal welfare, reducing the risk of injuries among livestock, eliminating the need for dehorning, and lowering the risk of accidents involving people who work directly with the animals (Schafberg & Swalwe, 2015).

Regarding the polled Nellore, 826,314 records have been counted according to ABCZ (2021), and this large number of animals without horns facilitates selection for this characteristic. Additionally, genetic selection for obtaining polled cattle can be carried out concurrently with other economically relevant traits (Oliveira et al., 2023), such as sexual precocity and longevity. This research opens new possibilities for the future of cattle breeding, offering a more efficient and humane approach to horn development. Temp et al. (2024) obtained heritability estimates ranging from 0.44 to 0.84 in the Nellore breed, where the highest heritability estimate was obtained for the binary classification (presence or absence of horns) of the phenotype, aligning with the value found by Stafuzza et al. (2018) of 0.90. According to the same authors, when only two categories were used in the evaluation, the true phenotype of some individuals was probably masked, resulting in biased variances and an overestimation of heritability. In contrast with three or four categories, it is possible to obtain a more robust estimation of genetic variation through an appropriate phenotypic classification of the animals.

In Bos indicus animals, Stafuzza et al. (2018) conducted the first research on the polled trait, identifying a 3.11 Mb region on chromosome 1 involved in horn development in Nellore cattle. In the literature, the most widely accepted and recognized model for the inheritance of the polled gene is described at three loci, each with two alleles: the Polled locus, the Scurs locus, and the African horn locus (Gehrke et al., 2020). However, according to these same authors, there is a gap in the literature regarding studies elucidating the inheritance mechanism of these genes and how the alleles at the three loci interact with each other to determine the phenotype of the trait, in addition to the influence of sex on horn development (Simon et al., 2022).

The inheritance patterns of horn development, particularly regarding sex influence, remain a complex puzzle in our field. One hypothesis suggests that male horns may serve multiple purposes, including self-defense, territorial disputes, and intra-male competition for mating success (Gehrke et al., 2020; Simon et al., 2022). However, distinguishing postnatal development of scurs early on, before yearling, poses significant challenges for selection decisions aimed at propagating horn absence (Randhawa et al., 2020). According to Temp et al. (2024) the binary coding of horn development is an unsuitable oversimplification of polled phenotype, and the genetic background of horn development is more complex than previously proposed. On practical field experience with Nellore breeders indicates that a four-category system—comprising polled, polled born of horned parents, scurs, and horns—could enhance genetic evaluation accuracy for horn development (Temp et al., 2024),

Implementing breeding values and Expected Progeny Difference (EPD) for horn development, facilitated by large-scale SNP genotyping and genomic selection, offers a promising approach. This method, particularly useful for traits difficult to assess early on, like horn development in indicine cattle, could leverage single-step genomic best linear unbiased prediction (ssGBLUP) assuming a polygenic model. Despite limited research on the genetic parameters of horn presence and absence in cattle, understanding such parameters, including heritability, across different classifications of horn development, is vital for evaluating genetic gain and guiding selection strategies in indicine cattle (Temp et al., 2024).

1.3.4 Single-step genomic best linear unbised prediction: Metafounders and Unknown parent groups

In a scenario where the application of technology and resources is scarce, obtaining reliable information on the genetic evaluation of sires and dams is limited, as is the use of technologies for animal reproduction. As a result, the common practice is to use multiple bulls in commercial herds to reduce costs and increase the likelihood of confirmed pregnancies. However, this mating method compromises paternity identification, resulting in the absence of pedigree records, which hinders the participation of these herds in genetic improvement programs. In contrast, herds with animals registered in breeders' associations enjoy greater access to advanced technologies and a broader range of phenotypic, genotypic, and genealogical information (Ferraz & Felício, 2010; Araújo et al., 2014; Londoño-Gil et al., 2023).

Genetic merit is predicted from phenotypic records and the pedigree-based relationship matrix (**A**) using the traditional BLUP program (Henderson, 1988; Tonussi et al., 2021). In practice, the pedigrees used in these evaluations may refer to various base populations considered unrelated due to lack of information (Kluska et al., 2018), as well as animals from different generations with missing data in the pedigree (Tsuruta et al., 2014). Furthermore, there are situations where the pedigree has gaps, especially related to the sex of the animals. For example, in beef cattle, there is often more information available about the parents of males than females. (Misztal et al., 2013; Garcia-Baccino et al., 2017).

Over the years, several methods such as hierarchical animal model (HIER), unknown parental groups (UPGs) and single-step genomic BLUP (ssGBLUP) have been developed to increase the reliability of genetic evaluations of animals with uncertain pedigrees. The HIER model, developed by Cardoso and Tempelman (2003), combines phenotypic records and prior information to infer the posterior probability of the genetic merit of a candidate bull, considering the uncertainty of paternity associated with the animal and its respective bull. UPGs, incorporated into mixed models, aim to explain genetic discrepancies among predefined groups of animals (Tsuruta et al., 2014).

Quass (1988) proposed this methodology to account for missing pedigree in a population that can be formed bases on different criteria of missing parents like a year of birth, breed composition, sex, among others (Legarra et al., 2007; Kluska et al., 2021). Additionally, assuming that all unknown parents belong to the same base population with the same genetic level in the presence of selection is incorrect (Misztal et al., 2013; Garcia-Baccino et al., 2017). Compatibility between the pedigree-based relationship matrix and genomic information is crucial to avoid bias in genomic predictions and estimation of variance components, as well as to reduce prediction accuracy (Vitezica et al., 2011). According to the literature, some studies reported reduction of bias with and without genomic information using UPGs (Tsuruta et al., 2019), although Phocas and Laloë (2004) and Kluska et al. (2021) found an increase in bias, that can be related with groups that have a small number of animals and insufficient quantity of phenotypes per trait (Tsuruta et al., 2019).

The redefinition of groups with the objective to get better connectivity and increase the number of animals and phenotypes can help to obtain a correct genetic trend and increase the reliability of GEBV estimates (Tsutura, et al., 2014; Kluska et al., 2021). Legarra et al. (2009), Misztal et al. (2009), and Aguilar et al. (2010) proposed the ssGBLUP methodology that integrates the pedigree relationship matrix (**A**) with the genomic relationship matrix (**G**) into a single matrix (**H**) to predict the genomic estimated breeding values of animals. This methodology requires genomic and pedigree relationships to refer to the same base (Legarra et al., 2014), where pedigree relationships can be projected for non-genotyped animals (Legarra, Aguilar, Misztal, 2009). This represents a strategy that not only reduces computational costs but also significantly enhances the accuracy of evaluations compared to models relying solely on phenotypic information (Aguilar et al., 2010; Christensen et al., 2012). The UPG has been incorporated into ssGBLUP through a modification of H^{-1} and in some models have decreased the bias and inflation of GEBV (Tsutura et al., 2011; Masuda et al., 2021).

The ssGBLUP methodology presents significant advantages such as ease of inverting the **H** matrix, the ability to group genotyped and non-genotyped animals, the absence of the use of derepressed values, and the appropriate weighting of information from genotyped bulls and females, thus avoiding double counting of animals in the evaluation to reduce bias (Legarra et al., 2014). According to Misztal et al. (2013), ssGBLUP, when compared to multi-step methods, is straightforward to execute and applicable to more complex models, such as multi-trait models.

However, in some situations, the **G** and **A** matrix can be incompatible resulting in biased estimates using ssBLUP (Misztal and Legarra, 2017) because not all animals of the pedigree have genotypes (Legarra et al., 2014; Legarra et al., 2015), and to solve this point the matrices need to refer to the same base population (Legarra et al., 2015). Traditionally, it has been assumed that individuals in the base population lack kinship ties, stemming from an extensive ancestral population. However, with the introduction of molecular data, it has become evident that this assumption does not hold true for most real populations (Legarra et al., 2015). Additionally, Christensen (2012) demonstrated that individuals from the base populations are indeed related to each other.

Legarra et al. (2015) proposed a method in which it is possible to include multiple base generations through metafounders (MF) that are fictitious individuals (pseudo-individuals) considered simultaneously as both the father and mother of the base population, aiming to work with common ancestry among individuals who, based on commonly available information, would not be related (Legarra et al., 2014). The aim of this methodology is resolving the incompatibility between matrices **G** and **A**, considering relationships within (γ^{i}, γ^{j}) and between ($\gamma^{i,j}$) base population, modeled by a gamma matrix ($\mathbf{\Gamma}$) which is using to form the relationship matrix ($\boldsymbol{A}^{\mathbf{\Gamma}}$) (Garcia-Baccino et al., 2017). If the differences between the matrices are not considered when relating them, genetic value predictions may be biased (Meyer, Tier, Swan, 2018; Misztal et al., 2013). The MF approach is similar to UPG, but allow a related base population with nonzero inbreeding coefficients (Kudinov et al., 2020).

MFs allows for considering a base population and then an MF, as well as multiple base populations with several MF’s likely related to each other. These are indicated in two situations: (1) combining genomic and genealogical information among animals, as in the ssGBLUP method; and (2) considering the existence of multiple base populations simultaneously (Legarra et al., 2015), such as multiracial populations. With the inclusion of MF, one of the main advantages observed is the increased compatibility between the genomic and pedigree relationship matrices, consequently reducing bias in the GEBV (Garcia-Baccino et al., 2017).

Genomic prediction, using MF and ssGBLUP, demonstrated lower bias, higher accuracy, and provided more consistent estimates of heritability in a simulated population of dairy cattle subject to selection, based on a trait recorded only in females with a heritability of 0.30, compared to evaluations without MF (Garcia-Baccino et al., 2017). In another study, Bradford et al. (2019) obtained more accurate and less biased genomic predictions for two sex-limited traits with heritabilities of 0.30 and 0.10 in young dairy cattle when employing MF and ssGBLUP, as well as adequately estimating genetic trends. Aldridge et al. (2020) observed that when using MF in real data on maternal traits of sows such as average litter weight at birth, litter variation defined as the standard deviation of birth weight within the litter, stayability, maximum number of cycles, total number of born, number of born expressed as log10, litter mortality, extended interval between weaning and first insemination, gestation length, and farrowing rate, genomic and pedigree relationships were consistent. Xiang et al. (2017) and Van Grevenhof et al. (2019), when working with a real population for total number of piglets born and crossbred pigs for the performance of purebred selection candidates, both found positive results when using the methodology associated with ssGBLUP. Moreover, the incorporation of MF in analyses using simulated sheep data for a trait with a heritability of 0.30 proved effective in preventing bias in genetic trend estimates (Meyer; Tier; Swan, 2018).

The development of tools capable of increasing the accuracy and reducing the bias of genomic evaluations in young animals is of utmost importance to achieve more significant response to selection, as well as being effective strategies for reducing genotyping and phenotyping costs. Studies with MF in Nellore beef cattle for traits related to reproduction, longevity, and production are still scarce in the literature. Furthermore, the evaluation of metafounders may clarify discrepancies between pedigree and genomic matrices, allowing for a more precise definition of the models employed in estimating genetic values for economically important traits in beef cattle production, even in complex populations with pedigree data limitations.

1.3.5 Adjust in genomic relationship matrix

The animals’ genetic value is commonly predicted from phenotypic records and pedigree-based kinship matrix using the traditional BLUP (Best Linear Unbiased Prediction) program (Tonussi et al., 2017). However, in practice, the pedigrees used in these evaluations may refer to several unrelated base populations due to a lack of information (Kluska et al., 2018), as well as animals from different generations with missing pedigree data (Tsuruta et al., 2019). The compatibility between the pedigree-based relationship matrix and genomic information is crucial to avoid bias in genetic predictions and variance component estimation (Vitezica et al., 2011).

The single-step genomic BLUP (ssGBLUP) method allows the combination of genomic and pedigree-based kinship matrices in genetic evaluation through the **H** matrix, which reduces the computational cost and increases the accuracy of evaluations (Legarra et al., 2014). Among the advantages of this method is the ease of inverting the **H** matrix and allowing the grouping with and without genotype animals, since genotyping the entire population becomes impractical due to the high cost of the technique.

Considering that the genomic kinship matrix used in genomic evaluations is centered and scaled based on the animal’s allele frequencies, it is important to note that the heterogeneity of these frequencies in certain populations can represent potential complications in the process. Lourenco et al. (2016) devised a strategy to enhance the compatibility between matrices **G** and **A**. In their approach, they utilized breed-specific allele frequencies to construct matrix **G**. This matrix is centered to fix the mean values of the allele effects as zero, and it is adjusted to the specific allele frequency of the founding breed, providing greater consistency between the coefficients of relationship of genotyped and non-genotyped individuals (Makgahlela et al., 2013).

Additionally, it is important to note that the impact of considering specific allele frequencies for each breed in the **G** matrix seems to be more significant in younger animals than in older animals. This is because, in the absence of phenotypes, the genotype is the only information used to estimate genetic values (Lourenco et al., 2015). According to Warburton et al. (2021) the use of genomic relationship matrices adjusted by breed allowed for the capture of additive genetic effects to a greater extent. On the other hand, Kluska et al. (2021), when using different relationship matrices adjusted for allele frequencies of the genetic groups that make up the Montana breed, did not observe benefits in improving the reliability, stability, dispersion, and trends of reduced genomic values. The **G** matrix adjusted to the allele frequency of the founding race should provide greater consistency between the coefficients of the relationship of genotyped and non-genotyped individuals (Makgahlela et al., 2013).

In the literature, little is discussed about the implementation and use of weighted genomic matrices on the prediction of genetic parameters in the Nellore breed. Even so, these approaches mainly aim to improve the robustness and predictive ability of genomic information in genetic evaluations.

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CHAPTER 2 - EFFECT OF GENETIC AND SEX EFFECT ON GENOMIC PREDICTION FOR HORN DEVELOPMENT IN NELLORE CATTLE

Larissa Bordin Temp[1*], Ludmilla Costa Brunes[2], Letícia Silva Pereira[3], Sabrina Thaise Amorim[4], Cláudio Ulhôa Magnabosco[2], Raysildo Barbosa Lobo[5], Ovidio Carlos de Brito[6], Ricardo Viacava[7] and Fernando Baldi[1]

[1]Universidade Estadual Paulista, Jaboticabal, SP, 14884-900, Brazil
[2]Embrapa Cerrados Planaltina, DF, 73310-970, Brazil
[3]Universidade Federal de Goiás, Goiânia, GO, 75680-900, Brazil
[4]Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061, USA
[5]National Association of Breeders and Researchers (ANCP), Ribeirão Preto, 14020-230, Brazil
[6]Marca OB, Pontes e Lacerda, MT, 78250-000, Brazil
[7]Nellore Mocho CV, São Paulo, SP, 05428-000, Brazil

Published in Livestock, doi: https://doi.org/10.1016/j.livsci.2024.105478
Received: 26 January 2024, Accepted: 2 May 2024.

Abstract: This study aimed to evaluate the influence of phenotypic classification of horn development, animal sex effect, and non-autosomal SNP (single nucleotide polymorphism) markers on the genetic parameters and genomic prediction ability for horn development in Nellore cattle using the single-step genomic best linear unbiased prediction method. The polled phenotype was evaluated in two (presence and absence of horns), three (scurs and polled offspring from a horned parent, and the polled and horned animals), and four (absence of horn, polled born to a parent with horn, scurs, and presence of horn) phenotypic categories. A total of 12 statistical models were evaluated. The variance components were estimated using the THRGIBBS1F90 software, and a threshold animal model was used for genomic prediction analyses with the single-step genomic BLUP (ssGBLUP) procedure. Accuracy, bias, and dispersion parameters were evaluated based on the linear regression (LR) method. The highest heritability (0.84) was obtained when the polled character was evaluated as a binary trait. The lowest heritability estimates (0.44 to 0.45) for horn development were obtained when the phenotype was classified into three categories. For the same horn development classification method, the heritability estimates were similar regardless of the genomic evaluated models and fixed effects included in the model. For models considering four and three phenotypic categories for horn development, the inclusion of the sex effect as a fixed effect within the CG did not improve the accuracy, bias, and dispersion of genomic predictions for horn development. Analyzing the trait with binary expression, the highest prediction accuracy was observed when the effect of sex was not included in the CG and without the SNPs in the sex chromosomes. These models displayed the highest dispersion, pointing out the low robustness of genomic prediction. In addition, models that use less than four categories to classify the horn development phenotype, with no discrimination between polled and homozygous polled displayed lower prediction ability. The inclusion of non-autosomal SNPs in the analyses for the models considering four phenotypic categories leads to an improvement in prediction accuracy in 5,26%, bias, and dispersion reduction, 37% and 4,55%, respectively, compared with models that only considered autosomal SNPs. The selection using genomic information for the polled trait is feasible, and it is an alternative to obtaining polled Nellore animals. The binary coding of horn development is an unsuitable oversimplification of polled phenotype, and probably, the genetic background of horn development is more complex than previously proposed. The most adequate prediction model to evaluate the horn development in Nellore cattle was considering four phenotypic categories and including non-autosomal SNP in the analyses for genomic prediction purposes of naturally genetically polled animals. Genetic dehorning can be adopted on a large scale as a low-cost and non-invasive approach to increase the frequency of hornless animals using genomic information and mating strategies.

Keywords: *Bos indicus*, polled, sex chromosomes, scurs, ssGBLUP

2.1 INTRODUCTION

Beef production in tropical environments is based on the use of indicine cattle, which display high ability to convert low-quality forage into protein, high resistance to parasites and high temperature, and high rates of productive and reproductive performance in tropical conditions (Ferraz & Felicio, 2010; Cooke et al., 2020). There is a worldwide trend for reducing horns to avoid accidents related to herd management

(Stafuzza et al., 2018). In this context, the presence of naturally polled Nellore is beneficial for the beef industry, due to economic losses caused by damage to the leather and injuries to muscle tissue (Mendonça et al., 2016; Youngers et al., 2017). To work around this problem, the first alternative is to carry out the dehorning process, preventing the growth of horns. However, it is an invasive procedure, harmful to well-being, and difficult to adopt on a large scale (Bates et al., 2016; Knierim et al., 2015). Another option is the production of animals that naturally lack horns, increasing the incidence of polled alleles, as with the polled variety of the Nellore breed.

The first research with polled phenotype in indicine cattle was carried out considering the presence and absence of horns, polled/horned phenotype (Stafuzza et al., 2018). These authors described networks of genes mainly in a 3.11 Mb region in chromosome 1 involved in horn development in Nellore cattle. Considering the phenotypic variation observed, the polled development assessment approach with not only the presence and absence of horns seems more realistic and would contribute to a more comprehensive understanding of genetic variations for the Nellore breed. Thus, considering also scurs would be more adequate for elucidating the inheritance pattern of horn development in indicine cattle, aiming to obtain animals without scurs, for example.

To date, the most accepted and recognized model reported in the literature for polled inheritance describes three loci, each with two alleles, controlling the polled development. First, the Polled locus with the P (polled) allele dominant to the p (horned) allele; the Scurs locus with Sc (development of scurs) and sc (absence of scurs) alleles, and the African horn locus with Ha (horned) and ha (polled) alleles. Nevertheless, no studies have been able to fully elucidate the inheritance mechanism, and how alleles in these three loci interact with each other determining the phenotype for this trait, which may be under the influence of many genes or oligogenic genetic background (Gehrke et al., 2020; Stafuzza et al., 2018).

The patterns of inheritance of horn development have not yet been fully elucidated about sex influence, but one hypothesis related to males is that the horns could be used for self-defense and in territorial fights, as well as in sexual selection through intra-male competition and mating success (Gehrke et al., 2020; Simon et al., 2022).

There is difficulty in differentiating the postnatal development of scurs at an early age, before yearling, not being a suitable determinant for selection decisions aimed at propagating the absence of horns (Randhawa et al., 2020). Furthermore, it has been suggested that both the presence of horns and polled homozygous can mask the scurs phenotype (Ketel & Asai-Coakwell, 2020). Based on practical field experience acquired in collaboration with Nellore breeders and the phenotypic variation observed in Nellore breed animals, it seems that a four-category system, considering the phenotypic categories such as polled, polled born of horned parents, scurs, and horn, for genetic evaluation purpose of horn development would be more appropriate. Thus, the approach of this study aims to reflect the observed field experience, thereby contributing to a more comprehensive understanding of genetic variations within the Nellore breed and develop and propose a feasible phenotype system to assess horn development in Nellore cattle.

In addition to validating the phenotype assessment method for horn development, implementing Expected Progeny Difference (EPD) for horn development is one possible by-product of releasing new selection criteria for horn development in the Nellore breed. Large-scale SNP (single nucleotide polymorphism) genotyping has enabled the application of genomic selection, a type of selection that predicts the genomic estimated breeding values (GEBV) of the individual from a large set of SNP-type genetic markers, increasing the accuracy of predicted genetic value estimates (Berry et al., 2016). This method is particularly useful for traits difficult to evaluate at an early age and those that depend on progeny tests, like horn development in indicine cattle. Considering a possible oligogenic architecture and a phenotypically quantitative basis on the polled trait, the single-step genomic best linear unbiased prediction (ssGBLUP) can be suitable when assuming a polygenic model. In cattle, there is limited research on the genetic parameters of horn presence and absence. It is important to understand the genetic parameters, such as heritability, for different classifications of horn development. This understanding can help evaluate the feasibility of genetic gain and direct selection for horn development in indicine cattle.

This study aims to estimate genetic parameters and evaluate the influence of phenotype classification of horn development, animal sex effect, and non-autosomal SNP markers on genomic prediction ability for horn development in Nellore cattle using the single-step genomic best linear unbiased prediction method. This study may increase the use of polled traits in indicine cattle as selection criteria and improve prediction ability by evaluating more animals.

2.2 MATERIAL AND METHODS

2.2.1 General data information

The phenotypic records and genotypic information of Nellore animals belonging to the Guaporé Pecuária and Nellore CV farms located in Mato Grosso do Sul and São Paulo states and evaluated by the National Association of Breeders and Researchers (ANCP, Ribeirão Preto, Brazil) were used. The pedigree consisted of 48,527 animals, of which 19,136 had registered phenotypes for the researched trait (6,722 with the absence of horn phenotype, 9,833 polled animals that were the progeny of sires or dam with horn, 708 scurs, and 1,873 horned (Table 2)**.** The phenotyping was performed at about 12 months of age by four trained technicians.

Table 2. Number of records of the complete population based on sex.

		Categories		
Models	**Polled**	**Polled born to a parent with a horn**	**Scurs**	**Horned**
1, 2, 3 e 4	6,722	9,833	708	1,873
♂	2,349	3,672	143	480
♀	4,373	6,161	565	1,393

	Polled	**Polled born to a parent with a horn + Scurs**	**Horned**
5, 6, 7 e 8	*6,722*	*10,541*	*1,873*
♂	2,349	3,815	480
♀	4,373	6,726	1,393

	Polled + *Polled born to a parent with a horn + Scurs*	**Horned**
9, 10, 11 e 12	17,263	1,873
♂	6,164	480
♀	11,099	1,393

♂: number of males in each category; ♀: number of females in each category.

2.2.2 Models, genotyping process and quality control analysis

A total of 12 statistical models considering different phenotypic classifications of horn development, animal sex effect, and non-autosomal SNP markers were evaluated to estimate variance component and genomic prediction ability for horn development. Table 3 shows the genetic models evaluated for genetic parameter estimation and genomic prediction for horn development. The effects listed in Table 3, marked with an X, indicate that they were used to compose the contemporary group (CG) alternately among the tested models as well as the models with non-autosomal SNPs in the analyses.

Table 3. Structure of the evaluated models for polled trait in Nellore cattle varying according to the inclusion of effects within CG, and SNPs present on sex chromosomes.

Models	**Number of Categories**	**Effects within CG**			**Non-autosomal SNPs**
		Farm	**Year of Birth**	**Sex**	
1	4	X	X	X	
2	4	X	X	X	X
3	4	X	X		
4	4	X	X		X
5	3	X	X	X	
6	3	X	X	X	X
7	3	X	X		
8	3	X	X		X
9	2	X	X	X	
10	2	X	X	X	X
11	2	X	X		
12	2	X	X		X

Model 1: four phenotypic categories with sex effect in the CG; Model 2: four phenotypic categories with sex effect in the CG and non-autosomal SNPs; Model 3: four phenotypic categories without sex effect in the CG; Model 4: four categories without sex effect in the CG but with non-autosomal SNPs; Model 5: three phenotypic categories with sex effect in the CG; Model 6: three phenotypic categories with sex effect in the CG and the SNP's of the sex chromosomes; Model 7: three phenotypic

categories with no sex effect in the CG; Model 8: three phenotypic categories without sex effect in the CG but with non-autosomal SNPs; Model 9: two phenotypic categories with sex effect in the CG; Model 10: two phenotypic categories with the effect of sex on the CG and non-autosomal SNPs; Model 11: two phenotypic categories with no sex effect in the CG; Model 12: two phenotypic categories without sex effect in the CG but with non-autosomal SNPs.

Different phenotypic classifications of horn development were evaluated based on practical field experience acquired in collaboration with Nellore breeders. Initially, four models (models 1 to 4) were tested considering four phenotypic categories of horn development, absence of horn (1), polled born to at least one parent with a horn (2), scurs (3), and presence of horn (4). described as follows: a) Figure 1 represents animals from the polled category (total absence of horn or scurs) and polled with horned sire (total absence of horn, but offspring of a horned sire or dam). The polled progenies of horned sire or dam were classified based on their own phenotype and the phenotype of their parents. Polled progeny of horned parents would probably be heterozygous polled animals, carrying alleles capable of causing the presence of horns and scurs; b) Figure 2 is an example of the scurs category. They represent animals of scurs with a protuberance at the birthplace of the horns, respectively. Scurs are hornlike formations in the horn area, loosely attached to the skull by soft tissue or frontal bumps with keratinization of the covering skin.; c) Figure 3 represents animals of the horned category or the presence of regular horns firmly attached to the skull.

Figure 1. Polled category and polled born to a parent with a horn in the Nellore breed.

Figure 2. Scurs category in the Nellore breed.

Figure 3. Horned category with the presence of regular horns firmly attached to the skull in the Nellore breed.

Additionally, we evaluated four models (models 5 to 8) considering three phenotypic categories for horn development, where the scurs phenotype and polled offspring from a horned parent were merged into a single category, and the polled (animals born from polled parents) and horned animals remained separate. The polled born from horned parents was merged with the scurs category because the polled progeny of horned parents would probably be heterozygous polled animals, carrying alleles capable of causing the presence of horns, and having a lower likelihood of being homozygous for the polled condition. In addition, to elucidate the most adequate phenotypic classification for horn development, four binary models (models 9 to 12) were evaluated considering the presence or absence of horns, the latter consisting of polled animals (without horns), polled offspring from a parent with horns, and scurs. Thus, categories 1, 2, and 3 previously described were grouped for this phenotypic classification.

The fixed effects of the farm, birth year, and sex of the animal were concatenated to define the CG (N=235) in models 1, 2, 5, 6, 9, and 10. In models 3, 4, 7, 8, 11, and 12, the fixed effects of farm and birth year were concatenated to define the CG (N=197) without including the animal sex effect. For all scenarios, the animal threshold model included the fixed effects of CG, the animal's age as covariable (linear effect), and the random direct additive genetic and residual effects. The general animal model used was:

$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e},$ (1)

where $\boldsymbol{y}$ is a vector of dependent variables; $\boldsymbol{\beta}$ is a vector of fixed effects including the CG, animal age at phenotypic evaluation; $\mathbf{u}$ are the random direct additive genetic effects and $\boldsymbol{e}$ are the random residual effect. $\mathbf{X}$ is the incidence matrix associating $\boldsymbol{\beta}$ with $\mathbf{y}$; $\mathbf{Z}$ is the incidence matrix associating $\mathbf{u}$ with $\mathbf{y}$.

The variance components and genetic parameter were estimated with a threshold animal model (Mrode 2014), assuming an underlying scale with a normal distribution:

$\mathbf{u}|\boldsymbol{\theta}\sim\mathbf{N}(\mathbf{W}\boldsymbol{\theta}, \mathbf{I}\boldsymbol{\sigma}_{\mathbf{e}}^{2}),$ (2)

where $\mathbf{u}$ is a vector of underlying scale with order r (r is the number of animals); $\boldsymbol{\theta}' = (\boldsymbol{\beta}', \mathbf{u}',)$ is the parameter vector with order s, where s is the numbers of effects in the model; $\mathbf{W}$ is the incidence matrix with order r by s ; $\mathbf{I}$ is the identity matrix with order r by r; and σ_e^2 is the residual variance. For binary models, the residual variance was fixed at $\sigma_e^2 = 1$ (Sorensen and Gianola, 2002). The link between base and underlying scale was made by a probit link function (Gianola and Foulley, 1983; Thompson and Baker, 1981).

The variance components were estimated using the THRGIBBS1F90 software, version 2.108 of the BLUPF90 family of programs, using a pedigree-based single-trait and best linear unbiased prediction (BLUP), without genomic information. Gibbs chains were generated with 400,000 iterations, burn-in 40,000, and a sampling interval of 100 iterations. For the convergence analysis, visual inspection with density and trace plots of variance components and a Geweke test were used, with the aid of BOA package (Smith, 2005) in R program (2023). After the Geweke test, the chain convergence was confirmed. A threshold animal model was used for genomic prediction analyses with the single-step genomic BLUP (ssGBLUP) procedure, implemented in the cblup90iod2OMP1 program, a commercial version.

The ssGBLUP is a modification of the traditional BLUP model, where the numerator relationship matrix $\mathbf{A^{-1}}$ is replaced by $\mathbf{H^{-1}}$ (GEBV) (Aguilar et al., 2010).

$$\mathbf{H^{-1}} = \mathbf{A^{-1}} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G^{-1}} - \mathbf{A_{22}^{-1}} \end{bmatrix}, \text{(3)}$$

where $\mathbf{A^{-1}}$ is an inverse of the pedigree relationship matrix, $\mathbf{G^{-1}}$ is an inverse of the genomic relationship matrix, and $\mathbf{A_{22}^{-1}}$ is an inverse of the pedigree matrix – for genotyped animals only. The heritability estimates were present in Table 3.

A total of 20,000 animals were originally genotyped with 70,092 SNPs (70k; ZBN, Zoetis, Kalamazoo). These animals were used as a reference for genomic imputation of 1,180 animals genotyped with CLARIFIDE® Nellore 3.0 (29k; Zoetis, Kalamazoo, MI) and 1,220 animals genotyped with GGP Indicus (54k; Neogen, Lincoln, Nebraska, USA). The animals were then imputed to a commercial panel (ZBN, Zoetis, Kalamazoo) containing 70k markers using FImpute software (Sargolzaei et al., 2011). Genotype quality control excluded SNPs with unknown genomic position according to the ARS-UCD1.3 bovine genome set, located on sex chromosomes (except for models 2, 4, 6, 8, 10, and 12), monomorphic, with minor allele frequency (MAF) less than 5% and call rate less than 90%. Samples with a call rate greater than 90% were also excluded. After quality control, a total of 21,425 genotyped animals, 1,133 SNPs from sex chromosome X (non-autosomal SNPs), and 36,161 autosomal SNPs were retained for genomic analysis. A total of 19,136 animals had both genomic information and phenotypic records. The number of records of the complete dataset are presented in Table 2.

2.2.3 Single step genomic BLUP and prediction ability

All scenarios were compared using the linear regression method (LR) proposed by Legarra and Reverter (2018). The “whole” dataset or training dataset included all sources of information for the animals, genotyped and phenotyped animals with progenies and phenotypes of non-genotyped animals, whereas the “partial” dataset or validation dataset omitted phenotypic information of validation animals born in 2020. The validation animals were young (N=904), genotyped individuals without their own or progeny records in the validation dataset but with complete records in the training dataset. This validation strategy simulates the practical situation of genomic selection, where the genomic breeding value GEBV prediction of the youngest animals and selection candidates depend on the phenotypic and genotypic information of the tested or older animals of the complete population. The LR estimators of accuracy, bias, and dispersion for the validation animals were computed as follows (Legarra and Reverter, 2018):

1) Accuracy ($\boldsymbol{\rho}_{p,w}$) was computed as the correlation of predicted GEBV of validation animals obtained in the training and validation dataset (Legarra & Reverter, 2018).

$$\boldsymbol{\rho}_{p,w} = \frac{cov(\hat{u}_p,\hat{u}_w)}{\sqrt{var(\hat{u}_w)var(\hat{u}_p)}} \text{ ,(4)}$$

where: $\hat{u}_p$ and $\hat{u}_w$ is the vector of GEBV for validation animals using the validation and training dataset, respectively.

2) The expected bias ($\mu_{w,p}$) is estimated from the difference between the mean GEBV of validation animals obtained in the validation and training dataset as Legarra & Reverter (2018):

$$\mu_{w,p} = \bar{\hat{u}}_{\boldsymbol{p}} - \bar{\hat{u}}_{\boldsymbol{w}} \text{ , (5)}$$

the bias refers to the difference between the estimated EBV and the true value, that is, the more accurate the model, the closer to zero it will be.

3) The dispersion ($b_{w,p}$) was calculated as the slope of the regression of the predicted GEBV of validation animals obtained in the training and validation dataset (Legarra & Reverter, 2018):

$$b_{w,p} = \frac{\boldsymbol{cov(\hat{\mu}_w - \hat{\mu}_p)}}{\boldsymbol{var(\hat{\mu}_p)}} \text{, (6)}$$

the dispersion indicates the degree of inflation/overdispersion (values below 1) or deflation/underdispersion (values above 1). $b_{w,p}$ deviations of up to 5% of a unit are considered good estimates, while deviations of up to 15% are considered acceptable (Tsuruta et al., 2011). Spearman's correlation coefficient was obtained after classifying the animals according to their expected genomic breeding values and calculating the correlations between the training dataset and the validation dataset.

2.3 RESULTS AND DISCUSSION

The heritability estimates for horn development ranged from 0.44 to 0.84, as shown in Table 4, indicating that selection for this trait is feasible in indicine cattle. The highest heritability was obtained when the horn development trait was considered binary. In contrast, the lowest heritability estimates were obtained by classifying the phenotype into three categories. When only two categories were used in the evaluation, the true phenotype of some individuals was probably masked, resulting in a more biased genetic and phenotypic variance and an overestimation of heritability estimates as observed. In contrast with three or four categories, it is possible to obtain a more robust estimation of genetic variation through an appropriate phenotypic classification of the animals (Stafuzza et al., 2018).

Table 4. Estimated variance components, heritability, bias, dispersion, and prediction accuracy for horn development in Nellore cattle applying different models.

Models	**σ^2a**	**σ^2e**	**h^2 (HPD)**	$\boldsymbol{\mu_{w,p}}$	$\boldsymbol{b_{w,p}}$	$\boldsymbol{\rho_{p,w}}$
1	1.45 (1.26;1.64)	0.96 (0.78;1.19)	0.60 (0.54;0.65)	0.13	1.21	0.76
2	1.48 (1.26;1.64)	0.96 (0.78;1.19)	0.60 (0.55;0.65)	0.09	1.15	0.80
3	1.37 (1.19;1.59)	0.97 (0.79;1.20)	0.58 (0.53;0.64)	0.14	1.21	0.76
4	1.40 (1.21;1.61)	0.97 (0.79;1.21)	0.59 (0.53;0.64)	0.08	1.16	0.80
5	0.64 (0.56;0.73)	0.76 (0.74;0.79)	0.45 (0.45;0.48)	0.08	1.19	0.74
6	0.62 (0.54;0.71)	0.76 (0.74;0.79)	0.45 (0.41;0.48)	0.08	1.20	0.74
7	0.62 (0.54;0.70)	0.76 (0.74;0.78)	0.44 (0.41;0.48)	0.07	1.18	0.75
8	0.61 (0.53;0.69)	0.76 (0.74;0.79)	0.44 (0.41;0.47)	0.08	1.22	0.73
9	5.52 (3.73;7.62)	0.99 (0.96;1.03)	0.84 (0.79;0.88)	0.01	0.73	0.56
10	5.20 (3.42;7.16)	0.99 (0.96;1.03)	0.83 (0.78;0.88)	0.14	1.19	0.69
11	5.33 (3.76;7.33)	0.99 (0.96;1.03)	0.83 (0.79;0.88)	0.14	1.22	0.72
12	5.04 (3.49;6.84)	0.99 (0.96;1.03)	0.83 (0.78;0.87)	0.14	1.20	0.70

σ^2a: additive genetic variance; σ^2e: residual variance; h^2: heritability; HPD: highest posterior density region. $\mu_{w,p}$: bias; $b_{w,p}$: dispersion; $\boldsymbol{\rho_{p,w}}$: accuracy. Model 1: four categories with sex effect in the CG; Model 2: four categories with sex effect in the CG and non-autosomal SNPs; Model 3: four categories without sex effect in the CG; Model

4: four categories without sex effect in the CG but with non-autosomal SNPs; Model 5: three categories with sex effect in the CG; Model 6: three categories with sex effect in the CG and the SNP's of the sex chromosomes; Model 7: three categories with no sex effect in the CG; Model 8: three categories without sex effect in the CG but with non-autosomal SNPs; Model 9: two categories with sex effect in the CG; Model 10: two categories with the effect of sex in the CG and non-autosomal SNPs; Model 11: two categories with no sex effect in the CG; Model 12: two categories without sex effect in the CG but with non-autosomal SNPs. Models 1 to 4 considered four categories: absence of horn, polled born to a parent with a horn, scurs, and presence of horn. Models 5 to 8 considered three categories: polled born to a parent with a horn and scurs, polled, and presence of horn. Models 9 to 12 considered two categories: polled, polled born to a parent with a horn and scurs, and presence of a horn.

Horn development heritability estimates were evaluated in four categories, and three categories were not reported for the Nellore breed. However, when evaluated as a binary trait (presence or absence of horns), Stafuzza et al. (2018) reported an estimate of 0.90, which is like our study. Despite the variation observed in the heritability estimates as a function of phenotype classification, the low and high posterior density intervals were close to each other, indicating the precision of the estimate obtained. The bias, dispersion, and accuracy for horn development in Nellore cattle obtained with the 12 genomic evaluated models are presented in Table 4. The highest prediction accuracies were obtained with models 2 and 4, which considered four horn classification categories with sex effect in the CG and non-autosomal SNPs (Model 2) or without fixed sex effect in the CG but with non-autosomal SNPs (Model 4). The model 9 (0.01) which considered two horn classification categories with sex effect in the CG showed the lowest prediction bias. Furthermore, the models 3, 9, 10 and 12 that considered four categories without sex effect in the CG and two categories with sex effect in the CG, sex effect in the CG and non-autosomal SNPs, and no sex effect in the CG showed the highest (0.14) prediction biases. Models 9 and 8 (two horn classification categories with no sex effect in the CG) showed the smallest and largest dispersions, with values of 0.73 and 1.22, respectively.

The lower bias and dispersion in the models using sex information with the inclusion of non-autosomal SNPs suggested that this information is essential to model the effects of the genomic prediction of the polled trait, especially when the horn development was analyzed considering four categories. Although the genetic basis of the polled trait has not yet been fully elucidated, the influence of sex has been reported (Gehrke et al., 2020; Simon et al., 2022). Johnston et al. (2011) related that the inheritance pattern of horns can be dominant in males and recessive in females. The phenotype distribution for the presence and absence of horns, or scurs also demonstrates that it is a sex-dependent trait, since scurs are significantly more prevalent in males than in females (61% vs. 10%) (Gehrke et al., 2020). These results allow us to infer that there would be a relationship between sexual aspects and horn development and/or that there is a possible relationship between the character evaluated and non-autosomal chromosomes, with inheritance patterns varying between sexes.

Differences in prediction model robustness, including markers present on sex chromosomes, may also be related to their biological role and not necessarily to the increase in the number of markers. Considering the results of bias and dispersion obtained and the influence of sex on the inheritance pattern mechanism of the horn development reported in the literature, the classification of this phenotype into two or three categories masked and biased the genetic background for polled development for genomic prediction purpose of naturally genetical polled animals.

For models considering four and three phenotypic categories for horn development, including the sex effect as a fixed effect within the CG did not improve the accuracy, bias, and dispersion of genomic predictions for horn development. Analyzing the trait with binary expression, the highest prediction accuracy was observed when the effect of sex was not included in the CG and without the SNPs in the sex chromosomes (model 11). These models displayed the highest dispersion and pointed out low robustness of genomic prediction. In addition, models that use less than four categories to classify the horn development phenotype, with no discrimination between polled and homozygous polled displayed lower prediction ability. Previous studies have addressed the hypothesis of an oligogenic inheritance to explain the genetic pattern inheritance of polled and scurs in cattle and the opportunity to propose genomic prediction for horn development using thousands of SNPs spread across the genome. Aldersey et al. (2020) outlined four intergenic DNA sequence variants associated with polled in cattle, while Gehrke et al. (2020) pinpointed four key loci influencing scurs development, including one on BTA5 and three on BTA12.

Genomic predictions need to be highly correlated with the phenotype for successful selection. The results obtained demonstrate that the expression of polled phenotype is dependent on sex, and its effect should be included in the prediction model, mainly using non-autosomal SNPs. Furthermore, these results allow us to infer that there are sex-specific influences on horn development that are not free to evolve independently. Thus, there may be differences between the sex chromosomes that harbor different genetic variations for the polled trait in males and females.

Including non-autosomal SNPs in the analyses for the models considering four phenotypic categories leads to an improvement in prediction accuracy in 5.26%, bias, and dispersion reduction in 37% and 4.55%, respectively, compared with models that only considered autosomal SNPs. When the information of non-autosomal SNPs is included in the genomic prediction, including sex effect as a fixed effect within contemporary groups is irrelevant for polled traits evaluated with four categories. On the other hand, when grouping polled born to sires with horns and scurs, using three categories, the information on non-autosomal SNPs did not improve the prediction ability, elucidating a possible interaction effect between the influence of non-autosomal SNPs with the phenotype classification of horn development. This outcome underscores the necessity of adapting the SNPs contained within the 1,133 SNPs originating from the X sex chromosome (non-autosomal SNPs) to avoid masking the genetic differences when the phenotype is classified into four categories.

Sex chromosomes are commonly excluded from genetic analyses due to the compensation mechanism of X-linked gene dosage in females and because males are hemizygous for the X chromosome (Couldrey et al., 2017). However, the X chromosome is one of the largest chromosomes in the genome, harboring many genes that can affect different phenotypes (Berry et al., 2017), and part of the mechanism involved in embryonic development comes from how this chromosome is established in the population (Ferreira & Franco, 2011) and how the sex chromosomes act in the production of gametes (Arnold et al., 2016). With the exclusion of sex chromosomes from genomic analyses, the direct and epistatic effects of the genes present in them are not accounted for, which may overestimate the effects of autosomal markers for horn development. Stafuzza et al. (2018) and Utsunomiya et al. (2019) identified genomic regions associated with the polled variant in *Bos indicus* animals using genome sequencing. Nonetheless, the existing literature lacks studies that combine genome-wide association (GWAS) methodology with the examination of sex chromosomes in the Nellore breed, highlighting a significant research gap that should be addressed in future investigations.

Prediction ability and response to selection depended on how the animals are classified according to the phenotype, being higher for the phenotype evaluated in four phenotypic categories. Although the models with two and three phenotypic categories have a lower prediction bias, the accuracy was also lower. Thus, it is expected that the production of polled animals through selection would be more effective by classifying the phenotype into four categories. It is necessary to discriminate the polled (homozygous) phenotype of the polled offspring of a sire with horn, scurs, and presence of horn instead of evaluating this phenotype as presence and absence of horn. It is important to emphasize that although it seems a simple trait, classifying between horns, scurs, and polled is still a challenge (Duijvesteijn et al., 2018), due to the difficult differentiation of the postnatal development of this phenotype at an early age. Therefore, a well-defined standardized guideline for the collection of this phenotype is necessary to improve the genetic prediction of horn development, as shown in Figure 1. The challenges in establishing the inheritance pattern of scurs are due to issues with phenotyping, inconsistent timing of scur development, the influence of sex, interaction with POLLED loci through epistasis, and genetic diversity within breeds. (Asai et al. 2004; Tetens et al. 2015; Grobler et al. 2018).

Animal welfare, sustainability, and profitability have driven the progress of beef cattle production systems, especially in efforts to minimize or eliminate commercially undesirable traits such as the presence of horns (Randhawa et al., 2020). The results obtained in the study pointed out that genetic dehorning can be adopted on a large scale as a low-cost and non-invasive approach to produce hornless animals using genomic prediction and mating strategies. In addition, the results demonstrated that including non-autosomal SNPs improved the prediction accuracy for horn development using four phenotypic categories to classify the horn development. Thus, although the genotype-phenotype relationships of horn development are complex, the genomic prediction for horn development allowed reliable identification of animals that would produce polled progenies.

2.4 CONCLUSION

The selection using genomic information for the polled trait is feasible, and it is an alternative to obtain polled Nellore animals and consequently avoid accidents related to herd management, reduce economic losses caused by damage to the leather and injuries to muscle tissue, and improve animal welfare. The binary coding of horn development is an unsuitable oversimplification of polled phenotype, and the genetic background of horn development is more complex than previously proposed.

The most adequate prediction model to evaluate the horn development in Nellore cattle was considering four phenotypic categories and including non-autosomal SNP in the analyses for genomic prediction purposes of naturally genetically polled animals. Genetic dehorning can be adopted on a large scale as a low-cost and non-invasive approach to increase the frequency of hornless animals using genomic information and mating strategies.

2.5 ACKNOWLEDGEMENTS

The authors thank to National Association of Breeders and Researchers (ANCP) for providing data set and genealogical data. The Regalito Farm for providing the photos of the animals used in the article. Grant 2022/12134-0, São Paulo Research Foundation (FAPESP).

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CHAPTER 3 - GENOMIC EVALUATION IN NELLORE CATTLE FOR REPRODUCTIVE TRAITS: MULTIPLE WAYS TO ACCOUNT FOR MISSING PEDIGREES

Larissa Temp[1]*, Gabriel Gubiani[1], Ludmilla Brunes[2], Claudio Magnabosco[2], Fernando Bussiman[3], Jorge Hidalgo[3], Daniela Lourenco[3], Fernando Baldi[1]

[1]Departamento de Zootecnia, Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brasil
[2]Centro de Desempenho Animal, Embrapa Cerrados, Planaltina, DF, Brasil
[3]Department of Animal and Dairy Science, University of Georgia, 30602 Athens, GA, USA

*Correspondence: larissabordintemp@gmail.com

Abstract: Missing pedigree is a common problem in most populations. Animals with unknown ancestors are usually treated as founders; however, this can underestimate inbreeding, not properly account for different base populations, and bias breeding values. We aimed to assess the use of unknown parent groups (UPG) or metafounders (MF) to model missing pedigrees in a beef cattle population. Phenotypic and genotypic data from the Nellore improvement program of the Brazilian Breeders and Researchers Association were used. The pedigree contained 3.8 M animals born between 1970 and 2022, of which 51,752 were genotyped. Records for scrotal circumference at 365 days of age (SC365, N = 239,806), age at first calving (AFC, N = 560,785) and accumulated cow productivity (ACP, N = 269,330) were used. Four models were implemented: single-step GBLUP without explicitly dealing with missing pedigree (G0), with MF (G1), with UPG (G2), and with G accounting for group-specific allele frequencies (G3). UPG and MF were assigned based on commercial and registered herds (S1), uncertain paternity (S2), and patriarchs (S3). The accuracy and bias of predictions were assessed using the linear regression (LR) method. Single-trait animal models were used for SC365 and AFC, and multi-trait for ACP. Heritability estimates ranged from 0.07 to 0.40. Compared to G0, accuracy was slightly higher in $G2_{S2}$ and $G2_{S3}$ (0.70 vs. 0.71) for SC365, $G2_{S3}$ (0.49 vs. 0.51) for AFC, $G1_{S2}$ for ACP (0.67 vs. 0.71). Bias was small in all the scenarios (≤ 0.06 SD), except of ACP that presented a great bias including MF. Overall, G1 and G2 had similar accuracy, possibly because of the limited number of genotyped animals linked to MF. Centering the genomic relationship matrix by patriarchs’ allelic frequencies resulted in similar accuracy and bias to the MF models. Replicating the study with a larger database containing more genotyped animals connected to MF could help improve the MF estimates, and thus, prediction accuracy and bias control.

Keywords: *Bos indicus*, accuracy, metafounders, unknow parent groups, ssGBLUP

3.1 INTRODUCTION

Missing pedigrees are common in livestock populations; animals whose parents are missing in the pedigree are assumed unrelated and non-inbred founders (with an expected breeding value of zero) of the population regardless of their generation in the pedigree. However, animals with unknown parents come from different generations and refer to different base populations (Tsuruta et al., 2011; Kluska et al., 2018). Assuming animals with missing pedigrees are founders is a wrong assumption leading to underestimation of inbreeding and additive relationships, incompatibility between pedigree and genomic relationships in single-step GBLUP models, and ultimately, biased predictions.

For pedigree-based models, Quaas (1988) proposed to replace unknown parents for group effects (also known as unknown parent groups; UPG) able to account for the change in the genetic trend that is not accounted for by incomplete pedigree relationships. In practice, UPG can be defined based on birth year, sex, and breed composition and proved to redue bias of pedigree-based breeding values (Legarra et al., 2007). The single-step GBLUP method (Legarra et al., 2009; Aguilar et al., 2010) is the most used method for breeding values prediction because of its flexibility to incorporate genotyped and non-genotyped animals in a combined relationship matrix ($\mathbf{H}$). The UPG methodology may not eliminate the bias in breeding values derived from single-step GBLUP models due to differences between the pedigree and genomic relationships (Masuda et al., 2022). Furthermore, the UPG are assumed to be unrelated as this theory was derived under pedigree-based relationships (Legarra et al., 2015).

The UPG have been incorporated into single-step GBLUP through a modification of $\mathbf{H}^{-1}$ and in some cases have decreased the bias and inflation of GEBV

(Tsutura et al., 2011; Masuda et al., 2021). However, in some situations, incompatibility between the genomic ($\mathbf{G}$) and pedigree ($\mathbf{A}$) relationship matrices can result in biased predictions (Misztal and Legarra, 2017). Because not all animals of the pedigree have genotypes, and to solve the incompatibility, the matrices need to refer to the same base population. Legarra et al. (2015) proposed the metafounders (MF) theory that generalizes and extends the concept of UPG. The main idea is to modify $\mathbf{A}$ to ensure compatibility with $\mathbf{G}$. Metafounders are fictitious individuals treated as both the father and mother of the base population, having within and across relationships derived from genomic information, facilitating the handling of common ancestry among individuals who would otherwise appear unrelated based on pedigree information.

A fundamental assumption of the MF approach is the construction of $\mathbf{G}$ with allele frequencies equal to 0.5, as stipulated by Christensen (2012), and their utility has been demonstrated across various datasets, including those in livestock (Koivula et al., 2021), sheep (Granado-Tajada et al., 2020), pig breeding (Xiang et al., 2017), and simulated data in dairy cattle (Garcia-Baccino et al., 2017).

Another strategy to enhance the compatibility between matrices $\mathbf{G}$ and $\mathbf{A}$ was devised by Lourenco et al. (2016); in this approach, breed-specific (or group-specific) allele frequencies are used to construct $\mathbf{G}$. This matrix is centered to fix the mean values of the allele effects to zero, and it is adjusted to the specific allele frequencies of each founder breed, providing greater consistency between the coefficients of relationship of genotyped and non-genotyped individuals (Makgahlela et al., 2013).

Using tools capable of increasing accuracy and reducing the bias of genomic evaluations in young animals is of utmost importance to achieve the desired response to selection, as well as being effective strategies for reducing genotyping and phenotyping costs. Studies with MF, UPG and group-specific **G** in Nellore beef cattle for traits related to reproduction, longevity, and production are still scarce in the literature. Our aim in this study was to evaluate the effect of using one or multiple metafounders and unknown parent groups in predicting the estimated genomic breeding values using single-step GBLUP and verify the impact of building **G** with patriarch-specific allele frequencies on genomic predictions for scrotal circumference at 365 days of age (SC365), age at first calving (AFC), and accumulated cow productivity (ACP) in Nellore breed.

3.2 MATERIAL AND METHODS

3.2.1 Data

Phenotypic and genotypic data from the Nellore improvement program of the Brazilian Breeders and Researchers Association (ANCP) were used. ANCP provided us with records for scrotal circumference (SC365), age at first calving (AFC) and accumulated cow productivity (ACP). Because data were obtained from existing databases, the approval of the Animal Care and Use Committee was not needed for this study. SC365 was measured in centimeters with a measuring tape, from 9 to 18 months of age in intervals of 3 months, followed by linear adjustment for 365 days. AFC was simply the age in months at the cows' first calving. ACP represents the average calf weaning weight per year per cow, calculated as in Lôbo et al. (2000): $\mathbf{ACP} = \frac{\mathbf{WW\ x\ n_c\ x\ Ca}}{\mathbf{ALC - Ci}}$ (7), where $\mathbf{WW}$ is the mean calf weaning weight (kg), $\mathbf{n_c}$ the total number of calves producted, $\mathbf{Ca}$ is the constant equal to 365 days to express fertility on an annual basis, $\mathbf{ALC}$ is the age of cow at last calving (days) and $\mathbf{Ci}$ is the constant equal to 450 days, reflecting the expectation that first calving will occur at 30 months of age.

The pedigree contained information on 3,835,379 animals born between 1970 and 2022. For all traits, contemporary groups (CG) were created by concatenating farm, birth year, birth season and weaning management group classes. The CG with less than five records were removed from the dataset with the aim of increasing variability within groups. Additionally, any phenotypic records (within CG) deviating from CG mean ± 3 standard deviations were also removed. A total of 51,752 animals were genotyped with a panel containing 65,436 single-nucleotide polymorphisms (SNP) (CLARIFIDE® Nelore 4.0). Genotype quality control excluded SNPs monomorphic, minor allele frequency (MAF) less than 5%, call rate less than 90% (SNPs & animals) and parent-progeny conflicts (SNPs & animals) performed using preGSF90 software (REF) leaving 35,871 markers for subsequent analyses. These evaluations were standardized by subtracting the mean EBV of animals born in 2015 from all breeding values. A summary of the data descriptive statistics is presented in Table 5.

Table 5. Number of phenotypic records (N), contemporary groups (GC), mean, standard deviation (SD), minimum and maximum for scrotal circumference at 365 days (SC365), age at first calving (AFC) and accumulated cow productivity (ACP)traits in Nellore breed.

Traits	N	GC	Mean (SD)	Min	Max
SC365 (cm)	239,806	7032	21.16 (2.69)	9.5	34.6
AFC (months)	560,785	15247	35.86 (6.09)	21	49
ACP (kg/year)	269,330	17054	148.20 (33.95)	43	466

3.2.2 Statistical model for variance components estimation and breeding values prediction

A linear model was used to analyze SC365, AFC, and ACP, considering fixed effects of CG and random additive animal effects. Under matrix notation this model can be written as:

$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$, (8)

where: $\mathbf{y}$ is the vector of phenotypic observations (SC365, AFC, or ACP); $\boldsymbol{\beta}$ is the vector of fixed effects (CG); $\mathbf{u}$ is the vector of random additive animal effects; $\mathbf{e}$ is the vector of random residuals; $\mathbf{X}$ is the incidence matrix relating the effects in $\boldsymbol{\beta}$ to observations in $\mathbf{y}$; and $\mathbf{Z}$ is the incidence matrix relating the levels in $\mathbf{u}$ to observations in $\mathbf{y}$.

Under multivariate normality assumption, the (co)variance matrix for the random effects is given by:

$Var\begin{bmatrix}\mathbf{u}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\sigma_{\mathrm{u}}^2 & \mathbf{0}\\ \mathbf{0} & \mathbf{I}\sigma_{\mathrm{e}}^2\end{bmatrix}$, (9)

where: $\mathbf{A}$ is the pedigree-based relationship matrix; $\mathbf{I}$ is an identity matrix of proper order; σ_{u}^2 is the additive genetic variance for the trait being analyzed; and σ_{e}^2 is the residual variance. σ_{u}^2 and σ_{e}^2 for SC365, AFC, and ACP were obtained through average information restricted maximum likelihood using the BLUPF90+ sotware (Lourenco et al., 2022) without genomic information. Single trait analyses were conducted for SC365 and AFC, while a multi-trait analysis was performed for ACP using SC365 and AFC as a base. The objective was to enhance the prediction of ACP by leveraging the larger amount of information available for the other two traits. The multi-trait analysis aimed to benefit ACP, a trait selectively measured in females and correlated with SC365 and AFC, which are measured earlier in the animals and have more data.

3.2.3 Genomic predictions

For all traits, four predictions were obtained: 1) regular ssGBLUP (G0); 2) ssGBLUP with MF (G1); 3) ssGBLUP with UPG as fixed effects (G2); and 4) regular ssGBLUP but **G** was constructed using patriarch specific allele frequencies (G3).

Without UPG or MF (G0), the covariance structure among animals (**H**) in ssGBLUP was as in Aguilar et al. (2010) and the **G** matrix was constructed according to VanRaden (2008).

With MF (G1), allele frequencies were assumed to be 0.5 to center **G**, and **A** was modified with a matrix $\mathbf{\Gamma}$ whose entries are relationship coefficients among MF. $\mathbf{H}^{-1}$ was as follows:

$$(\mathbf{H}^{\mathbf{\Gamma}})^{-1} = (\mathbf{A}^{\mathbf{\Gamma}})^{-1} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G}^{-1} - (\mathbf{A}_{22}^{\mathbf{\Gamma}})^{-1} \end{bmatrix}, (10)$$

where: $\mathbf{A}^{\mathbf{\Gamma}}$ and $\mathbf{A}_{22}^{\mathbf{\Gamma}}$ are equivalent to $\mathbf{A}^{-1}$ and $\mathbf{A}_{22}^{-1}$ modified by $\mathbf{\Gamma}$, a positive-definite matrix given by:

$$\mathbf{\Gamma} = \begin{bmatrix} \gamma_1 & \gamma_{1,2} & \cdots & \gamma_{1,n} \\ \gamma_{2,1} & \gamma_2 & \cdots & \gamma_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ \gamma_{n,1} & \gamma_{n,2} & \cdots & \gamma_n \end{bmatrix}, (11)$$

where: γ_i is the relationship within the ith MF and $\gamma_{i,j}$ is the relationship between the ith and jth MF. $\mathbf{\Gamma}$ was estimated using pedigree and genomic information by generalized least-squares according to Garcia-Baccino et al. (2017).

When UPG were considered (G2), the altered Quaas-Pollak (QP) transformation (Tsuruta et al., 2019; Masuda et al., 2022) was used to consider groups in **A** and in **G**. In this case, $\mathbf{H}^{-1}$ is given by:

$$\mathbf{H}^{*} = \mathbf{A}^{*} + \begin{bmatrix} \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} & -(-\mathbf{A}_{22}^{-1})\mathbf{Q_2} \\ \mathbf{0} & -\mathbf{Q'_2}(-\mathbf{A}_{22}^{-1}) & \mathbf{Q'}(-\mathbf{A}_{22}^{-1})\mathbf{Q_2} \end{bmatrix}, (4)$$

where: $\mathbf{A}^{*}$ is the inverse of the additive relationship matrix based on QP transformation; $\mathbf{G}^{-1}$ is the inverse of the genomic relationship matrix, constructed based on method I of VanRaden (2008); $\mathbf{A}_{\mathbf{22}}^{-\mathbf{1}}$ is the inverse of the pedigree relationship matrix among genotyped animals; and $\mathbf{Q}$ is a matrix relating genotyped animals with

UPG. MF and UPG were assigned based on missing pedigrees from commercial and registered herds (S1), if the animals had one or both parents' unknown (S2), and patriarchs (animals born in Brazil, sons of imported animals) (S3).

Ten patriarchs were chosen based on ANCP producers' demand, who are more likely to use bulls descended from the first animals brought from India to Brazil, and who have a greater influence on genetic evaluations, e.g., Kavardi, Taj Mahal, Golias, and Godhavari lineages (Oliveira, Magnabosco and Borges, 2002). A summary of the evaluation scenarios is presented in Table 6.

When **G** accounted for patriarch-specific allelic frequencies (G3), it was constructed according to Lourenco et al. (2016) as follows:

$\mathbf{G} = \mathbf{ZZ}', \text{with } \mathbf{Z} = (\mathbf{M} - \mathbf{P}_{\mathrm{k}}) / \left[2\sum_{\mathrm{j}=1}^{\mathrm{m}} \mathrm{p}_{\mathrm{jk}}(1 - \mathrm{p}_{\mathrm{jk}})\right]$, (12)

where: $\mathbf{M}$ is the gene content matrix; $\mathbf{P}_{\mathrm{k}}$ contains twice the allele frequency of the k$^{\mathrm{th}}$ MF; and p_{jk} is the allele frequency at the j$^{\mathrm{th}}$ locus for the k$^{\mathrm{th}}$ MF. It is worth noting that when allele frequencies are considered homogeneous across MF, this results in the **G** as in VanRaden (2008).

Table 6. Scenarios tested for longevity and reproductive traits in the evaluation of Nellore breed using UPG and MF.

	Definition
G0	G default with the current allele frequencies, constructed as VanRaden (2008)
G3	G matrix was centered and scaled by MF patriarchs specific allele frequencies as in Lourenco et al. (2016)
S1	Commercial and registered herd
S2	Paternity: both parents unknown and just one of the parents is unknown
S3	Patriarchs: Ludy de Garça, 1646 da M.N., Riacho da OB, Rambo da Mundo Novo, Zefec Abdala, Rapilho da SI, Fajardo da GB, Nurmahal Col, Paysandu de Nav, and Voltaire TE J.

G0: without MF and UPG

3.2.4 Evaluation of model performance

Scenarios were compared using the linear regression (LR) (Legarra and

Reverter 2018) method (Table 7).

Table 7. Number of records in the whole and partial datasets and validation animals used in LR method for the different scenarios for longevity and reproductive traits in Nellore breed.

	SC365	**AFC**	**ACP**
Whole	239.806	560.785	269.330
Partial	222.318	546.595	251.266
Validation[1]	17.488	14.190	18.064
Years of validation	2020-2021	2019-2020	2017-2019

[1]: young animals with genotypes and phenotypes.

The whole dataset (represented by subscript w) included all sources of information for all animals (records from 1970 to 2021). Two partial datasets were created removing either the last two years of data (only for SC365 and AFC), or the last three years of data (only for ACP). This was done to ensure enough number of focal animals for validation. Focal animals were young, genotyped individuals without their own (or progeny) records in the partial dataset. The whole data set can be interpreted as the posterior confirmation of the validity of the selection decisions. In contrast, the partial data set represents the evaluation conducted during selection decisions (Macedo et al., 2020).

The validation statistics were accuracy, bias, dispersion, and correlation, computed as follows:

$$\mathrm{accuracy} = \widehat{\mathrm{acc}} = \sqrt{\frac{cov(\hat{\mathbf{u}}_{\mathrm{w}},\hat{\mathbf{u}}_{\mathrm{p}})}{(1-\bar{\mathrm{F}})\hat{\sigma}^2_{\mathrm{u}}}}, \quad (13)$$

$$\mathrm{bias} = \hat{\Delta}_{wp} = \frac{\bar{\hat{\mathbf{u}}}_{\mathrm{p}}-\bar{\hat{\mathbf{u}}}_{\mathrm{w}}}{\hat{\sigma}_{\mathrm{u}}}, \quad (14)$$

$$\mathrm{dispersion} = \hat{\mathrm{b}}_{\mathrm{w,p}} = \frac{cov(\hat{\mathbf{u}}_{\mathrm{w}},\hat{\mathbf{u}}_{\mathrm{p}})}{var(\hat{\mathbf{u}}_{\mathrm{p}})}, \quad (15)$$

$$\mathrm{correlation} = \mathrm{corr} = \frac{cov(\hat{\mathbf{u}}_{\mathrm{w}},\hat{\mathbf{u}}_{\mathrm{p}})}{\sqrt{var(\hat{\mathbf{u}}_{\mathrm{w}})var(\hat{\mathbf{u}}_{\mathrm{p}})}}, \quad (16)$$

where: $cov(\cdot)$ is the sample covariance; $\hat{\mathbf{u}}_{\mathrm{w}}$ is the vector of GEBV from the whole dataset; $\hat{\mathbf{u}}_{\mathrm{p}}$ is the vector of GEBV from the partial dataset; $\bar{\mathrm{F}}$ is the average pedigree-inbreeding coefficient for focal animals; $\hat{\sigma}^2_{\mathrm{u}}$ is the estimated additive genetic variance;

$\bar{\hat{\mathbf{u}}}_{\mathrm{p}}$ is the average of $\hat{\mathbf{u}}_{\mathrm{p}}$ (likewise for $\bar{\hat{\mathbf{u}}}_{\mathrm{w}}$); and $var(\cdot)$ is the sample variance.

3.3 RESULTS AND DISCUSSION

3.3.1 Variance components and heritability

Variance components and genetic parameters are presented in Table 8. Direct heritability for SC365 (0.40) was similar to those reported by Kluska et al. (2018) (0.48), Silva Neto et al. (2020) (0.33), Carvalho Filho et al. (2020) (0.47) and Negreiros et al. (2022) (0.36), suggesting that it is possible to achieve genetic progress when selecting for SC365. Additionally, the heritability estimates for AFC (0.07) and ACP (0.12) indicate a larger environmental effect on these traits supporting prior research on Nellore cattle (Kluska et al., 2018; Silva Neto et al., 2020; Negreiros et al. 2024). Moreover, the heritability estimates were consistent with those found in other studies

Table 8. Variance components estimates and heritabilities for reproductive and longevity traits in Nellore breed.

Traits	$\boldsymbol{\sigma_d^2}$	$\boldsymbol{\sigma_e^2}$	$\boldsymbol{h_d^2}$ ±SD
SC365	1.24	1.87	0.40 ± 0.08
AFC	1.41	17.81	0.07 ± 0.03
ACP	72.68	551.74	0.12 ± 0.05

$\boldsymbol{\sigma_d^2}$: additive variance in liability scale; $\boldsymbol{\sigma_e^2}$**:** residual variance in liability scale, ACP = Accumulated Cow Productivity; AFC = Age at First Calving; SC365 = Scrotal Circumference at 365 days of age.

The relatively low heritability observed, particularly in reproductive traits, may be attributed to factors such as population structure, the statistical model employed for variance component estimation, and the gradual reduction of genetic variance in populations subjected to selection (Hidalgo et al., 2020).

3.3.2 Relationship within and across MF ($\boldsymbol{\Gamma}$)

Within-group relationships (diagonal of $\boldsymbol{\Gamma}$) were smaller than one, while between-group relationships (off-diagonal of $\boldsymbol{\Gamma}$) were different from zero across all three scenarios (S1, S2, and S3). In $\boldsymbol{\Gamma}_1$, relationships within the group ranged from 0.71 to 0.72, whereas between the two groups it was 0.70. $\boldsymbol{\Gamma}_2$ represents the relationships for S2, with within-group values ranging from 0.70 to 0.73 and a between-group value of 0.70. Furthermore, $\boldsymbol{\Gamma}_3$ depicts the relationships for S3, with within-group values ranging from 0.71 to 0.77 and between-group values ranging from 0.68 to 0.72:

$$\boldsymbol{\Gamma}_1 = \begin{bmatrix} 0.71 & 0.70 \\ \text{symm.} & 0.72 \end{bmatrix}, \text{(17)}$$

$$\boldsymbol{\Gamma}_2 = \begin{bmatrix} 0.73 & 0.70 \\ \text{symm.} & 0.70 \end{bmatrix}, \text{(18)}$$

$$\boldsymbol{\Gamma}_3 = \begin{bmatrix} 0.77 & 0.69 & 0.71 & 0.70 & 0.72 & 0.72 & 0.71 & 0.72 & 0.71 & 0.70 \\ & 0.71 & 0.69 & 0.69 & 0.70 & 0.69 & 0.69 & 0.69 & 0.69 & 0.68 \\ & & 0.72 & 0.70 & 0.71 & 0.71 & 0.71 & 0.71 & 0.70 & 0.69 \\ & & & 0.71 & 0.70 & 0.70 & 0.69 & 0.70 & 0.70 & 0.69 \\ & & & & 0.73 & 0.71 & 0.71 & 0.71 & 0.71 & 0.69 \\ & & & & & 0.74 & 0.71 & 0.71 & 0.70 & 0.70 \\ & & & & & & 0.75 & 0.71 & 0.70 & 0.70 \\ & & & & & & & 0.74 & 0.71 & 0.69 \\ & & & & & & & & 0.72 & 0.69 \\ \text{symm.} & & & & & & & & & 0.72 \end{bmatrix}, \text{(19)}$$

The values observed on the diagonal are considered high because the individuals within each MF share many alleles identical by descent (IBD). Additionally, the off-diagonal values indicate that the MFs also share a significant proportion of their genetic base, as evidenced by their highly similar allele frequencies. This pattern can be attributed to the origin of the first animals imported from India, which gave rise to the patriarchs used as MFs. These animals likely descended from a limited number of founders, resulting in a narrow genetic base.

In commercial and registered herds, there is commonly an intense genetic flow between these groups due to the shared use of sires and reference genetics. This interconnection reduces differences in allele frequencies among MFs, thereby increasing their genetic correlation, as indicated by the off-diagonal values. Finally, regarding individuals with unknown paternity, even though they lack parental information in the pedigree, they belong to the same general population. As a result, they share a significant proportion of allele frequencies with other MFs, which explains the high correlation values observed off the diagonal.

Legarra et al. (2015) discussed the significance of relationships within MF, where a value less than one suggests negative inbreeding, indicating divergence from the base population. Conversely, values exceeding one denote inbreeding within the base population, reflecting a higher degree of relatedness. Positive relationships observed across MF pairs imply overlapping ancestor populations. On the other hand, negative values signify population divergence, highlighting distinct genetic line. Moreover, the base populations are unrelated when the relationship between MF pairs equals zero. In genetic evaluations, a gamma value different from zero between MF is crucial for incorporating MF into the system. This is because a gamma equal to zero implies that MF is equal to UPG, as highlighted by Bradford et al. (2019) and Kluska et al. (2021).

We identified substantial relationships in the gamma matrix within and across MF, indicating a robust association significantly different from zero. Bradford et al. (2019) illustrated relationships within MF ranging from 0.54 to 0.71 in a simulated dairy cattle population. Similarly, Legarra et al. (2015) observed relationships of 0.55 and 0.77 in Holstein and Jersey breeds, respectively. Exploring the relatedness of parental lines, van Grevenhof et al. (2018) uncovered correlations between MF of 0.17 and 0.74. Kluska et al. (2021) conducted a study with the Montana composite cattle, revealing relationships within four and ten MF ranging from 0.15 to 0.38 and 0.15 to 0.65, respectively. Furthermore, relationships across MF ranged from 0.09 to 0.18 and -0.11 to 0.23. Negative values in a composite population are expected once they indicate genetic divergence from the base. In our study, regardless of the MF definition, strong association between the different populations were found. This could be interpreted as commercial and registered populations being genetically one single population, or that regarding the use of different genetic lines (i.e. patriarchs) there is no different genetic base in the population. Under this reasoning, little effect of MF in the validation statistics could be expected, once treating all population as having a single base population seems to be adequate.

3.3.3 Validation

Accuracy and bias of genomic predictions for the validation animals in each scenario and trait are shown in Figure 4 and Table 9. An effective model must accurately predict breeding values or future phenotypes to generate genetic gains. The G0 model resulted in accuracy of 0.70, 0.49, and 0.67 for SC365, AFC, and ACP, respectively. Among these, AFC showed a decrease in the model G1$_{S1}$ (0.45) incorporating MF compared to G0. The accuracy for SC365 remained consistent at 0.70 across all three MF models.

Table 9. Accuracy and bias of non-genotyped and genotyped animals comparing the ssGBLUP predictions between models and traits.

Models	**SC365**		**AFC**		**ACP**	
	$\widehat{\mathbf{acc}}$	$\hat{\Delta}_{wp}$	$\widehat{\mathbf{acc}}$	$\hat{\Delta}_{wp}$	$\widehat{\mathbf{acc}}$	$\hat{\Delta}_{wp}$
G0	0.70	0.06	0.49	-0.05	0.67	0.06
G1S1	0.69	0.03	0.45	-0.03	0.67	0.19
G1S2	0.69	0.03	0.50	-0.03	0.70	0.20
G1S3	0.69	0.03	0.49	-0.03	0.67	0.16
G2S1	0.70	0.04	0.48	-0.04	0.64	-0.02
G2S2	0.70	0.04	0.50	-0.04	0.62	-0.02
G2S3	0.70	0.04	0.51	-0.04	0.62	-0.02
G3	0.70	-0.20	0.49	0.04	0.68	0.40

Abbreviations: $\widehat{acc}$: accuracy; $\hat{\Delta}_{wp}$: bias; ACP = Accumulated Cow Productivity; AFC = Age at First Calving; SC365 = Scrotal Circumference at 365 days of age; G0: without MF and UPG; G1: MF; G2: UPG; G3: G accounted for patriarchs MF specific allele frequency; S1: commercial and registered herd; S2: Paternity; S3: Patriarchs

The model G1$_{S2}$ led to improved accuracy for both AFC (0.50) and ACP (0.70). The accuracy for AFC and ACP in the model G1$_{S3}$, as well as ACP in the model G1$_{S1}$, remained unchanged with the inclusion of MF. When considering the use of UPG relative to the model G0, SC365 remained stable and AFC decreased to 0.48 in the model G2$_{S1}$, while ACP decreased to 0.62 in models G2$_{S2}$ and G2$_{S3}$. In the G3 model, where patriarchs-specific allelic frequencies were accounted for **G**, there was an increase in accuracy for ACP (0.68), while the accuracy for SC365 and AFC remained unchanged.

Several studies have highlighted the advantages of using MF in genomic evaluations. Bradford et al. (2019) found a significant increase ($p<0.01$) in accuracy when using MF compared to the UPG in a dairy cattle population. Similar results were observed by Kudinov et al. (2022) in red dairy cattle, and by Macedo et al. (2020) in sheep. Londoño-Gil et al. (2024), employing a multibreed approach (Nellore, Brahman, Guzerat, and Tabapua), and Kluska et al. (2021) working with Montana composite cattle found considerable increases in accuracy when using MF for weight and SC365. Garcia-Baccino et al. (2017) and van Grevenhof et al. (2018) tested models with and without MF and found a small or no difference in accuracy.

The contribution of **G** to the breeding value estimates is reduced when the number of genotyped animals is limited, and the increase in accuracy with genomic information tends to be modest (Lourenco et al., 2015). The accuracy of genomic predictions is influenced by the number of genotyped animals connected with MF, quantity of markers, size of training and validation and heritability (Gondro et al., 2013; Cesarani et al., 2021; Melo et al., 2024).

Although the models remain accurate, our results suggest that using MF does not necessarily increase the accuracy of GEBV. Bradford et al. (2019) argued that GEBV accuracy is more closely related to trait heritability than to the inclusion of MF or UPG in ssGBLUP. They observed that traits with higher heritability could exhibit improved prediction accuracy. For example, using simulated data for a trait with a heritability of 0.30, they reported an increase in accuracy from 0.36 to 0.77 when applying ssGBLUP with MF compared to models that did not account for missing pedigree information using BLUP traditional. However, our findings did not align with this trend. Interestingly, two low-heritability traits, AFC and ACP, showed considerable improvements. Bradford et al. (2019) also found that, for a simulated trait with a heritability of 0.10 (low), the ssGBLUP model with MF was among the top-performing models, achieving an accuracy of 0.64 and a bias of -0.01.

Low-heritability traits generally respond less effectively to direct selection due to a lower proportion of additive genetic variance. However, the use of methodologies such as MF and UPG enhances the capture of information on the animals' genetic origins, enabling bias correction in GEBV. Additionally, by introducing MF, a relationship is assumed among founders that share similar genes, which facilitates the capture of additional variability and provides a more robust foundation for traits with challenging genetic prediction, such AFC and ACP. This network of related founders contributes to a more consistent and robust genetic evaluation across generations.

Model G0 resulted in no bias for all studied traits (0.06 for SC365, -0.05 for AFC and 0.06 for PAC). Models using MF for SC365 and AFC or UPG for SC365, AFC and ACP presented bias close to zero, ranging from -0.02 to 0.04. Negative bias indicates an underestimation of GEBV (Legarra and Reverter, 2018; Kluska et al., 2021) and were found for AFC using models with MF and UPG. According to Bradford et al. (2019), animals are more impacted by UPG when recent pedigree information is lacking, as $\mathbf{A}_{22}^{-1}$ reflects only known pedigree relationships, whereas the inverse of **G** accounts for all genetic relationships, even in the absence of complete pedigree data.

Therefore, it was expected that models incorporating MF would exhibit less bias compared to those using UPGs. The highest values of bias were found for ACP, showing an increase from -0.02 to 0.20 between models with UPG and MF. The increase observed with the use of MF may be attributed to an insufficient number of genotyped animals to accurately estimate MF effects, particularly since the number of genotypes and phenotypes varied across traits and with the dataset structure.

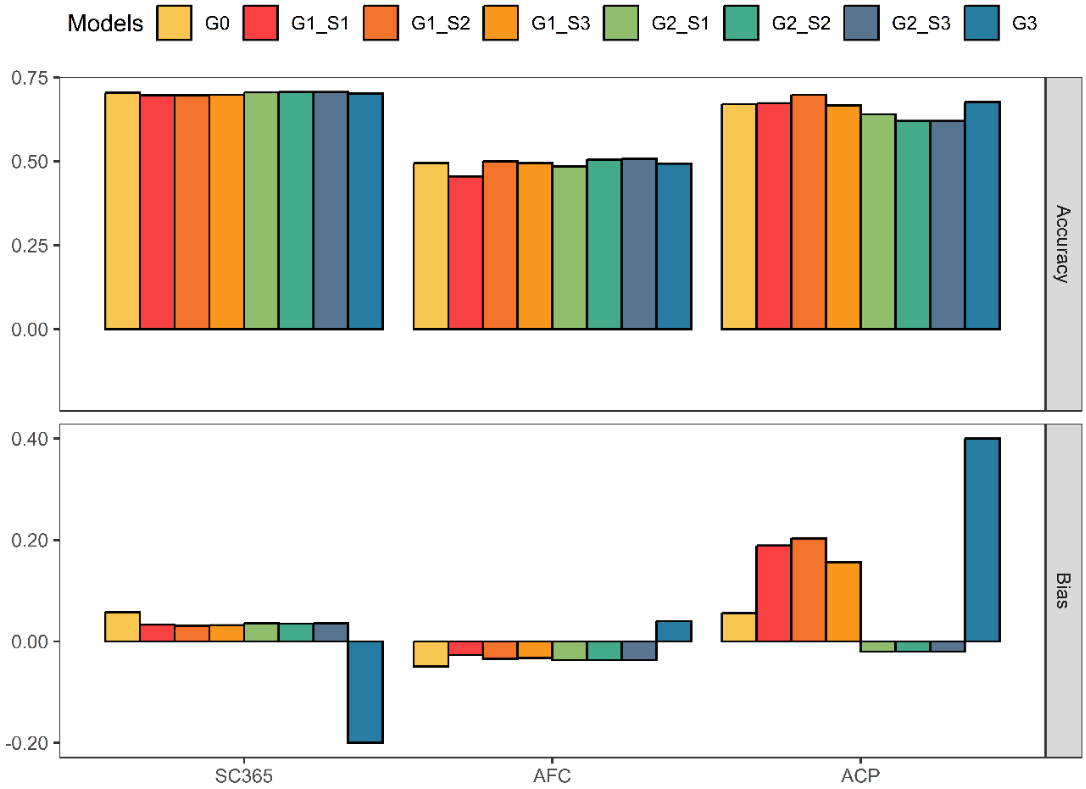

Figure 4. Accuracy and bias for genomic predictions of the validation animals according to the studied trait. Abbreviations: ACP = Accumulated Cow Productivity; AFC = Age at First Calving; SC365 = Scrotal Circumference; G0: without MF and UPG; G1: MF; G2: UPG; G3: G accounted for patriarchs MF specific allele frequency; S1: commercial and registered herd; S2: Paternity; S3: Patriarchs.

MF interconnects the animals through genomic utilization, unlike UPG where solutions are primarily related to the number of phenotypes and individuals in each group (Tsuruta et al., 2014). Another possible explanation for the increased bias in ACP could be selective phenotyping, considering the trait's low heritability and the fact that not all animals have available data, as females must complete the production cycle to be phenotype. This trait is not directly measured but is based on a calculation that includes the calving index, number of calves, and weaning weight during the female's time in the herd. Additionally, it depends on the age at first calving, the interval between calvings, and how long the cows stay in the herd (Lôbo et al., 2000).

Overall, compared to the G0 model, MF helped reduce bias for SC365 and AFC which are less influenced by selective phenotyping, whereas UPG benefited all traits. However, using MF in the multi-trait model increased the bias for ACP. In the G3 model, which incorporates patriarch-specific allele frequencies into the matrix **G**, an increase in bias was observed for both SC365 (-0.20) and ACP (0.40). This increase in bias could be attributed to the effects of allele frequency weighting, which may fluctuate due to genetic drift and selection. When allele frequencies in the base population are accounted for, particularly under the assumption that founders in this population are non-genotyped, but connected through pedigree information, these effects can become more pronounced (Neshat et al., 2023). The same authors also noted that inaccuracies in pedigree information can make it challenging to accurately trace founders and establish the true base allele frequencies.

However, genotypic data can still capture substantial information about these frequencies, providing a more reliable estimate than pedigree data alone, though the absence of precise pedigree records may still introduce some uncertainty. Based on the results obtained and in comparison, with findings from the literature, defining MF based on patriarchs may not improve accuracy and could increase the bias, especially for ACP, as observed in the G3 model where the patriarchs specific allele frequencies were used.

Londoño-Gil et al. (2024) found an increase in bias for weight at 450 days for Brahman and Guzerat, as well as SC365 for all breeds when using MF. The authors also mentioned that if the animals are related solely through $\mathbf{A}_{22}$ rather than **G**, the mismatch between these matrices can introduce bias and result in a loss of accuracy. In contrast to our findings, Bradford et al. (2019), in a simulation study, found an increase in bias in pedigree-based models when UPG were used to fill in pedigree gaps. Kluska et al. (2021), working with the Montana composite beef cattle, found that including UPG in pedigree-based models resulted in higher bias. The authors explained that the complex population structure is the main factor causing bias. In general, lower bias estimates indicate that MF and UPG effectively capture the population's genetic structure. This robustness suggests the model can accurately manage incomplete pedigree information, leading to more accurate genetic evaluations. It is important to highlight that the positive or negative effects observed from the different methods are highly influenced by the data structure, which should be considered when interpreting the results (Himmelbauer et al., 2024). This explains why different studies reached different conclusions about the same method (Bradford et al., 2019). Additionally, the criteria chosen for defining MF and UPG may not have been the most suitable for the studied traits, as the literature generally uses the year of birth, animal sex, country of origin, and breed as the basis for establishing MF and UPG.

Table 10. Correlation and dispersion of non-genotyped and genotyped animals comparing the ssGBLUP predictions between models and traits.

Models	**SC365**		**AFC**		**ACP**	
	corr	$\hat{\mathbf{b}}_{\mathbf{w,p}}$	**corr**	$\hat{\mathbf{b}}_{\mathbf{w,p}}$	**corr**	$\hat{\mathbf{b}}_{\mathbf{w,p}}$
G0	0.81	0.93	0.80	0.70	0.88	0.91
G1S1	0.80	0.94	0.95	0.86	0.87	0.94
G1S2	0.80	0.94	0.96	0.86	0.88	0.94
G1S3	0.81	0.94	0.96	0.86	0.89	0.95
G2S1	0.81	0.94	0.96	0.86	0.85	0.93
G2S2	0.81	0.93	0.96	0.87	0.85	0.94
G2S3	0.81	0.93	0.96	0.87	0.85	0.94
G3	0.81	0.94	0.96	0.87	0.89	0.91

Abbreviations: corr: correlation $\widehat{b}_{w,p}$: dispersion; ACP = Accumulated Cow Productivity; AFC = Age at First Calving; SC365 = Scrotal Circumference at 305 days; G0: without MF and UPG; G1: MF; G2: UPG; G3: G accounted for patriarchs MF specific allele frequency; S1: commercial and registered herd; S2: Paternity; S3: Patriarchs

Correlation between GEBV from different models, as well as dispersion, are shown in Figure 5 and Table 10. The dispersion (i.e. slope), which can be observed, particularly when selection candidates span across various generations or possess varying degrees of information (Piccoli et al., 2018; Kluska et al., 2021). Correlations ranged from 0.81 to 0.96, whereas dispersion had a smaller amplitude, between 0.86 and 0.95. Dispersion close to 1 indicates that there is a little over and under dispersion in the analyzed data set.

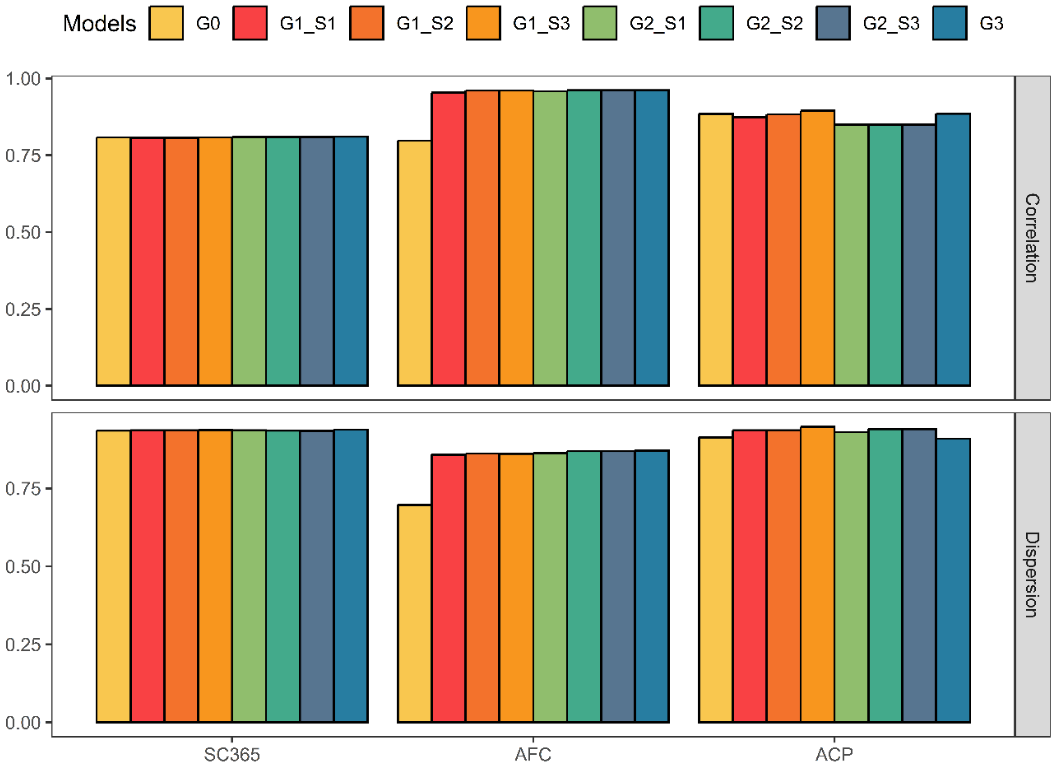

Figure 5. Correlation and dispersion for genomic predictions of the validation animals according to the studied trait. Abbreviations: ACP = Accumulated Cow Productivity; AFC = Age at First Calving; SC365 = Scrotal Circumference; G0: without MF and UPG; G1: MF; G2: UPG; G3: G accounted for patriarchs MF specific allele frequency; S1: commercial and registered herd; S2: Paternity; S3: Patriarchs.

For the SC365 trait, the models show similar correlations, ranging from 0.80 to 0.81, and dispersion between 0.93 and 0.94. This pattern suggests that the inclusion of MF or UPG does not bring significant changes in accuracy for this trait. This stability may be attributed to the nature of this trait, which appears less dependent on different relationship parameterizations. For AFC, the biggest impact of modeling MF and UPG effects is observed, with correlations ranging from 0.80 (G0) to 0.96 ($G1_{S2}$, $G1_{S3}$, $G2_{S1}$, $G2_{S2}$, $G2_{S3}$, and G3). These results indicate that the inclusion of MF or UPG substantially improves predictive accuracy for AFC, possibly due to the higher genetic and environmental complexity associated with this reproductive trait. The dispersion for AFC also increases in models with MF or UPG, rising from 0.70 in G0 to up to 0.87 in G3, suggesting an improvement in the match between predictions and observed values. For ACP, the correlation is high across all models, with slight variations between 0.85 ($G2_{S1}$, $G2_{S2}$, $G2_{S3}$) and 0.89 ($G1_{S3}$ and G3). However, models with lineage-specific MF ($G1_{S3}$ and G3) show the highest correlations, suggesting that including information on lineage-specific alleles can capture important genetic nuances for ACP. The correlations obtained between GEBV from the whole dataset and GEBV from the partial dataset are essential to understanding the efficiency and robustness of genomic prediction models. High correlations suggest that the prediction model captures genetic variability well and may be effective in predicting traits in the validation population. High correlations indicate that the model is transferable between populations and that the training captured relevant genetic information. Low correlations may suggest that the model is overfitting to the training data and does not generalize well to the validation population. Thus, this parameter is important to indicate whether genomic prediction can effectively select superior and inferior individuals, demonstrating the model's usefulness. Regardless of the approach used, the estimates of correlations between the GEBVs of the complete and partial populations obtained demonstrate that the models can be used for genomic prediction of SC365 and AFC.

Kluska et al. (2021) showed that including genomic information helped reduce inflation for the weaning weight trait in the Montana composite cattle. However, in this study, the overall impact using UPG or MF for dispersion was low. Bradford et al. (2019) noted that dispersion for BLUP models without considering the absence of pedigrees may be greater than in models with genomics. Kluska et al. (2021) found a lower correlation between genomic models than between BLUP models, and the correlation was higher between BLUP models and $ssGBLUP_{MF}$. The authors also commented that if animals with and without phenotypes in the same group are unrelated, group effects are not estimable, and the model is similar to ignoring UPG. Additionally, if genotyped animals are unrelated to non-genotyped animals, $\mathbf{H}^{-1}$ will not contribute to estimating the group solution, and it will be similar to ignoring UPG (Tsuruta et al., 2019). In single-breed evaluations, Londoño et al. (2024) observed minor changes in Pearson correlations between non-genotyped and genotyped animals. They explained that this occurs because alterations in **G** and $\mathbf{A}_{22}$ directly affect these individuals based on the propagation of genomic information to non-genotyped animals in the **H** matrix. The dispersion is relatively stable across models, ranging from 0.91 to 0.95, indicating good consistency in prediction for this trait. Regarding correlations, higher values are associated with greater precision in the model when identifying genetically superior animals. This precision facilitates selecting individuals with superior reproductive performance, promoting accelerated genetic progress, which is crucial for traits of high economic relevance, such as those related to reproduction and longevity.

Improvement in EBVs can be observed through increased prediction accuracy by including additional phenotypic information and molecular marker data. In the present study, the criteria for defining MF and UPG did not improve over the default model, particularly for patriarchs. This aspect interested producers, who wanted to assess whether including patriarchs would benefit genetic evaluations. These results may be attributed to the limited number of animals associated with these patriarchs. With increased phenotypes, genotypes, and pedigree information related to one another, greater accuracy and reduced bias would likely be evident.

3.4 CONCLUSION

MF and UPG provided similar GEBV for all traits, with no apparent increase in prediction accuracy. Dispersion and correlation remained close to one, indicating no inflation or deflation in the GEBV for younger animals, regardless of the methodology used. Centering and scaling **G** by the allelic frequency of the patriarchs yielded similar accuracy and bias compared to MF, except for APC, which showed higher bias, possibly due to the selective phenotyping. Over time, adding more genotypes and phenotypes to the database has the potential to improve estimates using MF, particularly for reproductive, longevity, and productivity traits in Nellore cattle.

ORCIDS and email address
Larissa Bordin Temp: https://orcid.org/0000-0003-1003-7977,
larissabordintemp@gmail.com
Gabriel Gubiani https://orcid.org/0000-0002-3810-7319, gabriel.gubiani@unesp.br
Ludmilla Costa Brunes: https://orcid.org/0000-0001-9012-520X,

ludmillabrunes@gmail.com
Cláudio Ulhôa Magnabosco: https://orcid.org/0000-0002-7274-0134, claudio.magnabosco@embrapa.br
Jorge Hidalgo: https://orcid.org/0000-0002-0783-381X , jh37900@uga.edu
Fernando Bussiman: https://orcid.org/0000-0001-5417-5816 , fob@uga.edu
Daniela Lourenco: https://orcid.org/0000-0003-3140-1002 , danilino@uga.edu
Fernando Baldi: https://orcid.org/0000-0003-4094-2011, fernandobaldiuy@gmail.com

3.5 DATA AVAILABILITY

The data that support the findings of this study are available from the National Association of Breeders and Researchers (ANCP). The data sets generated and/or analyzed during the current study are available through the corresponding author upon reasonable request with the permission of National Association of Breeders and Researchers (ANCP).

3.6 FUNDING

This work was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grants # 2022/12134-0 and # 2023/08817-8)

3.7 CONFLICT OF INTEREST

The authors of this work declare that there are no conflicts of interest that jeopardize the validity of the results obtained.

3.8 ETHICAL APPROVAL

Not applicable**.**

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