SÃO PAULO STATE UNIVERSITY
SCHOOL OF AGRICULTURAL AND VETERINARY SCIENCES
JABOTICABAL CAMPUS

GENOMIC REACTION NORM MODELS AND GENETIC NETWORK ANALYSIS EXPLORING THE EFFECTS OF GENOTYPE × ENVIRONMENT INTERACTION ON FEED EFFICIENCY INDICATOR TRAITS IN NELLORE CATTLE

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POTENTIAL IMPACT OF THIS RESEARCH

First study: This review provides a comprehensive and up-to-date synthesis of the main methodologies used to quantify genotype-by-environment interaction (G×E) in beef and dairy cattle, highlighting their implications for genetic evaluation and selection decisions across diverse production systems. By summarizing evidence from 116 studies and showing that most of them reported genetic correlations below 0.80 across environmental gradients, this work reinforces that ignoring G×E can compromise the accuracy of breeding values and reduce expected genetic gains, particularly for complex traits related to adaptation and robustness. In addition, the review emphasizes the strategic value of integrating genomic and phenotypic information to better characterize environmental sensitivity, identify candidate genes and biological pathways linked to resilience, and support the development of more efficient selection and mating strategies tailored to target environments. Overall, the findings serve as a practical reference for researchers and breeding programs aiming to improve productivity, animal welfare, and sustainability under variable climatic, nutritional, and management conditions, while also outlining key gaps and priorities for future research, including nutrigenomics and the incorporation of resilience-related indicators into routine evaluations.

Second study: As the first study of G×E for feed efficiency in Nellore cattle, this research demonstrates that genetic expression for dry matter intake (DMI) and residual feed intake (RFI) changes when animals are evaluated under contrasting management and nutritional conditions. By applying bi-trait reaction norm models across an environmental gradient derived from average daily gain (ADG), the study demonstrates that genetic parameters and breeding values are not constant across environments, leading to meaningful reranking of sires and reduced selection coincidence between extreme conditions. These results highlight the importance of incorporating G×E-aware genetic evaluation models to improve the accuracy of selection decisions and optimize genetic gain for feed efficiency under tropical beef production systems. From a practical point of view, when animals are selected for residual feed intake and dry matter intake under feeding trials that allow an average daily gain of approximately 1 kg/day (i.e., from 0.9 to 1.4 kg/day), a slight change in animal performance is expected. However, when animals are selected for both traits

in feeding trials with an ADG that is far from the average value, an increase in reranking is observed, which may be caused by the difference in nutritional levels masking the genetic potential and biasing the genetic evaluations for feed efficiency in progeny that are fed for a different ADG. Ultimately, this work contributes to more sustainable and profitable beef production by enabling the identification of animals that combine high feed efficiency with greater robustness across variable production settings.

Third study: This study represents one of the first genome-wide association analyses for feed efficiency traits in Nellore cattle that explicitly accounts for G×E using single-step genomic reaction norm models. By jointly investigating the genetic architecture of both baseline performance (intercept) and environmental sensitivity (slope) for DMI and RFI, the research provides a more realistic and biologically meaningful understanding of how cattle respond to environmental and management variability across large-scale feeding trials. The identification of major genomic windows and positional candidate genes involved in key mechanisms such as insulin/leptin signaling, glucose and lipid metabolism, energy balance, heat stress response, feeding behavior, digestion, and nutrient absorption reinforces the complex and dynamic regulation of feed efficiency. Importantly, the study highlights that SNP effects and genomic estimated breeding values can differ across environmental gradients, supporting the need for environment-aware genomic selection strategies.

Fourth study: This study provides one of the most comprehensive assessments of how heat stress modulates the genetic expression of feed efficiency traits in tropical beef cattle by investigating G×E for DMI and RFI in Nellore cattle, using single-step genomic reaction norm models and the temperature–humidity index (THI) as an environmental descriptor. Based on large-scale data from 296 feed-efficiency trials, including 22,838 animals across 21 farms and multiple years, the study demonstrates that heritability and additive genetic variance for both traits vary along the THI gradient, with a clear reduction in genetic control under intense heat-stress conditions. The results also indicate that G×E becomes more pronounced when THI exceeds approximately 76, leading to genetic correlations below 0.80 across environments and substantial sire re-ranking, suggesting that selection decisions may change depending on thermal conditions. Overall, these findings directly support climate-responsive breeding strategies by enabling the identification of animals that are not only efficient,

but also genetically more robust (less sensitive) under heat stress, contributing to improved productivity, resilience, and sustainability of beef production systems in tropical regions under increasing climate variability.

Fifth study: This study substantially expands the understanding of the genomic mechanisms underlying feed efficiency under varying levels of heat stress in Nellore cattle by identifying genomic regions, candidate genes, biological processes, and functional networks associated with DMI and RFI across contrasting environments along a THI gradient. Based on a robust dataset (22,838 animals evaluated in 296 feed efficiency trials conducted from 2011 to 2023 across 21 farms, including 18,567 genotyped animals), the study shows that the genomic architecture of these traits is not fully stable across thermal conditions, revealing both shared and environment-specific signals. Furthermore, network analyses indicate a marked functional reorganization as THI increases, particularly for RFI, with reduced gene connectivity under more challenging conditions, suggesting greater biological sensitivity of this phenotype to heat stress. From an applied perspective, these findings provide a direct scientific basis for climate-responsive genomic selection strategies, enabling the identification of animals that combine feed efficiency with greater robustness under heat stress, thereby supporting more consistent genetic gains, improved resilience, and the sustainability of beef production systems in tropical regions under increasing climate variability.

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AUTHOR'S BIOGRAPHY

João Barbosa da Silva Neto, son of Geasi Barbosa da Silva and Valéria Cristina Borges dos Santos, was born in Cachoeira Paulista, São Paulo, Brazil, on September 27, 1996. In September 2014, he began his undergraduate studies in Animal Science at the Federal Technological University of Paraná (UTFPR), Dois Vizinhos campus (PR), where he engaged in undergraduate research and teaching assistantship activities, including projects focused on the genetic improvement of *Apis mellifera* honey bees. In December 2018, he obtained his bachelor's degree in Animal Science, completing his undergraduate thesis entitled "Study of classification and selection coincidence of *Apis mellifera* L. queens (Hymenoptera: Apidae)". In March 2019, he began his MSc in Animal Breeding and Genetics at São Paulo State University (UNESP/FCAV), Jaboticabal (SP), supported by a FAPESP scholarship (Process no. 2019/06736-5). He completed his MSc in 2021 with the dissertation "Weighted genomic prediction for economically important traits in beef cattle using candidate genomic regions," under the guidance of Prof. Dr. Fernando Baldi. In the same year, he started his PhD in Animal Breeding and Genetics at UNESP/FCAV, funded by FAPESP (Process no. 2022/15385-4) and also supervised by Prof. Dr. Fernando Baldi. Between 2023 and 2024, he carried out a Research Internship Abroad at Purdue University (USA), supported by FAPESP (Process no. 2023/13417-9), under the supervision of Prof. Dr. Luiz F. Brito. Throughout his academic trajectory, he has actively contributed to multiple research projects and has authored/co-authored papers published in high-impact journals. In 2023, he received the Top Cited Article Award (*Animal Genetics*, Wiley), in recognition of being among the journal's 10 most-cited papers that year. In 2024, he was selected for and successfully completed the Higher Education Challenge Program - Communicating Agriculture Beyond Academia, supported by the USDA in collaboration with South Dakota State University, the University of Nebraska–Lincoln, the University of Minnesota, and North Dakota State University.

“He has made everything beautiful in its time”

Ecclesiastes 3:11

“If I have seen further, it is by standing on the shoulders of giants”

Isaac Newton

I dedicate this work to my parents, Geasi Barbosa da Silva and Valéria Cristina Borges dos Santos, in recognition of their unwavering support, encouragement, and strength throughout my journey.

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TABLE OF CONTENTS

GENOMIC REACTION NORM MODELS AND GENETIC NETWORK ANALYSIS EXPLORING THE EFFECTS OF GENOTYPE × ENVIRONMENT INTERACTION ON FEED EFFICIENCY INDICATOR TRAITS IN NELLORE CATTLE

Abstract: Feed efficiency is a major determinant of profitability and sustainability in beef cattle production systems, as feed accounts for the largest share of production costs and improvements in efficiency can reduce resource use and greenhouse gas emission intensity per unit of product. However, indicator traits such as dry matter intake (DMI) and residual feed intake (RFI) are expensive to measure and are strongly affected by management, nutrition, and climatic conditions. In tropical production systems, this environmental heterogeneity increases the likelihood of genotype-by-environment (G×E) interactions, potentially leading to re-ranking of selection candidates and reduced predictability of genetic progress. In this context, integrating reaction norm methodology with genomic information represents a promising approach to quantify genetic robustness and to support selection strategies aligned with specific production systems. Therefore, this thesis aimed to characterize the genetic architecture of feed efficiency in Nellore cattle under contrasting environmental conditions by integrating complementary approaches: reaction norm models to quantify G×E for DMI and RFI using alternative environmental descriptors related to production conditions and management, with emphasis on nutritional variation and climate; and genome-wide association studies (GWAS) within a genomic reaction norm framework, coupled with functional annotation and network-based interpretation, to identify genomic regions and biological processes associated with both baseline performance (intercept) and environmental sensitivity (slope). Collectively, the studies demonstrate that the genetic control of feed efficiency is not constant across environments, with changes in (co)variance components, genetic parameters, and sire re-ranking under more nutritionally and climatically challenging conditions. Genomic analyses further indicate the involvement of mechanisms related to energy metabolism, endocrine regulation, immune function, and cellular stress responses, reinforcing the complex and environment-dependent nature of these traits. Overall, this thesis contributes to advancing quantitative-genomic tools that support the selection of Nellore cattle that are not only more feed-efficient, but also more robust to environmental variability, promoting more consistent and sustainable genetic gains in tropical beef production systems.

Keywords: Feed efficiency; residual feed intake; genotype-by-environment interaction; reaction norm models; Nellore cattle.

MODELOS DE NORMA DE REAÇÃO GENÔMICA E ANÁLISE DE REDES GENÉTICAS EXPLORANDO OS EFEITOS DA INTERAÇÃO GENÓTIPO × AMBIENTE EM CARACTERÍSTICAS INDICADORAS DE EFICIÊNCIA ALIMENTAR EM BOVINOS NELORE

Resumo: A eficiência alimentar é um dos principais determinantes da rentabilidade e da sustentabilidade em sistemas de produção de bovinos de corte, uma vez que a alimentação representa a maior parcela dos custos de produção e melhorias na eficiência podem reduzir o uso de recursos e a intensidade de emissão de gases de efeito estufa por unidade de produto. No entanto, características indicadoras como o consumo de matéria seca (DMI) e o consumo alimentar residual (RFI) são custosas de mensurar e são fortemente influenciadas por manejo, nutrição e clima. Em sistemas tropicais de produção, essa heterogeneidade ambiental aumenta a probabilidade de ocorrência de interação genótipo × ambiente (G×E), podendo levar ao re-rank de candidatos à seleção e reduzir a previsibilidade do progresso genético. Nesse contexto, a integração da metodologia de normas de reação com informações genômicas constitui uma abordagem promissora para quantificar robustez genética e apoiar estratégias de seleção alinhadas ao sistema de produção. Assim, esta tese teve como objetivo caracterizar a arquitetura genética da eficiência alimentar em bovinos Nelore sob diferentes condições ambientais, integrando abordagens complementares: modelos de norma de reação para quantificar a G×E para DMI e RFI, utilizando diferentes definições ambientais relacionadas às condições de produção e manejo, com ênfase em variações nutricionais e clima; e análises de associação genômica ampla (GWAS) em um arcabouço de norma de reação genômica, acopladas à anotação funcional e à interpretação baseada em redes, para identificar regiões genômicas e processos biológicos associados tanto ao desempenho basal (intercepto) quanto à sensibilidade ambiental (inclinação). Em conjunto, os estudos demonstram que o controle genético da eficiência alimentar não é constante entre ambientes, com alterações nos componentes de (co)variância, parâmetros genéticos e reclassificação de reprodutores sob condições nutricionais e climáticas mais desafiadoras. As análises genômicas também indicam a participação de mecanismos ligados ao metabolismo energético, regulação endócrina, função imune e respostas celulares ao estresse, reforçando a natureza complexa e ambiente-dependente dessas características. De forma geral, esta tese contribui para o aprimoramento de ferramentas quantitativo-genômicas que apoiam a seleção de bovinos Nelore mais eficientes e, ao mesmo tempo, mais robustos frente à variabilidade ambiental, favorecendo ganhos genéticos mais consistentes e sustentáveis em sistemas tropicais de produção.

Palavras-chave: eficiência alimentar; consumo alimentar residual; interação genótipo × ambiente; modelos de norma de reação; bovinos Nelore.

CHAPTER 1: GENERAL CONSIDERATIONS

1.1. Introduction

Among livestock production costs, feed represents the predominant component, particularly in intensive systems, where it may represent approximately 55% to 75% of total expenditures (Greenwood, 2021; Pulina et al., 2021). Consequently, improving feed efficiency is a central strategy to enhance profitability and long-term competitiveness (Herd et al., 2003; Savietto et al., 2014). Feed efficiency reflects the ability to convert nutritional inputs into productive outputs and is commonly described as the relationship between performance (e.g., weight gain) and dry matter intake (DMI) (Herd et al., 2004; Kenny et al., 2018). Beyond economic benefits, improved efficiency is also associated with sustainability outcomes, as reduced feed intake for a given level of production may decrease the intensity of waste and greenhouse gas emissions per unit of product (Arthur and Herd, 2008; William et al., 2019; Manzanilla-Pech et al., 2021; Silva et al., 2025).

In this context, residual feed intake (RFI), first proposed by Koch et al. (1963), has become a widely adopted indicator trait for genetic improvement of feed efficiency. RFI is defined as the difference between observed DMI and expected DMI predicted from maintenance and performance covariates, such as metabolic body weight and average daily gain. Because it is constructed to be largely independent of production level and body size during the test period, RFI is generally considered a robust selection criterion compared with ratio-based measures (Berry and Crowley, 2013; Kenny et al., 2018). Animals with negative RFI are classified as more efficient, consuming less feed than expected to achieve the same performance, whereas animals with positive RFI require greater intake for comparable outputs (Herd and Arthur, 2009; Kava et al., 2023). In Nellore cattle, moderate heritability estimates for RFI have been documented, indicating potential for selection response and genetic improvement in feed efficiency (Santana et al., 2014; Grigoletto et al., 2017; Bonamy et al., 2018; Polizel et al., 2018; Kava et al., 2023).

Despite its value as an indicator trait, the estimation and interpretation of feed efficiency remain highly sensitive to nutritional and operational conditions. Under heterogeneous systems, environmental variation may affect not only mean performance but also the genetic expression of feed efficiency, increasing the relevance of genotype-by-environment interaction (G×E) and the risk of reranking

across production settings. This issue is particularly critical in Brazil, where feed efficiency tests are frequently decentralized across producers and testing centers, intensifying heterogeneity in infrastructure, management, and diet formulation (Mendes and Campos, 2016; Silva Neto et al., 2023). In addition, regional and seasonal variability in ingredient composition and nutrient availability can lead to substantial differences in dietary energy density even under similar formulations, ultimately impacting DMI, performance, and efficiency metrics (Ferreira et al., 2015; Ferreira and Brown, 2016; Dykier et al., 2020). Evidence of phenotypic reclassification for RFI following nutritional changes further supports that shifting environments can alter animal rankings, with direct implications for selection consistency (Durunna et al., 2011; Potts et al., 2015).

Collectively, these factors make the investigation of G×E particularly relevant. As formalized by Falconer (1996), G×E occurs when the relative performance of genotypes changes across environments, such that genetic superiority depends on the environmental context. From a practical standpoint, G×E may compromise the predictability of selection, induce reranking of sires across production conditions, and reduce the consistency of genetic progress in populations evaluated under broad environmental diversity. A robust approach to investigate G×E on a continuous scale is reaction norm modeling, in which phenotypes are described as a function of an environmental gradient, enabling the quantification of genetic components associated with performance in a reference environment and sensitivity to environmental change (Kirkpatrick et al., 1990; de Jong, 1995; Pégolo et al., 2009; Corrêa et al., 2010; Santana et al., 2014).

Beyond productive and nutritional factors, climatic variables have gained increasing relevance as environmental descriptors, particularly given the rising frequency and intensity of heat events across production regions and the effects of heat stress on intake, metabolism, and performance (Bernabucci, 2019). Under high temperature and humidity, cattle may display physiological and behavioral responses that alter feeding patterns and efficiency, reinforcing the importance of evaluating performance and genetic merit across climatic gradients (Neto et al., 2025; 2026). In this regard, biometeorological indices such as the temperature–humidity index (THI) have been widely used to objectively characterize thermal challenge and to enable comparisons across regions and time periods when integrated with meteorological and performance records (Silva Neto et al., 2024).

The expansion of genomic technologies has further strengthened the capacity to dissect and predict complex traits, particularly when phenotyping is costly or limited to subsets of the population (Meuwissen et al., 2001; Hayes et al., 2007; da Silva Neto et al., 2023). Under G×E, integrating genomics with environmental descriptors becomes even more relevant, as it may improve connectedness across environments, refine the prediction of genetic merit under specific conditions, and support inferences on the genetic architecture underlying performance and environmental sensitivity (Chen et al., 2011; Hayes et al., 2016; Zhang et al., 2019; Mota et al., 2020a;b). Although numerous studies have reported genetic parameters and genomic regions associated with feed efficiency without explicitly modeling G×E (Santana et al., 2014; Grigoletto et al., 2016; Polizel et al., 2018; Brunes et al., 2021), the literature highlights the importance of accounting for this component in populations exposed to pronounced environmental heterogeneity (Silva Neto et al., 2024).

In this context, it is essential to develop and apply quantitative and genomic approaches capable of characterizing feed efficiency more realistically under tropical conditions, explicitly incorporating the environmental heterogeneity that defines many production systems. Therefore, the objective of this thesis is to develop and implement an integrated framework of quantitative and genomic models to investigate indicator traits of feed efficiency in Nellore cattle across relevant environmental gradients, with emphasis on G×E and on productive and climatic environmental descriptors. Consistent with this goal, the thesis aims to: (i) discuss key foundations and methodological challenges related to feed efficiency and G×E in beef cattle; (ii) quantify the genetic sensitivity of efficiency-related traits across representative environmental gradients; (iii) explore reaction norm models as a framework to describe performance, robustness, and plasticity in heterogeneous environments; and (iv) integrate genomic information to support prediction of genetic merit and to deepen understanding of the genetic basis underlying performance and environmental sensitivity.

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CHAPTER 2: GENOTYPE-BY-ENVIRONMENT INTERACTIONS IN BEEF AND DAIRY CATTLE POPULATIONS: A REVIEW OF METHODOLOGIES AND PERSPECTIVES ON RESEARCH AND APPLICATIONS

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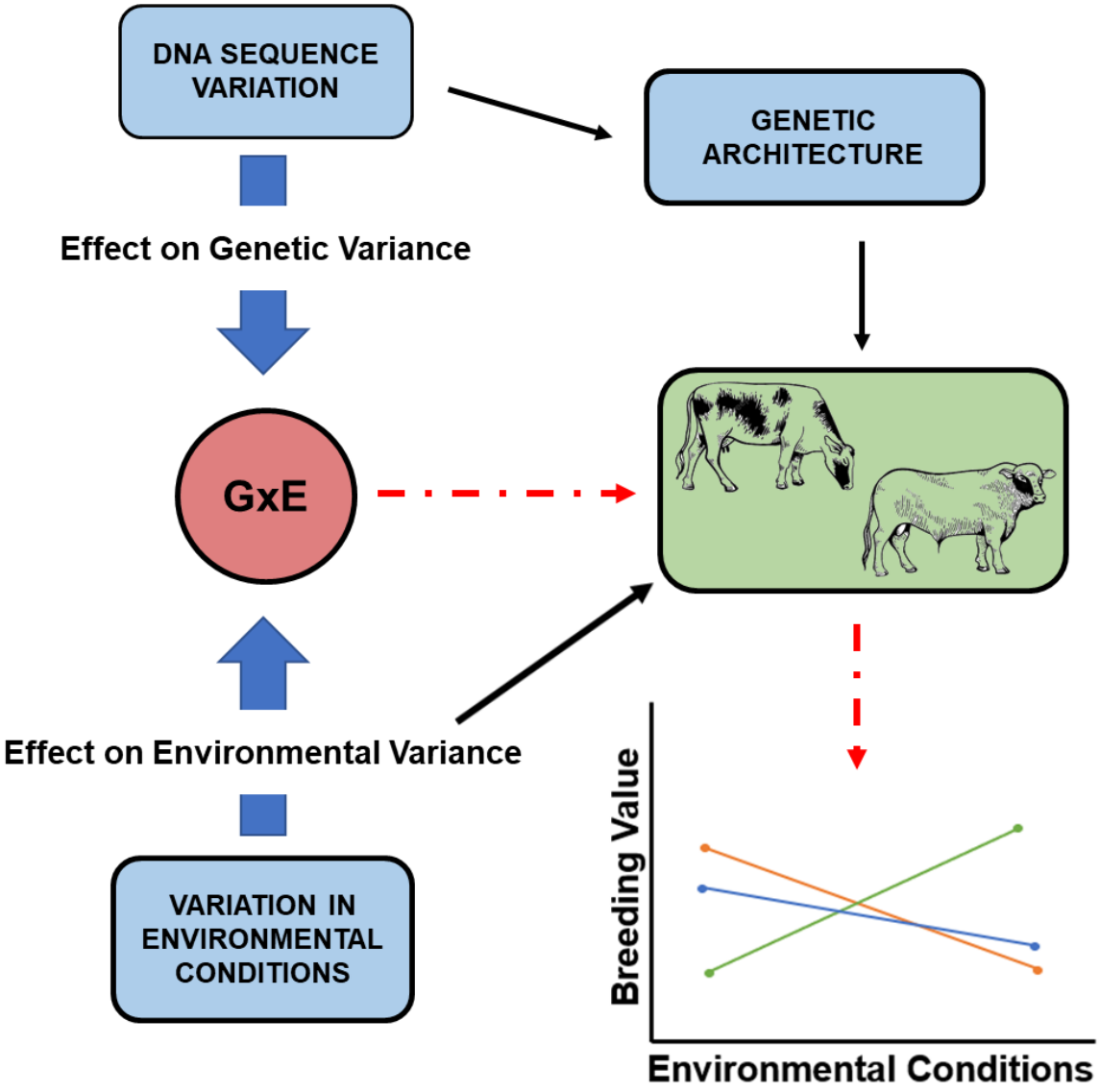
Abstract: Modern livestock production systems are characterized by a greater focus on intensification, involving managing larger numbers of animals to achieve higher productive efficiency and animal health and welfare within herds. Therefore, animal breeding programs need to be strategically designed to select animals that can effectively enhance production performance and animal welfare across a range of environmental conditions. Thus, this review summarizes the main methodologies used for assessing the levels of genotype-by-environment interaction (G × E) in cattle populations. In addition, we explored the importance of integrating genomic and phenotypic information to quantify and account for G × E in breeding programs. An overview of the structure of cattle breeding programs is provided to give insights into the potential outcomes and challenges faced when considering G × E to optimize genetic gains in breeding programs. The role of nutrigenomics and its impact on gene expression related to metabolism in cattle are also discussed, along with an examination of current research findings and their potential implications for future research and practical applications. Out of the 116 studies examined, 60 and 56 focused on beef and dairy cattle, respectively. A total of 83.62% of these studies reported genetic correlations across environmental gradients below 0.80, indicating the presence of G × E. For beef cattle, 69.33%, 24%, 2.67%, 2.67%, and 1.33% of the studies evaluated growth, reproduction, carcass and meat quality, survival, and feed efficiency traits, respectively. By contrast, G × E research in dairy cattle populations predominantly focused on milk yield and milk composition (79.36% of the studies), followed by reproduction and fertility (19.05%), and survival (1.59%) traits. The importance of G × E becomes particularly evident when considering complex traits such as heat tolerance, disease resistance, reproductive performance, and feed efficiency, as highlighted in this review. Genomic models provide a valuable avenue for studying these traits in greater depth, allowing for the identification of candidate genes and metabolic pathways associated with animal fitness, adaptation, and environmental efficiency. Nutrigenetics and nutrigenomics are emerging fields that require extensive investigation to maximize our understanding of gene–nutrient interactions. By studying various transcription factors, we can potentially improve animal metabolism, improving performance, health, and quality of products such as meat and milk.

Keywords: animal adaptability, *Bos taurus indicus*, *Bos taurus taurus,* environmental sensitivity, resilience.

Graphical overview



2.1. Introduction

The accurate identification of genetically superior individuals for economically relevant traits is crucial for the success of animal breeding programs (Goddard, 2009; Hayes et al., 2007). This involves assessing the genomic background of populations and evaluating the phenotypic variability from interactions between genetic and environmental factors using classical animal models (Burrow, 2012; Dickerson, 1962). The environmental effect encompasses non-genetic factors that contribute differentially to phenotypic variability. Several studies have consistently shown that most traits of economic relevance to cattle breeding are complex traits largely influenced by a large number of genes and environmental conditions (Carvalho Filho et al., 2022; Mota, Fernandes Jr, et al., 2020; Santana Jr et al., 2017). To achieve successful production intensification, it is important to identify and select animals with lower environmental sensitivity and enhanced adaptive traits (Brito et al., 2021; Henry et al., 2018). Typically, less environmentally sensitive genotypes are preferred, although, in some contexts (e.g., more environmentally controlled production systems), more productive and probably more sensitive may be more advantageous. Investigating the implications of genotype-by-environment (G × E) is essential for designing mating strategies and selection decisions to increase productivity across diverse production systems.

In traditional breeding programs, animals are often selected under more favorable or less stringent environmental conditions (e.g., nucleus breeding farms), allowing them to express more of their genetic potential. However, the offspring of the selected animals are frequently raised under more challenging conditions. As a result, in situations where G × E occurs, the reproductive and productive performance of specific genotypes may become unpredictable in certain environments, potentially impacting the overall genetic progress of the population (Cardoso & Tempelman, 2012; Rauw & Gomez-Raya, 2015). Thus, to minimize these effects, breeding programs should focus on selecting animals that are more adapted to various environmental and management conditions (Hermesch et al., 2015; Kolmodin & Bijma, 2004; Nirea & Meuwissen, 2017) or select animals that perform better in the environmental conditions where they will be raised.

A key goal of breeding programs is to comprehensively characterize and understand the impact of G × E to minimize or even eliminate the unpredictability associated with such interactions. The occurrence of G × E can introduce challenges

in the design of breeding programs. However, it also presents opportunities to strategically identify and combine the most appropriate genotypes for given production systems aiming to optimize the industry profitability and improve animal health and welfare. Despite being a significant source of variation in production systems, often leading to reduced responses to selection (Hayes et al., 2016; Mota, Fernandes Jr, et al., 2020), G × E is often ignored in routine genetic and genomic evaluations. One of the primary challenges in G × E modeling lies in disseminating and effectively implementing the results at the producer level. Furthermore, fitting G × E leads to additional complexity in the statistical models, particularly when relying solely on pedigree information. This is due to the increased number of parameters needed to account for interaction effects, the heterogeneous variance caused by different environments, the need for including environmental covariates, and the creation of complex correlation structures among traits. Additionally, G × E may exacerbate non-additive effects, which are already present in the population, making them more challenging to model accurately with pedigree data alone. This often results in less accurate genetic evaluations, especially in more extreme environmental conditions (De Leon et al., 2016; Mulder, 2016; Sae-Lim et al., 2016; Tiezzi et al., 2017).

G × E can be broadly categorized into two main types: (1) changes in the ranking of genotypes across different environments; and (2) variations in the dispersion of genetic values across environments without a simultaneous shift in the ranking of genotypes (Falconer, 1990; Lynch & Walsh, 1998). The impact of G × E is typically quantified based on the genetic correlation of a given trait measured in different environmental conditions (and considered as a separate trait depending on the environmental category). Genetic correlation values falling below 0.80 are considered as an indication of substantial G × E effects on animal performance (Robertson, 1959).

The occurrence of G × E, particularly when there is a reranking of genotypes, has been documented for several traits in cattle (Raidan et al., 2016; Ruiz-Sánchez et al., 2007; Santana Jr et al., 2017, 2018; Tsuruta et al., 2015). In such cases, substantial discrepancies in the genetic variance and shifts in the estimated breeding values (EBVs) of the selection candidates are observed (Mota et al., 2019). In other words, the genotypes that perform best in one environment may not necessarily maintain better performance in other environments (Mulder & Bijma, 2005). Advancements in omics technologies and statistical methods, particularly with the

implementation of genomic selection, have significantly enhanced the performance of genetic evaluations, leading to increased selection accuracy, especially for young animals (Misztal et al., 2020; Mrode et al., 2019; Silva Neto et al., 2023). Adding genomic information to the analyses plays a pivotal role in increasing the accuracy of genetic variation and breeding value estimates across environmental conditions (Carvalheiro et al., 2019; Mota et al., 2018; Mota, Fernandes Jr, et al., 2020; Mota, Lopes, et al., 2020; Oliveira et al., 2018).

This review exploits the main methods used to quantify G × E levels and their implications for important traits within cattle breeding programs. We aimed to discuss the implications of the reported findings for breeding programs and identify possible knowledge gaps. Additionally, we discuss the contribution of genomic information when estimating and accounting for G × E in cattle breeding schemes. Before doing so, we provide an overview of the cattle breeding program structure and their relationship with key challenges associated with harnessing G × E to optimize genetic progress in cattle populations. We also summarize key contributions of the nutrigenomics field and the role of nutritional strategies in the phenotypic variability of complex traits. We explore the relationship between resilience and productivity in livestock production systems and outline potential opportunities for future research and practical applications.

2.2. Structure of cattle breeding programs

Most cattle breeding programs are structured hierarchically, with the most intensive genetic selection of animals performed in *nucleus* or elite herds. Subsequently, the superior genetic material is disseminated to commercial herds through artificial insemination or other reproductive technologies (Raidan et al., 2016; Schenkel, 2000; Simm, 1998) (Figure 1). In nucleus herds, animals are selected under more favorable conditions, including better nutritional and management practices, enabling them to express their genetic potential fully (Raidan et al., 2016). However, this scenario contrasts with the reality of most commercial herds, where significant variation in environmental conditions exists, including factors related to nutritional practices, climatic conditions, and incidence of diseases. Consequently, the average performance of the offspring sired by the bulls selected in more favorable environmental conditions may deviate from expectations, especially when the

commercial conditions differ from those where the sires were initially evaluated (Burrow, 2012).

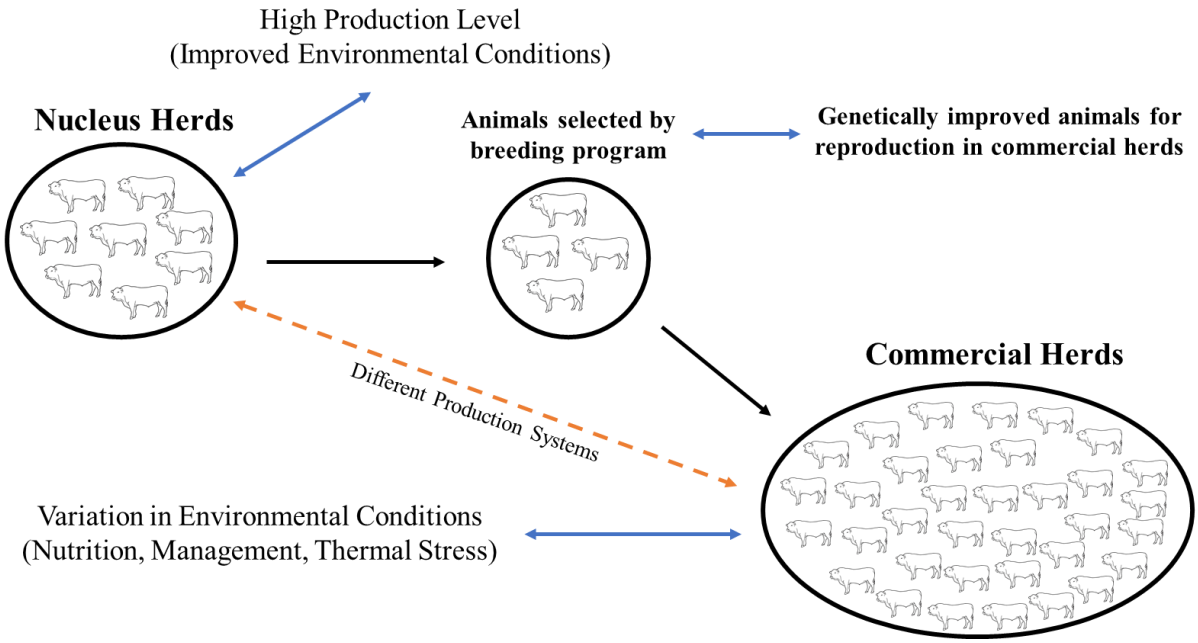


Figure 1. Hierarchical structure of cattle improvement programs with selection is usually done in elite herds with better nutritional and management conditions for later dissemination of the superior genetic material in commercial herds with less ideal environmental conditions.

A study by Raidan et al. (2016) evaluating the performance of young Nellore bulls in both nucleus and commercial herds for reproductive and growth traits, revealed significant variations in the estimates of genetic parameters. The highest additive genetic variance and heritability estimates were observed in more favorable environmental conditions. Haile-Mariam et al. (2008) reported evidence of G × E interaction in calving to first service interval and nonreturn rate in Australian dairy cattle considering different calving systems and regions. Some studies evaluating African Holstein cattle also observed G × E interaction for age at first calving and milk production, considering the performance of daughters of bulls raised in intensive and extensive production systems (Neser et al., 2014). The substantial differences between selection and breeding conditions often lead to genetic correlations between the same trait measure in different environments being close to or below 0.80, indicating a substantial G × E effect on the trait evaluated.

Dairy cattle breeding programs face a major challenge when using genetic material imported from countries with different environmental conditions and production systems. The market for artificial insemination is dominated by companies from the USA, Europe, and Canada (Araújo et al., 2016; Santos et al., 2020). In Brazil, around 80% of the semen used by farmers is imported into the country (Araújo et al., 2016). Studies investigating G × E in dairy cattle populations in Brazil have confirmed that these animals are more environmentally sensitive. This is expected given the countries' production system differences (Cardoso & Tempelman, 2012; Cooke et al., 2020). For instance, Sub-Saharan Africa smallholder dairy cattle farmers rely on the importation of sire semen (Chawala et al., 2021). This dependence contributes to slower genetic progress, partly due to the effects of G × E interactions.

To minimize the G × E effect, the Interbull organization conducts an international genetic evaluation of dairy bulls based on specific procedures (www.interbull.org; Philipsson, 1987, 2011). In these multi-trait across-country genetic evaluations, traits such as milk yield and milk composition, are fitted as different traits across countries, and bulls have EBV for different countries participating in the genetic evaluations. This approach is feasible in dairy cattle due to the reduced number of breeds used and high level of genetic connectedness in worldwide dairy cattle populations due to the widespread use of a small number of sires (Hayes et al., 2016).

The impact of choosing sires for different production systems from where their offspring will be raised depends on their genetic potential and adaptability to the environment. Breeders must carefully assess the performance of sires and their offspring in the target environmental conditions before making selection or semen purchase decisions to avoid adverse effects. By considering G × E interaction in breeding programs, breeders can select more adapted genotypes that perform well in diverse environments and production systems. Nonetheless, genetic evaluations that consider G × E have yet to be adopted in commercial breeding companies. This is because the G × E effects vary significantly across breeds, strains, and production systems, making it challenging to generalize genetic models for all situations. Additionally, accurate breeding values require substantial amounts of well-structured phenotypic and environmental data recorded across environmental levels. As more environmental variables and phenotypic traits are included in the models, the analyses become more parameterized, and convergence issues may arise in multivariate

evaluations. As a result, livestock breeding programs typically use simpler genetic evaluation models and only consider G × E in specific situations.

2.3. Genetic aspects of uniformity of production

The demand for enhanced productive efficiency and more uniform animal performance is essential for the profitability of livestock production systems (Júnior et al., 2022). Given that uniformity holds great importance in various stages of animal production, any lack of homogeneity within the production chain can directly impact the producers' profitability. In intensive production systems, such as group-housed pigs, poultry, and fish, variability in size and growth rate among animals can increase competition for resources, such as space, feed, and water. This excessive competition can lead to agonistic interactions, resulting in social stress, which in turn can negatively affect growth, increase mortality and morbidity, reduce milk production in dairy species, and impair feed intake in group-housed animals (Gilmour et al., 2005; Janhunen et al., 2012; Milligan et al., 2002). Therefore, a lack of uniformity impacts both meat and milk production, as it can result in lower production efficiency and higher management costs. One practical approach to address this need for greater uniformity is through genetic improvement, considering G × E and selecting more robust animals, i.e., animals that adapt and perform more consistently across environments.

The primary focus of this review was related to factors associated with macro-environmental effects, which are regular, persistent, and global factors (e.g., climatic conditions, average herd production, heat stress, and varying nutritional levels) that can lead to G × E interactions (Berghof et al., 2019). However, there are micro-environmental factors that are sporadic, conditional, and specific to the individual, such as animal age, health status, and social hierarchies. Although these micro-environmental factors can influence production uniformity and contribute to G × E interactions, few studies have evaluated their potential impact on G × E, with most focusing on the variance of genotypes in a common environment (Mulder et al., 2013; Neves et al., 2012).

Production homogeneity in livestock is somewhat influenced by genetic factors (Hill & Mulder, 2010; Iung et al., 2018; Mulder, 2016; Mulder et al., 2007; Sell-Kubiak et al., 2015). This genetic variability can be used to enhance animal uniformity through genetic selection. In traditional quantitative genetics, models often assume that the

residual variance is homogeneous, implying that genotypes primarily differ in their average effect (Falconer, 1996; Lynch & Walsh, 1998). However, genetic heterogeneity indicates how animals respond to environmental disturbances and their environmental sensitivity as reported in beef cattle studies (Iung et al., 2018; Mulder et al., 2013; Neves et al., 2011).

Differences in animal uniformity for a specific trait can be characterized as variations in residual variance (Iung et al., 2020). The genetic heterogeneity of residual variance has been examined through two primary methods. Mulder et al. (2009) employed a two-step approach to estimate the genetic heterogeneity component of residual variance in female and male broiler chickens. Several studies have used this methodology to evaluate G × E in cattle (Berghof et al., 2019; Ehsaninia et al., 2020). In the first step, they assessed the variance components for the trait's mean and subsequently employed a log transformation on the squared residuals, using these as the response variable in their analyses. Alternatively, Rönnegård et al. (2010) and Felleki et al. (2012) introduced the Hierarchical Dual Generalized Linear Model, which concurrently fits two sets of mixed model equations, one for the mean level and another one for the residual variance level. Elucidating the relationship between uniformity of production and G × E is crucial for advancing our understanding of the genetic mechanisms influencing integrated production systems' performance. This knowledge, in turn, can aid in developing or enhancing selection and mating schemes in the cattle industry.

2.4. G x E interactions

G × E refers to the phenomenon in which genotypes exhibit varying phenotypic responses due to variations in environmental conditions (Falconer, 1996). In the context of breeding programs, two primary forms of G × E are of major importance, given their far-reaching implications for selecting superior genotypes for performance in target environments (Figure 2B,C).

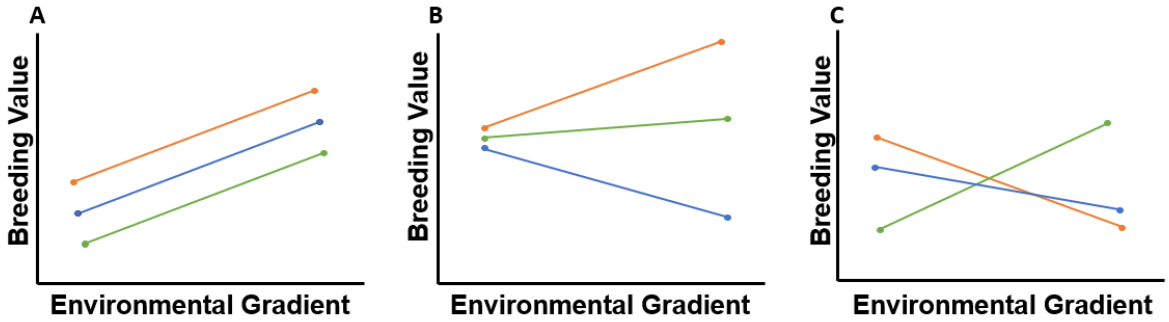


Figure 2. Environmental effect on genotype variation, breeding value can change depending on environment, often leading to complex interaction between genotype and environment. (A) additive effect of environment on phenotypic response, genotype by environment interaction (G x E) not significant (B) example of environmental effect leading to scale effect on the genetic value of the genotype and (C) significant rearrangement of genetic value in response to environmental change.

The first G × E scenario is typically considered non-significant, occurring only as an additional effect of the environment on the phenotypic response that can be adjusted for in the classical model of genetic evaluation (BLUP, Best Linear Unbiased Prediction; Figure 2A). The second type of G × E is characterized by variations in the magnitude of the genetic additive variance across the environments evaluated, as shown in Figure 2B.

The accuracy of selection in breeding programs can be affected by changes in genetic variance across environments, leading to more biased EBV when G × E is ignored in the genetic models, mainly due to the selection of sires from herds with high phenotypic variability (Meuwissen et al., 1996). This means that the selection of animals in one environment does not guarantee the desired improvement in other environments. The genetic parameters of the trait may differ between environments, which may affect the correlation between phenotypic values and EBV when G × E is ignored. This is due to changes in additive genetic (σ_a^2) and/or residual (σ_e^2) variances.

G × E can differently affect the phenotypic expression of complex traits, in which genotypes may have different responses when exposed to contrasting environmental conditions (Figure 2). In situations where genotypes exhibit differences in EBV between environmental conditions, yet without re-ranking, the environmental effect can result in an EBV scale adjustment when G × E is ignored (Figure 2B). G × E plays

a substantial role in this scale change, even though the best-performing genotype in one environment remains the best in other environments. However, when genotypes exhibit different degrees of response according to the environmental conditions, re-ranking of animals based on their EBV can occur (Figure 2C). This significant G × E effect highlights that the evaluated trait expression cannot be considered the same across environmental conditions (Strandberg et al., 2002). For animal breeding purposes, the reclassification of animals based on their EBV across environments represents the most critical form of G × E. In other words, there may not be a single superior genotype that stands out in all environments, and selection for performance in one environment may result in smaller genetic gains than anticipated in other environments (Mulder & Bijma, 2005).

Robertson (1959) suggested that a genetic correlation for the same trait evaluated in different environments lower than 0.80 indicates potential reclassification of animals and the need for selection schemes tailored to specific environments (Mulder & Bijma, 2005, 2006). However, given the current structure of cattle genetic evaluations, breeding programs with specialized selection schemes for each distinct production environment are not anticipated to be widely implemented in the near future. Nonetheless, the evaluation of G × E remains a crucial element in cattle breeding and management.

2.5. Environmental sensitivity and phenotypic plasticity

Environmental sensitivity or reaction norms can be defined based on differences in a genotype's or population's performance across environments (Falconer, 1990). This sensitivity can be assessed using reaction norm models (RNMs), employing random regression models to estimate how each individual responds to environmental changes. These models map trajectories influenced by a continuous environmental descriptor (Carvalheiro et al., 2019; Kirkpatrick et al., 1990; Mota et al., 2019; Schaeffer, 2004). Random regression models (RRMs) for reaction norms began to gain prominence in the 1990s (Oliveira et al., 2019). Henderson (1984) established the theoretical foundation with his mixed models. The direct application of RRMs to reaction norms was first demonstrated by Kirkpatrick et al. (1990). In 2004, Schaeffer (2004) further advanced the field by refining and expanding the use of RRMs in genetic evaluation, enhancing their effectiveness

across various environmental conditions. These developments were pivotal for advancing the modeling of G × E using reaction norms.

In general, the sensitivity of animals to environmental changes is assessed using linear models, as higher-order polynomials can be more complex to estimate and make biological interpretations. However, higher-order RNM may be indicated when changes in phenotypic expression do not exhibit a linear pattern along the environmental gradient (Hayes et al., 2016; Schaeffer, 2004). Animals with a steeper slope in their reaction norms are more sensitive to environmental changes, leading to greater variations in phenotypes. Conversely, animals with flatter slopes exhibit lower sensitivity to environmental variations, resulting in smaller phenotypic differences across diverse environmental conditions (Carvalho et al., 2019; Oliveira et al., 2018). Consequently, more resilient animals, i.e., those with a greater ability to be minimally affected by environmental variations or rapidly return to the unperturbed state (Colditz & Hine, 2016), are particularly valuable for breeding purposes in more challenging conditions.

Understanding the genetic factors that underlie the sensitivity of the animals to environmental variations holds significant importance in optimizing cattle breeding programs. In this context, incorporating genomic data in the analyses has expanded the application of RNM, transitioning from individual-level analyses to the level of single nucleotide polymorphisms (SNPs). This can be achieved by leveraging extensive genotypic data from animals exposed to diverse environmental conditions that might not be necessarily connected at the pedigree level (Carvalho et al., 2019; Hayes et al., 2009; Mota, Lopes, et al., 2020; Silva et al., 2014).

An important term to be addressed is phenotypic plasticity, defined by De Jong & Bijma (2002) and Sommer (2020) as the ability of a genotype to exhibit different phenotypic responses in different environments. This includes morphological, physiological, and behavioral variations of an animal's phenotype (Pelster & Burggren, 2018; Sommer, 2020) and is considered a fundamental mechanism for animal adaptation to environmental changes (Murren et al., 2015). Although genotype and gene expression determine an individual's phenotype, phenotypic plasticity allows the same genotypes to present considerable variation in their performance (West-Eberhard, 2003). Like environmental sensitivity, phenotypic plasticity can be measured by RNM, and factor-analytic (FA) models can also be used. As the multi-trait animal model that will be covered in the next session, the FA model is limited to

discrete environments and does not assume a scaled classification of environments or physiological continuity across different environments (De Jong & Bijma, 2002).

Although we focused on exploring genotype plasticity in macro-environments, Pelster & Burggren (2018) highlight that the conventional definition of phenotypic plasticity typically overlooks ‘response time.’ This refers to the period required for a phenotypic modification to manifest, with time intervals for these changes varying significantly, from minutes to years. Furthermore, the authors highlighted that when considering the temporal aspect of phenotypic plasticity, it must be clear whether the observed change is a real change in phenotype or a change in performance due to homeostatic adjustment.

2.6. Assessing the relationship between resilience and productivity in livestock systems

It has been known for a long time that the environment can influence the phenotype of animals (Lamarck, 1914). However, as suggested by Lerner (1954), populations can balance their genetic background and resist environmental changes. In livestock production systems, there has been an emphasis on animal resilience, which involves both crossbreeding to introduce suitable genetic variations and selecting animals with higher genetic potential for desired traits, as well as the ability to adapt to environmental conditions.

Resilience, understood as an animal's ability to adapt and maintain its performance in the face of challenges or environmental changes, is critical in ensuring productive stability (Berghof et al., 2019; Colditz & Hine, 2016). Resilient animals are characterized by their ability to recover from a disturbance quickly. Despite experiencing a sharp initial decline in productivity, these animals demonstrate a remarkable ability to return to pre-disturbance levels swiftly, while less resilient animals may take longer to recover and may not fully return to their previous state (Berghof et al., 2019). Thus, the intrinsic relationship between animal resilience and productivity in agricultural systems is a fundamental element of sustainability. Resilient animals have shown a remarkable ability to adapt to climate variations, resource scarcity, and environmental stress, maintaining productivity or minimizing losses (Poppe et al., 2021, 2022; Poppi et al., 2018). This adaptive capacity provides a more favorable scenario for maintaining consistent production levels in agro-industrial systems. Furthermore, animal resilience has a genetic correlation with the efficiency

in resource use, health, fertility, and longevity of animals (Chen et al., 2023; Poppe et al., 2020).

Measuring resilience can be difficult, and the mechanisms involved strongly depend on the nature of the disturbance, that is, the results are dependent on the disturbance investigated in the study (Berghof et al., 2019; Colditz & Hine, 2016). Colditz & Hine (2016) proposed a diverse set of response variables that enables the measurement of resilience for disturbance events. These variables encompass deviations between expected and observed production over the duration of the disturbance, aiming to assess animals' response after a severe drop in production (Van der Waaij et al., 2000).

Precision livestock farming technologies, such as automated feeding systems and automated milk sensors in milking parlors, have emerged as a strategic solution for longitudinal data sampling to assess resilience indicators based on variability in animal performance (Brito et al., 2020). With repeated records of frequently measured traits, estimating an individual's performance without environmental challenges is possible using linear and nonlinear statistical models (the differences between expected and observed production yield contain valuable information about the animal's adaptability to known and unknown macro- and micro-environmental disturbances). Based on these deviations, Berghof et al. (2019) suggested using skewness, residual variance, and autocorrelation of deviations in productive performance as resilience indicators in livestock production systems. This finding is based on the aspect that repeated measures over time for the same production trait contain relevant insights into an animal's ability to cope with micro-environmental challenges.

Residual variance is a measure calculated based on the analysis of variability patterns originating from repeated measurements over time, reflecting the impact of environmental disturbances at an individual level (Berghof et al., 2019; Chen et al., 2023; Elgersma et al., 2018; Poppe et al., 2020, 2021, 2022; Rodrigues et al., 2024; Scheffer et al., 2018). Animals lowly affected by environmental fluctuations tend to have low residual variance, while the opposite is observed in animals more susceptible to environmental disturbances. The autocorrelation (Lag-one) of the deviations signals the duration of the impact of the disturbances (Chen et al., 2023). For animals without disturbances or with rapid recovery, the autocorrelation approaches zero. For animals influenced by disturbances, with a

slower recovery, the autocorrelation approaches +1, indicating that subsequent deviations are similar. In cases of rapid and overcompensation responses to perturbations, such as compensatory growth, the autocorrelation approaches −1, indicating that subsequent deviations are the opposite (Berghof et al., 2019; Chen et al., 2023). In turn, the deviations' skewness highlights the direction of these variations. For animals without disturbances or with little influence, the asymmetry approaches zero. However, a positive skewness suggests positive deviations, mainly due to favorable responses to environmental improvements, while a negative skewness indicates predominantly negative deviations resulting from disturbances (Poppe et al., 2020).

In a study conducted by Chen et al. (2023) to assess resilience indicators based on daily milk yield variability, moderate genetic correlations were found between residual variance and cow's productive life in months, with estimates of −0.30. This suggests that more resilient cows (i.e., less affected by environmental challenges) tend to have a longer productive life. The same authors also reported genetic correlations of −0.20 between the autocorrelation of deviations and productive life in months. However, Poppe et al. (2020), evaluating the skewness of deviations, found insufficient genetic variability for this indicator. Furthermore, the authors also did not identify any significant genetic correlation between skewness and health, longevity, fertility, or metabolic traits.

Efficient adaptation to variable environments is an intrinsic characteristic of resilient animals, positively impacting agricultural production's sustainability (Berghof et al., 2019). Resilience is also closely related to animals' welfare and tolerance to disease and stress (Doeschl-Wilson et al., 2021). The animals' ability to quickly recover after stressful events contributes to maintaining productivity, as it reduces the duration of periods of production loss (Colditz & Hine, 2016; Doeschl-Wilson et al., 2021). This relationship between welfare, tolerance, and resilience emphasizes the importance of these attributes in the sustainability of agro-industrial production systems.

2.7. Statistical models for quantifying GxE

The analysis of G × E interactions has undergone significant evolution over time. Initially, these interactions were primarily examined through analysis of variance (ANOVA) in experiments with small, balanced datasets, focusing on the identification

of simple interaction effects. As statistical analysis advanced, the introduction of mixed models in animal breeding (Henderson, 1984) laid the foundation for more sophisticated methods. In the 1990s, Kirkpatrick et al. (1990) pioneered the application of RRMs to capture the nuances of reaction norms, overcoming the limitations of traditional ANOVA methods. Subsequently, Jensen (2001) further advanced the application of RRMs in dairy cattle breeding, while Schaeffer (2004) refined and expanded these models for genetic evaluations under diverse environmental conditions. The transition from basic analyses to more complex and precise models has significantly enhanced the understanding of G × E interactions, enabling a more detailed analysis of genotypic responses to varying environmental contexts.

The models for assessing G × E include two main components, the genotype (G) and environmental (E) effects, as well as the interactions among them (Falconer, 1996; Lynch & Walsh, 1998). To evaluate a trait and obtain (G)EBV across environments, three main statistical models can be used: (1) analytical factorial mixed models; (2) multi-trait models (MMs); and (3) RNMs (or RRMs). Among these options, 2 and 3 are the most used methods for assessing G × E in beef and dairy cattle populations (Crossa et al., 2022; Hayes et al., 2016; Mota, Fernandes Jr, et al., 2020; Mota, Lopes, et al., 2020; Santana Jr. et al., 2014). Each of these models has unique characteristics, which will be discussed in detail below.

2.7.1. FA mixed models

The FA models were initially proposed for analyzing multi-environment trials in plant breeding (Li et al., 2017). Meyer (2009), when examining FA models in a standard linear mixed model framework, showed that the models are applicable in animal breeding. FA models can identify environmental factors that cause G × E by structuring the genetic (co)variance matrix into two main components, i.e., common factor and specific effect. Common factors establish correlations between variables, whereas specific factors aim to identify those factors that explain the maximum variation (Meyer, 2009). Factor analysis is a valuable multivariate tool for assessing interrelated traits and streamlining redundant information inherent in multiple variables (Corrales et al., 2011; Vukasinovic et al., 1997; Xu et al., 2022). This, in turn, enhances the accuracy of variance estimates and reduces computational demands,

especially when working with large-scale datasets (Mazza et al., 2015; Olasege et al., 2019).

The mixed animal FA model can be described as (Meyer, 2009; Sae-Lim et al., 2014):

$$\boldsymbol{P_{ijk}} = \boldsymbol{\mu} + \boldsymbol{ef_k} + \boldsymbol{a_{c,i}} + \boldsymbol{a_{s,ik}} + \boldsymbol{e_{ijk}},$$

where, $\boldsymbol{P_{ijk}}$ is the phenotypic record of the *i*th individual of the *j*th genotype in *k*th environment, $\boldsymbol{\mu}$ is the overall trait mean, $\boldsymbol{ef_k}$ is the fixed environmental effect, $\boldsymbol{a_{c,i}}$ and $\boldsymbol{a_{s,ik}}$ are the random additive genetic effects of the i-th individual due to the common factor(s) and specific effects for the k-th environment, respectively, and $(\mathrm{a_c}, \mathrm{a_s})$ $\sim \mathrm{MVN}(\mathbf{0}, \mathbf{A} \otimes \mathbf{G_{FA}})$. The $\mathbf{G_{AF}} = \boldsymbol{\Gamma\Gamma'} + \boldsymbol{\Psi}$ is the matrix of genetic (co)variance for common and specific additive genetic effects; $\boldsymbol{\Gamma}$ is the matrix of factor loadings; and $\boldsymbol{\Psi}$ is the diagonal matrix of the specific variances ($\boldsymbol{\Psi_k}$), accounting for the additional variance, *i.e.*, the variation that is not explained by the common factor(s) of the $\boldsymbol{k}$-th environment**.** This additive genetic variance not explained by the common latent factor is captured by the specific effects. The matrix of loading factors, $\boldsymbol{\Gamma}$, is obtained from the analysis of the environmental variables, and its interpretation depends on the relationship of these loadings with the environmental gradients studied.

When the common factor delineates different proportions of genetic variance across different environments, it indicates the existence of G × E. The environmental factor responsible for G × E can be identified by correlating the estimated factor loadings with the observed environmental variables. For the FA model to adequately capture G × E interactions, it is essential that the animals are evaluated in multiple environments, allowing genetic and environmental variations to be captured in a multivariate way. Despite the possibility of using the FA model in livestock G × E studies, no studies using this methodology were found in the literature. One of the explanations for this scenario may be due to the greater complexity of animal data, involving a wider range of variables to be evaluated, making FA modeling more challenging to implement and interpret the results. For example, in plants, genetic structure often follows simpler patterns of inheritance, which can facilitate G × E modeling. In contrast, factors such as sex-linked inheritance, epistatic interactions, and more significant genetic variability can make modeling more challenging in animals.

2.7.2. MMs

In MMs in the context of G × E, the same trait recorded under different environmental conditions is considered as a potentially different trait (Falconer & Latyszewski, 1952). The MM is well-suited for situations where the environmental effects are considered to be categorical factors (Calus et al., 2002; Hayes et al., 2016; Kolver et al., 2002; Mulder et al., 2004). Such factors include diets, production systems, milking type, production levels, and geographical region. This model is a reliable tool for analyzing data containing multiple variables and can effectively capture their relationships. Therefore, the MM allows for quantifying the genetic variance of the trait and ranking of genotypes in each pair of environments. In livestock production, the genotype is usually defined as the individual animal, given that the same animals are not recorded across multiple environments. Consequently, the genetic performance of an animal can be defined based on the degree of relatedness among related animals raised in different environments (Hayes et al., 2016; Henderson, 1984).

For two environments (two traits), the MM can be described as (Hayes et al., 2003, 2016; Mulder et al., 2004):

$$\begin{bmatrix} \mathbf{y_1} \\ \mathbf{y_2} \end{bmatrix} = \begin{bmatrix} \mathbf{I_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{I_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{\mu}_1 \\ \boldsymbol{\mu}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z_2} \end{bmatrix} \begin{bmatrix} \mathbf{g_1} \\ \mathbf{g_2} \end{bmatrix} + \begin{bmatrix} \mathbf{e_1} \\ \mathbf{e_2} \end{bmatrix},$$

where, $\mathbf{y_1}$ and $\mathbf{y_2}$ are the phenotypic records for a certain trait in environments 1 and 2, respectively; $\mathbf{I_1}$ and $\mathbf{I_2}$ are identity matrices, $\boldsymbol{\mu_1}$ and $\boldsymbol{\mu_2}$ are the phenotypic means for the trait in environments 1 and 2; $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are the incidence matrices relating the (G)EBV (additive genetic values) to the response variables; $\mathbf{g_1}$ and $\mathbf{g_2}$ are the additive genetic values for the genotypes in environments 1 and 2, and $\mathbf{e_1}$ and $\mathbf{e_2}$ are the residual vectors for environments 1 and 2. The residuals are assumed $\begin{bmatrix} \mathbf{e_1} \\ \mathbf{e_2} \end{bmatrix} \sim \mathrm{N}(\mathbf{0}, \mathbf{I} \otimes \mathbf{R})$, where, $\mathbf{R} = \begin{bmatrix} \boldsymbol{\sigma}^2_{\mathbf{e_1}} & \boldsymbol{\sigma}_{\mathbf{e_{12}}} \\ \boldsymbol{\sigma}_{\mathbf{e_{12}}} & \boldsymbol{\sigma}^2_{\mathbf{e_2}} \end{bmatrix}$ is the matrix of residual variances and covariances for environments 1 and 2 and $\otimes$ is the Kronecker product (Hayes et al., 2016).

The genetic values based on the relationship between the animals can be assumed through the pedigree-based relationship matrix **A** (Henderson, 1984) as follows:

$$\begin{bmatrix} \mathbf{g_1} \\ \mathbf{g_2} \end{bmatrix} \sim \mathrm{N}(\mathbf{0}, \mathbf{A} \otimes \mathbf{T}),$$

where $\mathbf{T} = \begin{bmatrix} \boldsymbol{\sigma}^2_{\mathbf{g_1}} & \boldsymbol{\sigma}_{\mathbf{g_{12}}} \\ \boldsymbol{\sigma}_{\mathbf{g_{12}}} & \boldsymbol{\sigma}^2_{\mathbf{g_2}} \end{bmatrix}$ is the genetic variance and covariance matrix for environments 1 and 2.

The estimates of genetic correlations between phenotypic performance in two environments can be calculated as (Falconer & Latyszewski, 1952; Gilmour et al., 2006):

$$\mathbf{r_{g_{12}}} = \frac{\hat{\boldsymbol{\sigma}} g_{12}}{\hat{\boldsymbol{\sigma}} g_1 \hat{\boldsymbol{\sigma}} g_2},$$

where, $\hat{\boldsymbol{\sigma}} g_{12}$ is the genetic covariance between the same trait measured in environments 1 and 2; $\hat{\boldsymbol{\sigma}} g_1$ is the square root of genetic variance for trait measured in environment 1; and $\hat{\boldsymbol{\sigma}} g_2$ is the square root of genetic variance for trait measured in environment 2.

Genomic-based MM can also be obtained by replacing the **A** by the **G** or $\mathbf{H}$ matrices, which enables the calculation of GEBV for each environment. The $\mathbf{H}$ matrix was defined by Legarra et al. (2009) and its inverse $\mathbf{H^{-1}}$ can be calculated as (Aguilar et al., 2010):

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

where $\mathbf{A^{-1}}$ is the inverse of the pedigree-based relationship matrix; $\mathbf{A_{22}^{-1}}$ represents the inverse of the pedigree-based relationship matrix for the genotyped animals, and $\mathbf{G^{-1}}$ is the inverse of the genomic relationship matrix obtained according to VanRaden (2008).

An illustration of the use of MM for the estimation of EBV across environments was demonstrated by Williams et al. (2012) for the growth of Angus cattle at high and low altitudes. The authors evaluated the weaning weight of over 77 000 cattle raised in Colorado (USA) farms located at different altitudes as two traits: (1) weaning weight at high altitudes; and (2) weaning weight at low altitudes. The genetic correlation for growth at high and low altitudes was 0.74 ± 0.07, suggesting a re-ranking of genotypes raised on farms located at different altitudes. Therefore, the authors recommended that genetic evaluations for growth in Angus cattle raised on farms located at different altitudes consider the influence of G × E within their statistical modeling. For Holstein cows raised in 805 Chilean herds, Chuma-Alvarez et al. (2021) reported genetic

correlations of 0.73 ± 0.06 between milk yield measured in the central region (low production technology level) and the southern region (high production technology level). This finding suggests a re-ranking of genotypes of animals raised in different locations in Chile.

2.7.3. RNMs

The The RNM is an alternative to MM when the environmental gradients are described on a continuous scale (Hayes et al., 2016). These aspects are often referred to as *infinite dimensional* resources because they allow for an infinite number of values along the environmental gradient trajectories and because these values are considered different (Meyer, 1998). G × E in beef and dairy cattle for several traits has been widely described in the literature (Calus et al., 2006; Corrêa et al., 2010; Pegolo et al., 2011; Santana Jr. et al., 2014; Nguyen et al., 2016; Silva Neto et al., 2023).

The expression of genotypes across different environmental conditions is often modeled as a linear function (reaction norm) of an environmental value or gradient (Kirkpatrick et al., 1990). RNM assumes that the phenotypic value is expressed as a polynomial function associated with the environmental gradient. In this framework, the polynomial coefficients indicate the average EBV of the animal (intercept), while the slope coefficient represents the animal's response to environmental changes (de Jong, 1995). The covariance estimates between the random regression coefficients yield estimates of covariance functions (Kirkpatrick et al., 1990). In this context, a covariance function describes the covariance between measurements obtained in given environments as a function of those environmental conditions (Figure 3).

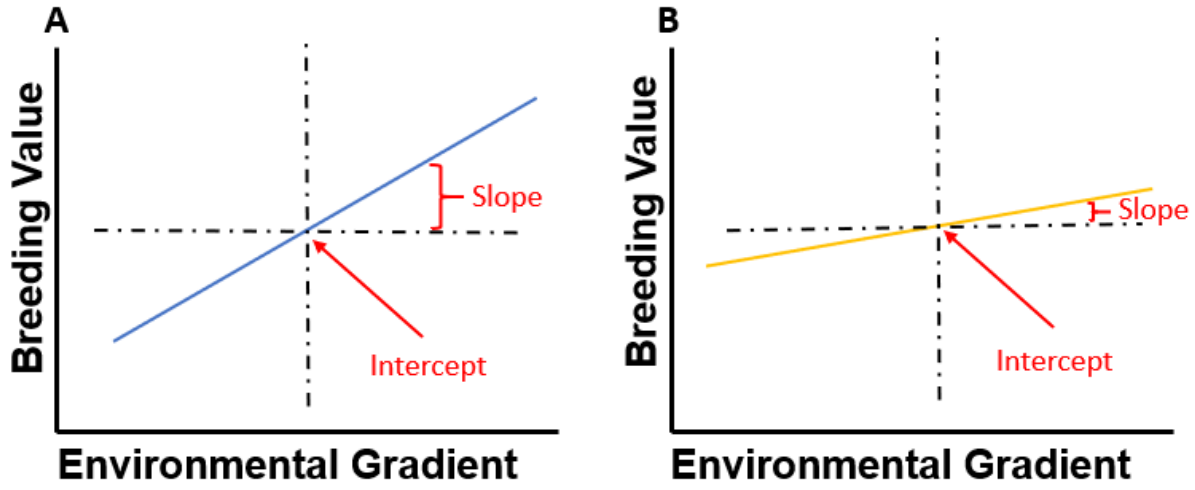


Figure 3. Reaction norms for genetic values in relation to environmental conditions. (A) represents the polynomial intercept and slope coefficients of an animal with lower resilience to variations in environmental conditions (higher slope coefficient). (B) represents the polynomial intercept and slope coefficients of an animal with higher resilience to variations in environmental conditions (lower slope coefficient).

The RNM can be applied in two steps (Calus & Veerkamp, 2003; Mota et al., 2020a). In the first step, BLUE (Best Linear Unbiased Estimates) solutions are obtained from an animal model and subsequently used to define the environmental conditions. In the second step, the general RNM can be used to estimate genetic parameters across environments conditions (EC):

$$\boldsymbol{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e},$$

where, $\boldsymbol{y}$ represents the phenotypic information recorded at different EC; $\boldsymbol{b}$ is a vector of the fixed effects to adjust the phenotypic information; $\boldsymbol{X}$ is an incidence matrix linking the fixed effects to the phenotypic information (y); $\mathbf{a}$ is the vector of random animal additive genetic effects of RN parameters (intercept and slope) corresponding to the EC levels; $\boldsymbol{Z}$ is the incidence matrix relating the records to additive genetic effects of RN parameters and $\mathbf{e}$ is the random residual (Mota et al., 2020b). In RNM, RN parameters (intercept and slope) on phenotypic information can also be included as fixed effects.

In RNMs, the EC levels can be modeled using different polynomials such as ordinary, Legendre, or spline polynomials. Details about each of these models are

presented in Table S1. Ordinary polynomials are simple algebraic expressions of variables, often used as a linear form using directly EC information. Ordinary polynomials are typically used in simple models to establish an understanding of how an environmental variable affects a trait. For example, they can be used to model growth rates or milk yield as a function of temperature humidity index (THI). By contrast, Legendre orthogonal polynomials are a specific type of orthogonal polynomial, useful in RNM due to their mathematical properties. These properties reduce the correlation between polynomial terms, meaning that the different polynomial terms are uncorrelated with each other (Meyer, 1998). This reduces the multicollinearity problems in the model, which leads to more reliable estimates of coefficients and better model interpretation. Legendre orthogonal polynomials are often better at generalizing beyond the range of observed data compared to ordinary polynomials, particularly when the reaction norm must be extrapolated.

Spline polynomials consist of segmented functions that are connected at specific points known as knots, for modeling segmented regressions, where each segment are smoothly linked (Meyer, 2005). Splines provide high flexibility as they enable the model to adjust to local changes in the data (Misztal, 2006). This is particularly valuable when the relationship between the environment and the trait shows multiple inflection points offering local control (Carvalheiro et al., 2019; Carvalho Filho et al., 2022). Splines are especially beneficial in situations where the reaction norm is expected to vary nonlinearly across a range of environmental conditions. RNM can also be used in sire models, which can facilitate the selection of the most adaptable and efficient sires across various environments, particularly in systems where the available data are predominantly paternal.

The genetic correlation between EC levels ($\mathrm{r}_{\mathrm{EC_j},\mathrm{EC_k}}$) can be calculated as:

$$\mathrm{r}_{\mathrm{EC_{j,EC_k}}} = \frac{\sigma_{\mathrm{EC_{j,EC_k}}}}{\sqrt{\sigma^2_{\mathrm{aEC_j}} * \sigma^2_{\mathrm{aEC_k}}}},$$

where, $\sigma_{\mathrm{EC_{j,EC_k}}}$ is the covariance between the level of $\mathrm{EC_j}$ and the level of $\mathrm{EC_k}$; $\sigma^2_{\mathrm{aEC_j}}$ and $\sigma^2_{\mathrm{aEC_k}}$ are the genetic variance for the trait at each level of $\mathrm{EC_j}$ and $\mathrm{EC_k}$. These variances can be obtained through various modeling techniques, which may include the use of transformed covariates, such as orthogonal polynomials, or untransformed covariates, depending on the approach used to capture genetic variation across different environmental levels.

The EBVs associated with each level of EC are obtained using the following equation: $\hat{g}_{j\mathrm{EC}_j} = \widehat{\alpha_i}\phi_j$; where $\widehat{\alpha_i}$ is the estimated additive genetic value for intercept and slope estimates of animal *i* and ϕ_j is the vector representing the specific characteristics of the EC_j. Genomic information can also be considered into RNM by replacing the **A** matrix with a genomic (**G**) or hybrid genomic relationship matrix such as **H** (Legarra et al., 2009; Aguilar et al., 2010), resulting in GEBV for each individual and environment.

Cheruiyot et al. (2020) presented an application of RNM to explore G x E, specifically in relation to heat tolerance in milk yield and composition in Australian Holstein cattle. The study incorporated a large dataset, encompassing 6.7 million milk production records from 491,562 cows across three lactations and 6,410 sires with offspring across multiple environments. The authors used the Temperature and Humidity Index (THI) derived from climate data obtained from 163 meteorological stations as the environmental gradient. As a result, the authors observed that the heritability estimates decreased as THI increased throughout the trajectory for fat and increased for THI > 70 for milk and protein yield. The correlation estimates between the 5th and 95th THI percentiles (extremes) for milk, protein, and fat yield were close to 0.80, with values of 0.88 ± 0.01, 0.79 ± 0.02, and 0.86 ± 0.02, respectively. The percentage of bulls with EBV with a lower slope, with at least 100 daughters with multi-parity records, was 65%, 57%, and 64%, respectively, demonstrating the presence of G x E in that population.

Silva Neto et al. (2023b) and Mota et al. (2020a) applied RNM models in Nellore cattle to investigate G×E interactions, observing significant genetic reranking across different environmental gradients. Silva Neto et al. (2023b) identified G × E interactions for dry matter intake (DMI) and residual feed intake (RFI) using contemporary group solutions for average daily gain (ADG) as the environmental gradient. The lowest genetic correlations between extreme environmental levels were 0.22 for RFI and 0.26 for DMI, resulting in selection coincidences of 53.3% and 40.0%, respectively. Mota et al. (2020a) reported G × E interactions for early pregnancy and scrotal circumference, using yearling weight as the environmental gradient, with genetic correlations of 0.30 and -0.12 between more favorable and more unfavorable environments. Both studies confirmed variability in genetic variance and reranking of selection candidates, highlighting the importance of considering G × E in breeding programs.

The clear and comprehensive definition of the environmental gradient plays a crucial role in GxE analysis, significantly influencing the results obtained in scientific studies (Fikse et al., 2003; Freitas et al., 2021). When the definition of the environmental gradient is inadequate or imprecise, distortion can occur in G x E analyses (Fikse et al., 2003). If environmental variations are not correctly identified or quantified, the real effects of genotypes can be misinterpreted, resulting in mistaken conclusions about the adaptability or resilience of different genotypes across environments (Freitas et al., 2021). Therefore, a precise definition of the environmental gradient is essential to obtain accurate estimates of G x E.

2.8. G x E interaction studies published from 1967 to 2023

To evaluate trends over time and the degree of G × E interaction for traits measured in cattle breeding programs, we performed a literature search from October to December 2023 in six electronic databases (Scielo, PubMed, Google Scholar, Scopus, Science Direct, and Web of Sciences). A total of 116 beef and dairy cattle articles published from 1967 to 2023 were identified [see Tables S2 and S3]. The success of breeding programs hinges upon identifying the best-performing animals for the target production systems and environmental conditions. In the G × E studies summarized, various definitions of environmental gradients were used, including diets (Hay & Roberts, 2018), temperature and humidity indices (Santana Jr et al., 2018), weight gain (Santana Jr et al., 2015), somatic cell count (Calus et al., 2006), productive indices (Lillehammer et al., 2009), production systems (Raidan et al., 2016), milking type (Hammami et al., 2015), geographical regions (Fennewald et al., 2018), and countries (Hammami et al., 2009). Hence, the G × E levels were presented based on the magnitude of genetic correlations. Among the 116 studies (60 in beef cattle and 56 in dairy cattle, see Tables S2 and S3, respectively), a total of 97 studies (83.62%) reported genetic correlations below 0.80, indicating the presence of G × E. Furthermore, differences in protocols for measuring traits may result in lower genetic correlations.

The variability in the estimates of genetic correlation for the same trait evaluated in different environments could be due to the genetic differences between the herds evaluated; the models used to quantify G × E, and the definitions of the environmental gradients (Araujo Neto et al., 2018; Calus et al., 2004). Approximately 69.33% of the 60 studies on beef cattle (see Table S2) focused on growth traits, 24% on reproductive

and fertility traits, 2.67% on carcass and meat quality traits, 2.67% on survival traits, and 1.33% on feed efficiency traits. In contrast, 79.36% of the 56 studies on dairy cattle (see Table S3) focused on milk production and quality traits, 19.05% on fertility and reproduction traits, and 1.59% on survival and productive life traits. A notable divergence was evident in the geographical regions of the G × E studies, in which 25.33% of the G × E studies in beef cattle were published using datasets from countries with a more temperate climate, while 74.67% of the studies were conducted in tropical countries, in which there is more significant variability in nutritional practices, management, and weather and/or geographical conditions. In contrast, for the dairy G × E studies, 66.67% of the studies were performed in countries with more temperate climatic conditions and 33.33% in countries with more tropical climate conditions. RNMs and MMs were used most to evaluate G × E, which was approximately 50% for each model. More beef cattle studies applied RNM (~60%) than MM (~40%) to assess G × E. Conversely, the reverse trend was observed for dairy cattle, with MM being used more than RNM. Among the traits evaluated in the studies listed in Tables S2 and S3, traits with lower heritability estimates, such as reproduction, displayed a more pronounced effect of the G × E.

Although most of the traits recorded in breeding programs have been used to assess the effect of the G × E (see Tables S2 and S3), one study has evaluated feed efficiency traits in beef cattle and none in dairy cattle populations. This is a notable knowledge gap, considering that 55% to 80% of the total cost of cattle production is related to feeding, especially in more intensive production systems (Anderson et al., 2005; Herd et al., 2003; Ramsey et al., 2005; Rolf et al., 2010). Therefore, there is a need to investigate G × E on feed efficiency traits in cattle populations, especially in herds raised in countries with large variations in soil, climate, or other sources of heterogeneity in production environments. In such conditions, nutrition level and quality may restrict the expression of the full genetic potential of an animal, and this is even more pronounced in extensive production systems, particularly in tropical regions where animal diets may be suboptimal. In these situations, even if management is ideal for the environmental conditions, the lack of adequate nutrients can prevent the expression of superior alleles. Thus, it is crucial to clearly define the production system in question and assess whether the environment and management are optimized for animals to reach their full genetic potential.

According to the Food and Agricultural Organization (FAO, 2009), global meat consumption is expected to increase by 30% until 2050. To meet this demand, beef production needs to increase by approximately 72%. Beef represents a quarter (25%) of the overall global meat production and consumption is projected to rise from 60 million to 130 million tons. At least 70% of the anticipated rise in beef production essential to meet the escalating demand is projected to come from subtropical and tropical regions (FAO, 2009). In this context, G × E emerges as a critical factor, especially in subtropical and tropical regions where climatic and breeding conditions vary considerably and animals are raised in more extensive production systems. As ~80% of the world's livestock population is concentrated in these locations (Cooke et al., 2020), identifying genotypes adapted to these specific climatic contexts is essential to maximize production efficiency. Breeds and lineages genetically adapted to such environments have the potential to offer superior performance in animal production in these regions. Therefore, understanding the complex interaction between the genotype and the environment is fundamental in optimizing livestock production, ensuring an efficient response to growing global food needs, particularly in places where climatic conditions favor food production.

2.9. Inclusion of genomic information in the assessment of G x E

The integration of genomic information into genetic evaluations has resulted in major advancements in animal breeding. Genomic evaluations result in more accurate breeding values for younger animals and enable a considerable reduction in generation interval, to name some of the benefits of genomics (Oliveira et al., 2018; Silva Neto et al., 2023a). By including genomic information in G x E studies, researchers have better understood the genetic factors contributing to variation in complex traits across environmental conditions (Oliveira et al., 2018; Carvalheiro et al., 2019; Mota et al., 2020b). Quantifying and modeling the G x E in the context of genomic selection is a valuable approach for addressing the impact of G x E on selection decisions and investigating the genetic basis of animal adaptation and environmental sensitivity (Oliveira et al., 2018; Carvalheiro et al., 2019; Mota et al., 2020b).

Understanding the genomic background of G x E for traits under selection is highly relevant and contributes to improving the robustness of genetic evaluations. This is because an animal can have a reasonably accurate GEBV in an environment

where they have not been measured yet based on information from their relatives (Hayes et al., 2016; Cao et al., 2020). However, one of the main challenges in implementing genomic selection is the development of a reference population consisting of genotyped animals with phenotypic records in a wide range of environmental conditions (Mota et al., 2020a). Hence, genotyping and phenotyping animals widely distributed across environmental conditions can lead to more accurate GEBV predictions.

Incorporating genomic information into models for evaluating G x E can enhance breeding schemes by contributing to a more accurate selection of the most resilient animals based on genotypic performance consistency across environments. In addition to greater selection accuracy, genomic information also enable the identification of genomic regions that influence plasticity or stable phenotypic performance across environments (Mulder, 2016). The evaluation of G x E effects has been deepened by including genomic information combined with pedigree information for several traits evaluated in cattle breeding programs, such as body weight (Oliveira et al., 2018), tick resistance (Mota et al., 2016), traits related to sexual precocity (Mota et al., 2020a), and milk production (Toro-Ospina et al., 2023).

2.9.1. Genome-wide association studies in the identification of G × E

In the context of G x E, the single-step genome-wide association study (ssGWAS) approach has been used to detect SNP markers and genomic regions associated with resilience and environmental sensitivity in animals (Mota et al., 2018, 2020b; Carvalheiro et al., 2019). An important question in the G x E is whether the quantitative trait loci (QTL) that control a trait show consistent effects across different environments, varying only in magnitude and direction as environmental conditions vary, or whether these QTL change in each specific environment (Lillehammer et al., 2008, 2009). Certain QTL might be more favorable for particular environments. As a result, we expect a higher prevalence of these QTL in comparable environments (*i.e.*, genetic correlations > 0.80), while in more divergent environments, others have a greater effect on the trait (*i.e.*, genetic correlations < 0.80).

Mota et al. (2020a), using genomic RNM to assess G × E in traits related to sexual precocity in Nellore cattle, reported dependent SNPs of some environments, indicating strong SNP-by-environment interaction with changes in the magnitude and direction of the SNP effects on the trait. The authors suggested the use of these

genomic regions and SNPs in statistical models so that the process of genetic selection could obtain animals with greater genetic potential for sexual precocity and tolerance to variations in environmental conditions. For example, the weighted single-step genomic BLUP model can highlight regions that explain a large proportion of the genetic variance of the trait (Wang et al., 2012). This approach would allow the weighting of SNPs adjacent to these regions, informing the model about their greater relevance for the trait in a specific environment allowing adjustments in the weightings according to the interactions between SNP and environment. Lillehammer et al. (2008), using random regression models to identify QTL related to production traits in Australian dairy cattle, reported that the greatest variation in SNP effects occurred when environmental conditions became less restrictive. In addition, the authors observed that those genomic regions with the higher effect on the trait were not equally significant across environments.

Mota et al. (2018), evaluating the genomic background of tick resistance in Hereford and Braford cattle using reaction norms, observed that an allele can additively increase the value of the trait as much as decrease it in different environments. This is because gene expression can vary and, therefore, the function of a gene and the percentage of genetic variance explained by the SNP marker near this gene is expected to change (Gibson, 2008; Hayes et al., 2009; Des Marais et al., 2013; El-Soda et al., 2014; Mota et al., 2020a).

Oliveira et al. (2018) and Carvalheiro et al. (2019), using the intercept and slope of the RNM as the target traits for the GWAS analyses, identified SNP markers corresponding to different types of biological functions and regulatory genes associated with environmental plasticity. Biological mechanisms associated with animal environmental sensitivity depend on the extent of the environmental effect on the trait (Lillehammer et al., 2007; Streit et al., 2013). Lillehammer et al. (2009) reported that some QTL were not reported in previous GWAS that did not consider G × E interactions. They suggested that SNP-by-environment interaction effects could explain inconsistencies between QTL mapping studies, particularly when a QTL in one environment is not detected in another or has different effects.

Among the effects of G × E, the variation in SNP marker effects represents one of the main challenges for performing more accurate genomic selection in cattle. This is because the best subset of SNP markers might differ across environments (Des Marais et al., 2013; Nirea & Meuwissen, 2017; Oliveira et al., 2018; Mota et al., 2020b).

This variation in SNP effects underscores the importance of considering G × E in breeding programs, as the animals' ability to adapt to adverse conditions in production systems can be strongly influenced by these interactions. Therefore, it is crucial that breeding programs include strategies that integrate G × E to ensure that genetic selection is adaptive and effective under different environmental conditions.

A practical approach to addressing G × E in animal breeding programs is through progeny tests, which involve collecting data on animal performance in different locations, management systems, and climatic conditions. From these data, statistical models can be used to estimate the specific genetic effects of each environment, allowing a more precise understanding of how genotypes behave under different circumstances. However, it is crucial to keep it simple to make it easier for breeders to understand and make decisions. A viable strategy for this simplification is the development of selection indices that incorporate G × E (Mulder & Bijma, 2006). These indices would combine EBV from different environments into a single measurement, considering how animals respond in varying conditions (Mulder, 2016; Mulder & Bijma, 2006). This simplified approach can assist breeders in making more informed selection decisions, contributing to improved animal adaptation and performance in diverse environments.

2.10. Nutrigenetics, nutrigenomics, and GxE in livestock production

With advances in nutrition, biochemistry, molecular biology, and genomics are transforming nutritional studies into an integrated science (Osorio et al., 2017; Sato, 2016), where we are now better able to understand how nutrients interact with the animal genome, which is crucial to understanding G × E in animal production systems. Nutrition evaluates the effects of nutrients on animal physiology (Osorio et al., 2017). Nutrigenomics and nutrigenetics are new research approaches that aim to study the interaction between food nutrients and gene expression in individual animals (Neibergs & Johnson, 2012). Nutrigenetics focuses on the study of genetic variations in a genome and the response of those organisms to a given diet (Neibergs & Johnson, 2012). Nutrigenomics, in turn, investigates the relationship between nutrition and the genome at the molecular, cellular, and systemic levels. In other words, it focuses on how the nutrients present in the diet interact with genes and their effects on gene regulation processes, such as transcription factors, RNA, protein expression,

and metabolite production (Benítez et al., 2017; Gonçalves et al., 2009; Osorio et al., 2017; Trujillo et al., 2006).

There are various databases useful in these fields, including the dbSNP database (www.ncbi.nlm.nih.gov/projects/SNP), gene ontology (www.geneontology.org), the Kyoto Encyclopedia of Genes and Genomes (www.genome.jp/kegg), carbohydrate-active enzymes (www.cazy.org), peptidase database (http://merops.sanger.ac.uk), gene cards (www.genecards.org), and the bovine gene atlas (http://bovineatlas.msstate.edu), as well as websites hosting the cattle genome sequences (Neibergs & Johnson, 2012). These resources can enable the assessment of the impact of different dietary environments on gene expression levels, offering a better understanding of G x E in livestock.

The possibility of adapting the diet to the individual's genome has encouraged researchers worldwide to develop studies in this research area. According to Trujillo et al. (2006), nutrigenomics presents four basic premises: (1) diet and dietary components can alter the risk of disease development by modulating multiple processes involved with onset, incidence, progression, and severity of disease; (2) food components can act on the genome, either directly or indirectly, to alter the expression of genes and gene products; (3) diet could potentially compensate or accentuate the effects of genetic polymorphisms; and (4) the consequences of diets are dependent on the balance of health and disease states and on an individual's genetic background. These premises illustrate how diet, part of the environment, interacts with the genome to influence the phenotype, exemplifying the importance of G × E in animal performance and environmental adaptation. Thus, knowing that the diet and the nutrients present in the food are an important part of the environment in which the animals are raised and greatly affects variations in performance. Using new tools and results obtained in nutrigenomics would make it possible to understand how the genes of animals respond to different diets and how this interaction can influence their performance, health, and adaptation to the environment in which they are raised. This knowledge is valuable for developing schemes for specific diets or more efficient nutritional strategies and for selecting more genetically suitable animals for given production systems or environments.

DNA methylation is one of the most studied forms of epigenetics, which involves adding methyl groups to certain DNA regions, inhibiting or activating gene expression (Lesta et al., 2023; Xue et al., 2023). These epigenetic changes can be, in some

cases, heritable, affecting gene expression in future generations (Fitz-James & Cavalli, 2022; Van Cauwenbergh et al., 2020). Epigenetic changes, resulting from interactions between genes and the environment, are an essential component of G × E, showing how the environment can have lasting effects on gene expression and, consequently, on phenotype. Thus, environmental effects can permanently influence the animal's genotype, changing how genes are expressed and its impact on the target phenotype. This complex interaction between the environment and the genotype is fundamental for the adaptation of animals to different environmental conditions and for developing genetic improvement strategies that consider these epigenetic influences.

Several studies conducted in humans and mice have focused on exploring the molecular basis of multifactorial diseases, such as obesity, cardiovascular diseases, and cancer, interpreting them as a result of interactions between genes and diet (Ferguson et al., 2009; Ordovas & Corella, 2004; Šedova et al., 2004; Trujillo et al., 2006). Notably, they revealed significant interactions resulting from combining specific foods or dietary components. These studies demonstrate clearly the G × E, where different genotypes respond differently to the same nutritional conditions. For example, combined consumption of soy with black or green tea has demonstrated greater efficacy in preventing the growth and metastasis of prostate cancer in men than alone consumption (Gonçalves et al., 2009; Lyn-Cook et al., 1999; Zhou et al., 2003). Furthermore, in humans, an understanding of the implications of early maternal nutrition on epigenetic changes and how they translate into phenotypic changes has already been clarified (Burdge & Lillycrop, 2010).

In domestic animals, although part of the information about the genes that make up the genome and their respective locations on the chromosome, structure, and function have been identified (Barbosa et al., 2023; Marti et al., 2005), there are still scarce studies on how genes act in animal metabolism. Recent studies investigating the molecular interactions of dietary nutrients have revealed that gene expression undergoes modifications due to various nutritional elements. These include carbohydrates, proteins, fatty acids, vitamins, minerals, and phytochemicals such as flavonoids and isothiocyanates (Abete et al., 2012; Benítez et al., 2017; Mutch et al., 2005; Raqib & Cravioto, 2009).

In cattle, variations in dietary mineral requirements between breeds indicate that genetic factors influence the individual requirements of each animal. An example of this is the study carried out by Mullis et al. (2003) with Angus and Simmental heifers

housed together during pregnancy and early lactation, in which the authors observed differences in plasma concentrations between breeds, with Angus heifers having higher plasma copper concentrations, suggesting a lower copper requirement compared to Simmental heifers (Mullis et al., 2003). In a study conducted by Joseph et al. (2010), they examined the impact of diverse dietary supplements on the expression of genes associated with lipid metabolism in Angus steers. The study compared several animal groups: those solely consuming grass, pasture-fed steers supplemented with soy hulls and corn oil, pasture-fed steers supplemented with corn grain, and a group on a high-concentrate diet. The findings highlighted that alterations in diet, mainly through high-concentrate supplementation, influenced the transcription of genes linked to fat metabolism. This, in turn, impacted the fatty acid composition in carcass tissues, thereby influencing carcass quality. Notably, supplementing with corn, whether in oil or grain, influenced the expression of genes associated with fatty acid synthesis.

It has long been agreed that nutrition and genetic predisposition significantly influence reproductive performance and fertility in dairy cattle (Butler, 1998; Roche, 2006; Sammad et al., 2022). This association becomes particularly crucial during transitional phases and early lactation when the animals are highly sensitive to nutritional fluctuations (Laible, 2009). Du et al. (2010) state that adequate maternal nutrition during gestation increases the Wingless and Int (Wnt). This pathway, responsible for bolstering myogenesis and curbing adipogenesis in skeletal muscle, regulates body fat levels and reduces susceptibility to obesity. It facilitates heightened myogenesis during early and mid-gestation through an epigenetic mechanism (Yan et al., 2013).

Among livestock species, cattle have one of the most complete and detailed sets of comparative SNP arrays, mainly due to their economic relevance (Seo et al., 2013). Databases and livestock-specific web-based pathway genomics tools (cited earlier), facilitate the functional analysis of diverse ‘omics’ data types, such as transcriptome, proteome, and metabolome (Seo et al., 2013). For beef and dairy cattle production, the overall efficiency of nutrient use during growth and lactation hinges on a combination of management practices and environmental factors, which influence metabolic responses through an integrated system of genetics, nutrition, immunity, and physiological processes (Berry et al., 2011; Hocquette et al., 2010; Loor, 2010). Hence, information from nutrigenomics studies has the potential to guide the

formulation of more specific diets, taking into account the health status of the animals and the nutritional composition of the feed, which may result in improved metabolic responses and, therefore, higher production.

2.11. Perspectives on research and application

G × E interaction is relevant for production and many other complex traits such as heat tolerance, disease resistance, reproductive and feed efficiency, as demonstrated in this review. With the use of omics data, it is possible to advance significantly in researching these traits identifying genes and metabolic pathways associated with animal adaptation. It is expected that the results obtained in the present and those that will be obtained in the future directly benefit the genetic improvement programs in livestock production. Evaluating and elucidating the G × E in feed efficiency traits will provide knowledge of the complex genetic background regarding feed efficiency in cattle, allowing the selection of animals with greater genetic potential for feed efficiency under different production conditions. In addition, the results from GWAS for these traits in contrasting environments (low, medium, and high environmental gradient levels) can be used to identify important genomic regions associated with these traits. This information can then contribute to the development of more comprehensive SNP panels that cover all relevant genomic regions, improving the accuracy of genomic evaluations by focusing on genomic regions with significant associations. Ultimately, advancements in omics technologies will support the creation of more efficient selection strategies and tools, aiming to enhance production sustainability.

Studies on nutrigenetics and nutrigenomics are expanding areas of science that still require many studies to maximize the benefits of understanding gene–nutrient interactions. There are several transcription factors with great potential to be studied to improve animal metabolism for greater performance, health, and the quality of the inputs produced, such as meat and milk. Nutrigenomic analyses can prevent diseases linked to the individual's nutritional condition, formulating diets based on gene mapping. Diseases such as ketosis, a metabolic disease that mainly affects dairy herds with a body condition above that recommended at the time of parturition (Goodacre, 2007; Ingvartsen, 2006), can be partially prevented, reducing its incidence. The general results of the nutrigenomic experiments produced so far seem promising. However, practical applications are not yet available, partly due to the

complexity of the systems under study. The discoveries to be made in the coming decades using molecular tools will revolutionize our basic understanding of the physiology of herds and will help in the improvement and development of new methods for the nutritional control of animals.

2.12. Conclusions

Based on the literature results presented in this review, it is clear that G × E is a reality in cattle breeding programs leading to a loss in selection response, especially when genetic evaluations of sires are performed in different environments. In most studies, the genetic correlations were below 0.80, demonstrating the significant effect on the genetic reranking of the selection candidate, which results in changes in the residual and additive genetic variance of the traits. Consequently, there are expected changes in heritability estimates and breeding value across environments, which decreases the expected genetic gain. It is necessary conduct studies to understand the relationship between differences in animal performance and the effects of the G × E, as well as the best way to include them in the statistical models used in genetic evaluations. In the face of climate change, monitoring the G × E is essential, aiming to select resilient animals to ensure the sustainability of production systems.

2.13. Supplementary files

The supplementary file(s) supporting the analyses presented in this chapter are available online and can be accessed through the link provided in the published version of the corresponding paper: https://doi.org/10.1111/age.13483

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CHAPTER 3: GENOTYPE-BY-ENVIRONMENT INTERACTIONS FOR FEED EFFICIENCY TRAITS IN NELLORE CATTLE BASED ON BI-TRAIT REACTION NORM MODELS

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Abstract

Background

Selecting animals for feed efficiency directly impacts the profitability of the beef cattle industry, which contributes to minimizing the environmental footprint of beef production. Genetic and environmental factors influence animal feed efficiency, leading to phenotypic variability when exposed to different environmental conditions (i.e., temperature and nutritional level). Thus, our aim was to assess potential genotype-by-environment (G×E) interactions for dry matter intake (DMI) and residual feed intake (RFI) in Nellore cattle (*Bos taurus indicus*) based on bi-trait reaction norm models (RN) and evaluate the genetic association between RFI and DMI across different environmental gradient (EG) levels. For this, we used phenotypic information on 12,958 animals (young bulls and heifers) for DMI and RFI recorded during 158 feed efficiency trials.

Results

The heritability estimates for DMI and RFI across EG ranged from 0.26 to 0.54 and from 0.07 to 0.41, respectively. The average genetic correlations (± standard deviation) across EG for DMI and RFI were 0.83 ± 0.19 and 0.81 ± 0.21, respectively, with the lowest genetic correlation estimates observed between extreme EG levels (low vs. high) i.e. 0.22 for RFI and 0.26 for DMI, indicating the presence of G×E interactions. The genetic correlation between RFI and DMI across EG levels decreased as the EG became more favorable and ranged from 0.79 (lowest EG) to 0.52 (highest EG). Based on the estimated breeding values **from** extreme EG levels (low vs. high), we observed a moderate Spearman correlation of 0.61 (RFI) and 0.55 (DMI) and a selection coincidence of 53.3% and 40.0% for RFI and DMI, respectively.

Conclusions

Our results show evidence of GxE interactions on feed efficiency traits in Nellore cattle, especially in feeding trials with an average daily gain (ADG) that is far from the expected of 1 kg/day, thus increasing reranking of animals.

3.1. Background

In recent years, various worldwide beef cattle breeding programs have included feed efficiency-related traits as a selection criterion to increase the profitability of beef production and minimize the industry's environmental impact. Feeding represents approximately 70% of the total beef cattle production costs [1], and improving individual feed efficiency could potentially influence the profitability and sustainability of beef cattle production systems [2]. Feed efficiency-related traits are controlled by different physiological and biological processes that are associated with feed intake and energy expenditure [3, 4]. In this context, variation in feed composition and availability may result in different weight gain ratios, which could potentially be impacted by genotype-by-environment (GxE) interactions [5].

Both genetic and environmental factors affect individual feed efficiency traits, leading to phenotypic variability in response to exposure to divergent environmental conditions [6–8]. Previous studies indicated that animals that are fed high-energy diets had a significantly improved feed efficiency as compared to those fed low-energy diets [9]. In this context, evaluating the evidence of GxE interactions on economically important traits is essential to selecting breeding animals with progeny showing good performance even under challenging conditions [8, 10, 11].

In spite of several studies that have evaluated feed efficiency traits under the assumption of independent genetic and environmental effects in the models used to estimate genetic parameters [12–15], the effect of GxE interactions on the genetic parameters and breeding values for feed efficiency traits, such as dry matter intake (DMI) and residual feed intake (RFI), across environmental gradients (EG) remain unknown in Nellore cattle (*Bos taurus indicus*) populations. In taurine breeds (*Bos taurus taurus)*, Durunna et al. [9] reported evidence of GxE interactions for RFI and DMI in crossbred steers fed with growing and finishing diets.

GxE interactions occur when a genotype expresses different performances in different environmental or management conditions [6]. GxE interactions can be assessed based on genetic correlations for the same trait measured across environments. Genetic correlations lower than 0.80 have been suggested to indicate a significant effect of GxE interactions on the target traits [7], with potential reductions in selection response [8]. In this context, GxE interactions represent a major challenge for breeding programs, as they are an important source of phenotypic variation in animals raised across different environments. GxE interactions also affect the genetic

variance and re-ranking among selection candidates [16, 17].

In Brazil, feed efficiency trials have been carried out on experimental stations and commercial herds across diverse geographical and climatic regions or nutritional strategies [18]. Although an average daily gain (ADG) of approximately 1 kg/day is recommended for feeding trials [5], possible differences in diet composition and environmental conditions during different feeding trials may result in differences in ADG. In this context, animals from herds with a greater selection emphasis on growth traits tend to have a higher genetic merit for ADG, which influences the feeding intake needed to meet their energy requirements and, consequently, the RFI results. Thus, comparisons of the expected estimated breeding values (EBV) between feeding trials conducted under different management conditions, with different diet compositions, and with animals from multiple herds are often challenging [9, 19]. Although most feed efficiency trials follow standard nutritional recommendations for diet formulation, the chemical composition of the diets can lead to divergent nutrient intake [20].

In beef cattle, G×E interactions have been evaluated for several traits based on reaction norm (RN) models and continuous EG levels, including the estimated average performance of contemporary groups (CG) [21–23]. The RN model links the phenotypic variability to an environmental value through the polynomial function, where the polynomial coefficients indicate the expected average EBV of the animal (intercept) and the slope coefficient represents the animal response to environmental changes [24–26]. Evaluating GxE interactions is crucial to optimize the design of breeding programs and enhance the genetic improvement of feed efficiency-related traits measured across environments. Thus, the aim of this study was to assess the level of GxE interactions for DMI and RFI in Nellore cattle based on a bi-trait RN model and evaluate the genetic correlation between DMI and RFI across EG levels.

3.2. Methods

3.2.1. Field data

The phenotypic information for feed efficiency-related traits was measured on 12,958 Nellore animals (9170 males and 3788 females) and was provided by the National Association of Breeders and Researchers (ANCP, Ribeirão Preto, SP, Brazil; www.ancp.org.br). Animals were recorded during 158 feeding trials and belonged to three commercial herds (Nelore HoRa, Cornélio Procópio, PR; Rancho da Matinha,

Uberaba, MG; and AgroNova, Barra do Garças, MT, Brazil) and two research centers (Embrapa Cerrados, Goiânia, GO; and Federal University of Uberlândia, Uberlândia, MG, Brazil). The dataset used includes phenotypic information for ADG, DMI, and RFI, following the procedures for measuring individual feed intake in beef cattle [5]. The datasets are highly connected due to the use of common sires across herds through artificial insemination (AI), with at least five genetic links across the feeding trials, which were evaluated using the AMC program [27]. The animals were raised on pasture-based systems (*Urochloa brizantha cv*). The commercial herds adopt different nutritional practices with some farms providing protein and mineral supplementation, especially during the dry season, while others providing only urea supplementation.

3.2.2. Phenotypic information

The animals received an *ad libitum* mixed diet during the feeding trials, allowing refusals from 5 to 10%. The feeding trial was performed in group pens from 2011 to 2017 with animals grouped by sex. Feed intake was recorded automatically based on the GrowSafe (www.vytelle.com) and Intergado (www.intergado.com) feeding systems. The feeding trials comprised at least 21 days for adaptation to the feedlot diet and management and an average of 64.74 ± 29.6 days for the data collection period of DMI and ADG. Animals were weighed without fasting at the beginning and end of the feeding trial and every 14 days during the experimental period. Total mixed ration (TMR) was offered over the years but differed in composition and ingredients. Diets were formulated as described by Mendes et al. [5], based on corn silage and commercial concentrate, with an average of 64% of total digestible nutrients (TDN), 13% of crude protein (CP), 76% of dry matter (DM), and formulated for different weight gains/day. The diets were adjusted based on the percentage of dry matter (%DM) to guarantee 2.17 Mcal/kg for metabolizable energy (ME) and 1.3 MJ/kg for net energy for gain (NE_g). In addition, samples of roughage, concentrate, and diet refusals were collected to evaluate their chemical composition, such as %DM, which is crucial for evaluating DMI and feed efficiency. Thus, the %DM in the diet was determined from weekly samples of the diets offered and refused.

The DMI was estimated as the feed intake per animal recorded automatically by the GrowSafe or Intergado feeding system with subsequent adjustments for dry matter content and expressed as kg/day. ADG was defined as the slope from the linear

regression of body weight (BW) on feeding trial days. Finally, residual feed intake (RFI) was estimated within each contemporary group (CG), which was defined by year and season of the feeding trial, farm, sex (males and females were evaluated in different groups) and management groups, as the difference between the observed and expected feed intake considering each animal's average metabolic body weight (MBW) and ADG, using the equation proposed by Koch et al. [19] as follows:

$$\mathrm{DMI} = \mathrm{b}_0 + \mathrm{b}_1\mathrm{AGD} + \mathrm{b}_2\mathrm{MBW} + \mathrm{e},$$

where b_0 is the intercept, b_1 and b_2 are the linear regression coefficients for ADG and MBW, respectively, and e is the residual effect representing the RFI estimate. The MBW was calculated as:

$$\mathrm{MBW} = \left[\alpha + \mathrm{b} * \left(\frac{\mathrm{DFT}}{2}\right)\right]^{0.75},$$

where α is the intercept of the regression equation which represents the body weight at the beginning of the feeding trial test; and b is the linear regression coefficient which represents the ADG; and DFT is the number of days of the feeding trial. The descriptive statistics for DMI and RFI are in Table 1.

Table 1. Descriptive statistics for dry matter intake (DMI), residual feed intake (RFI), and average daily liveweight gain (ADG) in Nellore cattle and feeding trials information.

Variable	**RFI (kg/day)**	**DMI (kg/day)**	**ADG (kg/day)**
Average	-0.001	8.496	1.205
Standard deviation	0.720	2.063	0.350
Minimum	-4.931	3.171	-0.403
Maximum	4.698	18.748	4.335
Feeding trials information			
Number of trials with only males	118		
Number of trials with only females	40		
Animals in the pedigree	23,665		
Sires	802		
Dams	6,833		
Sires with progeny records	510		
Dams with progeny records	6,349		
Number of contemporary groups	505		

3.2.3. Reaction norm models

A reaction norm model with two steps [11, 28] was considered in the present study. In the first step, the ADG during the feeding trials was used to define the EG levels, given that the actual ADG shows significant variation from the recommended ADG of 1.0 kg per day [5]. The best linear unbiased estimates (BLUE) solutions of CG for ADG were used to quantify potential differences between the management and the environmental conditions (i.e., nutritional differences) during the feeding trials.

3.2.4. First step – estimation of the environmental gradient levels

The EG levels describing the environmental condition were based on the BLUE solutions of CG for ADG as they are expected to capture differences in management and environmental factors experienced by the animals during the feeding trials. The CG solutions were obtained considering an animal model via best linear unbiased prediction (BLUP) as follows:

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

where $\mathbf{y}$ is the vector of phenotypic information for ADG; $\mathbf{b}$ is the vector of fixed effects of CG and age at feeding trials as a linear covariate, $\mathbf{a}$ is the vector of additive genetic effects assumed to follow $N(\mathbf{0}, \mathbf{A}\sigma_a^2)$, where σ_a^2 is the additive genetic variance for ADG and $\mathbf{A}$ is the pedigree relationship matrix; and $\mathbf{e}$ is the vector of residual effects assumed $N(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where $\mathbf{I}$ is an identity matrix and σ_e^2 is the residual variance. $\mathbf{X}$ and $\mathbf{Z}$ are the incidence matrices related to the systematic and additive genetic effects, respectively. The EG levels were obtained by standardizing the BLUE solutions of CG to a mean of 0 and a standard deviation (SD) of 1.

3.2.5. Second step – reaction norm model

Genetic parameters for DMI and RFI across the EG levels were estimated based on a bi-trait reaction RN model as follows:

$$\mathbf{y_{ij}} = \mathbf{Xb} + \sum_{f=0}^{1} \boldsymbol{\omega}_\mathbf{f} \boldsymbol{\Phi}_\mathbf{f}(\mathbf{EG_j}) + \sum_{f=0}^{1} \boldsymbol{\alpha}_\mathbf{fi} \boldsymbol{\Phi}_\mathbf{f}(\mathbf{EG_j}) + \mathbf{e_{ij}},$$

where $\mathbf{y_{ij}}$ is the vector of phenotypic records for DMI and RFI of the animal i on EG level j, $\mathbf{b}$ is the vector of fixed effect of CG and age of animal as a linear covariate, $\mathbf{X}$ is the incidence matrix, $\boldsymbol{\omega}_\mathbf{f}$ is the vector of the f-th fixed regression coefficient for

intercept ($f = 0$) or slope ($f = 1$) on $\mathbf{\Phi_f(EG_j)}$; $\mathbf{\Phi_f(EG_j)}$ is the vector containing the f-th linear Legendre orthogonal polynomials corresponding to EG level j ($\mathrm{EG_j}$), $\boldsymbol{\alpha_{fi}}$ is the vector of the random regression coefficients for the additive genetic effects of the intercept ($f = 0$) or slope ($f = 1$) corresponding to animal i on EG level j, and $\mathbf{e_{ij}}$ is a vector of random residual effects. The bi-trait RN was evaluated considering the residual variances as homogeneous or heterogeneous across EG levels. For heterogeneous residual variances, the different classes of residual variance were determined using the K-means clustering approach [29] and the best model with different classes of residual variance was chosen based on the Bayesian information criterion (BIC) [30]. For this we considered 11, nine, seven, six, and five classes of residual variance (Table 2). In addition, a quadratic Legendre orthogonal polynomial was tested for the best linear model (Table 3).

Table 2. Group of environment gradient (EG) considered for each class of residual variance.

Class	EG1	EG2	EG3	EG4	EG5	EG6	EG7	EG8	EG9	EG10	EG11
Eleven	CL1	CL2	CL3	CL4	CL5	CL6	CL7	CL8	CL9	CL10	CL11
Nine	CL1	CL1	CL2	CL2	CL3	CL4	CL5	CL6	CL7	CL8	CL9
Seven	CL1	CL2	CL2	CL2	CL3	CL3	CL3	CL4	CL5	CL6	CL7
Six	CL1	CL2	CL2	CL2	CL3	CL3	CL3	CL4	CL5	CL6	CL6
Five	CL1	CL2	CL2	CL2	CL3	CL3	CL3	CL4	CL5	CL5	CL5

CL: class of residual variance

Table 3. Comparison of the reaction norm models according to the log-likelihood function (LogL) and Schwarz-Bayesian information criterion (BIC) for dry matter intake (DMI) and residual feed intake (RFI) in Nellore cattle.

Model	PO	CRV	LogL	BIC	NP
Lin_hom	2	1	2988.3	3054.1	13
Lin_het_11	2	11	2805.9	3023.5	43
Lin_het_9	2	9	2811.3	2998.6	37
Lin_het_7	2	7	2830.0	2986.9	31
Lin_het_6	*2*	*6*	*2819.0*	*2960.7*	*28*
Lin_het_5	2	5	2853.2	2979.7	25
Qua_het_6	3	6	2823.6	3021.0	39

Italic: model used; CRV: Residual variance classes; Lin_hom: linear model with homogeneous residual variance; Lin_het_11: linear model with eleven classes of residual variance; Lin_het_9: linear model with nine classes of residual variance; Lin_het_7: linear model with seven classes of residual variance; Lin_het_6: grouping the last two classes of residual variance; lin_het_5: model lin_het_7 grouping the

last three classes of residual variance; Qua_het_6: quadratic model with six classes of residual variance.

The genetic variance ($\hat{\sigma}^2_{aEG_j}$) and heritability ($\hat{h}^2_{EG_j}$) estimates for DMI and RFI across the EG levels were calculated based on the following equations: $\hat{\sigma}^2_{aEG_j}=\boldsymbol{\Phi_f K_{ab} \Phi_f}'$; where $\mathbf{K_{ab}}$ is the matrix of estimated (co)variances pertaining to the random regression coefficients for the additive genetic effects of the intercept and slope. The heritability ($\hat{h}^2_{EG_j}$) for each EG level was determined as follows: $\hat{h}^2_{EG_j}=\frac{\hat{\sigma}^2_{aEG_j}}{\hat{\sigma}^2_{aEG_j}+\hat{\sigma}^2_{eEG_j}}$; $\hat{\sigma}^2_{aEG_j}$ is the additive genetic variance and $\hat{\sigma}^2_{eEG_j}$ is the residual variance considering heterogeneous variance for EG level j. The genetic correlation across EG levels ($r_{EGj,EGj'}$) was determined as follows: $r_{EGj,EGj'} = \sigma_{EGj,EGj'} / \sqrt{\hat{\sigma}^2_{aEG_j} * \hat{\sigma}^2_{aEG_{j'}}}$, where $\sigma_{EGj,EGj'}$ represents the covariance between EG level j and EG level j′, which are estimated in the same way as the additive genetic variance for each EG level.

The EBV for animal i at each EG level were obtained using the following equation: $\hat{g}_{iEG_j}=\boldsymbol{\alpha_{fi}\Phi_f}'$; where $\boldsymbol{\alpha_{fi}}$ is the vector of the additive genetic values for the intercept and slope estimates of animal i and $\boldsymbol{\Phi_f}'$ is the transposed vector of the Legendre orthogonal polynomials for each EG level. Pearson's and Spearman's rank correlation coefficient for EBV across EG levels were used to assess the reranking of 50 sires that were selected based on the highest EBV values for a medium EG level (EG = 0) and with at least five progenies recorded at low, medium, and high EG levels. To evaluate the sire coincidence between the low, medium, and high EG levels, we selected 15 sires with the highest EBV in these EG levels.

The genetic analyses were performed using the Wombat software [31] based on the average information restricted maximum likelihood (AI-REML) algorithm. The models were compared using the BIC [30], according to the following equation: $BIC = -2\log L + p\log(N - r(X))$, where p is the number of parameters estimated for the model, N is the number of phenotypic records for DMI and RFI, $r(X)$ is the rank of the coefficient matrix of fixed effects in the analyzed model, and $\log L$ is the maximum log-likelihood.

3.2.6. Environmental sensitivity

A plasticity scale was assumed based on the absolute individual value of the slope *(f_1)* and standard deviation of the population slope (σ_{f1}). The animals were classified as robust ($|f_1| < \sigma_{f1}$), plastic ($\sigma_{f1} < |f_1| < 2\sigma_{f1}$), and highly plastic ($|f_1| > 2\sigma_{f1}$).

3.3. Results and discussion

3.3.1. Comparison of reaction norm models

Based on the BIC criteria, on the one hand, a linear model considering heterogeneous residual variances with six classes (Lin_het_6) was the most appropriate model to fit the residual structure of DMI and RFI, as presented in Table 3. On the other hand, a quadratic model considering six classes of residual variance (Qua_het_6) did not improve the model fit of the data. Among the models tested, the model that assumed homogeneous residual variances (Lin_hom) showed the highest (worst) BIC value. Thus, models considering heterogeneous residual variances fitted the data better than those considering a homogeneous residual variance. To select the optimal model, Meyer [32] recommended to balance the classes of residual variance and the amount of data available, especially when the data are irregularly distributed. In this context, Carvalheiro et al. [33], Mota et al. [11] and Carvalho Filho et al. [34], suggested that for the evaluation of GxE interactions for productive and reproductive traits in Nellore cattle, RN models with heterogeneous residual variances provided a better fit to the data than homogeneous residual variances.

3.3.2. Phenotypic means of RFI, DMI, and ADG across EG levels

The phenotypic means and standard deviation by EG for the traits studied are in Table 4. For DMI and ADG, as the environment became more favorable (or less restricted), the mean values showed an increasing pattern, ranging from 6.255 to 11.498 (kg of DM/day) for DMI and from 0.680 to 1.928 (kg/day) for ADG. Following the recommendations that a diet energy intake that allows an ADG of around 1.0 kg/day during the feeding trials should be provided [5], there was a large variability in ADG across EG (Table 4). The potential physicochemical differences in the ingredients used for the formulation of the diets, which were caused by the vast climatic variation and geographic regions across the Brazilian regions where the trials were conducted,

in addition to the greater selection emphasis on body weight for specific farms, might explain the considerable variation observed in the average ADG of the animals evaluated in this study.

Table 4. Number of records (N) and descriptive statistics for dry matter intake (DMI), residual feed intake (RFI) and average liveweight gain (ADG) by environmental gradient (EG) in Nellore cattle.

EG*	N	DMI (kg DM/day)	RFI (kg DM/day)	ADG (kg/day)
		Mean ± SD		
1 (-1.50)	254	6.255 ± 1.036	0.00 ± 0.512	0.680 ± 0.191
2 (-1.20)	1312	6.907 ± 1.027	0.00 ± 0.649	0.851 ± 0.224
3 (-0.90)	1938	7.379 ± 1.531	0.00 ± 0.652	0.978 ± 0.205
4 (-0.60)	2494	7.846 ± 1.278	0.00 ± 0.651	1.093 ± 0.208
5 (-0.30)	2117	8.472 ± 2.027	0.00 ± 0.724	1.201 ± 0.222
6 (0.00)	1646	9.419 ± 2.553	0.00 ± 0.758	1.313 ± 0.236
7 (0.30)	1512	9.677 ± 1.691	0.00 ± 0.726	1.445 ± 0.289
8 (0.60)	834	10.674 ± 1.690	0.00 ± 1.026	1.566 ± 0.298
9 (0.90)	499	10.349 ± 1.578	0.00 ± 0.616	1.698 ± 0.239
10 (1.20)	151	10.353 ± 1.746	0.00 ± 0.840	1.817 ± 0.225
11 (1.50)	201	11.498 ± 0.094	0.00 ± 0.980	1.933 ± 0.292

*Values in parentheses represent the environmental gradient solution standardized for mean zero and standard deviation of 1 for ADG BLUE (best linear unbiased estimate) solutions; DM: dry matter.

In pigs, differences in feed composition have been reported as a crucial source of environmental variation and GxE interaction [35], with ADG, DMI, and RFI showing sensitivity to these variations in environmental conditions. Furthermore, improving feed efficiency in production systems generally increases environmental sensitivity, whereby differences in dietary energy concentration significantly impact feed efficiency outcomes [36]. Therefore, it is important to monitor and manage the impact of selection across different feeding trials to improve these traits successfully.

3.3.3. Heritability, phenotypic, and additive genetic variance estimates

The heritability estimates obtained for RFI and DMI across EG ranged from 0.07 to 0.41 (Fig. 1a) and from 0.26 to 0.54 (Fig. 1c), respectively. Both RFI and DMI showed similar patterns for heritability estimates across EG, i.e., first decreasing from the lower EG level (-1.5) until a medium EG level (0.60) and then increasing for higher EG (0.90) levels. Differences in heritability estimates across different EG levels occur due to the effect of GxE interactions leading to changes in genetic and phenotypic variances between EG levels for RFI (Fig. 1b) and DMI (Fig. 1d). Based on these

results, it seems that environmental factors might have a greater impact on phenotypic variations than additive genetic effects. This could be due to the enhanced EG level leading to higher ADG or to the possibility of the animal's genetic potential being masked by the environment.

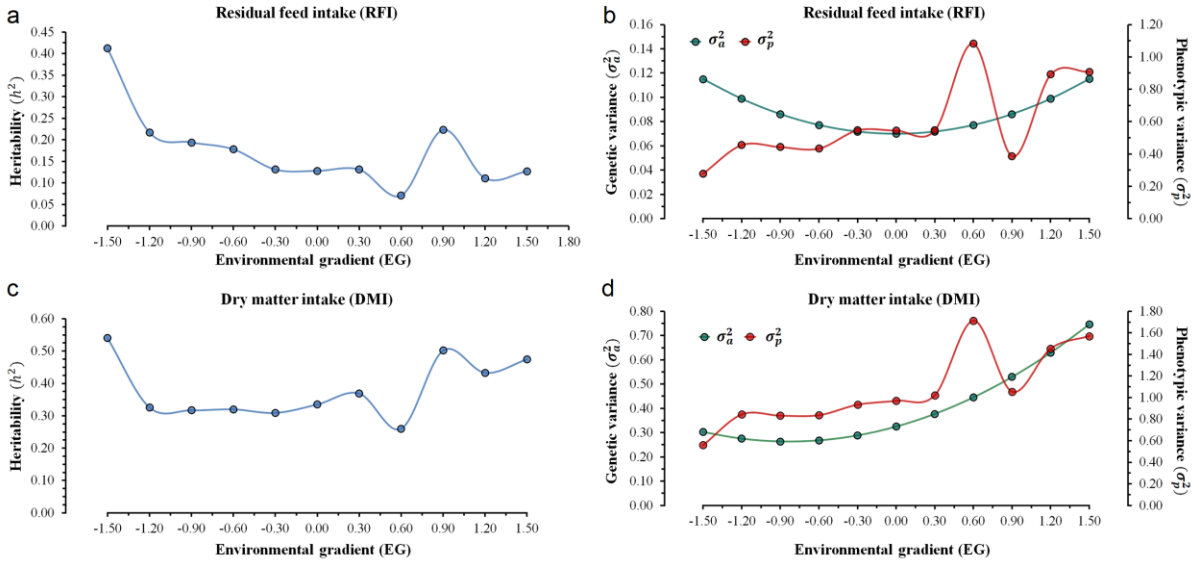

Figure 1. Heritability (h^2) for residual feed intake (RFI) (a) and dry matter intake (DMI) (c), and additive genetic variance (σ_a^2) and phenotypic variance (σ_p^2) estimates for RFI (b) and DMI (d) across environmental gradients.

The heritability estimates obtained for RFI (0.13) and DMI (0.34) for the medium EG level (0.0) were slightly different from those reported in the literature, with models that do not consider GxE interactions. However, the values across EG levels (Fig. 1a and 1c) corroborate with the expected values observed in the literature. The heritability estimates were lower than those described in the literature for RFI, ranging from 0.17 to 0.28 [4, 14, 37, 38]. For DMI, the heritability estimates were within the range observed in other Nellore cattle studies, with values ranging from 0.23 to 0.47 [4, 14, 37, 38]. However, the differences in heritability estimates are probably due to the differences between populations (as genetic parameters are population-specific due to differences in allele frequencies), environmental conditions, and the statistical model used to estimate the variance components. Higher genetic responses are expected at EG levels of 1 for RFI and at EG levels of 1 and 9 for DMI due to higher heritability estimates (Fig. 1).

In a study that estimated genetic parameters for feed efficiency traits in

crossbred cattle fed growing and finishing diets under successive feeding regimes, Durunna et al. [9] reported higher heritability values for DMI and RFI (0.43 and 0.36, respectively) with the finisher-fed regime than with the grower-fed regime (0.30 and 0.19, respectively). The authors justified these results by the greater additive genetic variation for DMI and RFI in the finisher-fed group than in the grower-fed group. The estimates of the additive genetic variance for DMI increased gradually as environmental conditions improved (0.26 to 0.75), i.e., better environments (assessed based on greater ADG) enhance the differences between animals for this trait. Regarding RFI, the additive genetic variance showed a constant behavior along the EG (0.07 to 0.11; Fig. 1).

The phenotypic variance estimated for RFI and DMI increased across EG levels, ranging from 0.27 to 1.08 and from 0.55 to 1.71, respectively. Thus, a less restricted environment (higher ADG) resulted in the largest phenotypic variability for both traits. However, for RFI, this increase was not due to the greater additive genetic variance, but it reflected the increase in environmental variance, and consequently, the heritability estimates decreased for RFI along the EG. Therefore, the influence due to variance heterogeneity was greater for RFI than for DMI, probably because RFI had a larger influence on environmental variance along the EG.

3.3.4. Genetic correlation estimates for RFI and DMI across environmental gradients

The genetic correlation estimates for the evaluated traits across EG levels ranged from 0.22 to 0.99 (0.81±0.21) for RFI and from 0.26 to 0.99 (0.83±0.19) for DMI, which indicates the presence of GxE interactions (Fig. 2). When the EG levels were more similar, we observed genetic correlation estimates higher than 0.80, and as the EG levels were more divergent, the genetic correlation decreased below 0.80, indicating the occurrence of GxE interactions across EG [7]. The lower genetic correlations between the extreme EG levels represent a significant effect of GxE interactions, potentially leading to reranking of breeding animals due to variation in EBV across EG levels [11, 39]. Genetic correlations close to 0.80 were obtained by Godinho et al. [35] for RFI and average daily feed intake in pigs during the early phase, and below 0.80 for RFI (0.74) in the grower phase with two diets. The authors reported that the genetic progress observed when applying selection under one diet might not be the same as that observed when selecting animals with another diet during these

phases due to a reranking of the genotypes. Thus, in pigs, these traits are sensitive to changes in the source of energy nutrients in the diets.

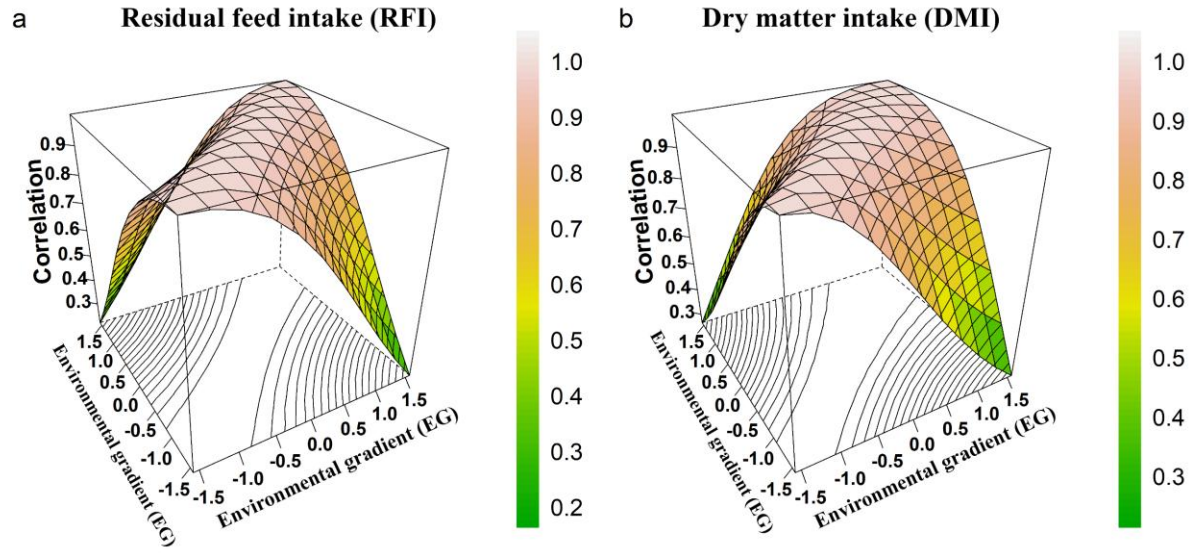

Figure 2. Genetic correlation estimates for residual feed intake (RFI) (**a**) and dry matter intake (DMI) (**b**) across environmental gradients (EG) in Nellore cattle. The colors indicate the magnitude of the genetic correlations.

Durunna et al. [9] evaluated GxE interactions for feed efficiency traits in crossbred cattle that were fed growing and finishing diets under successive feeding regimes and reported the existence of GxE interactions for DMI and RFI. Low genetic correlation estimates were observed between DMI (0.63) and RFI (0.39) measures with the two diets, indicating differential performance across environments, i.e., feed efficiency may depend on the diet provided. In this context, GxE models provide a new tool for the evaluation of traits measured in different environments where genetic heterogeneity might exist.

Genetic correlation estimates between the same trait evaluated in different environments have been used to evaluate the degree of the sensitivity of animals to environmental variations for many traits of economic importance in beef cattle [11, 16, 34, 39, 40]. When the genetic correlation between EG levels is below 0.80, the genes that control the additive genetic variance differ between environments or act differently [22]. Thus, the results presented here show evidence of GxE interactions on RFI and DMI, which reflect changes in genetic parameters according to environmental

fluctuations, i.e., different ADG in feeding trials influenced the additive genetic variance estimates of DMI and RFI.

3.3.5. Estimates of the genetic and phenotypic correlations between RFI and DMI across EG

The estimates of genetic and phenotypic correlations between RFI and DMI across EG were positive, ranging from 0.52 to 0.79 and from 0.63 to 0.81, respectively (Fig. 3). These results were expected since RFI is the residual of the regression equation between observed DMI, ADG, and MBW. Previous studies that estimated genetic and phenotypic correlations between DMI and RFI without considering GxE interactions obtained values ranging from 0.51 to 0.85 and from 0.70 to 0.81, respectively [37, 41–44]. This association between RFI and DMI has been widely explored since more efficient animals evaluated for RFI are usually animals that consume less feed, i.e., selecting animals with a negative RFI should lead to a decrease in DMI and consequently to a decrease in the nutritional requirements of the herd without changing the performance of the animals [44, 45]. Although the magnitude of the genetic correlations between RFI and DMI across environments indicates a similar genetic background between these traits, especially in more restricted environments (EG 1 to EG 3), there was a decrease in the magnitude of the correlations as the EG level increased (less restricted environmental groups).

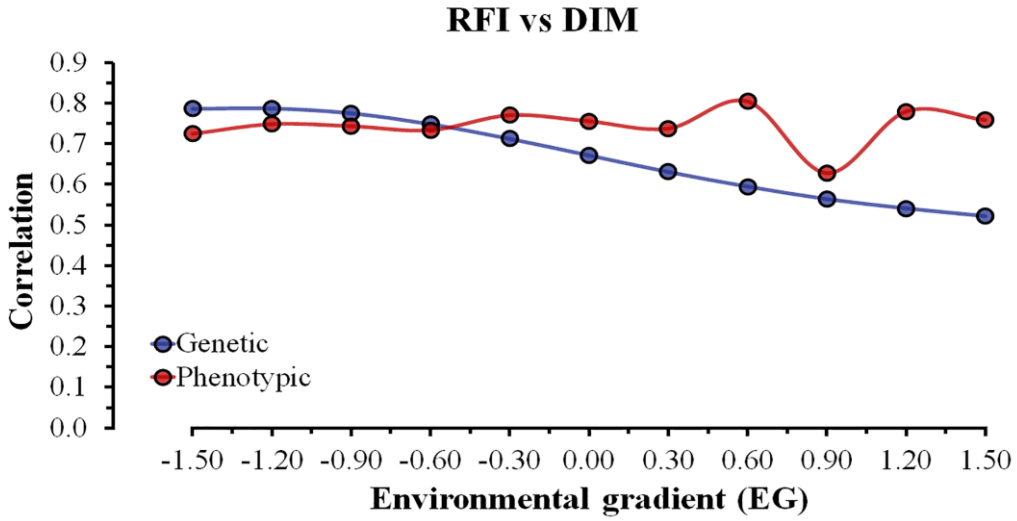

Figure 3. Genetic and phenotypic correlation estimates between residual feed intake (RFI) and dry matter intake (DMI) across environmental gradients (EG) in Nellore cattle.

In restricted EG levels (lower ADG), animals showed greater efficiency due to their lower DMI, resulting in lower RFI values. Based on these results, animals with a higher genetic potential for feed efficiency (i.e., lower RFI) tend to have lower DMI. In spite of the nutritional recommendation to provide a diet to attain an ADG of 1 kg/day during the feeding trial [5], differences in diet quality across tests can be expected due to the bromatological variation of the diets. The ability of an animal to use energy from the diet provided is associated with the rumen microbial population, converting the diet energy into body weight gain [46]. Allen [47] and Mertens [48] concluded that physical and physiological factors regulating feed intake change with increasing digestibility or diet quality. Thus, differences in diet quality across tests would affect the regulation of feed intake and consequently feed efficiency.

A reduction in feed efficiency was observed in less restricted EG conditions (Table 4), probably due to the larger residual effect produced by a higher proportion of animals from farms with greater selection emphasis on growth traits, which increases the maintenance requirements and phenotypic mean in the most favorable EG. However, for a better understanding of the genetic mechanisms that are involved in feed efficiency, the use of genomic information could provide a better explanation of the effects of GxE interactions on these traits. In the literature, there are few studies that have estimated the genetic correlations between traits related to feed efficiency, such as RFI and DMI, measured in different environments. Bi-trait RN models are a promising tool for genetic evaluation programs, as they allow the evaluation of the heterogeneity of variances in different environments for traits of economic importance. Therefore, the results presented in this study provide support and information to researchers and breeders to define appropriate selection criteria for specific environments for genetic improvement of feed efficiency-related traits in beef cattle populations that are raised in tropical conditions.

3.3.6. Genotype-by-environment interactions

The RN for 50 sires with the largest number of progeny (average of 86.04, ranging from 27 to 451) that were distributed in at least three EG levels, i.e., low, medium and high EG for RFI and DMI, showed reranking among these sires (Fig. 4 a). The effect of GxE interactions on the sensitivity of animals across EG levels, especially between extreme EG levels, was expected due to a genetic correlation

lower than 0.80 (Fig. 2). The average EBV for RFI and DMI were, respectively, -0.027 kg/DM/day and 0.103 kg/DM/day for the low EG level (-1.5), -0.053 kg/DM/day and 0.122 kg/DM/day for the medium EG level (0.0), and -0.085 kg/DM/day and 0.147 kg/DM/day for the high EG level (1.5) [see Additional file 1 Fig. S1]. The pattern of the RN reflects the significance of the GxE interactions with the genotypes that have a high plasticity, i.e., a greater sensitivity to environmental changes, being associated with steeper slopes, while the more robust genotypes have flatter slopes. Based on the slope ($f1$) solutions, 43.3% and 42.9% of the animals were classified as highly plastic to environmental changes, and 56.7% and 57.1% as more robust to environmental changes for RFI and DMI, respectively. Among the more robust animals, i.e., ($|f_1| < \sigma_{f1}$), at a medium EG level, 60% were considered better for both traits and 40% were considered better for one or neither of the traits, while 53% and only 40% were considered better for both traits at a low and high EG level, respectively.

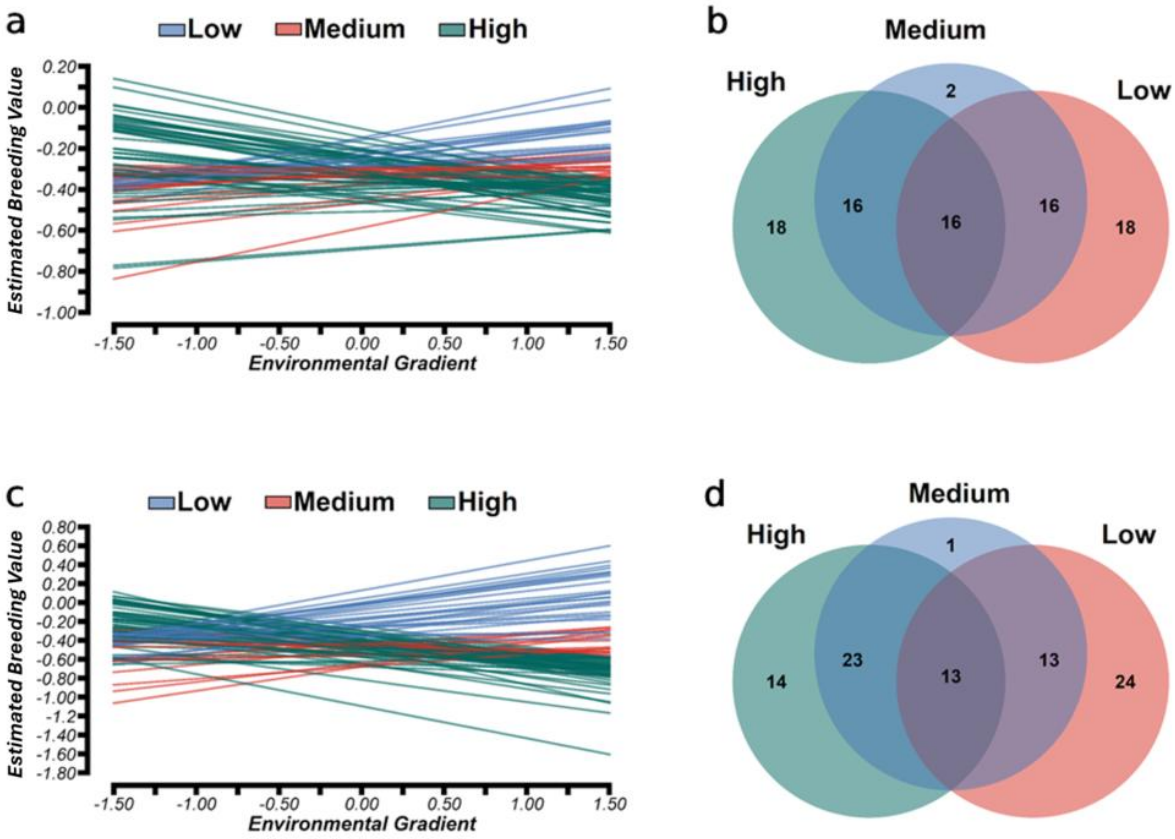

Figure 4. Reaction norms for residual feed intake (RFI) (a), and dry matter intake (DMI) (c), and the number of matching and specific sires in the environments for RFI (b) and DMI (d) considering the 50 sires with the highest number of progeny and top-ranked by EBV for RFI and DMI in the moderate environmental gradient (EG = 0.0).

The sire EBV for RFI and DMI across EG levels followed similar trends (Fig. 4 a). However, comparing the EBV of the top 15 sires for both traits [see Additional file 2, Table S1 and S2], larger differences in EBV for DMI were observed between EG levels [see Additional file 2 Table S2], as indicated by the lower genetic correlation between extreme EG levels (Fig. 2 b). Animal sensitivity to environmental variations plays a role in the phenotypic mean and the trait's genetic variance under different environmental conditions [49]. RFI and DMI showed changes in the phenotypic mean (Table 4) and genetic variance (Fig. 1) according to EG level, and it is important to carefully evaluate animal selection for feed efficiency traits as the environment becomes more divergent (e.g., greater diet variability). Spearman correlation and selection coincidence were estimated by selecting the 50 sires with at least five progenies raised at low, medium, and high EG levels and with the highest EBV to visualize the effect of GxE interactions (Table 5). When the top 50 sires that were ranked based on the EBV of animals with at least five progenies raised at low, medium, and high EG levels were considered, the Spearman's correlation values were highest between the medium EG level and either the low or high EG levels for both traits (Table 5). These results indicate that the selection of animals for feed efficiency based on data from feeding trials with an expected ADG around 1 kg/day had less impact on sires' rank across environments compared to selection based on data from extreme EG levels.

Table 5. Spearman's rank correlation and selection coincidence of top 50 sires for residual feed intake (RFI) and dry matter intake (DMI) with at least five progenies in three different environmental gradients.

Scenarios[a]	**Spearman's correlation**	**Selection coincidence**[b]	
Residual feed intake (RFI)			
Medium EG vs low EG	0.85	73.3%	
Medium EG vs high EG	0.89	80.0%	
Low EG vs high EG	0.61	53.3%	
Dry matter intake (DMI)			
Medium EG vs low EG	0.83	66.7%	
Medium EG vs high EG	0.90	70.0%	
Low EG vs high EG	0.57	40.0%	
Number of sires and progenies under different environmental gradients (EG)	Low EG	Medium EG	High EG
Number of sires with progeny	45	48	26
Number of average progeny/sire	28	58	9
Total number of progenies	1273	2792	237

[a]Medium environmental gradient (EG) is the comparison criterion for selection coincidence.

[b]Represents the percentage of sires in common between environments gradients evaluated.

The selection coincidence of the sires that were ranked based on the highest EBV and the largest number of progenies between the medium and low EG levels was 73.33% for RFI and 66.67% for DMI, and between the medium and high EG levels it was 80% for RFI and 70% for DMI (Table 5). These results indicate that most of the bulls with a superior genetic potential for feed efficiency based on the performance of their progeny maintain this potential for feed efficiency at better EG levels. However, we compared the selection of animals performed under extreme EG levels (low EG vs. high EG), and found lower Spearman correlations, 0.61 and 0.57, and lower selection coincidence percentages among the bulls, being 53.3% and 40.0%, for RFI and DMI (Table 5), respectively, which indicate a clear reranking of sires across extreme EG. These results indicate that selection decisions for feed efficiency-related traits based on EBV from more restricted environments would affect the ranking of sires in less restricted environments (and vice versa). Pearson’s correlations for the EBV of the 50 sires with the largest progeny number between low and high EG levels were equal to 0.61 for RFI (Fig. 5a) and 0.57 for DMI (Fig. 5b). These results indicate that these traits are influenced by GxE interactions when a smaller number of animals are shared among EG levels (Fig. 4). We observed that among the top 50 sires, only 16 for RFI (Fig 4a) and 13 for DMI (Fig 4b) were shared among the low, medium, and

high EG levels.

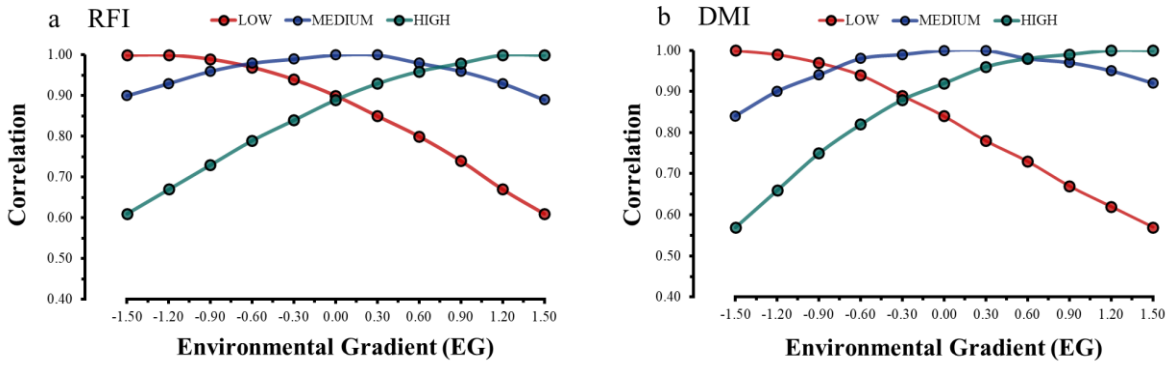

Figure 5. Pearson's correlation between the sire estimated breeding values (EBV) obtained in the low (red), moderate (blue), and high (green) environmental gradients (EG) across EG levels for residual feed intake (RFI) (a) and dry matter intake (DMI) (b).

The Pearson's correlations when comparing sire EBV for RFI and DMI at the medium EG level with those at the other EG levels ranged from 0.85 to 1.00 (Fig. 5a) and from 0.83 to 1.00 (Fig. 5b), respectively, which indicates a small change in EBV ranking. Thus, on the one hand, the top sires selected at the medium EG level are expected to maintain their genetic superiority across EG levels. On the other hand, when the sires' ranks were compared between extreme environmental conditions (i.e., low EG or high EG level), greater reclassification of sires was expected (Fig. 5). From a practical point of view, when animals are selected for RFI and DMI under feeding trials with ADG around 1 kg/day, small changes in animal performance, or in reranking of top sires for RFI and DMI are expected. The presence or evidence of GxE interactions for RFI and DMI caused small changes in the sires' EBV when selection was applied at a medium EG level, and major reranking of the top sires in extreme environments. Therefore, it is crucial to follow the recommendations proposed by Mendes et al. [5], i.e. to adequately measure or collect RFI and DMI records in feed efficiency trials under different conditions when performing national genetic evaluations for these traits.

3.4. Conclusions

Our results show clear evidence of genotype-by-environment interactions on

feed efficiency indicator traits in Nellore cattle. The breeding values for residual feed intake and dry matter intake were sensitive to environmental changes. This interaction was particularly clear in more divergent environments, e.g. when the variance in average live weight gain was substantial during the feeding trials. Furthermore, as the environmental conditions were less restricted (better environments), the expected correlated response in residual feed intake based on selection for dry matter intake is expected to decrease (and vice versa) since the genetic association between these traits was smaller in less restricted (better) environments. From a practical point of view, when animals are selected for residual feed intake and dry matter intake under feeding trials that allow an average daily gain of approximately 1 kg/day (i.e., from 0.9 to 1.4 kg/day), a slight change in animal performance is expected. However, when animals are selected for both traits in feeding trials with an ADG that is far from the average value, an increase in reranking is observed, which may be caused by the difference in nutritional levels masking the genetic potential and biasing the genetic evaluations for feed efficiency in progeny that are fed for a different ADG.

3.5. Supplementary files

The supplementary file(s) supporting the analyses presented in this chapter are available online and can be accessed through the link provided in the published version of the corresponding paper: https://doi.org/10.1186/s12711-023-00867-2

3.6. Declarations

3.6.1. Availability of data and materials

Phenotypic and genotypic information is available for academic use from the authors upon reasonable request (Dr. João Carlos G. Giffoni Filho, President of ANCP email: presidencia@ancp.org.br).

3.6.2. Funding

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CHAPTER 4: GENOME-WIDE ASSOCIATION STUDY FOR FEED EFFICIENCY INDICATOR TRAITS IN NELLORE CATTLE CONSIDERING GENOTYPE-BY-ENVIRONMENT INTERACTIONS

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Abstract

Introduction: Feed efficiency is a key factor in animal production sustainability, directly affecting production costs, environmental efficiency, and farmer profitability. The inclusion of feeding efficiency traits in cattle breeding programs has occurred later than other species due to longer life cycles and the high costs associated with measuring feed intake. However, genomic selection has facilitated the inclusion of difficult-to-measure traits in selection schemes. Thus, understanding the genetic basis of feed efficiency, particularly under varying environmental conditions, is essential.

Methods: This study aimed to identify genomic regions associated with dry matter intake (DMI) and residual feed intake (RFI) in Nellore cattle by performing a genome-wide association study (GWAS) based on single-step genomic reaction norm models that account for genotype-by-environment interactions (G×E). Phenotypic data from 23,170 young bulls and heifers were collected across 301 feed efficiency trials. Genomic windows explaining more than 1% of the total direct additive genetic variance were identified for both the intercept and slope components of the reaction norm for each trait.

Results: For RFI, ten and eleven genomic windows explained more than 1% of the genetic variance for the intercept and slope, respectively. For DMI, 12 windows were identified for the intercept and 17 for the slope. Within these regions, Multiple protein-coding genes were annotated (RFI: 66 for intercept and 47 for slope; DMI: 107 for intercept and 109 for slope), which are involved in key biological processes such as insulin, leptin, glucose, protein, and lipid metabolism; energy balance; heat stress response; feeding behavior; digestion; and nutrient absorption.

Discussion: The results highlight the functional diversity of genes involved in feed efficiency and their dynamic response to environmental variation. While certain genes remained central across environments, others were specifically important under more challenging conditions, emphasizing the role of G×E in regulating these traits. Furthermore, the magnitude and direction of SNP effects varied across environmental gradients, reinforcing the relevance of G×E. Consequently, genomic estimated breeding values for DMI and RFI also differed between environmental extremes. These findings underscore the adaptability of genetic networks to environmental

changes and are essential for refining strategies to improve feed efficiency in Nellore cattle.

Keywords: *Bos indicus*, beef cattle, dry matter intake, GWAS, residual feed intake, regulatory pathways.

4.1. Introduction

In animal production, the feed efficiency of individuals is one of the main determinants of production costs, environmental impact, and farm profitability (Nahm, 2002; Kenny et al., 2018). However, despite the strong influence of feed efficiency on the financial return of animal production (Herd and Arthur, 2009; Patience et al., 2015; McLoughlin et al., 2020), in cattle, its measurement and incorporation into selection indices began later compared to poultry and swine (Bottje and Carstens, 2009). This delay can be attributed to several factors unique to poultry and swine farming systems, including shorter life cycles (Tokach et al., 2016; Mottet and Tempio, 2017), easier management, and greater control over environmental and feeding conditions (Kyriazakis, 2011; Gilbert et al., 2015), facilitating standardization and measurement of feed efficiency with greater precision. Furthermore, these species tend to experience greater response to genetic improvement due to the shorter generation intervals and higher selection intensities due to the larger number of offspring per generation.

Given the diversity in cattle production systems and the high costs of accurately measuring individual feed intake, genomic selection (Meuwissen et al., 2001) represents a great opportunity for genetically improving difficult or expensive-to-measure traits such as feed efficiency (Pryce et al., 2014). The wide availability of genomic information has also contributed to a better understanding of the genetic architecture of complex traits, improving the accuracy of selection, particularly for traits with low heritability and more difficult or expensive to measure (Hayes et al., 2007; da Silva Neto et al., 2023), such as feed efficiency traits.

Feed efficiency is influenced by multiple underlying biological mechanisms, such as age, sex, locomotor activity, caloric increment, body composition, feeding behavior, and others (Basarab et al., 2003; Herd et al., 2004; Herd and Arthur, 2009; Haskell et al., 2019). A particularity when considering feed efficiency in ruminants is their ability to convert plant biomass into volatile fatty acids (VFA), proteins, and

vitamins due to the presence of microorganisms in the rumen that ferment and transform their feed (McLoughlin et al., 2020; Fregulia et al., 2021; Zhao et al., 2024). These microorganisms are responsible for producing most of the VFAs that serve as metabolizable energy sources for the host (Enjalbert et al., 2017; Zeineldin et al., 2018; McLoughlin et al., 2020; Zhao et al., 2024). Mechanisms related to ruminal function contribute to 23% of the variation in feed efficiency in cattle (Herd et al., 2004). Furthermore, the variability in ruminal microbiota has been associated with feed efficiency, with diet being one of the main components influencing the composition, diversity, and functionality of the rumen microbiome (Krause et al., 2013; Shabat et al., 2016; Ellison et al., 2017, 2019).

Metabolizable energy (ME) is another crucial determinant of feed efficiency in cattle, as it provides the energy needed for vital functions such as maintenance, growth and production (Reynolds et al., 2011; Marcondes et al, 2013; Arndt et al., 2015). Differences in ME utilization efficiency arise from interactions between diet composition, rumen activity and the animal's physiological processes (Moe, 1981; Reynolds et al., 2011; Hales, 2019). Diets with high energy density, such as those rich in grain, increase ME availability, improving feed efficiency by reducing losses associated with digestion and increasing nutrient assimilation (Reynolds et al., 2011, Hales et al., 2017). In contrast, fiber-rich diets often result in lower ME availability, which poses challenges for animals with higher genetic potential for growth (Nkrumah et al., 2006; Reynolds et al., 2011). Variations in microbiota, driven by diet or environmental factors and management practices, can significantly influence the efficiency of ME utilization (Shabat et al., 2016; Ellison et al., 2017, 2019). These complex interactions between ME, diet, and animal physiology highlight the challenges and opportunities in selecting cattle for greater feed efficiency in diverse production systems.

We have previously assessed genotype-by-environment interactions (G×E) for dry matter intake (DMI) and residual feed intake (RFI) in Nellore cattle using bivariate reaction norm models (RN) (Silva Neto et al., 2023). The environmental gradient (EG) was defined based on the Best Linear Unbiased Estimation (BLUE) solutions of the contemporary groups (CG) for ADG, which captures differences in nutritional, environmental, and management practices during the feed efficiency trials. Heritability estimates for DMI and RFI ranging from 0.26 to 0.54 and 0.07 to 0.41 across EG levels were obtained, respectively, with average genetic correlations for the same trait at

different EG of 0.83 and 0.81. The lowest correlations were observed between extreme levels of EG (i.e., 0.22 for RFI and 0.26 for DMI). These results indicated the presence of G×E interactions, particularly under extreme environmental conditions (low and high EG values), resulting in significant reranking of selected animals. These findings underscore the complexities involved in selecting for feed efficiency across varying environments.

Genome-wide association studies have been extensively conducted for traits related to feed efficiency traits in cattle, including DMI and RFI (de Oliveira et al., 2014; Olivieri et al., 2017; Seabury et al., 2017; Brunes et al., 2020). These studies have identified important genetic loci that influence these economically important traits in livestock (Brito et al., 2020). However, there is a notable gap in the literature regarding the inclusion of GxE interactions in these analyses, especially for beef cattle raised under varying environmental conditions, such as in the Nellore breed (Silva Neto et al., 2024). The lack of studies addressing this interaction for traits like DMI and RFI highlights the need for future research that incorporates environmental variation, enabling more precise and effective selection of animals adapted to diverse environmental scenarios.

Therefore, the primary objectives of this study were to: 1) conduct a genome-wide association study (GWAS) using a single-step genomic reaction norm model to identify specific genomic regions associated with dry matter intake and residual feed intake in Nellore cattle (*Bos taurus indicus*) considering G×E interactions; and 2) identify biological processes and metabolic pathways that regulate the expression of DMI and RFI across EG levels. The findings from this study have the potential to provide valuable information into the genetic mechanisms underlying feed efficiency in Nellore cattle, offering a deeper understanding of how environmental conditions modulate the expression of feed efficiency in Nellore cattle.

4.2. Methods

4.2.1. Field Data

Individual feed intake was measured on 23,170 Nellore animals (16,430 males and 6,740 females) from 2011 to 2023. The National Association of Breeders and Researchers (ANCP, Ribeirão Preto, SP, Brazil; www.ancp.org.br) provided the data. Animals were recorded during 301 feeding trials and belonged to 25 farms. The dataset used includes phenotypic information for ADG, DMI, and RFI, following the

procedures for measuring individual feed intake in beef cattle (Mendes et al., 2020). The herds involved are highly connected due to the use of common sires through artificial insemination (AI), with at least five genetic links across the feeding trials, which were evaluated using the AMC program (Roso and Schenkel, 2006). The animals were raised on pasture-based systems (*Urochloa brizantha cv*). The commercial herds adopted different nutritional practices with some farms providing protein and mineral supplementation, especially during the dry season, while others provided only urea supplementation.

4.2.2. Phenotypic Information

The feeding trial was performed in group pens with animals grouped by sex. Feed intake was recorded automatically based using the GrowSafe (www.vytelle.com) and Intergado (www.intergado.com) feeding systems. Detailed information on diet composition, management, and the description of the evaluated traits, i.e. ADG, DMI, and RFI, is provided in Silva Neto et al. (2023). Performance evaluations and feed intake measurements followed the recommended protocols for beef cattle, as described by Mendes et al. (2020). To ensure consistency across trials, it is recommended that the diet be provided *ad libitum* as a total mixed ration (TMR), with a homogeneous blend of forage and concentrate to prevent ingredient selection by the animals. The same standardized dietary formulation should be maintained across all trials conducted at the same facility, with only minimal adjustments in ingredient quantities. Feed refusals should be monitored and maintained between 5% and 10% of the total amount offered. The nutritional value of the diet should reflect that of high-quality pasture, with total digestible nutrient (TDN) levels aligned with the expected average daily weight gain for the animal category under evaluation. The dietary energy concentration should range from 2.4 to 2.8 Mcal of metabolizable energy per kilogram of dry matter, and the average daily gain of the group should not exceed 2.0 kg/day.

Across the feed efficiency trials, variations in dietary composition were observed among different farms, and in some cases, within the same farm across different years. In general, the forage fraction accounted for 70% to 80% of the total diet, consisting predominantly of corn or sorghum silage, although some farms used silage from *Brachiaria* grass species. The concentrate fraction primarily consisted of ground corn and ground sorghum, with the addition of protein sources in some trials, such as soybean meal, soybean hulls, and urea.

The dietary effect was indirectly accounted for by including the contemporary group (CG) as a fixed effect in the statistical model (see Section 4.2.4.1**)**. Information on geographic regions, climate conditions, and the number of animals per farm is available in Supplementary File 1 (Table S1). The descriptive statistics for these traits are reported in Table 1.

Table 1. Descriptive statistics for dry matter intake (DMI), residual feed intake (RFI), and average daily liveweight gain (ADG) during feeding traits in Nellore cattle.

Variable	RFI (kg/day)	DMI (kg/day)	ADG (kg/day)
Number of phenotypic records (and animals)	23,170	23,170	23,170
Phenotypic average	0.003	8.532	1.231
Standard deviation	0.840	2.153	0.378
Minimum	-7.109	2.519	-0.580
Maximum	6.940	20.658	3.460
Feeding trials information			
Number of trials with only males	211		
Number of trials with only females	90		
Animals in the pedigree	46,631		
Number of sires	2,833		
Number of dams	21,888		
Sires with progeny records	1,024		
Dams with progeny records	11,477		
Number of contemporary groups	760		

4.2.3. Genomic Data

A total of 18,567 animals born between 2014 and 2022 were genotyped with a SNP panel containing 65,414 markers (Clarifide® Nelore 3.0). The genotypes were imputed to a SNP panel containing 735,964 markers using the Fimpute 2.2 software (Sargolzaei et al., 2014). The reference population for genotype imputation consisted of 963 representative sires of the main Nellore lineages (i.e., Karvadi, Golias, Godhavari, Taj Mahal, Akasamu, and Nagpur). These reference sires were born between 1995 and 2015 and genotyped with the Illumina BovineHD BeadChip (Illumina Inc., San Diego, CA, USA). Before imputation, we removed non-autosomal markers and autosomal SNPs with GenCall < 0.6 to remove genotyping problems. We evaluated imputation accuracy by splitting the reference population into three folds, simulating the medium-density panel density (Mota et al., 2024), resulting in an

accuracy of 0.98 like da Silva Neto et al. (2023). The quality control of genotypes after the imputation was performed using the QCF90 software (Misztal et al., 2014). Samples and SNPs with a call rate lower than 0.90 were removed from the dataset. Markers with more than 1% of Mendelian conflicts, with unknown or redundant genomic positions, MAF lower than 0.05, and those located in non-autosomal chromosomes were also removed. After quality control, 18,567 genotyped animals and 452,283 SNPs were retained for further analyses.

4.2.4. Statistical Modelling

4.2.4.1. Reaction Norm Models

A two-step reaction norm model (Mota et al., 2020a; Silva Neto et al., 2023) was considered in the present study. In the first step, the ADG during the feeding trials was used to define the EG levels, given that the actual ADG shows significant variation from the recommended ADG of 1.0 kg per day (Mendes, 2020). The best linear unbiased estimates (BLUE) solutions of the CG for ADG were used to quantify potential differences between the management, nutritional, and environmental conditions during the feeding trials. Thus, differences in ADG among CG were used as an indirect indicator of better or worse environmental conditions, as higher ADG values were interpreted as being associated with more favorable environments. The CG was defined by year and season of the feeding trial, farm, sex (males and females were allocated to different batches). Age at the beginning of trials (415 ± 116 days of age) was considered a linear covariate in the model. The CG solutions were obtained with an animal model using the best linear unbiased predictions (BLUP) as follows:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z\alpha} + \boldsymbol{e}$$

where $\mathbf{y}$ represents the phenotypic information for ADG, $\boldsymbol{\beta}$ is a vector with the fixed effects of CG and age at feeding trails as a linear covariate; $\boldsymbol{\alpha}$ is a vector of additive genetic effects assumed to be normally distributed $\mathbf{N}(\mathbf{0}, \mathbf{A}\sigma_a^2)$, in which σ_a^2 is the additive genetic variance and **A** is the pedigree relationship matrix, and **e** is a residual vector assumed $\mathbf{N}(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where **I** is an identity matrix and σ_e^2 is the residual variance. **X** and **Z** are incidence matrices linking the records to the fixed and additive genetic effects, respectively. The EG levels were obtained by the BLUE solutions of the CG solutions standardized to a mean value of 0 and standard deviation (SD) of 1.

In the second step, to estimate the GEBV for DMI and RFI across the EG levels, a single step bi-trait genomic reaction norm model (ssBRN) was used as follows:

$$\boldsymbol{y_{ij}} = \boldsymbol{Xb} + \boldsymbol{\omega_f \Phi_f}\left(\boldsymbol{EG_j}\right) + \boldsymbol{\alpha_{fi} \Phi_f}\left(\boldsymbol{EG_j}\right) + \boldsymbol{e_{ij}}$$

where $\boldsymbol{y_{ij}}$ is the vector of phenotypic information for DMI and RFI of the animal *i* recorded at the level *j* of EG, $\boldsymbol{b}$ is the fixed effect of CG and age of animal as linear covariate, $\boldsymbol{X}$ is the incidence matrix, $\boldsymbol{\omega_f}$ are the *f*-th fixed regression coefficients (intercept and slope) on $\boldsymbol{\Phi_f}\left(\boldsymbol{EG_j}\right)$; $\boldsymbol{\Phi_f}\left(\boldsymbol{EG_j}\right)$ are the *f*-th Legendre orthogonal polynomials corresponding to *EG* level *j* ($\boldsymbol{EG_j}$), $\boldsymbol{\alpha_{fi}}$ are the random regression coefficients for additive effects of intercept and slope corresponding to animal *i* on EG level *j*, and $\boldsymbol{e_{ij}}$ is a random residual. The ssBRN was fitted considering heterogeneous residual variance across EG levels (Silva Neto et al., 2023).

The additive and residual genetic effects were considered normally distributed: $\boldsymbol{\alpha} = \{\boldsymbol{\alpha}\} \approx \mathbf{N}(\mathbf{0}, \mathbf{H} \otimes \mathbf{K})$ and $\boldsymbol{e} = \{\boldsymbol{e}\} \approx \boldsymbol{N}(\mathbf{0}, \mathbf{I} \otimes \boldsymbol{R})$, where $\boldsymbol{K}$ represents the additive genetic variance-covariance matrix attributed to the intercept and slope and $\boldsymbol{R}$ is a diagonal residual variance matrix considering heterogeneous classes; $\mathbf{I}$ is an identity matrix, $\otimes$ is the Kronecker product and $\mathbf{H}$ is a matrix combining pedigree and genomic relationship. The inverse $\mathbf{H^{-1}}$ was calculated as (Aguilar et al., 2010):

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

where, $\mathbf{A^{-1}}$ is the inverse of the pedigree-based relationship matrix, $\mathbf{A_{22}^{-1}}$ represents the inverse relationship matrix based on pedigree for the genotyped animals, and $\mathbf{G^{-1}}$ is the inverse of the genomic relationship matrix obtained according to the first method proposed by VanRaden (2008).

Posterior distribution samples of the (co)variance components were obtained through Bayesian inference using the Gibbs sampling algorithm, implemented in the GIBBSF90 software (Misztal et al., 2014). The Bayesian analyses consisted of a single chain of 500,000 cycles, a burn-in of 50,000 iterations, and storage of values every ten cycles. The convergence was evaluated through visual inspection using the Bayesian Output Analysis (Smith, 2007) and Geweke test (Geweke, 1992).

4.2.4.2. Estimates of SNPs effects in different environments

The SNP effects for the intercept and slope were obtained using weighted single-step GWAS (WssGWAS) (Wang et al., 2012). The breeding value of the genotyped animals (a_g) is a function of the SNPs effects:

$$a_g = \mathbf{Z}_g\mathbf{u}$$

where $\mathbf{Z}_g$ represents the incidence matrix of genotypes, and **u** is a vector of the SNPs effects. Thus, the variance of the genetic effects is given by:

$$\text{var}\left(a_g\right) = \text{var}\left(\mathbf{Z}_g\mathbf{u}\right) = \mathbf{Z}_g\mathbf{D}\mathbf{Z}_g'\sigma_u^2 = \mathbf{G}^*\,\sigma_a^2$$

where **D** represents the diagonal matrix of the weights for the SNP variances (**D = I** for GBLUP), σ_u^2 is the variance of the additive genetic effect obtained from each SNP when the same variance is assumed for all SNPs, σ_a^2 is the additive genetic variance of the trait, and **G*** is the weighted genomic relationship matrix:

$$\mathbf{G}^* = \frac{\text{var}\left(a_g\right)}{\sigma_a^2} = \frac{\text{var}(Zu)}{\sigma_a^2} = \mathbf{Z}_g\mathbf{D}\mathbf{Z}_g'\boldsymbol{\lambda}$$

where, $\boldsymbol{\lambda}$ is a ratio of variances $\left(\frac{\sigma_u^2}{\sigma_a^2}\right)$ or normalization constant (Vanraden et al., 2009). According to Stranden and Garrick (2009), the SNP effects ($\hat{\mathbf{u}}$) can be obtained as follows:

$$\hat{\mathbf{u}} = \frac{\sigma_u^2}{\sigma_a^2}\,\mathbf{D}\mathbf{Z}_g'\mathbf{G}^{*-1}\,\hat{a}_g = \mathbf{D}\mathbf{Z}_g'\left[\mathbf{Z}_g\mathbf{D}\mathbf{Z}_g'\right]^{-1}\hat{a}_g$$

In this way, the best predictor of the SNPs effects given by the genetic effect can be estimated. Estimates of the SNP effects can be used to estimate the individual variance of each SNP effect ($\sigma_{u,i}^2$), and apply a different weight to each SNP as follows:

$$\sigma_{u,i}^2 = u_i^2 2p_i\ (1 - p_i\ )$$

In summary, the SNP effects and weights for the WssGWAS were derived as follows (Wang et al., 2012):

1. Let **D** = **I** in the first step.
2. Calculate G = $\mathbf{Z}_g\mathbf{D}\mathbf{Z}_g'\boldsymbol{\lambda}$.
3. Calculate GEBVs for the entire data set using the ssGBLUP.

4. Convert GEBVs to SNP effects ($\hat{u}$): $\hat{u} = \boldsymbol{\lambda}\ \mathbf{DZ'}(\mathbf{Z_g}\mathbf{D}\mathbf{Z_g}'\ \boldsymbol{\lambda})^{-1}\hat{a}_{\mathbf{g}}$, where $\hat{a}_{\mathbf{g}}$ is the GEBVs of genotyped animals.
5. Calculate the weight for each SNP: $d_i = \hat{u}^2_i 2p_i(1-p_i)$, where *i* is the *i-th* SNP.
6. Normalize SNP weights to remain the total genetic variance constant.

The SNP weights were calculated iteratively through two iterations. The proportion of the genetic variance explained by moving genomic windows of 100 adjacent SNP were computed according to Wang et al. (2012):

$$\frac{\mathrm{Var}(a_i)}{\sigma^2_a} \times 100\% = \frac{\mathrm{Var}\left(\sum_{j=1} Z_j \hat{u}_j\right)}{\sigma^2_a} \times 100\%$$

where a_i is the genetic value of the i^{th} region of 100 SNP; σ^2_a is the direct additive genetic variance; Z_j is the vector with the genotype of the j^{th} SNP for all animals; and $\hat{u}_j$ is the estimated effect for the j^{th} SNP within the i-th region. Genomic windows that explained at least 1% of the genetic variance for the slopes were considered potentially associated with animals' specific responses to changes in EG. The application of SNP windows aims to approximate the structure of haplotype blocks, assuming that these windows can be inherited together (da Silva Neto et al., 2023). The choice of genomic windows consisting of 100 SNPs was based on studies in the literature conducted on Nellore cattle for economic interest traits (Fernandes Júnior et al., 2016; Marín-Garzon et al., 2021; Silva et al., 2024). However, the concept of SNP window has not been unified yet (Garcia et al., 2018).

4.2.4.3. Gene Enrichment Analyses

The proportion of the total direct additive genetic variance explained by each genomic window containing 100 SNPs was visualized using Manhattan plots, generated with the CMplot v4.3.0 package in R (Yin et al., 2021). The identified relevant genomic regions were annotated using the *Bos taurus* ARS-UCD1.2 assembly as the reference genome (Rosen et al., 2020). Candidate genes were identified based on the BioMart tool in the ENSEMBL platform (www.ensembl.org/biomart/martview/).

Gene Ontology (GO) and KEGG pathway enrichment analyses ($p < 0.05$) were conducted using the Database for Annotation, Visualization, and Integrated Discovery (DAVID; version 6.8) (Dennis et al., 2003). This was done to identify biological processes, molecular functions, cellular components, and metabolic pathways

associated with positional candidate genes. Interactions between protein-coding genes were predicted using the STRING database with default settings (Szklarczyk et al., 2015).

4.3. Results and Discussion

4.3.1. Phenotypic means of RFI, DMI, and ADG across EG levels

The phenotypic means and standard deviations by EG for the studied traits are presented in Table 2. For DMI and ADG, the mean values displayed an increasing trend as the environment became more favorable (or less restrictive), with DMI ranging from 7.14 ± 1.40 (EG 2) to 12.80 ± 3.00 (EG 17) kg of DM/day and ADG from 0.696 (EG 1) to 2.050 (EG 17) kg/day. RFI, an indicator of feed efficiency, remained relatively low and stable across the EG levels, ranging from 0.00 ± 0.687 (EG 2) to 0.30 ± 1.450 (EG 17) kg DM/day. This suggests that, in general, animals consumed more feed and grew faster in better environments, while their efficiency in converting feed to body mass remained largely consistent across EG levels. However, small fluctuations in RFI among EG levels might indicate a slight reduction in feed efficiency under highly favorable environmental conditions. This finding suggests that the animals might consume more feed than needed for growth and maintenance in highly favorable conditions, which could result in less efficient nutrient utilization. Furthermore, the phenotypic expression of RFI can also be influenced by genetic differences in feed utilization, management practices, or dietary composition across farms.

Considering the recommendation to provide a diet that supports an ADG of around 1.0 kg/day during feeding trials (Mendes et al., 2020), there was significant variability in ADG across the different EGs (Table 2). This variation may be attributed to the physicochemical differences in dietary ingredients, which likely resulted from the wide climatic and geographic diversity across the regions where the trials were held. Another important aspect is that differences in management practices (for example, individual or collective feed distribution systems and different animal densities in the pen), the genetic background of herds, and the genetic selection strategies employed by various farms also play a crucial role in ADG variability. The combination of all these factors underscores the complexity of GxE on feed efficiency traits measured in Nellore cattle (Silva Neto et al., 2023).

Table 2. Number of records (N) and descriptive statistics for dry matter intake (DMI), residual feed intake (RFI), and average daily liveweight gain (ADG) by environmental gradient level (EG) in Nellore cattle.

EG	N	DMI (Kg DM/day)	RFI (Kg DM/day)	ADG (Kg/day)
		Mean ± SD		
1	828	7.88 ± 1.76	0.18 ± 0.862	0.696 ± 0.210
2	1,371	7.14 ± 1.40	0.00 ± 0.687	0.839 ± 0.207
3	1,247	7.79 ± 1.77	0.00 ± 0.732	0.936 ± 0.241
4	1,703	7.28 ± 1.39	0.00 ± 0.626	0.960 ± 0.226
5	1,936	7.69 ± 1.42	0.00 ± 0.589	1.040 ± 0.223
6	1,923	7.72 ± 1.38	0.02 ± 0.652	1.100 ± 0.264
7	1,183	7.96 ± 1.69	0.00 ± 0.705	1.140 ± 0.215
8	2,465	8.19 ± 2.10	0.14 ± 1.030	1.210 ± 0.219
9	1,336	8.54 ± 1.69	0.02 ± 0.826	1.270 ± 0.233
10	1,031	8.48 ± 1.93	0.00 ± 0.711	1.300 ± 0.238
11	1,111	8.70 ± 2.02	0.00 ± 0.902	1.320 ± 0.251
12	1,409	9.49 ± 1.92	0.07 ± 0.798	1.410 ± 0.272
13	1,257	9.43 ± 1.84	0.00 ± 0.851	1.430 ± 0.283
14	1,915	9.42 ± 1.94	0.03 ± 0.811	1.490 ± 0.303
15	1,018	9.85 ± 1.82	0.10 ± 0.791	1.630 ± 0.276
16	812	10.80 ± 2.35	0.27 ± 1.380	1.770 ± 0.254
17	625	12.80 ± 3.00	0.30 ± 1.450	2.050 ± 0.377

4.3.2. Genome-wide Association Study and Functional Genomic Enrichment

In this study, we performed a GWAS that considered GxE interactions for DMI and RFI, an approach not yet explored in previously published work for these traits. The results are presented in terms of intercept and slope, providing a more detailed assessment of GxE interactions for these feed efficiency traits. The intercept represents the adjusted mean value of the trait, excluding environmental temporal influences (Mota et al., 2020a,b; Silva Neto et al., 2023; 2024). This can be interpreted as the genetic baseline of the trait under idealized conditions, where environmental effects are considered standard. In practice, the intercept captures the genetic variation of the trait before considering interactions with the environment or over time. On the other hand, the slope quantifies the rate of change in the trait as environmental or temporal factors vary (Oliveira et al., 2018; Mota et al., 2020a,b; Silva Neto et al., 2024). The slope measures how the trait responds to these changes, offering insight into the GxE interaction. This model allows for a deeper understanding of the dynamics

between genetic and environmental factors in animal performance, aiding in the selection of genetically more adaptable individuals to diverse environmental conditions (Silva Neto et al., 2024).

4.3.2.1. Intercept for RFI

In this study, ten genomic windows explained more than 1% of the intercept's total direct additive genetic variance for RFI (Table 3, Figure 1). These genomic windows are located on seven chromosomes: BTA1 (94.24–95.04 Mb and 95.05–95.90 Mb), BTA3 (79.33–80.65 Mb), BTA4 (71.07–72.11 Mb and 110.35–110.80 Mb), BTA5 (66.72–67.21 Mb), BTA12 (15.05–15.49 Mb and 42.95–43.49 Mb), BTA14 (10.43–10.64 Mb), and BTA18 (34.60–35.22 Mb). Within these genomic regions, a total of 71 genes were identified, including 2 miRNAs, 66 protein-coding genes, 1 snoRNA, and 2 snRNAs. These results highlight the polygenic architecture of RFI, a trait influenced by multiple genomic regions exerting additive effects on its phenotypic expression.

Brunes et al. (2020) also identified genomic windows that explained more than 0.5% of the total additive genetic variance for RFI on BTA3 (54.02 – 54.06 Mb) and BTA5 (70.28 – 71.12 Mb) in Nellore cattle. Similarly, Olivieri et al. (2017) found regions that explained more than 1.0% of the total additive genetic variance for RFI on BTA1 (100.01 – 100.02 Mb and 121.63 – 121.67 Mb), BTA4 (105.90 – 105.91 Mb and 118.56 – 118.60 Mb), and BTA18 (11.03 – 11.06 Mb). Additionally, Bolormaa et al. (2011) identified SNPs significantly associated with RFI on BTA3 (105 – 106 Mb), BTA4 (41 – 42Mb and 91 – 92 Mb), BTA5 (51 – 52 Mb, 75 – 76 Mb, 85 – 86 Mb and 110 – 111 Mb), BTA12 (55 – 56 Mb) and BTA18 (3 – 4 Mb) across seven different cattle breeds (Angus, Murray Grey, Shorthorn, Hereford, Brahman, Santa Gertrudis, and Belmont Red).

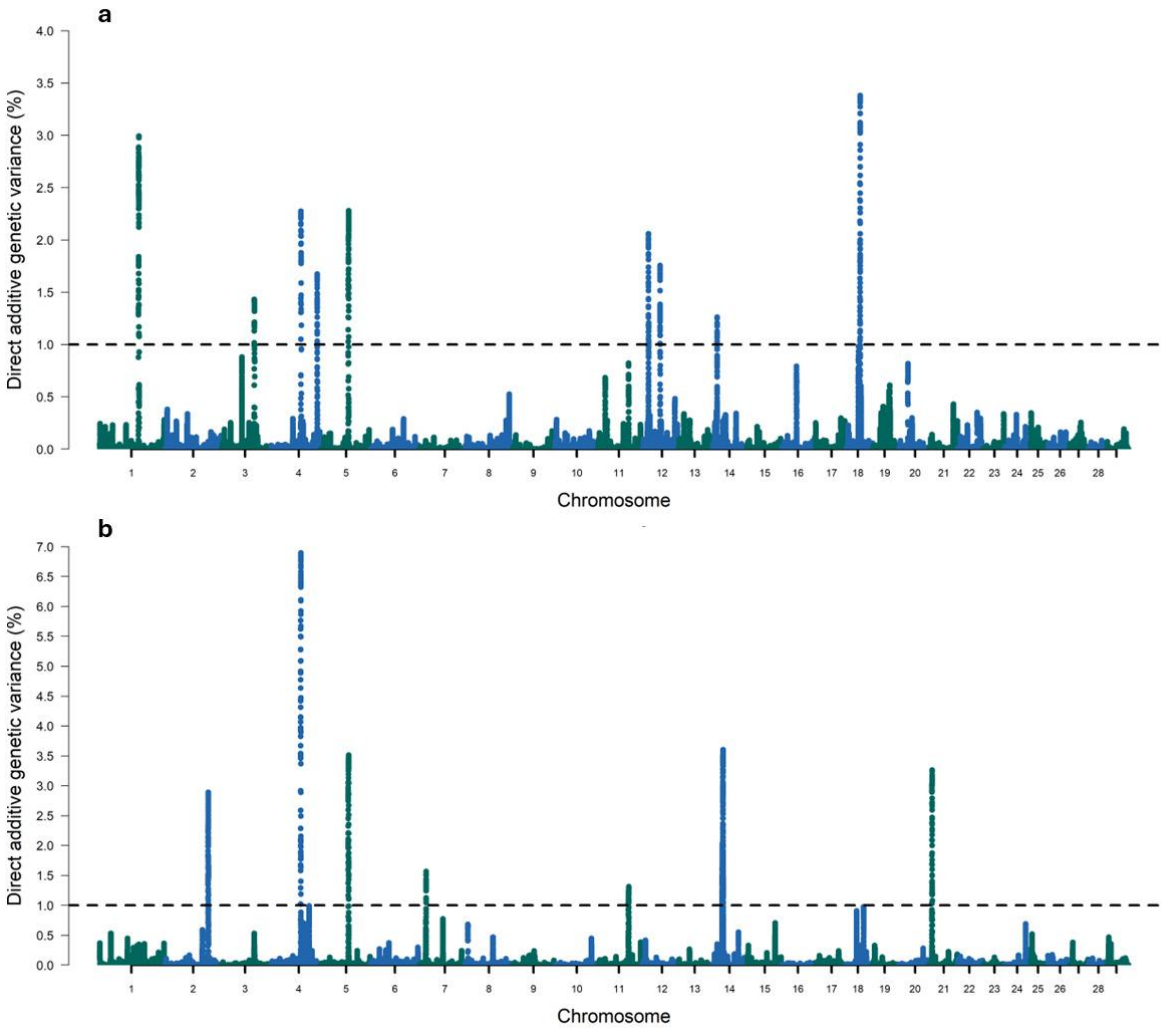

Figure 1. Manhattan plots for the proportion of the total additive genetic variance explained by each genomic window for the intercept (a) and slope (b) coefficients of the reaction norm model for residual feed intake (RFI) in Nellore cattle. The horizontal line represents the relevance threshold of 1% of the total additive genetic variance explained by each genomic window.

Table 3. List of the top genomic windows that explained more than 1% of the total direct additive genetic variance (σ_a^2) for residual feed intake (RFI - intercept).

BTA	Location (Mb)	Genes	σ_a^2 (%)
18	34.60 – 35.22	*CDH16, RRAD, CIAO2B, CES2, CES3, CES4A, CBFB, PHAF1, B3GNT9, TRADD, FBXL8, HSF4, NOL3, MATCAP1, EXOC3L1, E2F4, ELMO3, bta-mir-328, TMEM208, FHOD1, SLC9A5, PLEKHG4, KCTD19, LRRC36, TPPP3, ZDHHC1, HSD11B2, ATP6V0D1, AGRP, RIPOR1, CTCF, CARMIL2, ACD, PARD6A, ENKD1, C18H16orf86*	3.41
1	95.05 – 95.90	*GHSR, FNDC3B, TMEM212, PLD1*	3.02
5	66.72 – 67.21	*PAH, ASCL1, U1*	2.30
4	71.07 – 72.11	*GSDME, PALS2, NPY, STK31, FAM221A, ADAM22*	2.29
12	15.05 – 15.49	*NUFIP1, GPALPP1, GTF2F2, KCTD4, TPT1, SNORA31, SLC25A30*	2.08
12	42.95 – 43.49*	-	1.77
4	110.35 – 110.80	*CNTNAP2*	1.69
3	79.33 – 80.65	*LEPR, LEPROT, DNAJC6, AK4, bta-mir-101-1, JAK1, RAVER2, U2*	1.44
1	94.24 – 95.04	*SPATA16, ECT2, NCEH1, TNFSF10*	1.36
14	10.43 – 10.64	*ASAP1, CYRIB*	1.27

BTA: *Bos taurus* autosome.

Considering the genetic variance explained by the regions that accounted for at least 1% of the direct additive genetic variance, 20.63% of the total direct additive genetic variance was captured. The genomic window on BTA18 (34.60–35.22 Mb) explained the largest proportion of the total additive genetic variance for RFI, accounting for 3.41%, with 36 annotated genes identified within this region (Table 3). The genes located in this genomic window have important functions related to animal performance across different environmental conditions. For instance, the *Cadherin 16* (*CDH16*) gene is a protein primarily expressed in kidney epithelial cells (Lennartz et al., 2023). Cadherins are crucial for cell-cell adhesion, and in the kidney, *CDH16* influences nutrient reabsorption (Igarashi, 2003; Cali et al., 2012; Lennartz et al., 2023). This role is particularly relevant for RFI, as efficient nutrient utilization directly affects the energy balance and intake in cattle (Swanson and Miller, 2008). The *Ras-related glycolysis inhibitor and calcium channel regulator* (*RRAD*) gene is involved in glucose and fatty acid metabolism, which are essential for energy homeostasis (Wang et al., 2014; Lin et al., 2018; Astrain et al., 2022). Its role in regulating glucose levels and insulin signaling could affect how efficiently cattle utilize energy from feed.

Another important set of genes includes *carboxylesterase 2* (*CES2*), *carboxylesterase 3* (*CES3*), and *carboxylesterase 4A* (*CES4A*), which belong to the carboxylesterase family (Hosokawa et al., 2007; Lamego et al., 2013; Liu et al., 2021). These genes are involved in lipid metabolism and detoxification of xenobiotics (Lamego et al., 2013; Liu et al., 2021). The ability of animals to efficiently process and metabolize lipids and other dietary components is particularly important in environments with great variability in feed composition. Differences in carboxylesterase activity could influence how effectively nutrients are converted into energy, thereby affecting feed efficiency (Nawaz et al., 2024). The *heat shock factor 4* (*HSF4*) gene plays a crucial role in cellular responses to heat stress (Lang et al., 2021; Singh et al., 2024). *HSF4* regulates the expression of heat shock proteins, which are important for maintaining protein stability and cellular function under conditions of heat stress (Abbas et al., 2020; Hu et al., 2024; Lang et al., 2021; Tian et al., 2021). In tropical environments, where Nellore cattle are commonly raised, efficient heat shock protein response can preserve metabolic efficiency during hotter conditions. Therefore, genetic variations in the *HSF4* gene may account for differences in cattle responses to heat stress, potentially affecting their feed efficiency and overall energy expenditure.

In the functional enrichment analysis for the intercept of RFI, 17 processes were significantly associated (p-value < 0.05) with this trait (Table 4). These processes provide valuable information into the polygenic regulation of RFI and biological mechanisms influencing this trait. One of the biological annotated was the adult feeding behavior (GO:0008343), with the involvement of *growth hormone secretagogue receptor* (*GHSR*), *neuropeptide Y* (*NPY*), and *agouti-related peptide* (*AGRP*) genes. This process directly relates to the regulation of feeding behavior, which is crucial for determining how efficiently an individual converts feed into body mass (Muhammad, 2018; Chen et al., 2019). *GHSR* regulates energy balance by mediating the effects of ghrelin, a hormone that stimulates appetite (Klok et al., 2006; Muhammad, 2018). *NPY* and *AGRP* are also key regulators of hunger and energy homeostasis (Cansell et al., 2012; Chen et al., 2019). Variations in these genes could result in differences in feed intake and consequently, RFI. Furthermore, positive regulation of appetite (GO:0032100), with the genes *GHSR* and *NPY* (Chen et al., 2019; Zhang et al., 2019), further underscores the relationship between hunger regulation, energy intake, and RFI. In tropical environments, where feed availability

and quality may vary, the ability to regulate appetite and energy expenditure becomes critical for maintaining efficient growth and production.

The endocytosis (bta04144) was also annotated in the enrichment analyses, involving *par-6 family cell polarity regulator alpha* (*PARD6A*), *dnaj Heat Shock Protein Family* (HSP40) *Member C6* (*DNAJC6*), *ankyrin repeat and PH domain 1* (*ASAP1*), and *Phospholipase D1* (*PLD1*) genes (Table 4). This pathway plays a role in cellular nutrient uptake and signaling (Watts, 1992; Scita and Fiore, 2010). Endocytosis is essential for internalizing nutrients and cellular receptors (Grant and Donaldson, 2009), which may influence how cattle absorb and process nutrients from their feed, thus impacting feed efficiency. Another important pathway is the adipocytokine signaling pathway (bta04920), involving *TNFRSF1A-Associated via Death Domain* (*TRADD*), *NPY*, *leptin receptor* (*LEPR*), and *AGRP* genes. This pathway plays a major role in energy metabolism and the regulation of fat storage (Jiang et al., 2019; Ahmad et al., 2020). *LEPR* mediates the effects of leptin, a hormone that signals satiety and regulates energy expenditure and fat storage (Meier and Gressner, 2004; Gan et al., 2012; Suárez-Mesa et al., 2024). Disruptions or variations in this pathway could alter how efficiently cattle utilize energy from feed, influencing their feed efficiency and overall growth (Prihandini et al., 2024).

At the molecular function level, carboxylesterase hydrolase activity (GO:0052689) was also found as a significant process, with genes such as *Neutral cholesterol ester hydrolase 1* (*NCEH1*), *CES4A*, and *CES2* being annotated (Table 4). Carboxylesterases are enzymes that catalyze the hydrolysis of ester bonds, involved in lipid metabolism and the detoxification of xenobiotics (Lamego et al., 2013; Liu et al., 2021). Efficient lipid metabolism is essential for optimizing energy use, especially under varying environmental conditions where feed quality may differ. This process directly impacts how efficiently cattle convert feed into usable energy, which influences RFI.

Table 4. Significant Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses for residual feed intake (RFI - intercept).

Category	GO Term	Genes Symbol	p-value
BP	GO:0008343 - Adult feeding behavior	*GHSR, NPY, AGRP*	< 0.001
PWY	bta04920 - Adipocytokine signaling pathway	*TRADD, NPY, LEPR, AGRP*	< 0.001
MF	GO:0052689 - Carboxylic ester hydrolase activity	*NCEH1, CES4A, CES2*	0.002
CC	GO:0043231 - Intracellular membrane-bounded organelle	*HSD11B2, DNAJC6, TMEM208, PLD1, CES2*	0.005
BP	GO:0032100 - Positive regulation of appetite	*GHSR, NPY*	0.008
BP	GO:0060259 - Regulation of feeding behavior	*LEPR, AGRP*	0.013
R_PWY	R-BTA-109581 - Apoptosis	*TRADD, TNFSF10, GSDME*	0.016
R_PWY	R-BTA-3371378 - Regulation by c-FLIP	*TRADD, TNFSF10*	0.021
R_PWY	R-BTA-69416 - Dimerization of procaspase-8	*TRADD, TNFSF10*	0.021
PWY	bta04144 - Endocytosis	*PARD6A, DNAJC6, ASAP1, PLD1*	0.022
CC	GO:0005923 - Bicellular tight junction	*PARD6A, PALS2, ECT2*	0.024
R_PWY	KW-0970 - Cilium biogenesis/degradation	*TRADD, TNFSF10*	0.025
BP	R-BTA-140534 - Caspase activation via Death Receptors in the presence of ligand	*KCTD19, KCTD4, ECT2*	0.027
R_PWY	GO:0051260 - Protein homooligomerization	*TRADD, TNFSF10*	0.029
R_PWY	R-BTA-5357769 - Caspase activation via extrinsic apoptotic signalling pathway	*TRADD, TNFSF10, GSDME*	0.030
MF	R-BTA-5357801 - Programmed Cell Death	*DNAJC6, FHOD1, E2F4*	0.031
BP	GO:0019904 - Protein domain specific binding	*GHSR, TPPP3*	0.033

BP: biological process; CC: cellular component; MF: molecular function; PWY: metabolic pathway; R_PWY: Biochemical reactions and signaling.

4.3.2.2. Slope for RFI

Eleven relevant genomic windows were identified for the slope of RFI (Table 5 and Figure 1). These genomic regions were distributed across seven chromosomes, with four windows located on BTA14 (22.61–22.99 Mb, 22.99–23.45 Mb, 24.39–24.91 Mb, and 24.91–25.43 Mb), two on BTA2 (104.16–104.55 Mb and 104.65–105.41 Mb), and one genomic window each on BTA4 (70.83–71.85 Mb), BTA5 (66.51–67.03 Mb), BTA7 (16.07–16.44 Mb), BTA11 (74.02–74.67 Mb), and BTA21 (7.35–8.15 Mb). These regions overlap with genomic regions previously associated with RFI in Nellore cattle (Mota et al., 2022). These eleven genomic windows accounted for 29.65% of the total direct additive genetic variance for the slope of RFI. In total, 49 genes were mapped within these regions, of which 47 are protein-coding genes and 2 are snRNA genes.

Table 5. List of the top genomic windows that explained more than 1% of the total direct additive genetic variance (σ^2_a) for residual feed intake (RFI - slope).

BTA	Location (Mb)	Genes	σ^2_a (%)
4	70.83 – 71.85	*OSBPL3, GSDME, PALS2, NPY*	6.89
14	24.39 – 24.91	*RPL39, UBXN2B, CYP7A1, U1, SDCBP, NSMAF*	3.60
5	66.51 – 67.03	*U6, PAH, ASCL1, U1*	3.51
21	7.35 – 8.15	*LRRC28, TTC23, SYNM, IGF1R, PGPEP1L*	3.26
2	104.16 – 104.55	*XRCC5, MARCHF4, SMARCAL1*	2.89
14	22.99 – 23.45	*TMEM68, TGS1, LYN, RPS20, U1, MOS, PLAG1, CHCHD7, SDR16C5, FAM110B*	2.04
2	104.65 – 105.41	*IGFBP5, TNP1*	1.65
7	16.07 – 16.44	*INSR, ARHGEF18, PEX11G, TEX45, ZNF358, MCOLN1, PNPLA6*	1.56
14	24.91 – 25.43	*TOX*	1.49
14	22.61 – 22.99	*XKR4*	1.45
11	74.02 – 74.67	*DNMT3A, POMC, EFR3B, DNAJC27, ADCY3, CENPO, PTRHD1, NCOA1*	1.31

BTA: *Bos taurus* autosome.

The genomic window located on BTA4 (70.83–71.85 Mb) explained the largest proportion of the total additive genetic variance for the slope of RFI (6.89%), and the

Oxysterol Binding Protein-Like 3 (*OSBPL3*), *Gasdermin E* (*GSDME*), *Protein Associated with Lin Seven 2* (*PALS2*), and *NPY* genes were identified within this window. *OSBPL3* is involved in lipid metabolism and intracellular lipid transport, suggesting a potential effect in energy homeostasis and efficiency (Song et al., 2012), which are processes that have an influence in RFI. *GSDME* is known for its role in programmed cell death (pyroptosis), which could influence energy expenditure through tissue turnover and inflammatory responses, thereby impacting metabolic efficiency (Zhu et al., 2024). *NPY* is a key regulator of appetite and energy balance, making it a direct candidate for influencing feed intake and energy utilization (Cansell et al., 2012; Chen et al., 2019). The involvement of these genes in some metabolic pathways related to energy balance and tissue homeostasis could explain their contribution to the genetic variance in the slope of RFI. This suggests that variations in the expression or function of these genes might modulate how Nellore cattle adjust their feed intake and energy expenditure in response to environmental or nutritional changes, thereby affecting the slope of the reaction norm for RFI.

The functional enrichment analyses of genes found in the genomic windows that explained the largest proportion of the genetic variance of the slope of RFI in Nellore cattle are displayed in Table 6. Twenty-nine mechanisms were significantly associated with the slope of RFI (p-value < 0.05), including the positive regulation of the MAPK cascade (GO:0043410), which plays a crucial role in mediating cellular responses to environmental stimuli (Bardwell, 2006; Meng and Zhang, 2013). The MAPK signaling pathway influences growth, cell proliferation, and stress response, essential for maintaining metabolic balance in varying environmental conditions (Bardwell, 2006; Meng and Zhang, 2013; Yue and López, 2020). The *v-mos Moloney murine sarcoma viral oncogene homolog* (*MOS)*, *LYN proto-oncogene*, *Src family tyrosine kinase (LYN)*, *Insulin receptor (INSR)*, *Gasdermin E (GSDME)*, and *Insulin-like growth factor 1 receptor (IGF1R)* genes were annotated and involved in the positive regulation of the MAPK cascade (Gonzalez-Garcia et al., 2014; Werner, 2023). *MOS* is a key regulator of the MAPK pathway, primarily known for its role in cellular proliferation and differentiation (Gonzalez-Garcia et al., 2014). Changes in the regulation of the MAPK pathway by *MOS* could alter energy balance and metabolic rate (Okazaki and Sagata, 1995; Adhikari and Cullen, 2014), which are critical for feed efficiency. *LYN* plays a significant role in the activation of the MAPK pathway (Avila et al., 2012), and variations in the expression of *LYN* may influence how cattle respond

to feeding under different environmental conditions, possibly by affecting energy expenditure and metabolic adjustments. The *LYN* gene was also found in a GWAS study for growth traits in Nellore cattle (Terakado et al., 2017). The *INSR* gene is associated with the insulin signaling pathway, closely interacting with the MAPK cascade (Zhang et al., 2011; Werner, 2023). Insulin is a key regulator of glucose metabolism and energy homeostasis (Payankaulam et al., 2019). Genetic variation in *INSR* may influence how cattle manage nutrient absorption, storage, and overall energy balance. Given the importance of glucose metabolism on efficient feed use, *INSR* variants could impact feed efficiency by modulating energy use under different environmental conditions, thus influencing RFI. Alongside *INSR*, the *IGF1R* gene also plays a pivotal role in growth, development, and nutrient partitioning, all of which are integral to feed efficiency (Yang et al., 2019; Mota et al., 2022). The interaction of *IGF1R* with the MAPK pathway underlines its importance in mediating growth and metabolic responses, particularly in response to environmental changes (Yang et al., 2019). Mutations in *IGF1R* may alter the cattle's ability to utilize feed for efficient growth, affecting how well animals adapt their nutrient use in response to varying environmental conditions.

Other biological processes associated with *IGF1R* and *INSR,* including insulin-like growth factor I binding (GO:0031994), insulin receptor activity (GO:0005158), and insulin receptor complex (GO:0005899) were found in the enrichment analysis for the slope of RFI (Table 6). These processes are crucial for regulating energy balance and nutrient partitioning. Insulin-like growth factor I (IGF-I) is critical for growth and metabolic regulation, with its binding modulating activity in pathways central to nutrient efficiency (Pereira et al., 2016; Rieger and Connor, 2021; Díaz Del Moral et al., 2022). More efficient insulin receptor activity could allow cattle to optimize energy use, particularly in response to environmental challenges, ensuring consistent feed efficiency. This pathway may be important in determining how well animals adapt their nutrient utilization strategies in response to GxE interactions. The slope of RFI, which reflects the animal's efficiency in utilizing feed under different conditions, could thus be significantly influenced by the genetic variation within the insulin and IGF signaling pathways.

Table 6. Significant Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses for residual feed intake (RFI - slope).

Category	GO Term	Gene Symbol	p-value
BP	GO:0043410 - Positive regulation of MAPK cascade	*MOS, LYN, INSR, GSDME, IGF1R*	< 0.001
MF	GO:0031994 - Insulin-like growth factor I binding	*IGFBP5, INSR, IGF1R*	< 0.001
R_PWY	R-BTA-211976 - Endogenous sterols	*NCOA1, POMC, CYP7A1*	0.001
R_PWY	R-BTA-8957322 - Metabolism of steroids	*NCOA1, POMC, OSBPL3, CYP7A1*	0.001
R_PWY	R-BTA-192105 - Synthesis of bile acids and bile salts	*NCOA1, OSBPL3, CYP7A1*	0.001
R_PWY	R-BTA-194068 - Bile acid and bile salt metabolism	*NCOA1, OSBPL3, CYP7A1*	0.002
R_PWY	R-BTA-556833 - Metabolism of lipids	*NCOA1, POMC, OSBPL3, TGS1, PNPLA6, CYP7A1*	0.003
R_PWY	R-BTA-211897 - Cytochrome P450 - Arranged by substrate type	*NCOA1, POMC, CYP7A1*	0.003
CC	GO:0005899 - Insulin receptor complex	*INSR, IGF1R*	0.006
MF	GO:0005009 - Insulin receptor activity	*INSR, IGF1R*	0.006
PWY	bta04923 - Regulation of lipolysis in adipocytes	*INSR, NPY, ADCY3*	0.007
PWY	bta04213 - Longevity regulating pathway - multiple species	*INSR, ADCY3, IGF1R*	0.007
MF	GO:0043559 - Insulin binding	*INSR, IGF1R*	0.008
PWY	bta04913 - Ovarian steroidogenesis	*INSR, ADCY3, IGF1R*	0.008
R_PWY	R-BTA-211945 - Phase I - Functionalization of compounds	*NCOA1, POMC, CYP7A1*	0.009
MF	GO:0031995 - Insulin-like growth factor II binding	*IGFBP5, INSR*	0.014
MF	GO:0043560 - Insulin receptor substrate binding	*INSR, IGF1R*	0.016
PWY	bta04211 - Longevity regulating pathway	*INSR, ADCY3, IGF1R*	0.016
PWY	bta04914 - Progesterone-mediated oocyte maturation	*MOS, ADCY3, IGF1R*	0.017
R_PWY	R-BTA-193807 - Synthesis of bile acids and bile salts via 27-hydroxycholesterol	*NCOA1, CYP7A1*	0.020
MF	GO:0043548 - Phosphatidylinositol 3-kinase binding	*INSR, IGF1R*	0.025
R_PWY	R-BTA-193368 - Synthesis of bile acids and bile salts via 7alpha-hydroxycholesterol	*NCOA1, CYP7A1*	0.028
BP	GO:0030335 - Positive regulation of cell migration	*LYN, INSR, IGF1R*	0.028
PWY	bta04114 - Oocyte meiosis	*MOS, ADCY3, IGF1R*	0.030
BP	GO:0071333 - Cellular response to glucose stimulus	*CYP7A1, IGF1R*	0.035
PWY	bta04915 - Estrogen signaling pathway	*NCOA1, POMC, ADCY3*	0.035
R_PWY	R-BTA-211859 - Biological oxidations	*NCOA1, POMC, CYP7A1*	0.036
R_PWY	R-BTA-400206 - Regulation of lipid metabolism by PPARalpha	*NCOA1, TGS1*	0.036
MF	GO:0005184 - Neuropeptide hormone activity	*POMC, NPY*	0.048

BP: biological process; CC: cellular process; MF: molecular function; PWY: metabolic pathaway; R_PWY: Biochemical reactions and signaling.

Enriching pathways related to bile acid and salt metabolism (R-BTA-194068) further emphasizes the importance of lipid homeostasis in determining feed efficiency. Bile acids are essential for fat digestion and absorption, and the *cytochrome P450 family 7 subfamily A member 1* (*CYP7A1*) and *nuclear receptor coactivator 1* (*NCOA1*) genes are key regulators of bile acid metabolism (Jia et al., 2024). Variations in the efficiency of bile acid metabolism could influence the absorption of nutrients (Jia et al., 2021), particularly lipids, which are critical for energy balance. Animals with optimized bile acid metabolism may be better able to maintain feed efficiency under fluctuating environmental conditions, contributing to differences in the RFI slope.

The neuropeptide hormone activity (GO:0005184) was also one of the processes significatively associated with RFI. The ability of animals to regulate feed intake through neuroendocrine mechanisms may be a key determinant of how efficiently they convert feed into body mass, particularly when facing environmental variability. The *pro-opiomelanocortin* (*POMC*) and *adrenocorticotropic hormone* (*ACTH*) genes play fundamental roles in appetite regulation and energy balance (Millington, 2007; Hasenmajer et al., 2021). This regulation could explain variations in feed efficiency as environmental conditions change, influencing the slope of RFI.

The regulation of lipid metabolism by PPARalpha (R-BTA-400206) pathway, which includes *Nuclear Receptor Coactivator 1* (*NCOA1*) and *Trimethylguanosine Synthase* (*TGS1*), highlights the role of lipid metabolism in RFI expression. PPARalpha (peroxisome proliferator-activated receptor alpha) is a critical regulator of lipid metabolism, particularly in response to fasting or limited nutrient availability (Lefebvre et al., 2006; Bougarne et al., 2018; Fuior et al., 2023). This pathway may influence how animals utilize lipids for energy under stressful or nutrient-limited conditions, which could affect the slope of RFI by enabling animals to maintain energy balance and feed efficiency across different environments.

4.3.2.3. Intercept for DMI

Twelve genomic windows explaining more than 1% of the total direct additive genetic variance of the intercept for DMI were identified as shown in Table 7 and Figure 2. These genomic regions are located on seven chromosomes: BTA1 (95.09–95.95), BTA4 (70.88–71.88 Mb), BTA6 (36.02–36.57 Mb and 37.15–37.95 Mb), BTA8 (67.24–67.72 Mb), BTA14 (22.90–23.31 Mb and 23.33–23.89 Mb), BTA18 (34.83 – 35.42 Mb), BTA20 (9.15 – 9.83 Mb), BTA21 (68.47 – 68.77 Mb) and BTA29 (46.18 – 47.10 Mb

and 48.54 – 50.15 Mb). Some regions in these chromosomes were also identified as associated with DMI in other GWAS studies with cattle (Serão et al., 2013; de Oliveira et al., 2014; Brunes et al., 2020; Mota et al., 2022). A total of 112 genes were identified within these genomic windows, including 107 protein-coding genes, three miRNAs, and two snRNAs. These findings highlight the polygenic nature of DMI, a trait influenced by numerous genomic regions that collectively contribute to its phenotypic expression, as RFI.

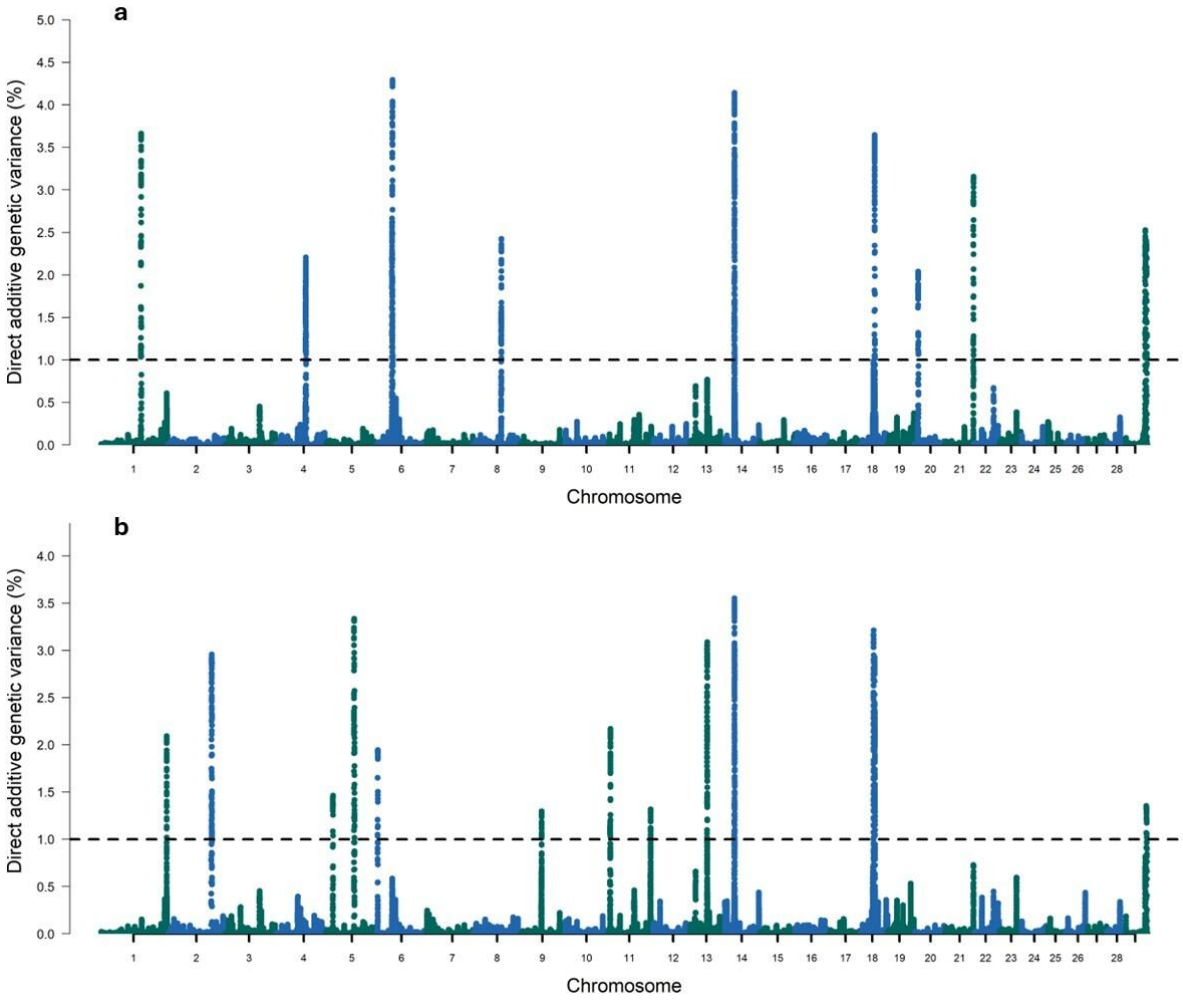

Figure 2. Manhattan plots for the proportion of the total additive genetic variance explained by each genomic window for the intercept (a) and slope (b) coefficients of the reaction norm model for dry matter intake (DMI) in Nellore cattle. The horizontal line represents the relevance threshold of 1% of the total additive genetic variance explained by each genomic window.

Table 7. List of the top genomic windows that explained more than 1% of the total direct additive genetic variance (σ_a^2) for dry matter intake (DMI - intercept).

BTA	Location (Mb)	Genes	σ_a^2 (%)
6	37.15 – 37.95	*LAP3, MED28, FAM184B, DCAF16, NCAPG, LCORL*	4.29
14	22.90 – 23.31	*XKR4, TMEM68, TGS1, LYN, RPS20, U1, MOS*	4.14
1	95.09 – 95.95	*FNDC3B, TMEM212, PLD1*	3.66
18	34.83 – 35.42	*E2F4, ELMO3, bta-mir-328, TMEM208, FHOD1, SLC9A5, PLEKHG4, KCTD19, LRRC36, TPPP3, ZDHHC1, HSD11B2, ATP6V0D1, AGRP, RIPOR1, CTCF, CARMIL2, ACD, PARD6A, ENKD1, C18H16orf86, GFOD2, RANBP10, TSNAXIP1, CENPT, THAP11, NUTF2, EDC4, NRN1L, PSKH1, PSMB10*	3.64
21	68.47 – 68.77	*TDRD9, RD3L, ASPG, MIR203B, KIF26A*	3.15
6	36.02 – 36.57	*FAM13A, HERC3, NAP1L5, PYURF, HERC5, HERC6, PPM1K, ABCG2, U6, bta-mir-10170*	2.66
29	46.18 – 47.10	*CPT1A, MRPL21, IGHMBP2, MRGPRF, TPCN2, CCND1, LTO1, FGF19, FGF4, FGF3*	2.52
8	67.24 – 67.72	*SLC18A1, ATP6V1B2, LZTS1*	2.42
29	48.54 – 50.15	*U6, CARS1, NAP1L4, PHLDA2, SLC22A18, CDKN1C, KCNQ1, TRPM5, TSSC4, TSPAN32, ASCL2, TH, INS, IGF2, TNNT3, LSP1, PRR33, TNNI2, SYT8, CTSD, IFITM10, DUSP8, MOB2, BRSK2*	2.40
4	70.88 – 71.88	*OSBPL3, GSDME, PALS2, NPY*	2.21
20	9.15 – 9.83	*ZNF366, PTCD2, MRPS27, MAP1B*	2.04
14	23.33 – 23.89	*PLAG1, CHCHD7, SDR16C5, SDR16C6, PENK, U6, BPNT2*	1.70

BTA: *Bos taurus* autosome.

The relevant genomic regions for DMI explained 34.83% of the overall direct additive genetic variance. The genomic window located on BTA6 (37.15–37.95 Mb) explained the largest portion of additive genetic variance, accounting for 4.29%, with six annotated genes identified within this region (Table 7). The *Leucine Aminopeptidase 3* (*LAP3*) is involved in protein degradation, processing and regulating peptide breakdown (Yao et al., 2021; Wang et al., 2024). It has been associated with growth traits in Holstein cattle and Yak (Bos grunniens) (Yao et al., 2019; Wang et al., 2024). Given its role in protein metabolism, *LAP3* may influence the efficiency of cattle utilizing nutrients, which is directly related to DMI. Therefore, efficient protein metabolism could enable animals to optimize their intake for growth and maintenance under varying environmental conditions. Another important gene widely associated with growth traits and feed intake in cattle is *Non-SMC Condensin I Complex Subunit G* (*NCAPG)* (Hoshiba et al., 2013; Lindholm-Perry et al., 2013). Studies have shown that polymorphisms in *NCAPG* are linked to ADG and DMI in cattle (Angus, Hereford, Simmental, Limousin, Cha rolais, Gelbvieh and Red Angus) (Lindholm-Perry et al., 2011; Lindholm-Perry et al., 2013; Seabury et al., 2017). This gene is involved in cell

cycle regulation and it has been associated with growth rate and body size in several cattle breeds (Setoguchi et al., 2011; Zhang et al., 2016). *NCAPG* influences feed intake by modulating growth demands, where larger or faster-growing animals require more feed to meet their energy needs. This makes it a strong candidate gene for influencing DMI in response to average environmental conditions.

The *Ligand Dependent Nuclear Receptor Corepressor Like* (*LCORL*) is a transcription factor associated with skeletal growth and body size in humans, horses, and cattle (Utsunomiya et al., 2013; Al-Mamun et al., 2015). *LCORL* has been linked to growth traits and feed efficiency in cattle, often acting in concert with *NCAPG* (La et al., 2019). Polymorphisms in *LCORL* have been correlated with feed intake and gain, particularly in beef cattle (Angus, Hereford, Simmental, Limousin, Cha rolais, Gelbvieh and Red Angus) (Lindholm-Perry et al., 2013). Its role in skeletal growth may be crucial for determining body size and the corresponding feed requirements, thereby influencing DMI. The *DDB1 and CUL4 Associated Factor 16* (*DCAF16*) is part of the ubiquitin-proteasome pathway, essential for protein degradation and cellular homeostasis (Zhang et al., 2021). By influencing protein degradation, it indirectly impacts growth rate and metabolic efficiency (Mistry et al., 2020; Zhang et al., 2021), potentially altering the energy requirements and feed intake of cattle. Since protein metabolism is energy-intensive, variations in this gene may affect how animals convert feed into growth.

In the functional enrichment analysis, 53 processes were found to be significantly associated (p-value < 0.05) with the intercept of DMI (Table 8). These processes offer important insights into the polygenic control of DMI and the biological pathways affecting its regulation. In comparison to the significant processes identified for RFI, DMI had a greater number of associated processes. This can be attributed to the larger number of relevant genomic windows identified for DMI, which also reflected in a higher number of genes found and involved in regulating this trait.

Table 8. Significant Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses for dry matter intake (DMI - intercept).

Category	GO Term	Gene Symbol	p-value
R_PWY	R-BTA-74752 - Signaling by Insulin receptor	*FGF19, ATP6V1B2, ATP6V0D1, CTSD, FGF3, FGF4, INS*	< 0.001
R_PWY	R-BTA-9006934 - Signaling by Receptor Tyrosine Kinases	*FGF19, ATP6V1B2, IGF2, ATP6V0D1, CTSD, FGF3, FGF4, INS*	< 0.001
R_PWY	R-BTA-77387 - Insulin receptor recycling	*ATP6V1B2, ATP6V0D1, CTSD, INS*	< 0.001
BP	GO:0009887 - Animal organ morphogenesis	*FGF19, E2F4, PHLDA2, FGF3, FGF4*	< 0.001
R_PWY	R-BTA-2428928 - IRS-related events triggered by IGF1R	*FGF19, IGF2, FGF3, FGF4*	< 0.001
R_PWY	R-BTA-2428924 - IGF1R signaling cascade	*FGF19, IGF2, FGF3, FGF4*	< 0.001
R_PWY	R-BTA-2404192 - Signaling by Type 1 Insulin-like Growth Factor 1 Receptor (IGF1R)	*FGF19, IGF2, FGF3, FGF4*	< 0.001
R_PWY	R-BTA-74751 - Insulin receptor signalling cascade	*FGF19, FGF3, FGF4, INS*	< 0.001
BP	GO:0043410 - Positive regulation of MAPK cascade	*MOS, LYN, IGF2, GSDME, INS*	0.001
CC	GO:0005737 - Cytoplasm	*RIPOR1, BRSK2, TSSC4, FHOD1, IGHMBP2, NCAPG, FGF3, FGF4, HERC5, HERC3, CCND1, TSNAXIP1, E2F4, PHLDA2, LZTS1, HERC6, LYN, TDRD9, DUSP8, NAP1L4, THAP11, MED28, MOS, TPPP3, TH, KIF26A, FGF19, MOB2, RANBP10, ELMO3, LAP3, CARS1, GSDME*	0.001
BP	GO:0001934 - Positive regulation of protein phosphorylation	*CCND1, FGF19, MOB2, FGF3, FGF4*	0.002
R_PWY	R-BTA-1257604 - PIP3 activates AKT signaling	*FGF19, FGF3, PSMB10, FGF4, INS*	0.003
R_PWY	R-BTA-199418 - Negative regulation of the PI3K/AKT network	*FGF19, FGF3, FGF4, INS*	0.003
R_PWY	R-BTA-6811558 - PI5P, PP2A and IER3 Regulate PI3K/AKT Signaling	*FGF19, FGF3, FGF4, INS*	0.003
R_PWY	R-BTA-9006925 - Intracellular signaling by second messengers	*FGF19, FGF3, PSMB10, FGF4, INS*	0.004
MF	GO:0005104 - Fibroblast growth factor receptor binding	*FGF19, FGF3, FGF4*	0.005
BP	GO:0010628 - Positive regulation of gene expression	*PLAG1, FGF19, CTCF, FGF3, FGF4, INS*	0.005
PWY	bta05218 - Melanoma	*CCND1, FGF19, FGF3, FGF4*	0.005
PWY	bta04014 - Ras signaling pathway	*FGF19, IGF2, PLD1, FGF3, FGF4, INS*	0.006
BP	GO:0030334 - Regulation of cell migration	*FGF19, PHLDA2, FGF3, FGF4*	0.007

R_PWY	R-BTA-109704 - PI3K Cascade	*FGF19, FGF3, FGF4*	0.007
R_PWY	R-BTA-112399 - IRS-mediated signalling	*FGF19, FGF3, FGF4*	0.008
MF	GO:0003779 - actin binding	*MAP1B, TNNT3, TNNI2, LSP1, MED28*	0.008
BP	GO:0051781 - Positive regulation of cell division	*IGF2, FGF3, FGF4*	0.009
CC	GO:0045121 - Membrane raft	*LYN, KCNQ1, CTSD, ABCG2*	0.014
PWY	bta04010 - MAPK signaling pathway	*FGF19, IGF2, DUSP8, FGF3, FGF4, INS*	0.014
BP	GO:0006006 - Glucose metabolic process	*KCNQ1, IGF2, INS*	0.015
R_PWY	R-BTA-917937 - Iron uptake and transport	*ATP6V1B2, ATP6V0D1, ABCG2*	0.016
BP	GO:0008543 - Fibroblast growth factor receptor signaling pathway	*FGF19, FGF3, FGF4*	0.016
R_PWY	R-BTA-162582 - Signal Transduction	*CPT1A, FGF19, NPY, PENK, ATP6V1B2, IGF2, ATP6V0D1, CTSD, FGF3, PSMB10, FGF4, INS*	0.019
PWY	bta04015 - Rap1 signaling pathway	*PARD6A, FGF19, FGF3, FGF4, INS*	0.020
MF	GO:0008083 - Growth factor activity	*FGF19, IGF2, FGF3, FGF4*	0.025
PWY	bta04810 - Regulation of actin cytoskeleton	*MOS, FGF19, FGF3, FGF4, INS*	0.025
BP	GO:0008343 - Adult feeding behavior	*NPY, AGRP*	0.026
R_PWY	R-BTA-190236 - Signaling by FGFR	*FGF19, FGF3, FGF4*	0.026
R_PWY	R-BTA-5658623 - FGFRL1 modulation of FGFR1 signaling	*FGF3, FGF4*	0.026
R_PWY	R-BTA-382551 - Transport of small molecules	*SLC9A5, SLC22A18, ATP6V1B2, ATP6V0D1, PSMB10, ABCG2*	0.031
CC	GO:0016324 - Apical plasma membrane	*PARD6A, KCNQ1, ATP6V1B2, PLD1, ABCG2*	0.032
CC	GO:0005861 - Troponin complex	*TNNT3, TNNI2*	0.035
PWY	bta05224 - Breast cancer	*CCND1, FGF19, FGF3, FGF4*	0.036
BP	GO:1902600 - Proton transmembrane transport	*SLC9A5, ATP6V1B2, ATP6V0D1*	0.037
PWY	bta05226 - Gastric cancer	*CCND1, FGF19, FGF3, FGF4*	0.038
BP	GO:0046628 - Positive regulation of insulin receptor signaling pathway	*IGF2, INS*	0.038
PWY	bta04151 - PI3K-Akt signaling pathway	*CCND1, FGF19, IGF2, FGF3, FGF4, INS*	0.039
BP	GO:0007218 - Neuropeptide signaling pathway	*NPY, PENK, AGRP*	0.043
MF	GO:0005159 - Insulin-like growth factor receptor binding	*IGF2, INS*	0.043
R_PWY	R-BTA-5683057 - MAPK family signaling cascades	*FGF19, FGF3, PSMB10, FGF4*	0.046
R_PWY	R-BTA-5654228 - Phospholipase C-mediated cascade; FGFR4	*FGF19, FGF4*	0.047
R_PWY	R-BTA-5654219 - Phospholipase C-mediated cascade: FGFR1	*FGF3, FGF4*	0.047

R_PWY	R-BTA-190242 - FGFR1 ligand binding and activation	*FGF3, FGF4*	0.047
R_PWY	R-BTA-190322 - FGFR4 ligand binding and activation	*FGF19, FGF4*	0.047
CC	GO:0030672 - Synaptic vesicle membrane	*ATP6V1B2, SYT8, SLC18A1*	0.048
PWY	bta04920 - Adipocytokine signaling pathway	*CPT1A, NPY, AGRP*	0.048

BP: biological process; CC: cellular component; MF: molecular function; PWY: metabolic pathway; R_PWY: Biochemical reactions and signaling.

One of the biological annotated was the Insulin Signaling and Pathways IGF1 (R_BTA-74752, R_BTA-77387, R_BTA-2428924), with the involvement of *Fibroblast Growth Factor 19* (*FGF19*), *ATPase H+ Transporting V0 Subunit D1* (*ATP6V0D1*), *ATPase H+ Transporting V1 Subunit B2* (*ATP6V1B2*), *Insulin-like Growth Factor 2* (*IGF2*), *Fibroblast Growth Factor 3* (*FGF3*) and *Insulin* (*INS*). These pathways are linked to insulin receptor signaling and IGF1 receptor activation, both of which are critical for energy metabolism and growth (Hakuno and Takahashi, 2018; Al-Massadi et al., 2022; Werner, 2023). The insulin pathway regulates glucose uptake, energy storage, and lipid metabolism (Hakuno and Takahashi, 2018), directly influencing feed efficiency and body weight gain. In cattle, variations in these processes can lead to differences in nutrient utilization, thereby affecting DMI and, consequently, feed efficiency.

The Cascata MAPK (GO:0043410, GO:0030334) was also annotated in the enrichment analyses, involving *Moloney Murine Sarcoma Viral Oncogene* (*MOS*), *LYN Proto-Oncogene, Src Family Tyrosine Kinase* (*LYN*), *Insulin-like Growth Factor 2* (*IGF2*) and *Gasdermin E* (*GSDME*) genes. Processes such as growth, differentiation, and response to environmental stress are regulated by this pathway (Avila et al., 2011; Adhikari and Cullen, 2014; Gonzalez-Garcia et al., 2014; Werner, 2023). The influence of these processes on DMI may be related to how animals respond to their environment, impacting their nutritional requirements and feed intake. Genes such as *LYN* and *IGF2*, which are associated with growth and development (Avila et al., 2011; Pereira et al., 2016; Terakado et al., 2017; Rieger and Connor, 2021), further highlight the importance of this pathway in managing energy demands. Another crucial pathway is the PI3K-AKT signaling pathway (R_BTA-1257604, R_BTA-6811558), involving the *FGF19*, *FGF3*, and *Fibroblast Growth Factor 4 (FGF4)*, as well as *INS* genes. The PI3K-AKT pathway is central to cell survival, growth, and metabolism, particularly in insulin response (Hardy et al., 2011; Toschi and Baratta, 2021; Yang et al., 2022). In cattle, this pathway is closely linked to feed efficiency and nutrient metabolism, influencing how animals efficiently convert feed into body mass (Cantalapiedra-Hijar et al., 2018; Toschi and Baratta, 2021; Yang et al., 2022). Variations in genes related to this pathway could alter how energy is allocated for growth, maintenance, and reproduction, thereby affecting DMI.

The Ras signaling pathway (bta04114, bta04015) was also identified, with genes such as *FGF19*, *IGF2*, *FGF3*, and *FGF4* involved in this process. This pathway regulates cell proliferation, differentiation, and survival (Huang et al., 2014; Nies et al., 2016). It can influence growth and metabolism in response to environmental stressors, directly impacting DMI. For instance, cattle exposed to adverse conditions may experience altered metabolic demands, and genes such as *FGF19* and *IGF2* can modulate these responses, leading to changes in feed intake (Mota et al., 2022). The regulation of the actin cytoskeleton (GO:0005824, GO:0008543) was also annotated in the enrichment analyses, involving genes such as *MOS*, *FGF3*, *FGF4*, *IGF2*. This process includes alterations in cellular structure, which are essential for various cellular functions such as growth and mobility (Illescas et al., 2021; Gao and Nakamura, 2022; Dehghanian Reyhan et al., 2023). It may affect muscle development and maintenance, key factors in determining the energy demands of cattle (Dehghanian Reyhan et al., 2023; Arikawa et al., 2024; Sacarrao-Birrento et al., 2024), and consequently, could influence DMI. In the context of DMI, this process may influence how animals metabolize nutrients and convert feed into body mass efficiently, affecting feed intake requirements. The genes and pathways identified for the DMI intercept are central to metabolic processes that regulate growth, energy balance, and nutrient utilization. These biological processes are particularly important for animals raised under variable environmental conditions, such as Nellore cattle, which may impact feed intake and efficiency.

4.3.2.4. Slope for DMI

For the DMI slope across EG levels, 17 relevant genomic windows were identified (Table 9, Figure 2). These genomic windows are located on ten chromosomes: BTA1 (155.72 – 156.03 Mb), BTA2 (104.16 – 104.55 Mb and 104.58 – 105.27 Mb), BTA5 (15.53 – 15.88 Mb and 65.97 – 66.93 Mb), BTA6 (2.32 – 2.80 Mb), BTA9 (49.80 – 50.31 Mb), BTA11 (4.85 – 5.21 Mb, 5.55 – 5.92 Mb and 100.94 – 101.52 Mb), BTA13 (41.40 – 41.97 Mb), BTA14 (22.90 – 23.31 Mb and 23.33 – 23.89 Mb), BTA18 (32.19 – 32.54 Mb and 35.62 – 36.07 Mb) and BTA29 (48.74 – 50.54 Mb). In other GWAS studies with Nellore cattle, some of these regions were also associated with DMI (Brunes et al., 2020; Mota et al., 2022). A total of 111 genes were identified within these genomic windows, including 109 protein-coding genes and two snRNAs.

Table 9. List of the top genomic windows that explained more than 1% of the total direct additive genetic variance (σ_a^2) for dry matter intake (DMI - slope).

BTA	Location (Mb)	Genes	σ_a^2 (%)
14	22.90 – 23.31	*XKR4, TMEM68, TGS1, LYN, RPS20, U1, MOS, PLAG1, CHCHD7, SDR16C5, SDR16C6, PENK, U6, BPNT2*	3.55
5	65.97 – 66.93	*PARPBP, PMCH, IGF1, U6, PAH, ASCL1, U1*	3.33
18	32.19 – 32.54*	-	3.21
13	41.40 – 41.97	*FOXA2, U6, THBD, CD93*	3.09
2	104.16 – 104.55	*XRCC5, MARCHF4, SMARCAL1*	2.96
18	34.95 – 35.60	*LRRC36, TPPP3, ZDHHC1, HSD11B2, ATP6V0D1, AGRP, RIPOR1, CTCF, CARMIL2, ACD, PARD6A, ENKD1, C18H16orf86, GFOD2, RANBP10, TSNAXIP1, CENPT, THAP11, NUTF2, EDC4, NRN1L, PSKH1, PSMB10, LCAT, SLC12A4, DPEP3, DPEP2, DDX28, DUS2, NFATC3, U6*	2.93
2	104.58 – 105.27	*IGFBP2, IGFBP5, TNP1*	2.87
11	5.55 – 5.92	*NMS, PDCL3, NPAS2*	2.17
1	155.72 – 156.03*	-	2.09
6	2.32 – 2.80	*NPY5R, NPY1R, NAF1, U6*	1.94
14	23.33 – 23.89	*PLAG1, CHCHD7, SDR16C5, SDR16C6, PENK, U6, BPNT2*	1.61
5	15.53 – 15.88	*NTS, MAGT4C, PARPBP, PMCH, IGF1, U6, PAH, ASCL1, U1*	1.46
29	48.74 – 50.54	*KCNQ1, TRPM5, TSSC4, TSPAN32, ASCL2, TH, INS, IGF2, TNNT3, LSP1, PRR33, TNNI2, SYT8, CTSD, IFITM10, DUSP8, MOB2, BRSK2, TOLLIP, MUC2, AP2A2*	1.35
11	100.94 – 101.52	*PRDM12, EXOSC2, ABL1, QRFP, FIBCD1, LAMC3, NUP214, FAM78A, PLPP7*	1.32
9	49.80 – 50.31	*MCHR2, PRDM13, U6, CCNC, TSTD3, USP45*	1.30
18	35.62 – 36.07	*ESRP2, PLAG2G15, SLC7A6, SLC7A6OS, PRMT7, SMPD3, ZPF90, CDH3, CDH1*	1.20
11	4.85 – 5.21	*AFF3*	1.19

BTA: *Bos taurus autosome*.

A total of 37.57% of the overall direct additive genetic variance was captured by the relevant genomic regions identified. The genomic window located on BTA14 (22.90 – 23.31 Mb) explained the largest portion of additive genetic variance, accounting for 3.55%, with 14 annotated genes identified within this window (Table 9). The *XK Related 4* (*XKR4*) gene encodes a protein involved in apoptosis and membrane remodeling (Chakraborty et al., 2024; Song et al., 2024). *XKR4* is expressed in a wide range of tissues, including the nervous system and muscles (Xu et al., 2020a; Yu et al., 2024). Given that DMI influences muscle growth and energy balance, the role of *XKR4* in muscle-related processes (Edea et al., 2020) may render it significant for energy metabolism, and consequently, for feed intake and utilization under varying environmental conditions. Another relevant gene, *Transmembrane Protein 68* (*TMEM68*), is implicated in lipid metabolism (Edea et al., 2020; Wang et al., 2023; Zeng et al., 2024). Genes involved in lipid metabolism are generally critical for

energy storage and utilization (Srivastava et al., 2020). Lipid metabolism plays a pivotal role in feed efficiency by regulating how energy is stored, mobilized, and used by the animal. In more feed-efficient cattle, enhanced lipid oxidation pathways and more effective lipid transport have been observed, leading to greater energy availability for growth and maintenance (Artegoitia et al., 2019, Yang et al., 2023). Additionally, these animals tend to exhibit reduced hepatic lipid synthesis and accumulation, further supporting the association between lipid metabolism and improved nutrient utilization (Taiwo et al., 2022). Altogether, these findings highlight the importance of lipid metabolic pathways in promoting feed efficiency in beef cattle. As lipid metabolism is closely linked to feed efficiency, *TMEM68* may influence the conversion rate of feed into energy, particularly under diverse environmental conditions, thereby influencing total feed intake. Another gene identified was *Pleomorphic Adenoma Gene 1 (PLAG1)*. Its role is involved in regulating growth and development, particularly influencing body size and stature (Hou et al., 2019; Zhang et al., 2020; Pan et al., 2022). The effect of *PLAG1* on growth potentially makes it a critical gene for feed efficiency. Cattle with variants of this gene that promote more efficient growth may exhibit different patterns of DMI, particularly under variable environmental conditions.

The *Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 7 (CHCHD7)* gene is involved in mitochondrial function, specifically in maintaining mitochondrial integrity (Li et al., 2020; Yan et al., 2024). This gene is also associated with growth and stature in several species, including cattle (Li et al., 2020; Xu et al., 2020b; Pan et al., 2022; Kolpakov et al., 2024). Mitochondria are central to energy production, and variations in genes affecting mitochondrial efficiency can influence energy metabolism, thereby impacting how much feed is required to maintain or support growth under different environmental conditions. Another gene, *Proenkephalin (PENK)*, encodes a precursor for enkephalins, which are neuropeptides involved in pain regulation and stress responses (Adhikari et al., 2022; Pierzchała-Koziec et al., 2023). Stress responses can influence appetite and metabolism in cattle (Fernandez-Novo et al., 2020; Sammad et al., 2020; Meneses et al., 2021). Variations in *PENK* may affect how cattle respond to environmental stressors, thereby influencing their feeding behavior and metabolic efficiency.

A total of 42 processes were found to be significantly linked (p-value < 0.05) with the slope of DMI (Table 10). One of the key processes identified is signaling by

the Insulin Receptor (R-BTA-74752), which is critical for glucose metabolism and overall energy homeostasis. The insulin receptor pathway controls how cells take up glucose from the bloodstream, a process essential for energy production (Pereira et al., 2016; Rieger and Connor, 2021; Díaz Del Moral et al., 2022). Genes like *FGF19*, *ATP6V1B2*, and *INS* are involved in this pathway, with *INS* directly regulating nutrient uptake and metabolism (Payankaulam et al., 2019). In the context of DMI slope, these genes may influence how cattle adjust their feed intake in response to energy needs, impacting their efficiency in converting feed into energy under variable environmental conditions.

Similarly, the *Receptor Tyrosine Kinases* (*RTK*) signaling pathway (R-BTA-9006943), which includes genes such as *FGF19*, *FGF3*, and *INS*, is involved in cellular growth, proliferation, and metabolism (Schlessinger, 2000). *RTKs* play a pivotal role in transmitting extracellular signals to the cell's interior, regulating growth and development processes (Schlessinger, 2000). Variations in these genes could affect how cattle respond to growth-related signals, potentially altering their feed intake based on growth demands in different environments, which could explain variation in the DMI slope.

The Insulin-like Growth Factor 1 Receptor (*IGF1R*) signaling pathway (R-BTA-2428924) also emerged as significant. This pathway is crucial for growth and development, influencing cell growth, differentiation, and survival (Pereira et al., 2016; Rieger and Connor, 2021; Díaz Del Moral et al., 2022). Genes like *FGFR3*, *FGFR4*, and *INS* are associated with this process. Given that IGF1R signaling regulates anabolic processes and energy usage, it is plausible that variations in this pathway could influence how efficiently cattle manage their energy resources when environmental conditions fluctuate, thus impacting the slope of DMI.

Table 10. Significant Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses for dry matter intake (DMI - slope).

Category	Terms	Gene Symbol	p-value
BP	R-BTA-74752 - Signaling by Insulin receptor	*FGF19, ATP6V1B2, ATP6V0D1, CTSD, FGF3, FGF4, INS*	< 0.001
BP	R-BTA-9006934 - Signaling by Receptor Tyrosine Kinases	*FGF19, ATP6V1B2, IGF2, ATP6V0D1, CTSD, FGF3, FGF4, INS*	< 0.001
BP	R-BTA-77387 - Insulin receptor recycling	*ATP6V1B2, ATP6V0D1, CTSD, INS*	< 0.001
R_PWY	GO:0009887 - Animal organ morphogenesis	*FGF19, E2F4, PHLDA2, FGF3, FGF4*	< 0.001
BP	R-BTA-2428928 - IRS-related events triggered by IGF1R	*FGF19, IGF2, FGF3, FGF4*	< 0.001
MF	R-BTA-2428924 - IGF1R signaling cascade	*FGF19, IGF2, FGF3, FGF4*	0.001
BP	R-BTA-2404192 - Signaling by Type 1 Insulin-like Growth Factor 1 Receptor (IGF1R)	*FGF19, IGF2, FGF3, FGF4*	0.001
CC	R-BTA-74751 - Insulin receptor signaling cascade	*FGF19, FGF3, FGF4, INS*	0.001
BP	GO:0043410 - Positive regulation of MAPK cascade	*MOS, LYN, IGF2, GSDME, INS*	0.001
BP	GO:0005737 - Cytoplasm	*RIPOR1, BRSK2, TSSC4, FHOD1, IGHMBP2, NCAPG, FGF3, FGF4, HERC5, HERC3, CCND1, TSNAXIP1, E2F4, PHLDA2, LZTS1, HERC6, LYN, TDRD9, DUSP8, NAP1L4, THAP11, MED28, MOS, TPPP3, TH, KIF26A, FGF19, MOB2, RANBP10, ELMO3, LAP3, CARS1, GSDME*	0.003
BP	GO:0001934 - Positive regulation of protein phosphorylation	*CCND1, FGF19, MOB2, FGF3, FGF4*	0.003
BP	R-BTA-1257604 - PIP3 activates AKT signaling	*FGF19, FGF3, PSMB10, FGF4, INS*	0.005
PWY	R-BTA-199418 - Negative regulation of the PI3K/AKT network	*FGF19, FGF3, FGF4, INS*	0.005
MF	R-BTA-6811558 - PI5P, PP2A and IER3 Regulate PI3K/AKT Signaling	*FGF19, FGF3, FGF4, INS*	0.006
MF	R-BTA-9006925 - Intracellular signaling by second messengers	*FGF19, FGF3, PSMB10, FGF4, INS*	0.006
BP	GO:0005104 - Fibroblast growth factor receptor binding	*FGF19, FGF3, FGF4*	0.007
R_PWY	GO:0010628 - Positive regulation of gene expression	*PLAG1, FGF19, CTCF, FGF3, FGF4, INS*	0.007
R_PWY	bta05218 - Melanoma	*CCND1, FGF19, FGF3, FGF4*	0.012
MF	bta04014 - Ras signaling pathway	*FGF19, IGF2, PLD1, FGF3, FGF4, INS*	0.014

MF	GO:0030334 - Regulation of cell migration	*FGF19, PHLDA2, FGF3, FGF4*	0.014
BP	R-BTA-109704 - PI3K Cascade	*FGF19, FGF3, FGF4*	0.016
PWY	R-BTA-112399 - IRS-mediated signaling	*FGF19, FGF3, FGF4*	0.017
BP	GO:0003779 - actin binding	*MAP1B, TNNT3, TNNI2, LSP1, MED28*	0.023
R_PWY	GO:0051781 - Positive regulation of cell division	*IGF2, FGF3, FGF4*	0.025
R_PWY	GO:0045121 - Membrane raft	*LYN, KCNQ1, CTSD, ABCG2*	0.032
BP	bta04010 - MAPK signaling pathway	*FGF19, IGF2, DUSP8, FGF3, FGF4, INS*	0.032
BP	GO:0006006 - Glucose metabolic process	*KCNQ1, IGF2, INS*	0.032
BP	R-BTA-917937 - Iron uptake and transport	*ATP6V1B2, ATP6V0D1, ABCG2*	0.033
MF	GO:0008543 - Fibroblast growth factor receptor signaling pathway	*FGF19, FGF3, FGF4*	0.033
MF	R-BTA-162582 - Signal Transduction	*CPT1A, FGF19, NPY, PENK, ATP6V1B2, IGF2, ATP6V0D1, CTSD, FGF3, PSMB10, FGF4, INS*	0.033
BP	bta04015 - Rap1 signaling pathway	*PARD6A, FGF19, FGF3, FGF4, INS*	0.036
R_PWY	GO:0008083 - Growth factor activity	*FGF19, IGF2, FGF3, FGF4*	0.037
CC	bta04810 - Regulation of actin cytoskeleton	*MOS, FGF19, FGF3, FGF4, INS*	0.037
MF	GO:0008343 - Adult feeding behavior	*NPY, AGRP*	0.038
R_PWY	R-BTA-190236 - Signaling by FGFR	*FGF19, FGF3, FGF4*	0.039
BP	R-BTA-5658623 - FGFRL1 modulation of FGFR1 signaling	*FGF3, FGF4*	0.041
BP	R-BTA-382551 - Transport of small molecules	*SLC9A5, SLC22A18, ATP6V1B2, ATP6V0D1, PSMB10, ABCG2*	0.041
PWY	GO:0016324 - Apical plasma membrane	*PARD6A, KCNQ1, ATP6V1B2, PLD1, ABCG2*	0.043
MF	GO:0005861 - Troponin complex	*TNNT3, TNNI2*	0.047
BP	bta05224 - Breast cancer	*CCND1, FGF19, FGF3, FGF4*	0.049
BP	GO:1902600 - Proton transmembrane transport	*SLC9A5, ATP6V1B2, ATP6V0D1*	0.049
R_PWY	bta05226 - Gastric cancer	*CCND1, FGF19, FGF3, FGF4*	0.049

BP: biological process; CC: cellular component; MF: molecular function; PWY: metabolic pathway; R_PWY: Biochemical reactions and signaling.

The *PI3K/AKT* signaling pathway (R-BTA-1257044) is another important metabolic pathway linked to the regulation of growth and survival, particularly under conditions of nutrient stress (Edinger, 2007). This pathway includes genes like *PI3K*, *AKT1*, and *PSMB10*, which are involved in cell survival, proliferation, and glucose metabolism (Edinger and Thompson, 2004; Edinger, 2007; Wu et al., 2016). As this pathway integrates signals related to nutrient availability, it likely plays a role in determining how cattle adjust their intake to optimize growth and energy storage under variable conditions, affecting the slope of DMI.

Additionally, pathways related to Fibroblast Growth Factor Receptor (FGFR) signaling (GO-0005104) and Ras signaling (GO-0043404) were highlighted. These pathways involve genes like *FGFR1*, *FGFR3*, and *FGF19*, which are critical for cell proliferation, differentiation, and metabolism (Itoh and Ornitz, 2004; Itoh, 2007). The Ras pathway is central in transmitting signals that regulate cellular growth and energy use (Huang et al., 2014; Nies et al., 2016). Variations in these genes might influence how cattle balance their growth and metabolic processes in response to environmental changes, impacting their feed efficiency and DMI slope.

Moreover, cytoplasmic processes (GO:0005737) and positive regulation of protein phosphorylation (GO:0001934) are involved in cellular signaling and metabolic regulation (Huttermann et al., 2007; Hermann et al., 2008; Humphrey et al., 2015; Zhu and Thompson, 2019). Genes such as *INS*, *FGFR4*, and *PSMB10* play crucial roles in modulating these processes (Huttermann et al., 2007; Zhu and Thompson, 2019). These pathways could influence the efficiency of nutrient metabolism and energy utilization, thereby affecting how animals adapt their feed intake to varying environmental conditions, which in turn affects the DMI slope.

In summary, the processes identified in the enrichment analysis, particularly those involved in insulin signaling, growth factor signaling, and cellular metabolism, suggest a strong connection between the regulation of energy balance and the slope of DMI in Nellore cattle. The genes involved in these pathways, such as *FGF19*, *FGFR3*, and *INS*, are likely to affect the cattle's ability to adjust their feed intake in response to changing environmental conditions, influencing their overall efficiency and adaptability. This genomic information can provide a foundation for improving feed efficiency and productivity in livestock through targeted breeding strategies.

4.3.3. Functional Networks for RFI

The functional networks of the candidate genes identified for RFI in Nellore cattle showed significant changes in connectivity and the central role of certain genes between the intercept and slope. These results illustrate the GxE interaction, highlighting how different gene networks are mobilized depending on the environmental influence on the phenotype. In the network related to the intercept (Figure 3), genes such as *LEPR*, *LEPROT*, *NPY*, *GHSR*, and *AGRP* exhibited high connectivity, forming a functional core with several interactions. These genes are closely associated with appetite regulation and energy metabolism, such as *GHSR* and *LEPR*, which are known for their roles in regulating feeding behavior and energy efficiency, suggesting that central metabolic pathways are key determinants in the baseline genetic variation of RFI. Additionally, several smaller sub-networks with fewer connections were identified, indicating that these genes may be linked to more specific processes or sub-functions within the regulation of RFI.

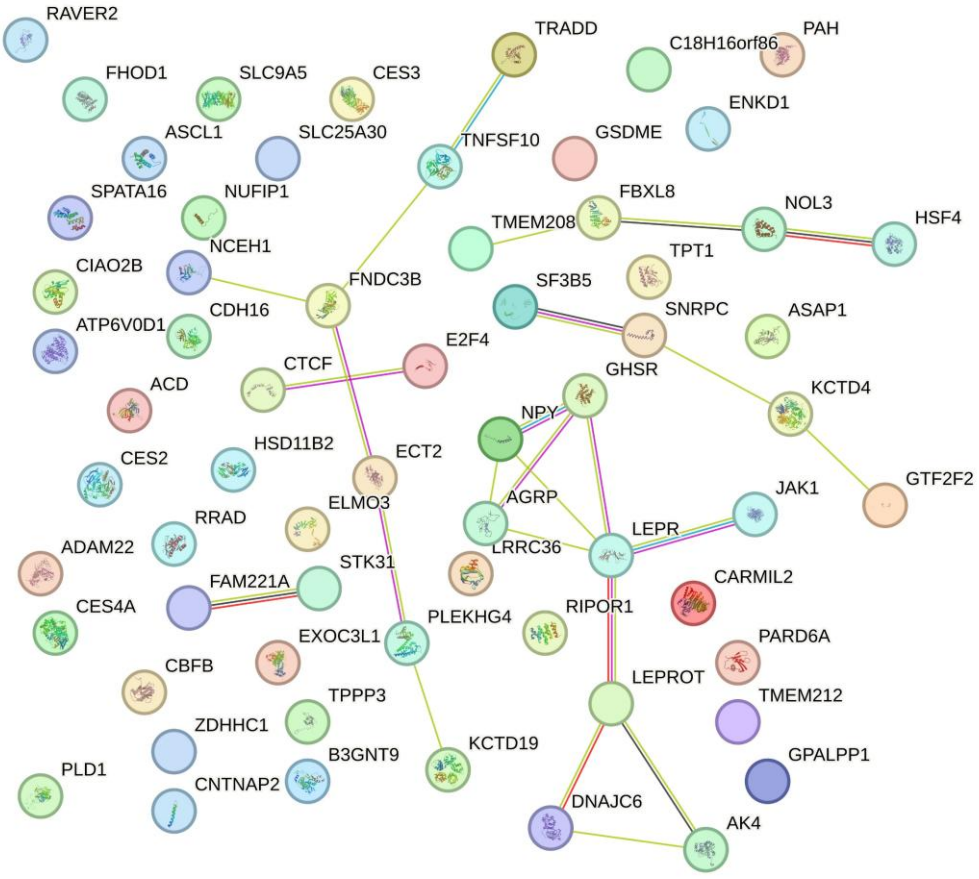

Figure 3. Functional network of genes identified in the genomic windows that explained more than 1% of the total direct additive genetic variance of the intercept for residual

feed intake (RFI) in Nellore cattle. Each node represents a gene, while the lines connecting the nodes indicate known functional interactions or associations between these genes. The different colors of the nodes and lines indicate distinct types of interactions or classifications of biological functions, based on the network analysis.

In contrast, in the network related to the slope of RFI (Figure 4), there was a shift in the most connected genes. Genes such as *TMEM68*, *XKR4*, *CHCHD7*, *RPS20*, *PLAG1*, and *FAM110B* emerged as central, exhibiting multiple interactions with other genes. This indicates that distinct genetic mechanisms may be involved in the variation of RFI over time, with feed intake regulation being mediated by different genetic pathways. The interaction between genes related to energy metabolism and growth is evidenced by the connection of *INSR* with *IGF1R* and *IGFBP5*, which are fundamental to insulin signaling. Additionally, *NPY* and *POMC* indicate the influence of appetite control pathways on feed efficiency. The network also includes genes such as *CYP7A1* and *SDR16C5*, involved in lipid metabolism, that interact with *CHCHD7* and *UBXN2B*, suggesting a role in lipid metabolism in the variation of the slope of RFI. Genes associated with mitochondrial function, such as *TMEM68* and *CHCHD7*, emphasize the importance of cellular health in feed efficiency. Finally, small subnetworks formed by genes like *PTRH1*, *DNMT3A*, and *MOS* indicate potential more specific functions, such as epigenetic regulation and response to stressors. This complex network highlights the interconnection of multiple biological processes that influence feed efficiency under different environmental conditions.

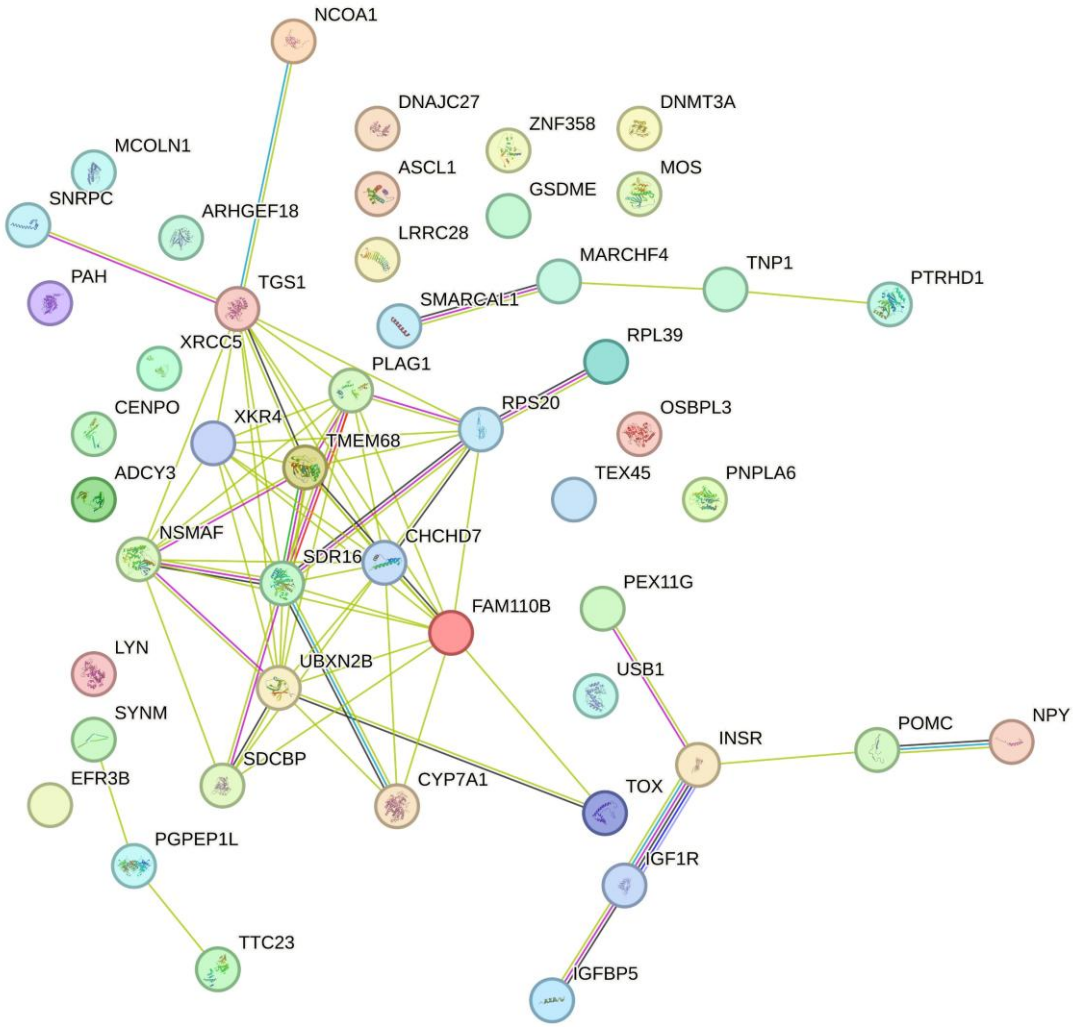

Figure 4. Functional network of genes identified in the genomic windows that explained more than 1% of the total direct additive genetic variance of the slope for residual feed intake (RFI) in Nellore cattle. Each node represents a gene, while the lines connecting the nodes indicate known functional interactions or associations between these genes. The different colors of the nodes and lines indicate distinct types of interactions or classifications of biological functions, based on the network analysis.

4.3.4. Functional Gene Networks for DMI

Figures 5 (intercept) and 6 (slope) present the functional gene networks identified based on the candidate genes identified for DMI in Nellore cattle. In Figure 5, the functional network consists of a dense web of interactions among genes, suggesting strong basal genetic regulation for DMI under controlled environments. Genes such as *PLAG1*, *IGF2*, *CHCHD7*, *CCND1*, and *NCAPG* show centrality, with several direct and indirect connections, indicating their crucial role in regulating this

trait. The interactions among these genes stand out as responsible for the genetic architecture of the phenotype in an average environment, with particular emphasis on *NCAPG*, a gene known for its association with growth traits and feed efficiency in cattle (Takasuga, 2015).

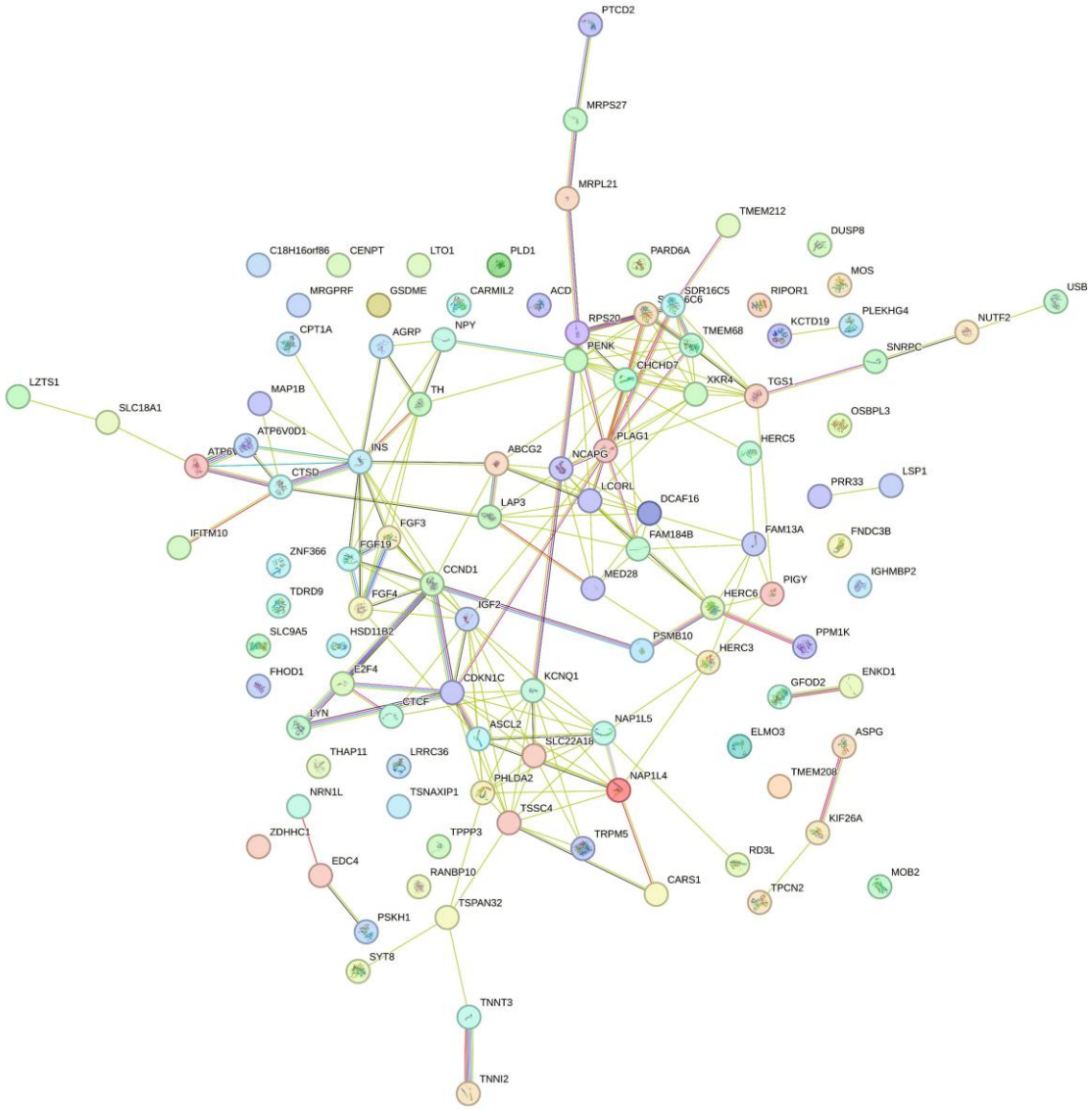

Figure 5. Functional network of genes identified in the genomic windows that explained more than 1% of the total direct additive genetic variance of the intercept for dry matter intake (DMI) in Nellore cattle. Each node represents a gene, while the lines connecting the nodes indicate known functional interactions or associations between these genes. The different colors of the nodes and lines indicate distinct types of interactions or classifications of biological functions, based on the network analysis.

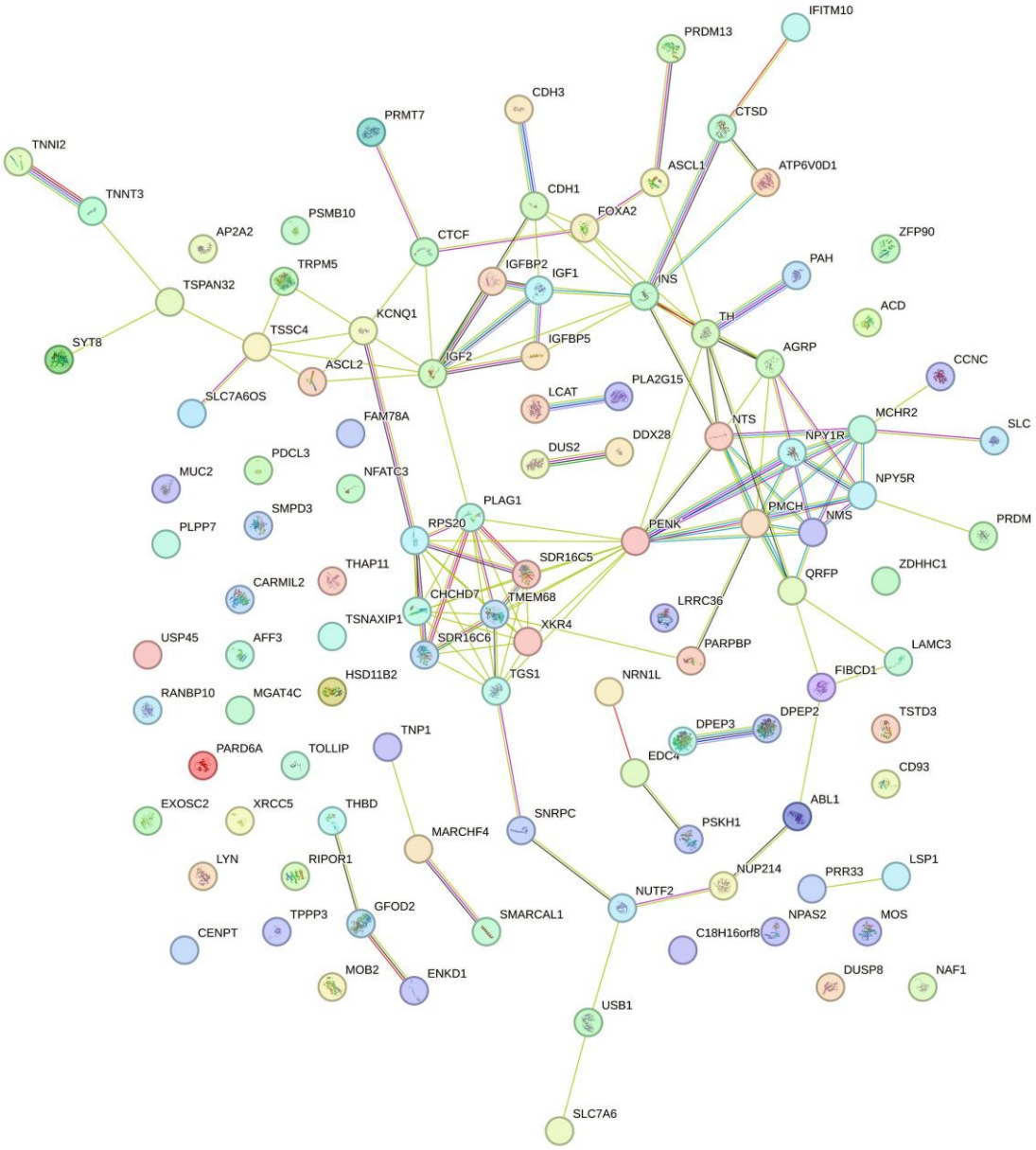

Figure 6. Functional network of genes identified in the genomic windows that explained more than 1% of the total direct additive genetic variance of the slope for dry matter intake (DMI) in Nellore cattle. Each node represents a gene, while the lines connecting the nodes indicate known functional interactions or associations between these genes. The different colors of the nodes and lines indicate distinct types of interactions or classifications of biological functions, based on the network analysis.

Figure 6 represents the genetic modulation in response to the environment in which a complex interaction between central and peripheral genes is observed, highlighting pathways associated with appetite regulation and energy metabolism. Genes such as *IGF2*, *INS*, *PLAG1*, and *PMCH* are strongly connected to other genes

related to energy homeostasis, such as *IGFBP5* and *ASCL2*, suggesting their involvement in growth regulation and response to changes in feed intake over time. The gene *NPY1R*, centralized in the network, reinforces its function in appetite regulation and variation of DMI, while less connected sub-networks, such as those involving the *TNNI2* and *SYT8* genes, may indicate specialized functions. The different colors in the connections between genes suggest varied gene interactions, potentially correlated with environmental and dietary factors. The gene *PENK*, which encodes precursors of enkephalins, stands out for its influence on neural signaling and appetite control, suggesting a crucial role in modulating feed intake and feeding behavior, thereby forming, along with the other genes, a complex regulatory network that affects the slope of DMI. Therefore, the differences observed between Figures 5 and 6 clearly demonstrate the plasticity of the genetic network in response to environmental changes. While some genes maintain central importance in both contexts, others emerge as key players in genetic modulation in the face of environmental variations, highlighting the role of GxE in regulating DMI in Nellore cattle.

4.3.5. SNP effects by environmental gradient

The graphs presented in Figure 7 demonstrate the reaction norms of 100 SNPs within the relevant genomic windows (panels "a" to "k") associated with RFI in Nellore cattle. The effects of the SNPs are plotted across low, medium, and high EG, allowing for the visualization of GxE interactions. The genomic windows are ordered according to their relevance, providing a comparative view of the environmental sensitivity of the SNPs within each genomic window.

SNP-environment interactions are evident in certain genomic windows. Panels such as "b", "d", "e", "f", "g", "h" and "j" (BTA14: 24.39–24.91 Mb; BTA21: 7.35–8.15 Mb; BTA2: 104.16–104.55 Mb; BTA14: 22.99–23.45 Mb; BTA2: 104.65–105.41 Mb; BTA7: 16.07–16.44 Mb and BTA14: 22.61–22.99 Mb, respectively) reveal variability in SNP effects as the environment shifts from low to high environmental conditions (extremes), highlighting the presence of GxE interactions (Figure 7). In these cases, the SNPs show differentiated effects depending on environmental conditions, with some alleles exhibiting higher or lower effects as the environmental gradient changes. This suggests that these genomic regions may harbor genes that are particularly sensitive to environmental factors affecting RFI. On the other hand, some genomic windows, such as those located in BTA4: 70.83–71.85 Mb; BTA5: 66.51–67.03 Mb;

BTA14: 24.91–25.43 Mb and BTA11: 74.02–74.67 Mb illustrated in panels "a", "c", "i", and "k", respectively, show relatively more stable SNP effects across the EG (Figure 7). These SNPs appear to be less affected by environmental variation, indicating they may play a more consistent role in RFI across different environments. The greater stability observed in these windows may make them valuable targets for selection when a robust genetic response across environments is desired.

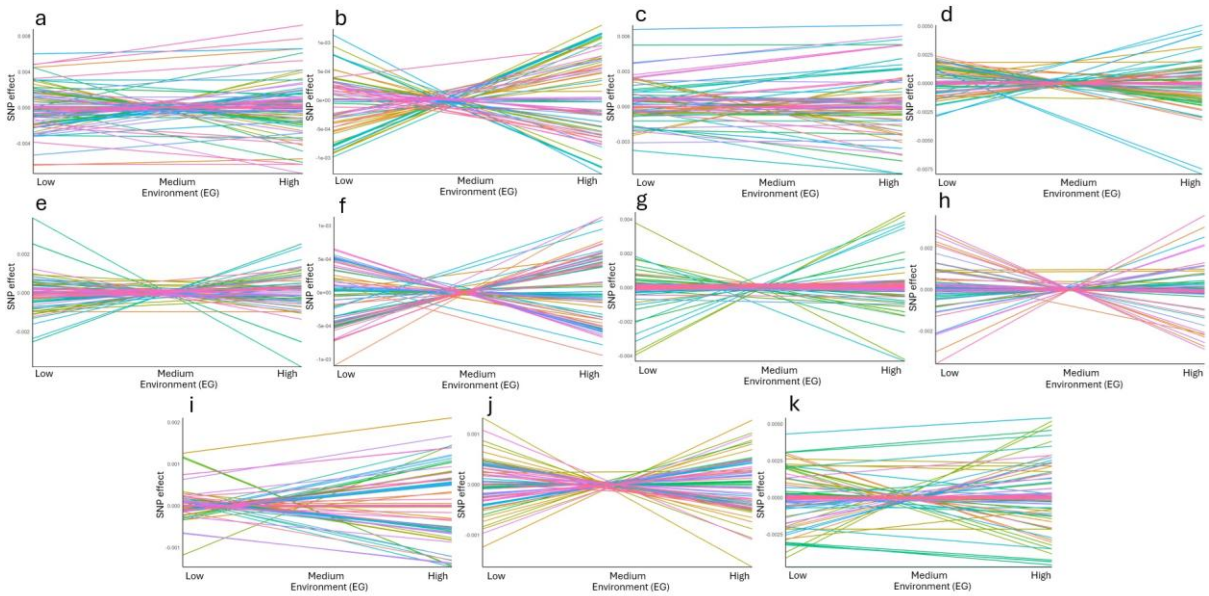

Figure 7. Reaction norms for the effects of all 100 Single Nucleotide Polymorphisms (SNP) comprising the genomic windows (a-k) that explained at least 1% of the total additive genetic variance associated with residual feed intake (RFI) in Nellore cattle across low, medium, and high environmental gradients (EG). The graphs are presented in order of the magnitude of additive genetic variance explained by each genomic window, as shown in Table 5, allowing for comparison of SNP impacts under different environmental conditions (EG). Each color of the lines represents a different SNP. Chromosomes and regions: (a) BTA4: 70.83–71.85 Mb; (b) BTA14: 24.39–24.91 Mb; (c) BTA5: 66.51–67.03 Mb; (d) BTA21: 7.35–8.15 Mb; (e) BTA2: 104.16–104.55 Mb; (f) BTA14: 22.99–23.45 Mb; (g) BTA2: 104.65–105.41 Mb; (h) BTA7: 16.07–16.44 Mb; (i) BTA14: 24.91–25.43 Mb; (j) BTA14: 22.61–22.99 Mb; (k) BTA11: 74.02–74.67 Mb.

An important pattern observed in several graphs is the crossing of reaction norms, where the effects of SNPs change not only in magnitude but also in direction

as the environmental conditions shifts. This highlights the complexity of GxE interactions. The crossed reaction norms underscore the need to consider the environmental context when selecting animals for traits related to RFI, as certain alleles may be beneficial only under specific conditions. Genomic windows with more pronounced changes in SNP effects likely capture a larger share of the genetic variability linked to environmental response. These windows are of particular interest for future research, as they may contain key genes that influence the adaptability of feed efficiency to environmental changes. Identifying these SNPs could lead to more precise genetic selection strategies, improving cattle resilience and performance across different environments.

The effects of SNPs located in the genomic regions associated with DMI in Nellore cattle are shown in Figure 8. Similar to what was observed for RFI, certain genomic regions exhibited interactions between SNPs and the environment. Panels such as "b" (BTA5: 65.97–66.93 Mb), "e" (BTA2: 104.16–104.55 Mb), "g" (BTA2: 104.58–105.27 Mb), "j" (BTA6: 2.32–2.80 Mb), "l" (BTA5: 15.53–15.88 Mb), "n" (BTA11: 100.94–101.52 Mb) and "o" (BTA9: 49.80–50.31 Mb) highlight the variation in SNP effects as the EG shifts from low to high (Figure 8). Furthermore, the crossing of reaction norms was also observed, indicating that the effects of SNPs not only vary in magnitude but also change direction with environmental alterations, suggesting that these genomic regions may harbor genes that are highly sensitive to environmental factors. In contrast, some genomic windows, such as those represented in panels "a" (BTA14: 22.90–23.31 Mb), "c" (BTA18: 32.19–32.54 Mb), "d" (BTA13: 41.40–41.97 Mb), "f" (BTA18: 34.95–35.60 Mb), "i" (BTA11: 155.72–156.03 Mb), "k" (BTA14: 23.33–23.89 Mb), "m" (BTA29: 48.74–50.54 Mb) and "p" (BTA18: 35.62–36.07 Mb) show more consistent SNP effects across EG (Figure 8), indicating that these SNPs are less influenced by environmental variations and may play a more stable role in DMI across different environments.

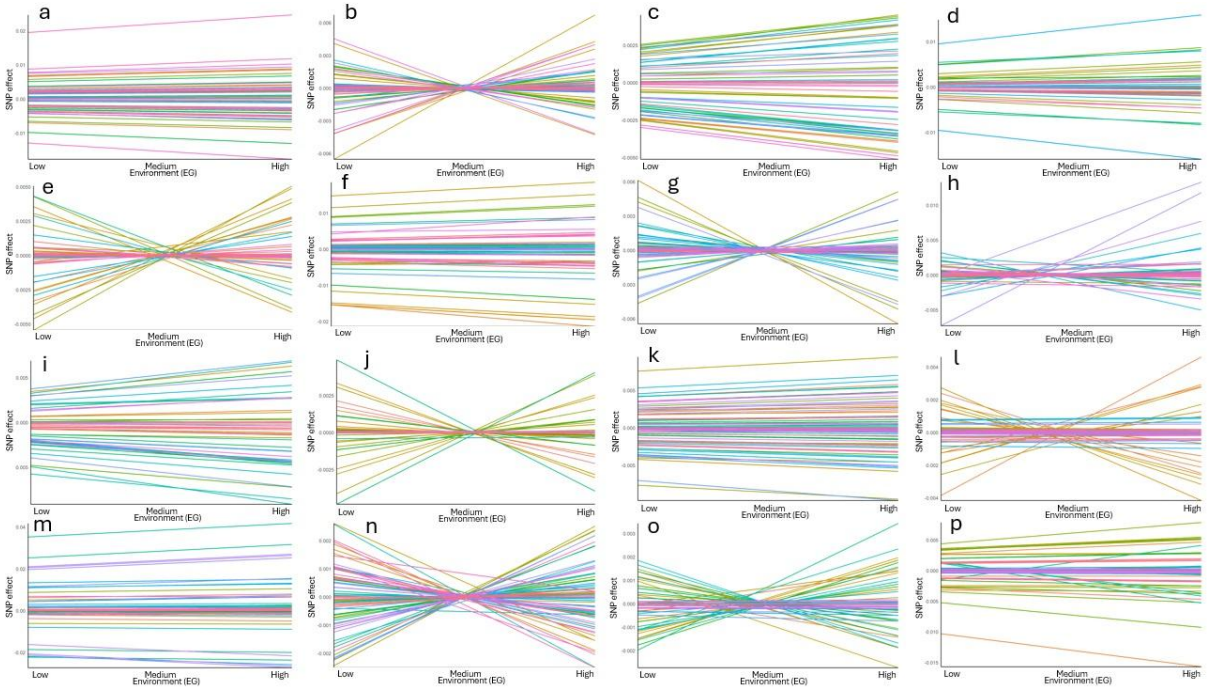

Figure 8. Reaction norms for the effects of all 100 Single Nucleotide Polymorphisms (SNP) comprising each genomic window (a-p) that explain at least 1% of the additive genetic variance associated with dry matter intake (DMI) in Nellore cattle across low, medium, and high environmental gradients (EG). The graphs are presented in order of the magnitude of additive genetic variance explained by each genomic window, as shown in Table 9, allowing for comparison of SNP impacts under different environmental conditions (EG). Each color of the lines represents a different SNP. Chromosomes and regions: (a) BTA14: 22.90–23.31 Mb; (b) BTA5: 65.97–66.93 Mb; (c) BTA18: 32.19–32.54 Mb; (d) BTA13: 41.40–41.97 Mb; (e) BTA2: 104.16–104.55 Mb; (f) BTA18: 34.95–35.60 Mb; (g) BTA2: 104.58–105.27 Mb; (H) BTA11: 5.55–5.92 Mb; (i) BTA11: 155.72–156.03 Mb; (j) BTA6: 2.32–2.80 Mb; (k) BTA14: 23.33–23.89 Mb; (l) BTA5: 15.53–15.88 Mb; (m) BTA29: 48.74–50.54 Mb; (n) BTA11: 100.94–101.52 Mb; (o) BTA9: 49.80–50.31 Mb; (p) BTA18: 35.62–36.07 Mb.

The variation in SNP effects across EG suggests that breeding programs to improve RFI and DMI should consider GxE interactions. SNPs that exhibit significant positive effects in low environmental gradients may not perform similarly in high gradients, which could impact the genomic selection efficiency of cattle in diverse environments. By identifying SNPs that maintain stable effects across different environments or that are advantageous under specific conditions, breeding strategies can be tailored to optimize feed efficiency. Understanding the genetic architecture of

these traits in relation to environmental variation will be crucial for enhancing feed efficiency and sustainability in cattle production, especially considering the increasing challenges posed by climatic variability.

4.3.6. Reaction Norms to GEBV for RFI

Figure 9 provides a comprehensive analysis of RFI, revealing the complexity of the interaction between GEBVs and EG. In panel “a”, the reaction norms for RFI indicate considerable variation in GEBVs across the EG. Different inclinations suggest that some individuals respond more to environmental variations, while others maintain stable performance, reflecting genetic plasticity. This suggests that the phenotypic response to feed efficiency depends on environmental conditions, emphasizing the importance of considering phenotypic plasticity in genetic improvement strategies.

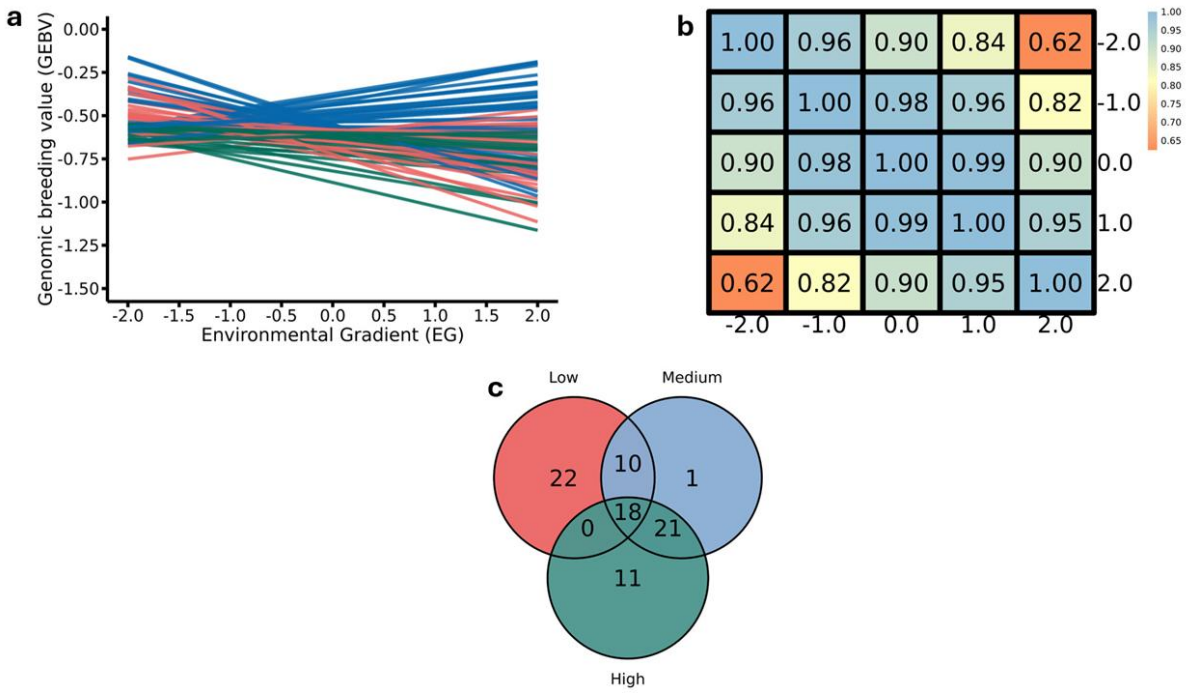

Figure 9. Reaction norms for residual feed intake (RFI) (a) Pearson correlation for genomic estimated breeding values (GEBVs) (b) and the number of common and specific sires with offspring in the environmental classes for RFI (c) considering the 50 sires with the highest number of progeny and top-ranked by GEBV in the moderate environmental gradient (EG = 0.0). The colors of the lines in panel “a” represent the EG, green for medium, red for high and blue for low.

In panel “b”, Pearson's correlation analysis highlights the similarity between GEBV values in different EGs, with most correlations exceeding 0.80 (Figure 9). This finding suggests that, while variations exist, sires with high GEBVs tend to maintain their ranking across environments, indicating consistency in the expression of the RFI. However, the lowest correlation (0.62) at the extremes (low and high) of the EG implies that environmental factors may more intensely influence feed efficiency under more divergent environmental conditions, which warrants attention in future selection programs.

Panel “c” illustrates the intersection of sires classified into different EG levels (Figure 9). The presence of 18 sires that stand out across all EG indicates that these individuals possess a robust genetic profile that translates into stable performance across varying environmental conditions. However, the significant number of sires exclusive to one environment (22 in the low EG and 11 in the high EG) suggests reduced precision in selection when contrasting environments are considered. These results provide a foundation for implementing genetic improvement programs aimed at sustainability and productivity, where GxE is considered crucial for optimizing sire selection and maximizing feed performance under diverse environmental conditions.

4.3.7. Reaction norm to GEBV for DMI

Figure 10 presents the results of the relationships between GEBVs and EG for DMI. In panel "a", the reaction norms illustrate the variation in GEBVs across the environmental gradient. The sires show different response patterns to environmental changes, with some animals exhibiting increasing GEBVs along the EG (upward lines), while others show a decrease (downward lines). Like for RFI, this indicates heterogeneity in the genetic response to the environment for DMI, highlighting the presence of GxE, as different sires perform variably across different EG.

The Pearson correlation matrix of GEBVs across different EGs for DMI (Figure 10, panel "b") presents correlations that exceed 0.90, demonstrating a strong consistency in the sires' GEBV rankings across EG levels. The decrease in the correlation (0.85) at the most different EGs (low and high) underscores a potential variability in DMI responses among sires, even if that is low. Higher Pearson correlations for DMI compared to RFI can be explained by the greater relative stability of the SNPs' effects present in the genomic windows that explain more additive genetic variance (Figure 8), thus reflecting the observed behavior in the GEBVs. The

intersection of sires classified across low, medium, and high EG levels is shown in Figure 10, panel “c”. The presence of 30 sires consistently ranked high across all environments, demonstrating genetic robustness under different EG. However, 17 sires were exclusive to the low EG, and four sires were unique to the high EG, suggesting that these animals may be better adapted to specific environmental conditions. Only three sires stood out in both low and medium EG, while one sire was shared between medium and high EG.

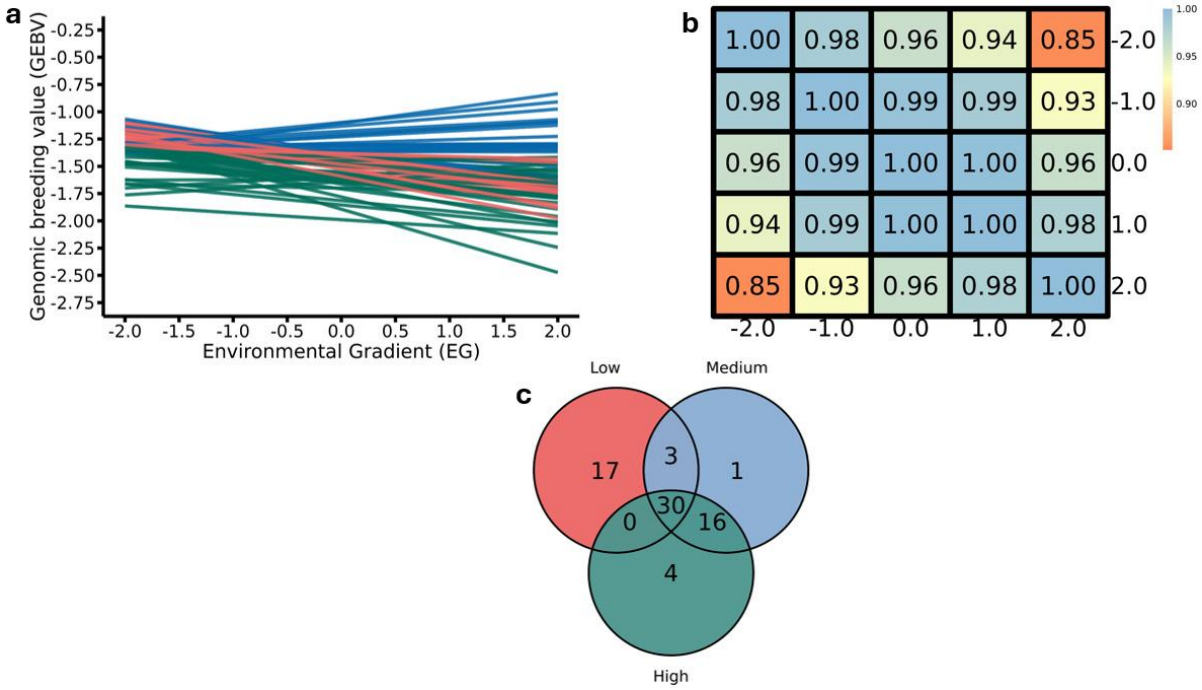

Figure 10. Reaction norms for dry matter intake (DMI) (a) Pearson correlation for genomic estimated breeding values (GEBVs) (b) and the number of common and specific sires with offspring in the environmental classes for DMI (c) considering the 50 sires with the highest number of progeny and top-ranked by GEBV in the moderate environmental gradient (EG = 0.0). The colors of the lines in panel “a” represent the EG, green for medium, red for high and blue for low.

4.4. Conclusions

This study identified key genomic regions associated with RFI and DMI in Nellore cattle, providing significant insights into the genetic background of feed efficiency traits across environmental gradients. For RFI, the intercept network pointed to biological processes crucial for appetite regulation and energy metabolism, emphasizing their role in the genetic variation of RFI in the average environment. The

slope network shifted focus to distinct genetic mechanisms influencing RFI variation across EG, including lipid metabolism and mitochondrial function. In the context of DMI, the intercept network featured processes involved in growth regulation, cellular proliferation, and energy metabolism, while the slope network emphasized pathways associated with appetite regulation and energy homeostasis. These findings underscore the adaptability of genetic networks in response to EG influences and highlight the importance of understanding these biological processes, which will be crucial for developing targeted breeding strategies to enhance feed efficiency in Nellore cattle, contributing to improved livestock production and sustainability.

4.5. Supplementary files

The supplementary file(s) supporting the analyses presented in this chapter are available online and can be accessed through the link provided in the published version of the corresponding paper: https://doi.org/10.3389/fgene.2025.1539056

4.6. Declarations

4.6.1. Funding

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4.6.2. Acknowledgments

The authors thank the National Association of Breeders and Researchers (ANCP, Ribeirão Preto, SP, Brazil) for providing the datasets for the research.

4.6.3. Data Availability Statement

The phenotypic and genotypic information are available for academic use from the authors upon reasonable request (Dr. João Carlos G. Giffoni Filho, President of ANCP email: presidencia@ancp.org.br).

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CHAPTER 5: EXPLORING THE IMPACT OF HEAT STRESS ON FEED EFFICIENCY IN TROPICAL BEEF CATTLE USING GENOMIC REACTION NORM MODELS

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Abstract: Global climate change poses significant challenges to livestock production, particularly in tropical regions where cattle frequently experience heat stress (**HS**). HS negatively impacts feed efficiency by reducing feed intake, altering metabolic processes, and increasing energy requirements, leading to decreased animal performance. Understanding how cattle respond to environmental stressors is essential for improving efficiency by breeding programs. In this context, we investigated genotype-by-environment interaction (**G×E**) for dry matter intake (**DMI**) and residual feed intake (**RFI**) in Nellore cattle using bi-trait genomic reaction norm models (**RNM**) and considering the temperature-humidity index (**THI**) as the environmental descriptor. Data from 22,838 animals collected between 2011 and 2023 across 21 Brazilian farms were analyzed. Meteorological data were obtained via NASA POWER and THI values were calculated based on the average temperatures and relative humidity recorded during feed efficiency trials. Genomic data were available for 18,567 animals, and the genetic parameters were estimated using the Single-step Genomic Best Linear Unbiased Prediction (**ssGBLUP**) approach. The genetic expression of feed efficiency traits was found to be influenced by climatic conditions, with heritability estimates for DMI (ranging from 0.22 to 0.39) and RFI (ranging from 0.08 to 0.28) varying across the THI gradient. Additionally, a reduction in additive genetic variance for both traits was observed under intense heat stress conditions, suggesting the important role of environmental factors on phenotypic variability of feed efficiency traits in Nellore cattle. The presence of G×E was more pronounced when THI exceeded 76, as genetic correlations for the same trait across different environmental gradients dropped below 0.80, leading to substantial sire reranking. Moreover, the genetic relationship between DMI and RFI also varied along the THI, with genetic correlations ranging from 0.64 to 0.72, highlighting alterations in the genetic expression of feed efficiency traits under different heat stress levels. These findings emphasize the need to consider genetic plasticity when selecting animals for improved feed efficiency in tropical regions. Overall, this study provides valuable insights for breeding programs aimed at improving beef cattle resilience to heat stress, ensuring sustainable production in the face of climate change.

Keywords: Feed intake; genetic plasticity; genotype ranking; Nellore cattle; thermotolerance.

5.1. Introduction

Global climate change significantly impacts livestock production, particularly in tropical environments (Bernabucci, 2019). In these regions, where cattle often face thermal stress, the relationship between feed efficiency traits and heat tolerance has become increasingly relevant (West, 2003; Lacetera, 2019). Thermal stress decreases feed intake, growth, reproduction, and overall productivity, leading to significant economic losses in livestock production systems (West, 2003; Marai et al., 2007). The temperature-humidity index (**THI**) is a widely used metric for quantifying heat conditions and estimating the severity of heat stress based on environmental temperature and relative humidity (Marai et al., 2001;Cheruiyot et al., 2020). High THI values increase thermoregulatory demands, such as elevated respiration rates and water intake, while reducing feed intake and, consequently, production efficiency (West, 2003; Marai et al., 2007). Genetic variability plays a critical role in individual responses to heat stress, with more heat tolerant animals displaying wider thermoneutral zones and smaller declines in performance under heat stress conditions (Carabaño et al., 2017). Identifying these animals is essential for breeding programs aimed at improving heat tolerance.

Reaction norm models (**RNM**) using THI as a continuous variable to estimate individual production responses to increasing heat loads has been proposed a few decades ago (Ravagnolo and Misztal, 2000). These models identify thermoneutral zones, where THI levels minimally affect animal performance, followed by linear declines in productivity as heat stress intensifies (Ravagnolo and Misztal, 2000). More heat tolerant animals exhibit wider thermoneutral zones (quantified by maintenance of performance) and smaller slopes of performance decline (Cheruiyot et al., 2022; Oloo et al., 2024). RNM have been widely applied to assess genotype-by-environment interaction (**G×E**) for various traits in beef cattle (Pégolo et al., 2009; Santana et al., 2014; Mota et al., 2020; Santos et al., 2020). However, most studies on feed efficiency traits, such as dry matter intake (**DMI**) and residual feed intake (**RFI**), have focused on genetic parameters and genomic regions without considering G×E (Grigoletto et al., 2017; Polizel et al., 2018;

Mota et al., 2022; Kava et al., 2023; Valente et al., 2024). Given the economic importance of feed efficiency in beef cattle production, there is a need to assess the impact of G×E on these traits, and, if substantial, it should be accounted for in breeding programs.

We have previously assessed G×E for DMI and RFI in Nellore cattle using bivariate RNM (Silva Neto et al., 2023). The environmental gradient (**EG**) was defined based on the Best Linear Unbiased Estimation (**BLUE**) solutions of the contemporary groups (**CG**) for average daily gain (**ADG**), which captures differences in nutritional, environmental, and management practices during the feed efficiency trials. We identified significant GxE, particularly under extreme environmental conditions (low and high EG values). However, GxE based on BLUE solutions of CG capture all different types of stressors, including diet composition, management, and climatic variables. Therefore, there is a need for evaluating G×E due to heat stress conditions for feed efficiency traits in beef cattle raised in tropical regions such as Nellore cattle in Brazil (Silva Neto et al., 2024).

Understanding these interactions across diverse climatic conditions is essential for developing effective Nellore cattle breeding programs tailored to the selection of more resilient individuals (Rodrigues et al., 2024). Therefore, the primary objectives of this study were: (i) to estimate genetic parameters, including heritabilities and genetic correlations, for DMI and RFI using a bi-trait genomic RNM, considering the THI (based on different equations) as the environmental gradient descriptor; (ii) to assess the presence of G×E for DMI and RFI across different climatic conditions; and (iii) to evaluate the genetic relationship between DMI and RFI under varying climatic conditions. The results of this study are expected to form foundation for selecting more heat tolerant and feed efficient animals under variable or extreme environmental conditions, such as those characterized by thermal stress.

5.2. Material and methods

5.2.1. Field data and phenotypic information

Individual feed intake was measured on 22,838 Nellore animals (16,233 males and 6,605 females) from 2011 to 2023. The datasets were provided by the National Association of Breeders and Researchers (ANCP, Ribeirão Preto, SP, Brazil; www.ancp.org.br). Animals were recorded during 296 feeding trials performed in 21 farms

located across all Brazilian regions. The dataset used included phenotypic information for DMI and RFI, following the procedures for measuring individual feed intake in beef cattle described by Mendes et al. (2020). The feeding trials were performed in group pens with animals grouped by sex and age. Feed intake was automatically recorded using the GrowSafe (www.vytelle.com) and Intergado (www.intergado.com) feeding systems. Each performance trial was conducted using a single feeding system brand, ensuring that all animals within the same group were evaluated under the same recording conditions. Detailed information on diet composition, management, and the description of the evaluated traits is provided in Silva Neto et al. (2023). Descriptive statistics for the studied traits are presented in Table 1.

The herds involved are highly connected at the genetic level due to the use of common sires through artificial insemination, with at least five genetic links across the feeding trials, which were evaluated using the Across-herd Mean Connectedness (**AMC**) package (Roso and Schenkel, 2006). The animals were raised on pasture-based systems, with a predominance of the *Urochloa brizantha cv* forage. The commercial herds adopted different nutritional practices with some farms providing protein and mineral supplementation, especially during the dry season, while others provided only urea supplementation (Silva Neto et al., 2023).

Table 1. Descriptive statistics for dry matter intake (DMI), residual feed intake (RFI), and temperature and humidity index (THI) during feed intake traits in Nellore cattle.

Variable	RFI (kg/day)	DMI (kg/day)	THI
Number of records	22,838	22,838	239
Average	0.000	8.530	74.37
Standard deviation	0.842	2.151	3.52
Minimum	-7.109	2.519	66.86
Maximum	6.940	20.658	81.66
Feeding trials information			
Number of trials with only males		209	
Number of trials with only females		87	
Animals in the pedigree		46,383	
Sires		2,816	
Dams		21,749	
Sires with progeny records		817	
Dams with progeny records		10,339	
Number of contemporary groups		742	
Distribution of trials by seasons			
Summer		19	
Fall		34	
Winter		45	
Spring		48	
*Summer and Fall		28	
*Fall and Winter		49	
*Winter and Spring		53	
*Spring and Summer		20	

Trials were distributed across seasons as follows: 15.02% in Summer, 24.89% in Fall, 32.96% in Winter, and 27.13% in Spring. Some trials spanned two consecutive seasons, as marked with an asterisk (*) in the table.

5.2.2. Genomic data

A total of 18,567 animals born between 2014 and 2022 were genotyped with a Single Nucleotide Polymorphism (**SNP**) panel containing 65,414 markers (Clarifide® Nelore 3.0). The genotypes were imputed to a high-density (**HD**) SNP panel considering the Illumina BovineHD BeadChip (Illumina Inc., San Diego, CA, USA), containing 735,964 autosomal markers using the Fimpute 3.0 software (Sargolzaei et al., 2014). Before imputation of the 18,567 animals' genomic data, we removed monomorphic markers, markers with duplicated coordinates, located on non-autosomal chromosomes, and with GenCall score lower than 0.60 (Mota et al., 2024). The reference population for genotype imputation consisted of 963 representative sires of the main Nellore lineages (i.e., Karvadi, Golias, Godhavari, Taj Mahal, Akasamu, and Nagpur). These reference sires were born between 1995 and 2015 and genotyped with the Illumina BovineHD BeadChip (Illumina Inc., San Diego, CA, USA). The genomic quality control was performed using the QCF90 software (Misztal et al., 2014). After genotype imputation, we removed samples and SNPs with call rate lower than 0.90, markers with Mendelian conflicts higher than 1%, extreme deviations (p-value $\leq 10^{-8}$) from Hardy-Weinberg equilibrium, minor allele frequency lower than 0.05, and those located in non-autosomal chromosomes. After quality control, 18,567 genotyped animals and 452,283 SNPs were retained for further analyses.

5.2.3. Weather data

Meteorological data corresponding to the days when the evaluated traits were recorded (2011–2023) were retrieved from NASA POWER (https://power.larc.nasa.gov/) based on each herd's geographical coordinates. The addresses for each herd were converted to latitude and longitude coordinates using Google Maps Geocoding (https://developers.google.com/maps/documentation/geocoding). NASA POWER weather data is freely available and can be accessed through its data access viewer or by integrating a POWER API URL into a scripting application.

To calculate the THI, four different approaches were tested:

I. Using temperature (**T**) and relative humidity (**RH**) data from three specific hours of the day (09:00 a.m., 03:00 p.m., and 09:00 p.m.), we calculated the average T and RH for each period (feed efficiency trials) and then computed THI.

II. Using T and RH data from all available hours of the day (24 hours), we calculated the average T and RH per period, and then computed THI.

III. We calculated THI for each hour of the day and then averaged the hourly THI values to obtain the period THI.

IV. We calculated THI directly for the three selected hours of the day and averaged these values to obtain the period THI.

The Pearson correlation coefficients for THI values derived from these four approaches using the (NRC, 1971) equation were all close to 1.0 (see Supplementary Table S1), indicating strong agreement across methods. Thus, any of these approaches can be used without significant differences in THI outcomes. Here, we used the "I" approach to compute the THI. In addition, seven equations for calculating THI were evaluated:

1. $THI = [(1.8\ x\ T_{db} + 32)] - (0.55 - (0.0055\ x\ RH)\ x\ (1.8\ x\ T_{db} - 26))]$ (NRC, 1971);
2. $THI = (T_{db} + T_{wb})\ x\ 0.72 + 40.6$ (NRC, 1971);
3. $THI = \left(0.55\ x\ T_{db} + 0.2\ x\ T_{dp}\right)\ x\ 1.8 + 32 + 17.5$ (NRC, 1971);
4. $THI = [0.4\ x\ (T_{db} + T_{wb})]\ x\ 1.8 + 32 + 15$ (Thom, 1959);
5. $THI = (0.35\ x\ T_{db} + 0.65\ x\ T_{dp})\ x\ 1.8 + 32$ (Bianca, 1962);
6. $THI = T_{db} + (0.36\ x\ T_{dp}) + 41.2$ (Youset, 1985); and,
7. $THI = (0.8\ x\ T_{db}) + [(RH/100)\ x\ (T_{db} - 14.4)] + 46.4$ (Mader et al., 2006).

where, T_{db} is the dry bulb temperature; T_{wb} is the wet bulb temperature, and T_{dp} is the dew point temperature. The temperature unit used was Celsius degrees.

Pearson correlation coefficients between THI values derived from these equations ranged from 0.992 to 0.999 (see Supplementary Table S2), confirming high consistency across methods. Similar findings were reported for these same models by Dikmen and Hansen (2009). The descriptive statistics for each tested model are presented in the Supplementary Table S3. The first equation (NRC, 1971) was selected for this study due to its frequent application in similar studies (Bohmanova et al., 2008; Santana et al., 2015; Menéndez-Buxadera et al., 2020) and no substantial difference as compared to the other ones.

The impact of heat stress on Nellore cattle was assessed considering a THI of 76 as the threshold for the onset of thermal stress in beef cattle. This value was defined based on literature. For instance, decline in conception rates in Nellore cattle were

observed when THI reached 75.7 (Cordeiro et al., 2020), as well as changes in feed intake, physiological parameters, and behavior in Nellore cattle exposed to a THI of approximately 76 (Valente et al., 2015). Furthermore, research on epigenetic patterns reported that THI values exceeding 75 already represented a moderate level of stress for the Nellore breed (Del Corvo et al., 2021). Therefore, a THI of 76 was used as a reference threshold for identifying the beginning of physiological and productive responses to heat exposure in Nellore cattle.

5.2.4. Statistical modelling

To estimate the genetic parameters for DMI and RFI across the EG levels (THI), a single step bi-trait genomic reaction norm model (**ssBRN**) was used. In this approach, the EG was modeled as a continuous covariate, and each trait was modeled as the same trait expressed across distinct points of the THI levels. This model can be described as follows:

$$\boldsymbol{y_{ij}} = \boldsymbol{Xb} + \boldsymbol{\omega_f \Phi_f}\left(\boldsymbol{EG_j}\right) + \boldsymbol{\alpha_{fi} \Phi_f}\left(\boldsymbol{EG_j}\right) + \boldsymbol{e_{ij}}$$

where $\boldsymbol{y_{ij}}$ is the vector of phenotypic information for DMI and RFI of the animal *i* recorded at the level *j* of EG, $\boldsymbol{b}$ is the systematic effect of CG, which was defined by concatenating the effects of farm, year and season of the feeding trial, and sex (males and females were evaluated in separate groups), and the linear covariate of the age of the animal at the beginning of the feed efficiency trials, $\mathbf{X}$ is the incidence matrix, $\boldsymbol{\omega_f}$ are the *f*-th fixed regression coefficients for intercept (*f* = 0) and slope (*f* = 1) on $\boldsymbol{\Phi_f}\left(\boldsymbol{EG_j}\right)$; $\boldsymbol{\Phi_f}\left(\boldsymbol{EG_j}\right)$ are the *f*-th Legendre orthogonal polynomials corresponding to *EG* level *j* ($\boldsymbol{EG_j}$), $\boldsymbol{\alpha_{fi}}$ are the random regression coefficients for additive genetic effects of the intercept (*f* = 0) and slope (*f* = 1) corresponding to animal *i* on EG level *j*, and $\boldsymbol{e_{ij}}$ is a random residual term.

The additive and residual genetic effects were considered normally distributed: $\boldsymbol{\alpha} = \{\boldsymbol{\alpha}\} \approx \mathbf{N}(\mathbf{0}, \mathbf{H} \otimes \boldsymbol{K_a})$ and $\boldsymbol{e} = \{\boldsymbol{e}\} \approx \boldsymbol{N}(\mathbf{0}, \mathbf{I} \otimes \boldsymbol{K_e})$, where $\boldsymbol{K_a}$ and $\boldsymbol{K_e}$ are the covariance-variance matrices for the additive genetic and residual effects, respectively for the reaction norm coefficients (intercept and slope); $\mathbf{I}$ is an identity matrix, $\otimes$ is the Kronecker product, and $\mathbf{H}$ is a matrix combining pedigree and genomic relationships. The $\mathbf{H^{-1}}$ was calculated as (Aguilar et al., 2010):

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

where $\mathbf{A^{-1}}$ is the inverse of the pedigree-based relationship matrix, $\mathbf{A_{22}^{-1}}$ represents the inverse of the relationship matrix based on pedigree for the genotyped animals, and $\mathbf{G^{-1}}$ is the inverse of the genomic relationship matrix obtained according to the first method proposed by VanRaden (2008).

The ssBRN was evaluated considering the residual variances as homogeneous or heterogeneous across EG levels. For heterogeneous residual variances, the different classes of residual variance were determined using the K-means clustering approach (Hartigan and Wong, 1979) and the best model with different classes of residual variance was chosen based on the Deviance Information Criterion (**DIC**) (Spiegelhalter et al., 2002). For this, we considered 25, 20, 15, 13, 11, nine, seven, and five classes of residual variance. THI was incorporated as an environmental variable using the first-order Legendre orthogonal polynomial coefficients. In addition, a quadratic Legendre orthogonal polynomial of second order was tested for the best linear model according to DIC values.

The genetic variance ($\hat{\sigma}^2_{aEG_i}$) and heritability ($\hat{h}^2_{EG_i}$) estimates for these traits across the EG level were calculated based on the following equations: $\hat{\sigma}^2_{aEG_i}=\boldsymbol{\Phi_f K_a \Phi_f}'$, where $\boldsymbol{K_a}$ is the matrix for the additive genetic effects, as define above, and $\boldsymbol{\Phi_f}$ is the vector of Legendre orthogonal polynomial estimate (intercept and slope) corresponding to each EG level. The heritability ($\hat{h}^2_{EG_i}$) for each EG level was calculated as: $\hat{h}^2_{EG_i}=\frac{\hat{\sigma}^2_{aEG_i}}{\hat{\sigma}^2_{aEG_i}+\hat{\sigma}^2_{eEG_i}}$, in which $\hat{\sigma}^2_{aEG_i}$ and $\hat{\sigma}^2_{eEG_i}$ are the additive genetic and residual variances, respectively, in the environment *i*. The genetic correlation across EG levels ($r_{EGi,EGi'}$) was determined as: $r_{EGi,EGi'} = \sigma_{EGi,EGi'} / \sqrt{\hat{\sigma}^2_{aEG_i} * \hat{\sigma}^2_{aEG_{i'}}}$, where $\sigma_{EGi,EGi'}$ represents the covariance between EG level *i* and EG level *i'*, estimated the same way as the genetic variance in each EG level.

Posterior distribution samples of the (co)variance components were obtained through Bayesian inference using the Gibbs sampling algorithm, implemented in the GIBBSF90 software (Misztal et al., 2014). The Bayesian analyses consisted of a single chain of 500,000 cycles, a burn-in of 50,000 iterations, and values saved at every ten cycles. The convergence was evaluated through visual inspection using the Bayesian Output Analysis (BOA) (Smith, 2007) and Geweke test (Geweke, 1992).

5.2.5. Genomic estimated breeding values

The genomic estimated breeding values (**GEBV**) associated with each level of EG were obtained as:

$$\hat{g}_{iEG_j} = \alpha_i \mathbf{\Phi'_f}$$

where α_i is the estimated additive genetic coefficient for the intercept and slope of animal *i* and $\mathbf{\Phi'_f}$ is the transposed vector of estimates of Legendre orthogonal polynomials for level *j* of EG.

5.2.6. Environmental sensitivity

The genetic variabilities observed for the slope of RNM offers the possibility to use the random slope to identify animals that are more robust to environmental changes (De Jong and Bijma, 2002; Mota et al., 2020). For this purpose, a plasticity scale was based on the absolute individual value of the slope (f_1) and standard deviation of the population slope (σ_{f1}). The animals were classified as environmentally robust ($|f_1| < \sigma_{f1}$), environmentally plastic ($\sigma_{f1} < |f_1| < 2\sigma_{f1}$), and extremely environmentally plastic ($|f_1| > 2\sigma_{f1}$).

5.3. Results and Discussion

5.3.1. Characterization of the environmental gradient

Figure 1 displays the annual mean variation in THI during the feed efficiency trials conducted between 2011 and 2023. The results indicate a gradual increase in the average THI values over the years, particularly from 2018 onwards (THI > 71), suggesting that climatic conditions during the feed efficiency trial periods became progressively warmer and/or more humid. The distribution of THI across seasons of the year exhibited a clear seasonal variation, with the highest values observed in Spring and Summer, followed by Fall. Winter, in contrast, displayed the lowest THI values. These patterns reflect typical seasonal variation, where Summer is characterized by warmer and more humid conditions, while Winter is milder and drier. Additionally, the higher values observed in Spring compared to Summer are due to the imbalance in the number of observations by season, with more feed efficiency trials conducted in Spring (27.13%) than in Summer (15.02%) (Table 1). This results in a higher absolute number of THI measurements in Spring, which could potentially

influence the mean and distribution of THI values due to the greater representation of this season in the data.

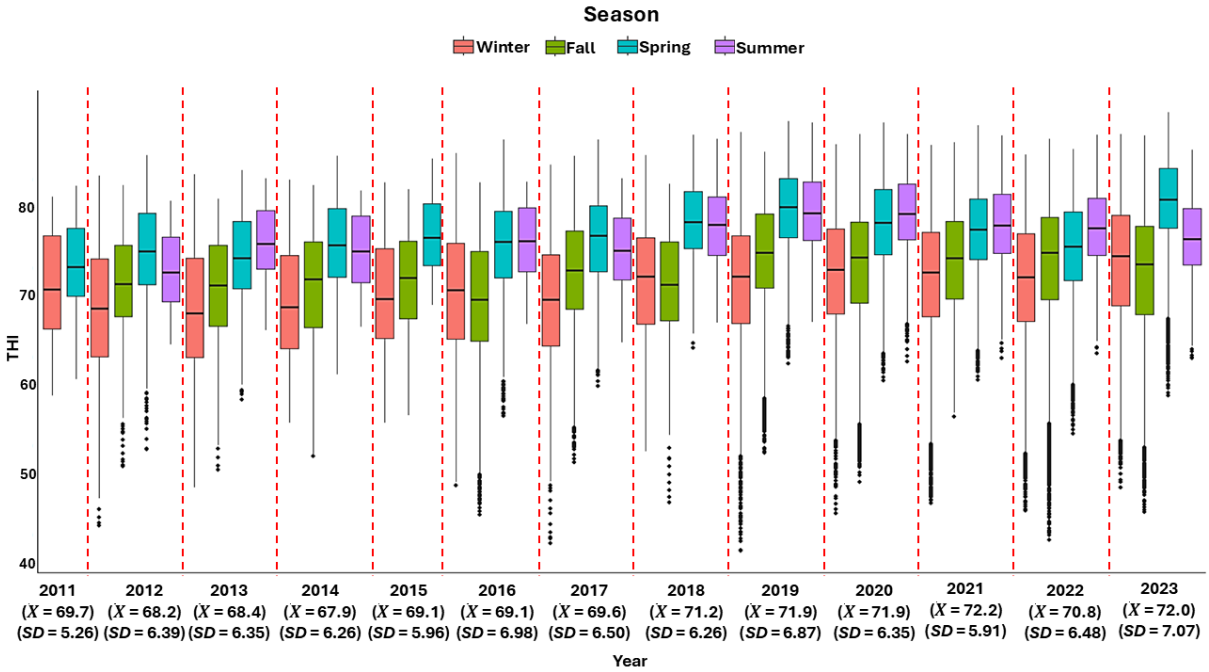

Figure 1. Distribution of the temperature-humidity index (THI) across seasons (Winter, Fall, Spring, Summer) from 2011 to 2023 during feed intake recording in Nellore cattle. Boxplots represent seasonal THI variation per year. Annual mean ($\bar{X}$) and standard deviation (SD) are shown below each year. Red dashed lines separate recording years.

Figure 2 illustrates the relative frequency of observations with THI values equal to or exceeding 76, a threshold indicating the onset of thermal stress conditions for the Nellore cattle breed (Valente et al., 2015; Cordeiro et al., 2020; Del Corvo et al., 2021), during feed efficiency trials. Between 2011 and 2017, the frequency of THI ≥ 76 remained below 34% across all seasons, with higher incidence during the Summer (from 10.3% to 31.4%) and Spring (from 14.8% to 33.7%) seasons. However, in years such as 2018, 2019, 2020, and 2023, an increase in the proportion of thermal stress conditions (THI ≥ 76) was observed across all seasons, with growing contributions from the warmer seasons, such as Summer (29.2% to 50.8%) and Spring (26.8% to 63.0%). The higher percentage of THI ≥ 76 observed in Spring compared to Summer may be linked to the greater concentration of feed efficiency trials conducted during periods of increased susceptibility to thermal stress in this season. Furthermore, the

trial location could distort the results if more trials were conducted in regions with a higher predisposition to thermal stress during spring. Additionally, variations in the years may have involved different farms or experimental facilities, which could impact the distribution of the data.

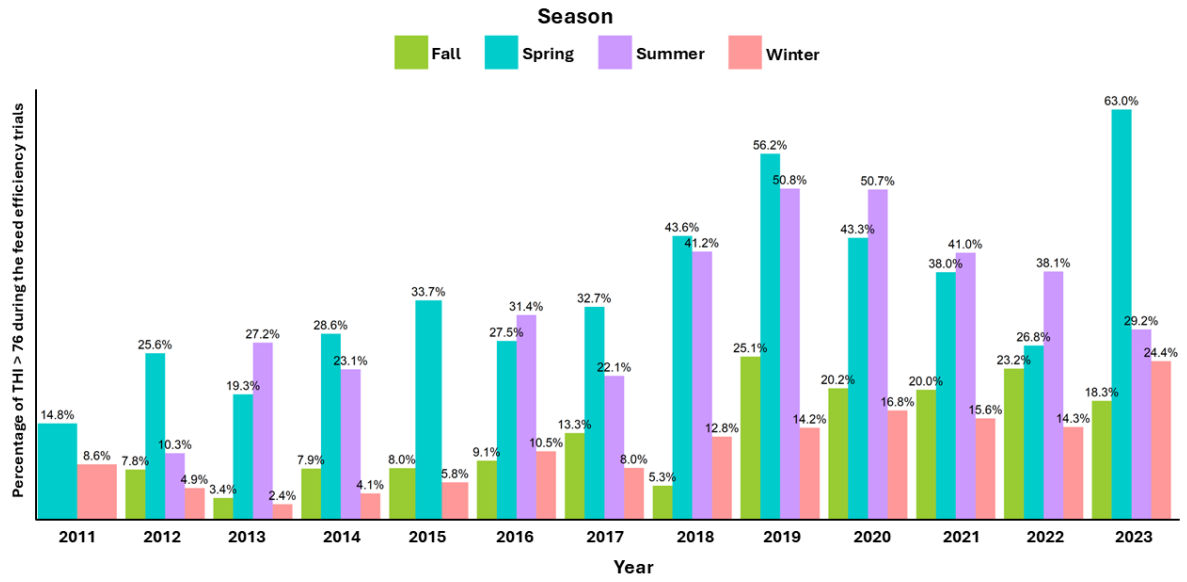

Figure 2. Proportion of days with temperature-humidity index (THI) ≥76 during feed efficiency trials from 2011 to 2023 in Nellore cattle. Bars are grouped by season (Winter, Fall, Spring, Summer) illustrating seasonal and annual exposure to heat stress.

The results highlight the impact of seasonal weather conditions on the thermal comfort of animals during feed efficiency trials. The increased occurrence of THI conditions ≥ 76 in recent years underscores the importance of management strategies and genetic selection for improved heat tolerance. Strategies such as adjusting management practices, implementing cooling technologies, and selecting more climatic resilient animals are essential to mitigate the adverse effects of increased heat stress conditions on animal productivity and welfare. These strategies are particularly relevant in the context of climate change, where the rising frequency and intensity of adverse conditions may compromise the sustainability of livestock production (Henry et al., 2018).

5.3.2. Phenotypic means of residual feed intake and dry matter intake across temperature-humidity index levels

Table 2 presents the descriptive statistics for DMI and RFI in Nellore cattle. Means and standard deviations are reported for each corresponding THI level. Regarding feed efficiency, as measured by RFI, it is observed that values are predominantly negative at lower THI levels (67 to 69), suggesting that cattle are more efficient in feed conversion under more thermoneutral conditions, consuming less feed than expected based on their production level. In physiological terms, this increase in feed efficiency may be related to a greater ability to maintain homeothermy and a more efficient use of metabolic energy, without the losses associated with thermal stress (Shephard and Maloney, 2023). Therefore, negative RFI may reflect a state of lower metabolic overload, where cattle are able to make better use of their feed for performance.

Table 2. Number of records (N) and descriptive statistics for dry matter intake (DMI) and residual feed intake (RFI) by temperature and humidity index (THI) in Nellore cattle.

THI	N	RFI (Kg DM/day)	DMI (Kg DM/day)
		Mean ± SD	
67	1,036	-0.23 ± 1.42	8.11 ± 1.89
68	1,335	-0.05 ± 0.68	8.36 ± 2.01
69	1,028	-0.01 ± 0.78	9.75 ± 2.42
70	1,182	0.39 ± 1.52	9.44 ± 2.84
71	1,321	-0.13 ± 0.82	8.75 ± 1.97
72	1,418	0.00 ± 0.74	8.32 ± 1.72
73	2,695	0.04 ± 0.85	8.92 ± 1.72
74	1,964	-0.02 ± 0.82	8.07 ± 1.95
75	3,233	0.01 ± 0.65	8.67 ± 2.57
76	1,167	0.00 ± 0.61	9.61 ± 1.47
77	2,657	0.00 ± 0.59	7.55 ± 1.47
78	1,362	0.00 ± 0.77	9.07 ± 2.96
79	1,701	0.00 ± 0.85	7.97 ± 1.60
80	511	0.00 ± 0.53	7.90 ± 1.21
81	228	0.00 ± 0.56	6.93 ± 1.02

As THI increases, particularly beyond 73, the mean RFI approached zero or became positive (Table 2), indicating a reduction in feed efficiency. This shift may be attributed to increased heat stress, which compromises the efficient utilization of nutrients by the animals. Elevated temperatures disrupt thermal balance mechanisms, negatively affecting health, welfare, growth, and production (Bernabucci, 2019; Chevin

and Hoffmann, 2017; Osei-Amponsah et al., 2019), ultimately impacting productivity and economic outcomes (West, 2003).

In terms of DMI, the feed intake reduced as the THI becomes more stressful, with the average values ranging from 9.75 kg/day (for THI 69) to 6.93 kg/day (for THI 81), with intermediate variations between 7.55 kg/day and 9.61 kg/day under more moderate THI conditions (Table 2). Feed intake tends to be lower under extreme heat stress conditions, such as at THI 81, possibly due to a reduction in appetite or motivation to consume feed under high temperature and humidity (Henry et al., 2018). On this case, environmental temperatures often approach or exceed the animal's body temperature, with mean radiant temperatures surpassing air temperatures, thereby limiting heat dissipation through convection and radiation (Lacetera, 2019). High humidity further diminishes the efficiency of evaporative heat loss mechanisms (Berman, 2011), preventing animals from dissipating excess heat and maintaining homeothermy, directly reflecting a decrease in feed intake to avoid the heat generated by the feed’s metabolic energy (Lees et al., 2019).

At moderate to mildly high THI levels (69 to 76), DMI appeared to stabilize, averaging between 8.07 and 9.75 kg/day (Table 2). Although these descriptive values suggest that cattle may maintain a relatively consistent intake under such conditions, it is important to note that no formal statistical tests were conducted in this analysis. Therefore, these observations should be interpreted as indicative trends rather than statistically validated effects. However, in support of this interpretation, the literature reports that feed intake and feeding behavior in crossbred cattle have been strongly influenced by thermal conditions and access to shade (Brown-Brandl et al., 2005), highlighting compensatory strategies employed by cattle to cope with heat stress. Brown-Brandl et al. (2005) reported that in intermediate climatic categories (THI 74 to THI 78), cattle adjusted meal frequency and size, maintaining a relatively stable daily feed intake, as observed in this study. Furthermore, under severe climatic conditions, these strategies were insufficient to overcome the adverse effects of extreme heat, resulting in significant reductions in feed intake, meal frequency, and total feeding duration (Brown-Brandl et al., 2005).

In dairy cattle, the negative impact of heat stress on feed efficiency is well documented. For instance, Chen et al. (2024) conducted a meta-analysis of 31 studies and found that each unit increase in THI led to a 4.13% reduction in DMI and a 3.25% decrease in energy-corrected milk (**ECM**) yield, particularly in mid-lactation cows.

Interestingly, despite the decline in feed intake, feed efficiency (kg ECM/kg DMI) remained relatively stable, suggesting that metabolic adjustments may partially compensate for reduced nutrient intake. Similarly, Gorniak et al. (2014) investigated the effects of mild heat stress (THI > 60) on mid-lactation Holstein cows in a temperate climate, finding that even moderate increases in THI led to significant reductions in DMI, affecting dairy cattle productive performance by the impairing in nutrient utilization efficiency. Moreover, Chang-Fung-Martel et al. (2021) conducted a global meta-analysis to assess the relationship between THI and DMI across cattle breeds and management systems. The authors reported a strong negative correlation (-0.82) between THI and DMI, with an estimated reduction of 0.45 kg/day in DMI for each unit increase in THI. These results provide robust evidence that reductions in feed intake under heat stress conditions are a widespread phenomenon, necessitating targeted interventions to minimize production losses. Furthermore, prolonged heat exposure (32°C) has been reported to influence energy utilization in Large White pigs divergently selected for RFI (Renaudeau et al., 2013). The authors found that metabolizable energy intake decreased by 38% and heat production by 20% under heat stress conditions, independent of genetic differences in feed efficiency.

These findings have significant implications for the management of animals in tropical environments, where heat stress can impact both feed intake and feed conversion efficiency. Management strategies, such as providing shade, fresh water, and other measures to mitigate heat stress, are crucial for optimizing the animals' feeding performance during periods of high temperature and humidity.

5.3.3. Comparison of reaction norm models

Table 3 presents a comparative analysis of RNM for DMI and RFI in Nellore cattle based on DIC values. This criterion assesses model fit while penalizing model complexity, with lower values indicating superior model performance (Spiegelhalter et al., 2002; Berg et al., 2004; Celeux et al., 2006). Additionally, Table 3 provides information on the number of estimated parameters (**NP**) and rankings of the models based on their DIC values.

The linear model incorporating five classes of residual variance (Lin_het_5) demonstrated optimal performance, exhibiting the lowest DIC value (-871,322.55) and the highest rank (Table 3). This model provides an effective balance between goodness of fit and model simplicity with 25 parameters, thereby ensuring a more

parsimonious representation of the data while maintaining an adequate fit. In contrast, the linear model with homogeneous residual variance (Lin_hom) ranked sixth, with a DIC of -186,813.35 and NP = 13. Although relatively simple and easy to interpret, the assumption of homogeneous residual variance across all environmental conditions failed to reflect the heterogeneous nature of variability inherent in the dataset. This structural limitation reduced the model's ability to accurately represent the intrinsic differences within the data, leading to a less precise fit of the data. Conversely, models that incorporate heterogeneous residual variances are better suited for capturing the complexity of data from Nellore cattle evaluated under diverse environmental and management conditions (Carvalheiro et al., 2019; Mota et al., 2020; Carvalho Filho et al., 2022).

Table 3. Comparison of the reaction norm models according to the deviance information criterion (DIC) for dry matter intake (DMI) and residual feed intake (RFI) in Nellore cattle.

Model	PO	CRV	DIC	NP	Rank
Lin_hom	1	1	-186,813.35	13	6
Lin_het_25	1	25	-424,824.88	85	3
Lin_het_20	1	20	-40,009.12	70	8
Lin_het_15	1	15	-560,332.90	55	2
Lin_het_13	1	13	-251,469.08	49	5
Lin_het_11	1	11	-378,719.06	43	4
Lin_het_9	1	9	-10,693.63	37	9
Lin_het_7	1	7	-41,440.74	31	7
Lin_het_5	1	5	-871,322.55	25	1
Qua_het_5	2	5	-4,071.03	36	10

The best-fitting model is Lin_het_5; PO: Legendre orthogonal polynomial order; CRV: Number of classes of residual variance; NP: number of estimated parameters; Lin_hom: linear model with homogeneous residual variance; Lin_het_25: linear model with twenty-two classes of residual variance; Lin_het_20: linear model with twenty classes of residual variance; Lin_het_15: linear model with fifteen classes of residual variance; Lin_het_13: linear model with thirteen classes of residual variance; Lin_het_11: linear model with eleven classes of residual variance; Lin_het_9: linear model with nine classes of residual variance; Lin_het_7: linear model with seven classes of residual variance; Lin_het_5: linear model with five classes of residual variance; Qua_het_5: quadratic model with five classes of residual variance; Rank: models ranking based on DIC values.

The quadratic model with five residual variance classes (Qua_het_5) demonstrated the poorest overall performance with a DIC of -4,071.03 (Table 3). This outcome suggests that the quadratic functional form is less effective in modeling reaction norms for DMI and RFI in Nellore cattle. This can be attributed to the negative linear expected phenotypic patterns for feed efficiency traits, such as DMI and RFI, in cattle under thermal stress conditions (Lees et al., 2019). The quadratic model assumes nonlinear curvatures, which, in this context, may lead to overestimation or underestimation of responses, deviating from the biological and statistical reality of the data.

The results indicate that models incorporating a greater number of residual variance classes, such as Lin_het_15 and Lin_het_25, generally exhibited improved performance, ranking second and third, respectively. However, increased model complexity does not always result in substantial improvements, as evidenced by the performance of models Lin_het_20 and Lin_het_9, which ranked eighth and ninth, respectively. To select the optimal model, Meyer (2005) recommended balancing the number of residual variance classes with the amount of available data, particularly when the number of records is unevenly distributed across EG levels. Conversely, more parsimonious models, such as Lin_het_5, demonstrated greater efficiency by striking a balance between model fit and simplicity.

Silva Neto et al. (2023) assessed G×E for DMI and RFI in Nellore cattle using BLUE solutions of the CG for ADG. According to the Bayesian Information Criterion, the authors concluded that a linear model considering heterogeneous residual variances with six classes was the most appropriate model to fit the residual structure of DMI and RFI. The quadratic model and the homogeneous residual variance model did not improve data fit, consistent with the findings of this study. It is important to note that the choice of the best-fitting model is strongly influenced by the data structure across the environmental gradient and by how the trait-specific variance components behave along this gradient. For instance, traits influenced by stage of growth, such as ADG, or by environmental adaptation, such as DMI and RFI, may exhibit linear patterns along certain gradients, which may justify the use of linear reaction norm models in such scenarios. These results underscore the importance of incorporating heterogeneous residual variance when modeling reaction norms, particularly in datasets with feeding trials conducted across multiple and diverse environments, such as in Brazil. This approach ensures a more accurate representation of G×E by

accounting for the intrinsic heterogeneity of the feed efficiency trials included in this study. Future studies should explore alternative residual variance structures to refine model selection and enhance the accuracy of genetic evaluations.

5.3.4. (Co)Variance components

Table 4 presents the posterior means of variance component estimates and posterior standard deviation (values in parentheses) for the parameters of the RNM selected as the best-fitting model (Lin_het_5, i.e., linear RNM with five heterogeneous residual classes) using genomic and pedigree information for feed efficiency traits in Nellore cattle. The slope/intercept ratio was low, with values of 0.205 for RFI and 0.102 for DMI, indicating a lower slope estimate and suggesting that Nellore cattle exhibit lower sensitivity to thermal stress for feed efficiency traits (Table 4). Animals with higher average values tend to respond more when environmental conditions become less challenging. Thus, increasing the slope/intercept ratio leads to a greater effect on animal sensitivity, as a high slope variation was observed, indicating a higher degree of G×E (Kolmodin and Bijma, 2004). Despite the low estimates for both traits, the values were different from zero, suggesting non-null environmental sensitivity and, therefore, some degree of GxE, with a greater intensity for RFI compared to DMI (Table 4). This finding is consistent with the nature of these traits, as DMI represents the feeding intake, while RFI represents an indicator of metabolic efficiency and may be more susceptible to physiological variations induced by heat stress. The non-zero slope/intercept ratio at the population level provides complementary evidence that animals differ in their genetic sensitivity to the EG, supporting the notion that genotypes do not respond uniformly across environments.

The genetic correlation between intercept and slope (Table 4) was negative for both traits (-0.514 for RFI and -0.212 for DMI), suggesting that animals with a higher intercept (better average performance) tend to be less sensitive to environmental variations (THI). Regarding residual variances, the values for RFI ranged from 0.382 (class 4) to 0.608 (class 2), while for DMI, the values were higher, ranging from 0.570 (class 1) to 0.857 (class 2).

Table 4. Posterior means of variance component estimates (posterior standard deviation) for the genomic reaction norm model parameters for residual feed intake (RFI) and dry matter intake (DMI) in Nellore cattle.

Variance component	RFI	DMI
Intercept ($\boldsymbol{\sigma_a^2}$)	0.124 (0.010)	0.467 (0.025)
Slope ($\boldsymbol{\sigma_b^2}$)	0.025 (0.008)	0.048 (0.011)
Covariance ($\boldsymbol{\sigma_{ab}}$)	-0.029 (0.008)	-0.032 (0.014)
Slope/intercept ratio	0.205 (0.071)	0.102 (0.026)
Correlation ($\boldsymbol{r_{ab}}$)	-0.514 (0.114)	-0.212 (0.094)
Covariance intercept RFI x DMI ($\boldsymbol{\sigma_{a1a2}}$)	0.159 (0.014)	
Covariance slope RFI x DMI ($\boldsymbol{\sigma_{b1b2}}$)	0.029 (0.008)	
Covariance intercept RFI x Slope DMI ($\boldsymbol{\sigma_{a1b2}}$)	-0.021 (0.009)	
Covariance intercept DMI x Slope RFI ($\boldsymbol{\sigma_{a2b1}}$)	-0.037 (0.011)	
Residual class 1 ($\boldsymbol{\sigma_{e1}^2}$)	0.392 (0.015)	0.570 (0.023)
Residual class 2 ($\boldsymbol{\sigma_{e2}^2}$)	0.608 (0.017)	0.857 (0.026)
Residual class 3($\boldsymbol{\sigma_{e3}^2}$)	0.557 (0.012)	0.837 (0.019)
Residual class 4 ($\boldsymbol{\sigma_{e4}^2}$)	0.382 (0.008)	0.618 (0.014)
Residual class 5 ($\boldsymbol{\sigma_{e5}^2}$)	0.499 (0.011)	0.792 (0.019)
Average heritability (h^2)	0.130 (0.051)	0.257 (0.048)
Average genetic correlations RFI x DMI (r_g)	0.667 (0.024)	
Average phenotypic correlations RFI x DMI (r_p)	0.708 (0.023)	

5.3.5. Heritability, phenotypic, and additive genetic variance estimates

The heritability estimates obtained for RFI and DMI across the EG values ranged from 0.08 to 0.28 and from 0.22 to 0.39, respectively (Figure 3). The heritability of DMI was higher under lower THI conditions, progressively decreasing as THI increased. This pattern suggests that additive genetic effects play a more pronounced role in determining DMI under less stressful environmental conditions. For RFI, heritability estimates were consistently lower than those for DMI across all THI levels but followed a similar trend, with the lowest heritability values observed under high THI conditions (Figure 3). This indicates a greater environmental influence on the trait under more challenging environments. As a reference, heritability estimates from the traditional model without accounting for G×E were 0.11 for RFI and 0.24 for DMI (Table S4), which fall within the range observed in the G×E model (RNM). However, heritability estimates were generally higher when G×E was modeled. Compared to the average heritability estimates obtained from the RNM (Table 4), the values from the traditional model were similar for both traits. These results indicate that the estimates obtained from the traditional model represent average values that may mask the underlying genetic variability expressed under different environmental conditions. For RFI, the traditional estimate was similar to those obtained at THI levels 72 to 74 and

76, higher than estimates under more severe heat stress (THI 77 to 81), and lower than those observed under more favorable conditions (THI 66 to 71 and 75). In the case of DMI, the pattern was similar, although less pronounced. The traditional estimate was close to those obtained at a broader range of THI levels (71 to 74 and 77 to 81), but lower than the estimates at THI 66 to 70, 75, and 76. This suggests that, even for highly correlated traits, the magnitude and pattern of G×E effects can differ.

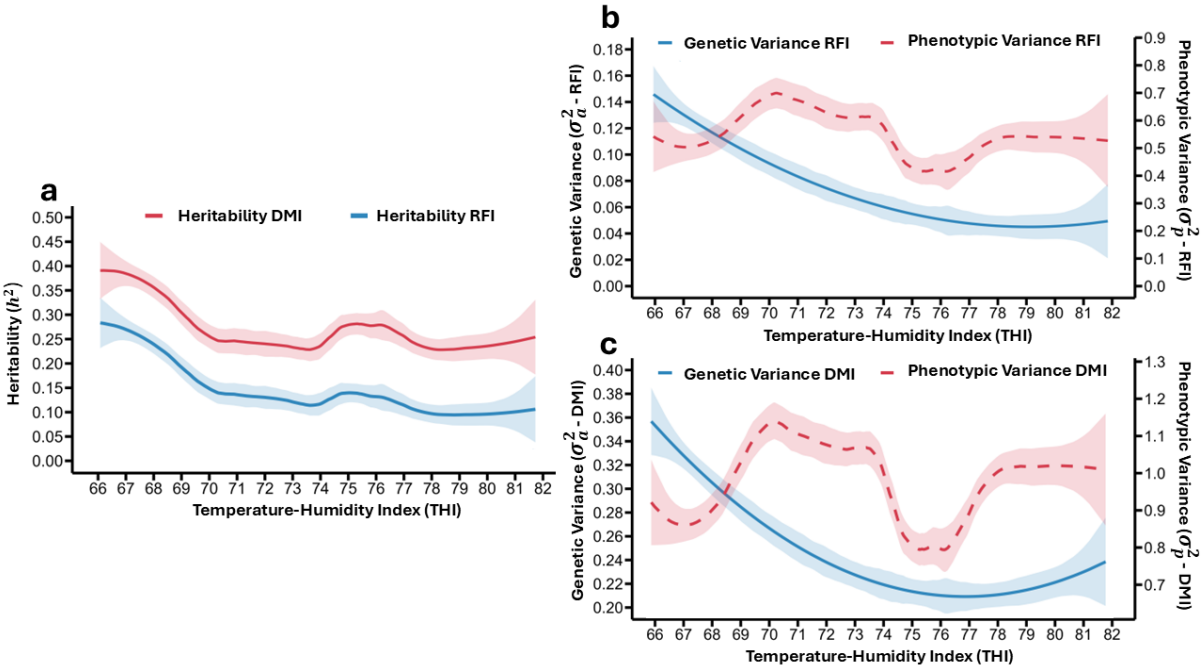

Figure 3. Estimates of heritability (h^2) (a), additive genetic variance (σ_a^2), and phenotypic variance (σ_p^2) for residual feed intake (RFI) (b) and dry matter intake (DMI) (c) in Nellore cattle across environmental gradients (THI). Shaded areas around the lines represent the posterior standard deviation (PSD), considering a 95% confidence interval.

Silva Neto et al. (2023) reported higher heritability estimates for RFI and DMI in Nellore cattle along the EG, ranging from 0.07 to 0.41 and 0.26 to 0.54, respectively. The authors used BLUE solutions of the CG for ADG. For both traits, heritability initially decreased from lower EG levels (lower environmental quality) to intermediate levels, then increased at higher EG levels (higher environmental quality). This pattern contrasts with the present study, where the lowest heritability estimates were observed under more challenging environments (high THI values). The difference in heritability

estimates between the two studies can be attributed to various factors, including the definition of the EG. In the present study, THI directly reflects heat stress conditions (temperature and humidity), whereas the EG based on BLUE solutions of ADG captures a broader range of environmental factors, such as feed availability and quality, management practices, and farm health conditions. Furthermore, differences between the populations studied, such as sample size, may also contribute to variability and subsequent differences in the heritability estimates.

The additive genetic variance for DMI (0.22 to 0.36) and RFI (0.04 to 0.15) across the THI gradient are presented in Figure 3. For DMI, higher values were observed under lower THI conditions, progressively decreasing as THI increased. This pattern suggests that the genetic potential for this trait is more efficiently expressed in less stressful environments. A similar trend was observed for the additive genetic variance of RFI, although with less variation across the THI levels.

The phenotypic variance estimates for both traits showed an increasing trend with rising THI (Figure 3). However, a notable decline was observed at THI levels 75 and 76, particularly for DMI. This temporary drop in phenotypic variance is supported by the residual variance estimates from the reaction norm model (Table 4), where the lowest posterior values for both DMI and RFI were observed in class 4, which corresponds to this THI range. The reduction in the residual component at this point decreases the total phenotypic variance without a proportional change in genetic variance, indicating a period of lower environmental heterogeneity or more uniform animal responses under those heat stress conditions. In general, for DMI (0.84 to 1.15), the increase observed was accompanied by a disproportionately smaller additive genetic variance, which did not rise at the same rate, reflecting a greater environmental influence as THI increased. For RFI (0.43 to 0.71), the increase in phenotypic variance was predominantly associated with greater environmental variance, thereby diluting the relative contribution of additive genetic effects. These findings emphasize the critical role of environmental factors in shaping the observed phenotypes, particularly under thermal stress conditions.

Comparatively, Silva Neto et al. (2023) reported additive genetic variance estimates for DMI progressively increasing in more favorable environmental conditions (0.26 to 0.75), suggesting that better environments amplify genetic differences among animals. However, for RFI, the values remained relatively stable (0.07 to 0.11) across EG levels. Similarly, the phenotypic variance for both traits increased with EG (0.27 to

1.08 for RFI and 0.55 to 1.71 for DMI), driven primarily by greater environmental variance. This effect was more pronounced for RFI, where heritability decreased as EG increased, indicating that variance heterogeneity had a greater impact on this trait than on DMI.

In conclusion, these findings highlight the dynamic nature of heritability and variance estimates for DMI and RFI across environmental gradients. The observed patterns reinforce the need for selection strategies that account for G×E, particularly in tropical production systems where environmental stressors, such as THI, play a significant role in modulating phenotypic expression and genetic potential of animals.

5.3.6. Genetic correlation estimates across temperature-humidity index levels

The genetic correlation estimates for the evaluated traits across THI levels ranged from 0.29 to 0.99 for RFI and from 0.55 to 0.99 for DMI, with mean (± SD) values of 0.83 ± 0.18 and 0.90 ± 0.11, respectively, indicating the presence of G×E (Figure 4). When EG levels were more similar, the genetic correlations were above 0.80, suggesting that a more similar set of genes is regulating performance in similar environments. However, as the environments became more divergent, the correlations dropped below 0.80, indicating more different genetic control and reflecting a higher genetic plasticity in response to environmental changes. The progressive decrease in genetic correlations below 0.80 was observed from THI 76 for both traits, suggesting significant G×E effects as environmental differences became more pronounced with increasing thermal stress. Similarly, Silva Neto et al. (2023) also observed G×E for these same traits in Nellore cattle, using BLUE solutions for ADG as EG. The genetic correlation for RFI across different EG levels ranged from 0.22 to 0.99, with an average (± SD) of 0.81 ± 0.21, while for DMI, the correlation ranged from 0.26 to 0.99, with an average (± SD) of 0.83 ± 0.19, showing a broader range compared to the present study, mainly for DMI. High genetic correlations were observed when the EG levels were more similar, indicating consistent genetic performance under similar conditions, which mirrors the findings in our study. However, as environmental conditions became more divergent, the genetic correlation decreased below 0.80, suggesting the presence of significant G×E. This decrease in genetic correlation implies that performance for feed efficiency and feed intake may vary across environments, potentially leading to a reranking of breeding animals by the variations in their estimated breeding values. This further emphasizes the need to account for

environmental variations in breeding programs to ensure consistent genetic progress, especially when dealing with different levels of thermal stress in the production systems.

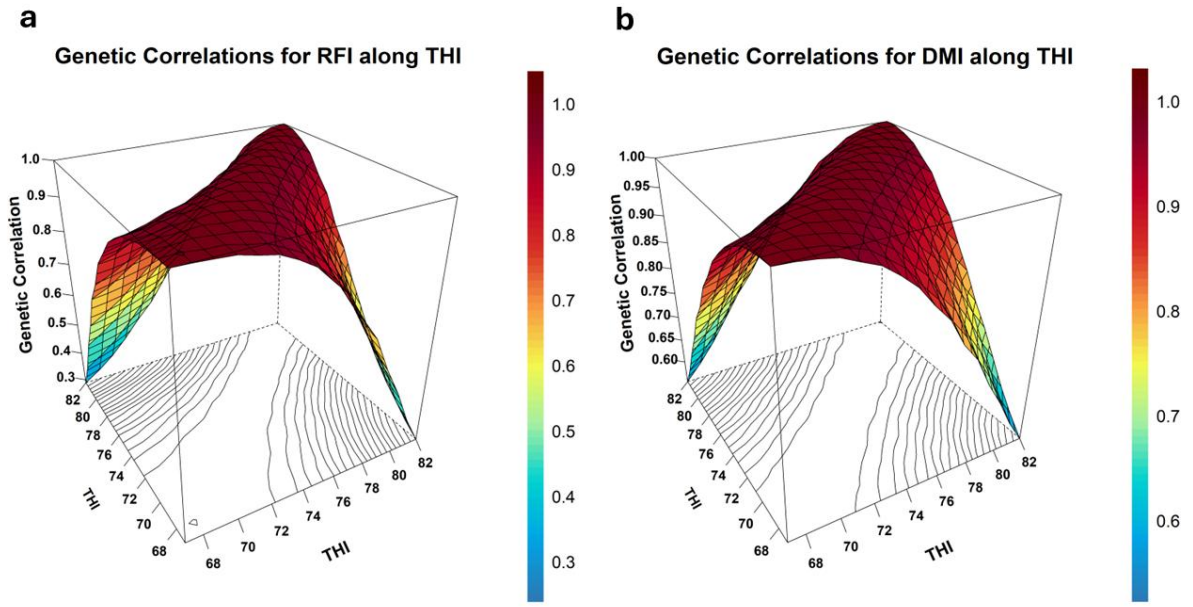

Figure 4. 3D surface plots of genetic correlation estimates for residual feed intake (RFI) (a) and dry matter intake (DMI) (b) across environmental gradients (THI – temperature humidity index) in Nellore cattle. Horizontal axes represent specific THI levels in pairwise comparisons, while the vertical axis shows the genetic correlation between those levels for the same trait. The surface illustrates variation in genetic correlations along the environmental gradient. The color scale indicates the magnitude of the correlations, ranging from low (blue) to high (red).

5.3.7. Genetic and phenotypic correlations between feed intake and efficiency trait

Estimated genetic and phenotypic correlations between RFI and DMI across the THI levels were consistently positive, ranging from 0.64 to 0.72 and 0.70 to 0.73, respectively (Figure 5). Compared to the traditional model, these estimates fell within the range observed in the RNM models and were also similar to their average values (Table 4), with approximately 0.65 (genetic) and 0.72 (phenotypic) (Table S4), reinforcing that traditional models represent average estimates across environmental conditions. The phenotypic correlation between RFI and DMI exhibited greater stability across the entire THI gradient. Although slight variations were observed at moderate

THI levels, the curve pattern suggests that non-genetic factors, such as management practices or specific environmental conditions, may play a crucial role in maintaining this relationship. Conversely, looking at the pattern of genetic correlations, a gradual decline was observed along increasing THI levels. The correlations remained relatively stable under thermoneutral conditions (THI < 76), but progressively decreased as heat stress intensified, suggesting that environmental heat load may influence the genetic regulation of feed efficiency traits. These reductions in genetic correlations indicate changes in genetic responses in more stressful environments, potentially driven by adaptive mechanisms or physiological trade-offs.

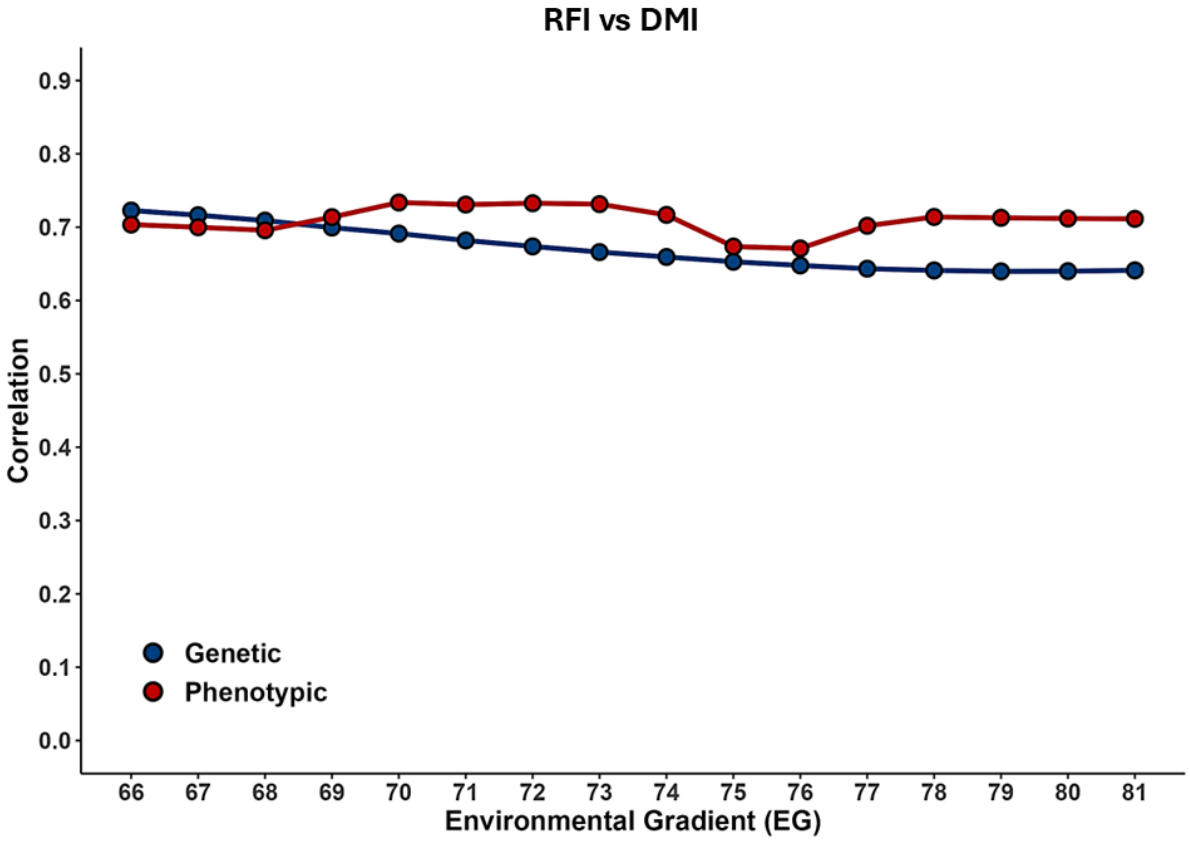

Figure 5. Genetic and phenotypic correlation estimates between residual feed intake (RFI) and dry matter intake (DMI) in Nellore cattle across environmental gradients (THI).

The stability of phenotypic correlations across the THI gradient indicates that, even under heat stress conditions, animals maintain a consistent relationship between

DMI and RFI. In contrast, the reduction in genetic correlations in high THI environments underscores the relevance of G×E in selection decisions. Therefore, incorporating environmental gradients into breeding programs is essential to more effectively capture G×E variation. Furthermore, a multivariate approach may be beneficial in identifying traits that are more stable and those requiring greater attention under extreme environmental conditions.

5.3.8. Reaction norms for genomic estimated breeding values

The reaction norms for the top 50 sires with the largest number of progeny (average of 285.78, ranging from 111 to 1,171 offsprings) for RFI and DMI, showed reranking among these sires (Figure 6). The effect of G×E on the sensitivity of animals across EG levels, especially between extreme THI levels, was expected due to a genetic correlation lower than 0.80 (Figure 4). The average GEBV for RFI and DMI were, respectively, 0.014 kg/DM/day and 0.548 kg/DM/day for the lowest EG level (THI = 67), − 0.001 kg/DM/day and 0.532 kg/DM/day for the intermediate EG level (THI = 74), and − 0.015 kg/DM/day and 0.516 kg/DM/day for the highest EG level (THI = 81). These values are part of internal results not shown in tables or figures.

The reaction norms pattern highlights the significance of the G×E interaction. Genotypes with high plasticity, characterized by greater sensitivity to environmental changes, are associated with steeper slopes, whereas more robust genotypes exhibit flatter slopes. Based on the slope (f_1) solutions, 18.0% and 20.0% of the top 50 sires were classified as highly plastic to environmental changes, 38.0% and 26% as plastic, and 44.0% and 54.0% as more robust to environmental changes for RFI and DMI, respectively (Table 5). When considering sire classifications for both traits simultaneously, 14.0% were classified as highly plastic, 14.0% as plastic, 34.0% as more robust, and 38.0% fell into different categories, meaning they were not consistently categorized for both traits. The desirability of each category depends on the production system and environmental challenges, particularly in the context of heat stress. In environments with frequent thermal stress, high plasticity may not be advantageous, as animals that drastically change their performance in response to environmental fluctuations could show unstable productivity under heat stress. On the other hand, in systems with stable or only moderately variable environmental conditions, high plasticity may represent an adaptive advantage by allowing animals

to maximize the expression of their genetic potential whenever conditions are favorable. Additionally, a moderate degree of plasticity might be advantageous even in changing environments, as it could allow for a certain degree of adaptability without the metabolic cost of maintaining strict homeostasis. Therefore, the optimal genotype depends on the specific environmental context, as initially mentioned.

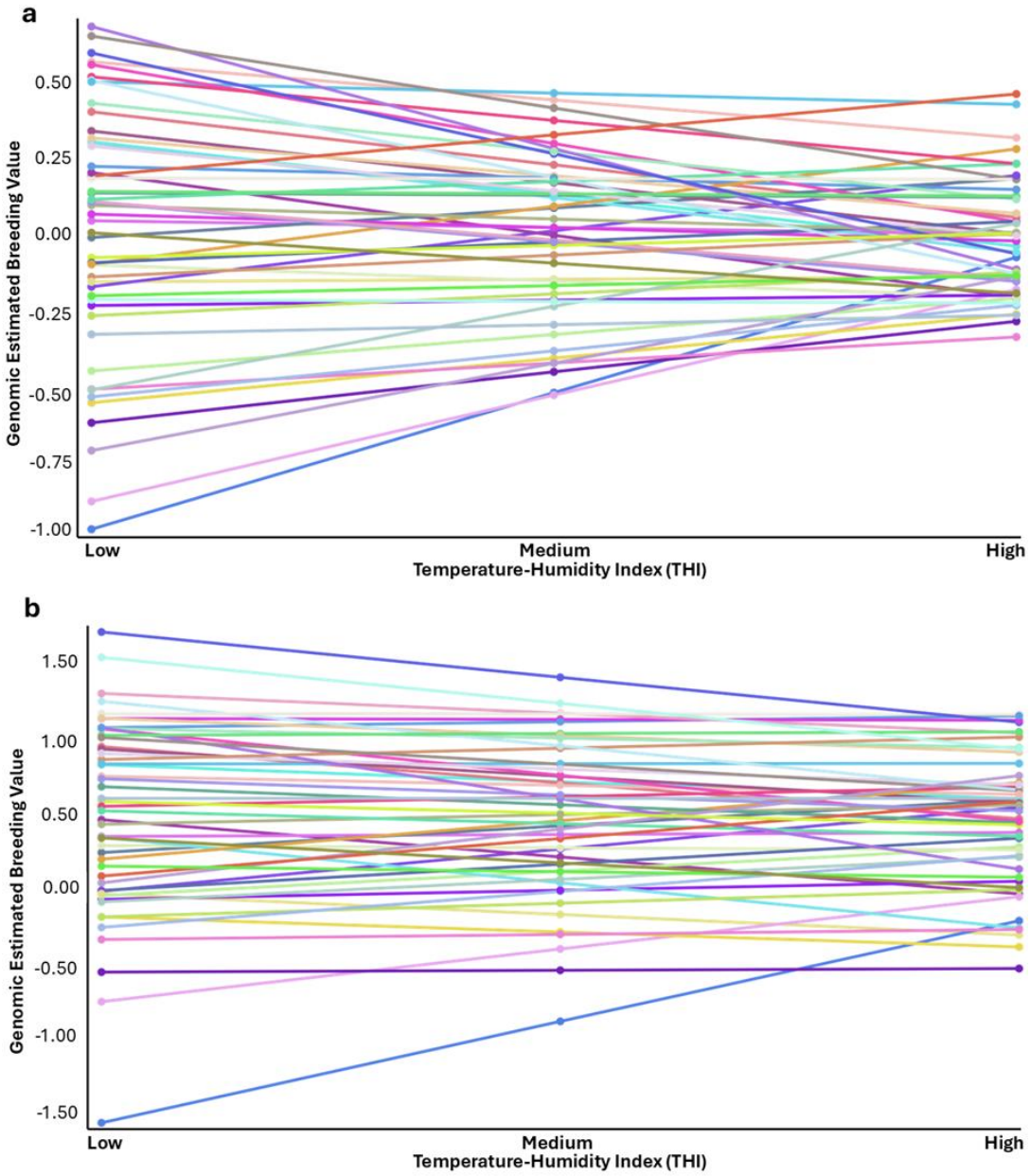

Figure 6. Reaction norms for the 50 Nellore sires with the highest number of progeny for residual feed intake (RFI) (a) and dry matter intake (DMI) (b). Each line represents a sire, with colors consistently assigned so that the same sire is represented by the same color across both traits.

Table 5. Proportion of animals by plasticity category for residual feed intake (RFI) and dry matter intake (DMI) in Nellore cattle: all individuals and top sires.

Classification	All individuals			Top sires		
	RFI	DMI	Both traits[1]	RFI	DMI	Both traits[1]
Robust	72.6%	73.7%	65.2%	44.0%	54.0%	34.0%
Plastic	21.2%	19.8%	10.6%	38.0%	26.0%	14.0%
High plasticity	6.2%	6.5%	3.9%	18.0%	20.0%	14.0%
Number of animals (%)	46,386 (100%)		36,970 (79.7%)[1]	50 (100%)		31 (62.0%)[1]

f_1: absolute individual value of the slope; σ_{f1}: standard deviation of the population slope; Robust: $|f_1| < \sigma_{f1}$; Plastic: $\sigma_{f1} < |f_1| < 2\sigma_{f1}$; Extreme plasticity: $|f_1| > 2\sigma_{f1}$; [1] 20.3% and 38.0% of the population and sires, respectively, fell into different categories for RFI and DMI, meaning they were not consistently classified across both traits and, therefore, were not included in the "Both traits" category.

At the population level, 6.2% and 6.5% of animals were classified as highly plastic to environmental changes, 21.2% and 19.8% as plastic, and 72.6% and 73.7% as more robust to environmental changes for RFI and DMI, respectively (Table 5). For both traits simultaneously, 3.9% were classified as highly plastic, 10.6% as plastic, 65.2% as more robust, and 20.3% fell into different categories, meaning they were not consistently classified for both traits.

The predominance of animals classified as robust reflects the inherent ability of Nellore cattle to maintain stable metabolic function despite environmental fluctuations. The breed's short, light-colored hair facilitates heat dissipation, while its well-developed sweat glands and loose skin enhance evaporative cooling, enabling animals to regulate body temperature more efficiently under high temperatures and elevated THI (Nonato et al., 2023). Beyond thermoregulation, Nellore cattle exhibit additional traits that contribute to their robustness and reduced plasticity. The relatively low proportion of highly plastic individuals (6.2% for RFI and 6.5% for DMI) suggests that most Nellore cattle do not rely on drastic physiological adjustments in response to environmental changes but instead maintain performance through adaptive mechanisms. While this lower plasticity may limit the breed's ability to capitalize on high-input systems to the same extent as more plastic breeds, it provides a crucial advantage in tropical climates where resilience to environmental challenges is essential towards more sustainable production (Rodrigues et al., 2024).

5.3.9. Spearman Correlation and Selection Coincidence

The top 50 sires were ranked based on their GEBVs for both traits, and 30% (15 sires) were selected to assess Spearman correlations and selection coincidence, defined as the proportion of sires that were simultaneously selected across different EG. Each of these top 15 sires had at least five progeny distributed across a minimum of five EG levels (i.e., THI 66, 71, 74, 77, and 81), with an average of 108 progeny per sire (ranging from 25 to 359). The rank, GEBVs, and number of progeny for each sire by trait are presented in Supplementary Tables S5 and S6.

The Spearman correlations and selection coincidence percentages for RFI and DMI among the top 15 sires are presented in Figure 7. The ranking correlation analyses indicate that the performance of sires for RFI is highly sensitive to environmental variations, with correlations across environmental gradients ranging from 0.55 to 0.99 (Figure 7a). The lowest estimates were observed between the extreme THI levels (66 and 81), suggesting that the ranking of sires for RFI is more prone to changes under extreme environmental conditions, such as higher or lower temperatures and humidity.

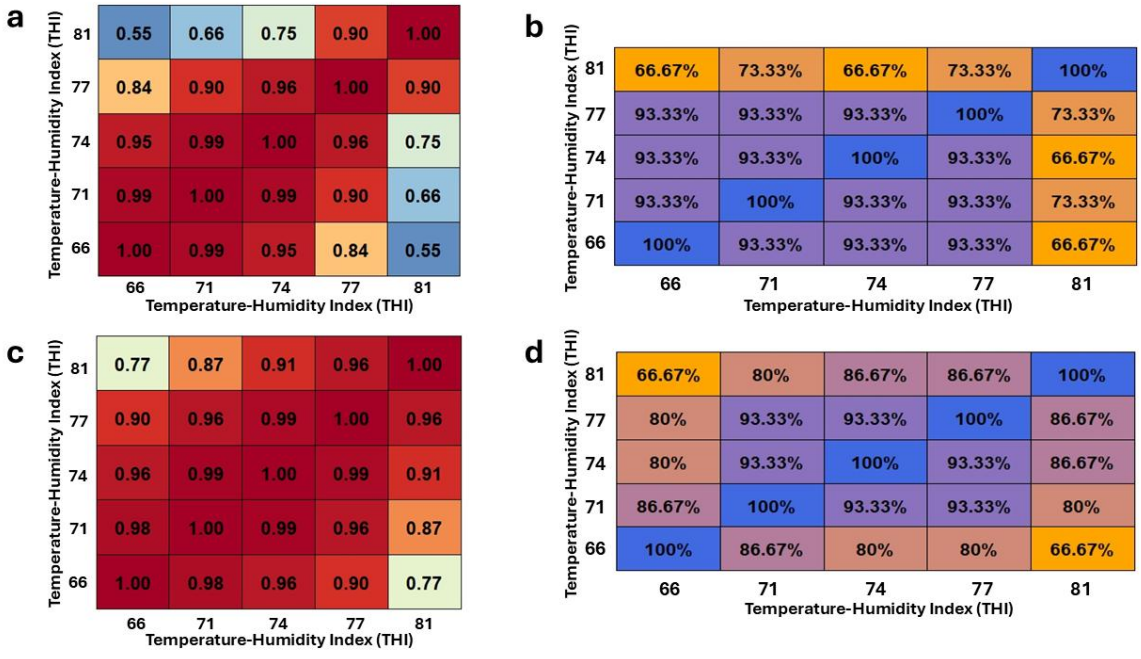

Figure 7. Spearman's rank correlations for residual feed intake (RFI) (a) and dry matter intake (DMI) (c), and selection coincidence for RFI (b) and DMI (d), among the top 15 Nellore sires with at least five progeny in each of the five temperature-humidity index (THI) levels.

In contrast, the ranking correlations for DMI showed greater consistency, remaining above 0.76 in all comparisons (Figure 7c). This result suggests that DMI is less influenced by environmental changes than RFI, reflecting lower variability in the ranking of sires under different environmental conditions. However, considering the trend of decreasing Spearman correlation values as the environments become more divergent, it suggests that extreme thermal stress conditions may still influence the ranking of sires.

The selection coincidence analyses revealed a pattern similar to that observed in the ranking correlations. For both traits, the selection coincidence percentages ranged from 66.67% to 93.33% (Figure 7b), with lower values observed between the extreme THI levels (e.g., 66.67% between levels 66 and 81). This reinforces that selection under extreme environmental conditions can lead to substantial differences in sire performance, suggesting that the effectiveness of RFI and DMI selection may be reduced under such conditions. Considering the magnitude of the values obtained between THI levels 66 to 77 compared with the extreme environment (THI 81), the results suggest that selection for RFI is more affected by environmental variation than DMI, which generally showed higher coincidence values. This is consistent with the ranking correlations, which indicate greater relative stability of DMI across environmental conditions.

In addition to comparisons across gradients, the Spearman correlations between the traditional model without G×E and each EG from the RNM model are reported in Supplementary Table S7. For RFI, Spearman correlations ranged from 0.78 (THI 81) to 0.99 (THI 74), and for DMI, from 0.91 (THI 81) to 1.00 (THI 74), highlighting a close alignment between the traditional model and intermediate environmental conditions. Similarly, the selection coincidence percentages between the traditional model and each EG ranged from 73.33% to 93.33% for RFI and from 80.00% to 100% for DMI, with the lowest values again observed in comparisons involving the most extreme environmental gradient (THI 81). This pattern consistently reinforces the results previously discussed in this study, indicating that the traditional model captures an overall average performance across environments.

Overall, these results indicate that RFI is more sensitive to environmental conditions than DMI, as evidenced by the lower correlations and lower selection coincidence observed at the extreme THI levels. This suggests that sire performance for RFI may be significantly altered by environmental variations, particularly under

more extreme conditions. The greater stability of DMI to environmental changes makes it a less variable metric for sire performance. On the other hand, the instability of RFI highlights the need to consider GxE in selection programs, to minimize the reranking of sires across different environmental conditions and thereby enhance selection efficacy. Incorporating models that account for these interactions can improve the accuracy of selection and ensure that the most suitable sires (or other breeding animals) are chosen for varying environmental conditions, such as THI.

5.4. Future research directions

In the present study, we used the phenotypic averages for each test period to assess the impact of heat stress on feed efficiency traits. While this approach facilitates the modeling of G×E and captures general trends, it may mask daily or even hourly variations in the effects of heat stress on DMI and RFI. Heat stress is a dynamic phenomenon, with substantial fluctuations throughout the day due to variations in ambient temperature, relative humidity, and solar radiation. Previous studies indicate that THI at specific times of the day can have distinct effects on metabolism and feeding behavior in cattle. For instance, animals tend to reduce feed intake during periods of higher temperatures and compensate for this reduction during cooler periods (Brown-Brandl et al., 2005). However, given the use of THI averages and phenotypic means for each feeding trial period, it was not possible to capture short-term variations and their direct implications on feed efficiency.

Future studies should use longitudinal data, allowing for a more precise assessment of how THI fluctuations affect DMI and RFI over time. Such an approach would enable the: i) identification of critical periods of the day during which heat stress has the greatest impact on feed intake; ii) evaluation of individual adaptation patterns to heat stress, determining whether certain animals adjust their feeding behavior throughout the day to mitigate heat stress effects; and iii) direct modeling of phenotypic responses based on daily THI variations, rather than relying solely on period-averaged values. Incorporating continuous records of feed intake and associated environmental variables could significantly enhance G×E modeling accuracy, providing more precise insights for selecting animals with greater resilience to heat stress. Future studies could greatly benefit from this approach to further elucidate the mechanisms that influence feed efficiency in tropical production systems and to support the development of more robust breeding programs in the face of climate change.

5.5. Conclusions

This study demonstrated the influence of heat stress on feed efficiency traits in Nellore cattle using genomic reaction norm models. Heritability estimates varied along the environmental gradient (THI), indicating that the genetic expression of these traits is sensitive to climatic variations. The detection of G×E was more evident at higher THI values, particularly above 76, where genetic correlations for the same trait across different environments dropped below 0.80 and sire reranking became more pronounced. However, progressive changes in genetic parameters were also observed across the full range of THI values, reinforcing the continuous nature of environmental influence. Additionally, the relationship between DMI and RFI was not constant along the environmental gradient, showing substantial genetic instability under extreme heat conditions, reinforcing the need to consider genetic plasticity when selecting beef cattle for improved feed efficiency in tropical regions.

5.6. Supplementary files

The supplementary file(s) supporting the analyses presented in this chapter are available online and can be accessed through the link provided in the published version of the corresponding paper: https://doi.org/10.1016/j.animal.2025.101612

5.7. Data availability statement

The data analyzed in this study were obtained from the National Association of Breeders and Researchers (ANCP). The phenotypic and genotypic information was provided to the authors for academic research purposes only. The following restrictions apply: the dataset is not publicly available and its use requires formal authorization. Requests to access these datasets should be directed to Dr. João Carlos G. Giffoni Filho, President of ANCP (email: presidencia@ancp.org.br).

5.8. Acknowledgments

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CHAPTER 6: MODELING GENOTYPE-BY-ENVIRONMENT INTERACTIONS ACROSS CLIMATIC CONDITIONS REVEALS ENVIRONMENT-SPECIFIC GENOMIC REGIONS AND CANDIDATE GENES UNDERLYING FEED EFFICIENCY TRAITS IN TROPICAL BEEF CATTLE

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Abstract

Background: Heat stress represents a major limitation for livestock production systems, negatively affecting feed efficiency, animal health and welfare, and overall performance. In this context, the objective of this study was to identify genomic regions, candidate genes, biological pathways, and functional networks associated with dry matter intake (DMI) and residual feed intake (RFI) in Nellore cattle exposed to varying levels of thermal stress. The dataset comprised records from 22,838 animals, with genotypes available for 18,567 individuals. The data was collected during 296 feed efficiency trials between 2011 and 2023 across 21 Brazilian farms. Genome-wide association studies (GWAS) were performed using the single-step GBLUP (ssGBLUP) approach to account for genotype-by-environment (G×E) interactions in Nellore cattle. Environmental variation was modeled using the temperature-humidity index (THI) as the environmental gradient, with analyses stratified across three environmental gradients (EG): low (THI = 66), medium (THI = 74), and high (THI = 81).

Results: Fifty-one SNPs were significantly associated with RFI, including 27 shared across all three EGs, 10 exclusives to the low EG, one to the high EG, and 13 shared between the moderate and high EGs. These associations were mapped to 44 candidate genes, with 19 genes commonly identified across all EGs, including key candidates such as *PIPOX*, *GTF2F2*, *KCTD4*, *MYO18A*, and *NFIA*. For DMI, 136 significant SNPs were identified: 12 and 39 exclusives to the low and moderate EGs, respectively; 28 shared across all EGs; and 57 shared between the moderate and high EGs. These variants were linked to 58 candidate genes, of which 19 were common to all EGs, including *NCAPG*, *LCORL*, *FAM13A*, *HERC3*, *CCND1*, and *FGF19*. Gene network analyses revealed a clear reconfiguration of interaction structures across thermal gradients, particularly for RFI, where gene connectivity declined with increasing THI levels. For DMI, gene networks remained highly integrated, especially in the lowest THI level. Functional annotation highlighted both conserved and environment-specific regulatory architectures, involving key biological processes such as growth regulation, lipid and protein metabolism, intracellular signaling, stress response, and neuroendocrine control.

Conclusions: These findings uncover the environmental sensitivity of RFI and DMI, highlight the complex and dynamic genomic basis of these traits under varying climatic

conditions, and support the identification of candidate genes for genomic selection programs aiming to enhance climatic resilience in tropical beef cattle.

Keywords: Climate resilience; dry matter intake; functional enrichment; Nellore cattle; residual feed intake; temperature-humidity index; tropical environments.

6.1. Background

Environmental stressors, particularly heat stress, impose significant challenges on livestock by triggering complex physiological and molecular responses that compromise animal health, welfare, and productivity [1, 2]. Among the various indicators to quantify heat stress, the temperature-humidity index (THI) remains the most widely adopted, as it integrates temperature and relative humidity into a single descriptor of environmental stress [3–5]. Exposure to elevated THI levels has been associated with altered gene expression patterns [5–7], dysregulation of metabolic pathways [8], and impairment of key physiological functions, including immune response [2, 9], reproductive performance [10–12], and nutrient metabolism [1, 13]. These stress-induced modifications occur at multiple biological levels, ranging from transcriptional and post-transcriptional regulation to endocrine signaling, contributing to phenotypic variability among animals [14–17]. Furthermore, there is growing evidence that thermal stress may modulate the expression of genetic merit, directly affecting the response to selection under varying environmental conditions [18, 19]. This highlights the importance of genotype-by-environment (G×E) interactions, in which the magnitude and direction of genetic effects vary depending on environmental conditions.

The intensification of climate change poses a major challenge to the sustainability of beef production systems, particularly in tropical environments where animals are continuously exposed to heat stress conditions [13, 20]. Among the traits most sensitive to heat stress is feed efficiency [11, 21], which directly influences profitability, environmental sustainability, and resource allocation. Heat stress can compromise feed intake, alter energy partitioning, and reduce metabolic efficiency, thereby amplifying phenotypic variability [1, 21, 22] and consequently variation in genetic merit. Consequently, identifying animals that maintain superior performance under thermal stress conditions becomes a strategic objective for breeding programs in tropical regions.

Genome-wide association studies (GWAS), when integrated with environmental descriptors such as THI, can enable the detection of genomic regions and candidate genes associated with resilience and adaptation to heat stress [23]. These approaches not only advance our understanding of the genetic background of feed efficiency under heat stress but also support the development of more precise genomic selection strategies, targeting animals that are both efficient and robust across diverse climatic scenarios. Furthermore, integrating environmental sensitivity into GWAS allows the identification of SNP-by-environment (SNP x E) interactions, unraveling the environment-dependent genetic effects that are often masked in conventional analyses [23–25]. Understanding how genomic regions influence response to environmental variability is therefore essential for advancing more precise and climate-resilient breeding programs.

In a previous study, Silva Neto et al. [19] investigated G×E interactions for feed efficiency traits in Nellore cattle using a bi-trait genomic reaction norm model, considering THI as an environmental descriptor. Their results demonstrated that the genetic expression of dry matter intake (DMI) and residual feed intake (RFI) is sensitive to heat stress, with both heritability estimates and additive genetic variance declining under high THI conditions. These findings highlight the importance of incorporating environmental sensitivity into genetic evaluations to improve the selection of animals that remain feed efficient under thermally stressful conditions. However, no previous GWAS have evaluated the genetic background of DMI and RFI in Nellore cattle while explicitly accounting for environmental variation through distinct THI levels [26]. Therefore, the main objectives of this study were to: i) perform a GWAS accounting for GxE interactions for RFI and DMI in Nellore cattle under varying levels of thermal conditions (low, medium, and high) according to the THI; and ii) to annotate candidate genes and conduct functional enrichment analyses to elucidate the biological processes and molecular mechanisms associated with thermal resilience in feed efficiency traits.

6.2. Material and methods

6.2.1. Field data and phenotypic information

Individual feed intake records were measured on 22,838 Nellore animals (16,233 males and 6,605 females) from 2011 to 2023. The datasets were provided by

the National Association of Breeders and Researchers (ANCP, Ribeirão Preto, SP, Brazil; www.ancp.org.br). Data originated from 296 feeding trials performed in 21 farms distributed in different Brazilian regions. Phenotypic information was available for DMI and RFI, following the standardized protocols for measuring individual feed intake in beef cattle described by Mendes et al. [27]. The feeding trials were performed in group pens with animals grouped by sex and age, with feed intake automatically recorded using the GrowSafe (www.vytelle.com) and Intergado (www.intergado.com) feeding systems. Each performance trial was conducted using a single feeding system brand and the same data collection protocol, ensuring that all animals within the same group were evaluated under the same recording conditions. Detailed descriptions of diet composition, management, and the evaluated traits are provided in Silva Neto et al. [28]. Descriptive statistics for the traits studied and environmental descriptor (THI) are reported in Table 1.

Table 1. Descriptive statistics for residual feed intake (RFI), dry matter intake (DMI), and temperature and humidity index (THI) during feed efficiency trials in Nellore cattle.

Variable	RFI (kg/day)	DMI (kg/day)	THI
Number of records	22,838	22,838	239
Average	0.000	8.530	74.37
Standard deviation	0.842	2.151	3.52
Minimum	-7.109	2.519	66.86
Maximum	6.940	20.658	81.66
Feeding trials information			
Number of trials with only males		209	
Number of trials with only females		87	
Animals in the pedigree		46,383	
Sires		2,816	
Dams		21,749	
Sires with progeny records		817	
Dams with progeny records		10,339	
Number of contemporary groups		742	

The herds are genetically connected through the extensive use of common sires via artificial insemination, with at least five genetic links across feeding trials, as confirmed using the AMC package [29]. The animals were raised on pasture-based systems, with a predominance of the *Urochloa brizantha cv* forage. The commercial

herds adopted different nutritional practices with some farms providing protein and mineral supplementation, especially during the dry season, while others provided only urea supplementation [28].

6.2.2. Genomic data

A total of 18,567 animals born between 2014 and 2022 were genotyped with a SNP panel containing 65,414 markers (Clarifide® Nelore 3.0, Zoetis, Kalamazoo, MI). The genotypes were imputed to a high-density (HD) SNP panel (Illumina BovineHD; San Diego, CA, USA) containing 735,964 autosomal markers using the Fimpute 3.0 software [30]. Before genotype imputation, we removed non-autosomal markers and autosomal SNPs with GenCall < 0.60 to remove genotyping problems [31].

The reference population for genotype imputation consisted of 963 representative sires from the main Nellore lineages in Brazil (i.e., Karvadi, Golias, Godhavari, Taj Mahal, Akasamu, and Nagpur), born between 1995 and 2015 and genotyped with the Illumina BovineHD BeadChip (Illumina Inc., San Diego, CA, USA). The quality control in imputed genotypes was performed using the qcf90 software [32], removing samples and SNPs with call rate < 0.90, markers with Mendelian conflicts > 1%, extreme deviations from Hardy-Weinberg equilibrium (p-value ≤ 10^{-8}), and minor allele frequency (MAF) < 0.05. After filtering, 18,567 genotyped animals and 452,283 SNPs remained for further analyses.

6.2.3. Weather data

Meteorological data corresponding to the days when the evaluated traits were recorded (2011–2023) were retrieved from NASA POWER (https://power.larc.nasa.gov/) based on each herd's geographical coordinates. The addresses for each herd were converted to latitude and longitude coordinates using Google Maps Geocoding (https://developers.google.com/maps/documentation/geocoding).

The Temperature-Humidity Index (THI) was calculated according to NRC [33]:

$$THI = [(1.8\ x\ T_{db} + 32)] - (0.55 - (0.0055\ x\ RH)\ x\ (1.8\ x\ T_{db} - 26))]$$

where, T_{db} is the dry bulb temperature (in Celsius degrees) and RH is the relative humidity. This equation has been frequently applied in similar studies to evaluate the GxE across heat stress conditions [19, 34–36]. As THI is a composite index that weights both temperature and relative humidity, different T–RH pairs can lead to the

same THI value. For example, combinations such as 33°C with ~20% RH, or 27°C with ~65% RH, yield THI values very close to 76. The annual mean variation of the THI during the years in which feed efficiency trials were conducted, the seasonal distribution of THI values, and the relative frequency of instances in which THI was equal to or exceeded 76 (threshold indicating the onset of thermal stress for the Nellore breed) are detailed in Silva Neto et al. [19]. These data provide important environmental context, emphasizing the intensity and frequency of heat stress exposure experienced by the animals throughout the feed efficiency trials.

Given the THI range observed during the feed efficiency trials (~66–81), and with the aim of facilitating the biological interpretation of the results, we selected three representative points along the environmental gradient to present and contrast the GWAS results: THI 66 (the mildest/thermoneutral condition available), THI 74 (close to the center of the gradient, where the first Legendre coefficient was ≈ 0 and very close to the reported onset of heat stress in Nellore cattle), and THI 81 (the condition of greatest thermal challenge within the dataset).

6.2.4. Genome-wide Association Analyses (GWAS)

GWAS were conducted independently for each EG (Low = THI 66, Medium = THI 74, and High = THI 81). The same population of 22,838 Nellore cattle, of which 18,567 were genotyped, was used in all analyses. No phenotypic stratification by EG was applied, instead, the environment-specific variance components previously estimated using a single-step genomic reaction norm model for the same population and described in detail by Silva Neto et al. [19], were used as inputs for the respective GWAS models. Integrating these variance components ensured methodological consistency between the genetic parameter estimates and the environmental conditions under which the phenotypes were expressed, so that association tests were carried out under the same environmental structure in which genetic parameters were obtained, making SNP detection consistent with the previously modeled G×E structure along the THI gradient and preserving coherence among phenotypic adjustments, environmental characterization, and marker detection. Additional descriptive statistics by THI classes and the distribution of records along the THI gradient, which were used to fit the reaction norm model in the previous study, are presented in Silva Neto et al. [19].

The single-step genome-wide association study (ssGWAS) method proposed by Wang et al. [37] was used for the analyses. The general linear mixed model used for the traits studied was:

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Za} + \boldsymbol{e}$$

where, $\boldsymbol{y}$ is the vector of phenotypic information for DMI and RFI; $\boldsymbol{X}$ is an incidence matrix relating the phenotypes to the fixed effects; $\boldsymbol{\beta}$ is the vector of fixed effect of CG, which was defined by concatenating the effects of farm, year and season of the feeding trial, and sex (males and females were evaluated in separate groups), and the age of the animal at the beginning of the feed efficiency trials as a linear covariate; $\boldsymbol{Z}$ is the incidence matrix relating the records to the additive genetic effects; $\boldsymbol{a}$ is the vector of random animal additive genetic effects with $\boldsymbol{a}$ ~N(0,$\mathbf{H}\sigma_u^2$), and $\boldsymbol{e}$ is the vector of residual effects with $\boldsymbol{e}$ ~N(0, $\mathbf{I}\sigma_e^2$).

The inverse of the hybrid relationship matrix $\mathbf{H^{-1}}$ was constructed as [38]:

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

where, $\mathbf{A^{-1}}$ is the inverse of the pedigree-based relationship matrix; $\mathbf{A_{22}^{-1}}$ represents the inverse of the relationship matrix based on pedigree for the genotyped animals; and $\mathbf{G^{-1}}$ is the inverse of the genomic relationship matrix obtained according to the first method proposed by VanRaden [39].

SNP effects were estimated by back-solving from the genomic estimated breeding values (GEBVs) of genotyped animals, following the procedure described by Wang et al. [37] and implemented in the postGSf90 [40] of the BLUPf90 suite. All SNPs were considered to contribute equally to the total additive genetic variance, and no weighting scheme was applied. The SNP effects were derived as:

$$\hat{\boldsymbol{u}} = \boldsymbol{Z'(ZZ')^{-1}}\hat{\boldsymbol{a}}$$

where, $\hat{\boldsymbol{u}}$ is the vector of estimated additive genetic effects for the SNP markers; $\hat{a}$ is the vector of GEBVs for the genotyped animals; $\boldsymbol{Z}$ is the centered genotype matrix (each genotype coded as 0, 1, or 2, centered by subtracting 2p, where p is the allele frequency of the reference allele). All computations were performed using postGSf90, which executes this back solving algorithm internally.

The *p*-value of the SNP effect was calculated based on the prediction error variance as [40]:

$$p_i = 2(1 - \Phi\left(\left|\frac{a_i}{sd(a_i)}\right|\right))$$

where α_i is the SNP effect estimate; *sd* is the standard deviation; and Φ is the standard normal cumulative distribution function. The *p*-values were generated by back-solving the SNP effects from the GEBVs.

After performing the GWAS, the genomic inflation factor (λ_{GC}) was calculated to assess potential biases in the statistics, such as those arising from population stratification. The λ_{GC} value was computed as the ratio between the median of the observed test statistic distribution and its expected median, with a 95% confidence interval subsequently derived [41]. Multiple testing correction was applied using the Bonferroni method ($\alpha = 0.05$) [42], resulting in a genome-wide significance threshold of at $P = 0.05 / 452{,}283$ ($P < 1.11 \times 10^{-7}$), equivalent to $-\log_{10}(P) \approx 6.96$. To avoid type I and II errors, a chromosome-wide significance threshold was considered based on the number of independent chromosomal segments (M_e) [43] as: $M_e = 2N_eLk / \log(N_eL)$, where M_e is a function of effective population size; L is the length of each chromosome in Morgans; and k is the number of chromosomes. N_e was set to 100 based on linkage disequilibrium patterns observed in the population [25]. Quantile-quantile (Q-Q) plots were created using the CMplot R package [44].

6.2.5. Gene Enrichment Analyses

The annotation of candidate genes was performed using the GALLO package [45] available in R (R Core Team). For that, a window of 100Kb up and downstream from the significant SNP marker was used considering the assembly *Bos taurus* ARS-UCD1.2 as the reference genome [46]. After annotation, the positional candidate genes were subjected to functional enrichment analysis using the “clusterProfiler” R package [47]. Gene Ontology (GO) terms including biological processes (BP), metabolic functions (MF), and cellular components (CC), as well as the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways ($p < 0.05$) were used to explore the biological relevance of the associated genomic regions. Interactions between protein-coding genes were predicted using the STRING database with default settings [48].

6.3. Results

6.3.1. Significant Markers

The significant SNPs associated with feed efficiency traits were evaluated across three EG levels: low (THI 66), medium (THI 74), and high (THI 81). For RFI (Figure 1, Table 2), 51 genome-wide significant SNPs were identified across chromosomes BTA3, BTA4, BTA9, BTA11, BTA12, BTA13, BTA19, BTA20, BTA24, and BTA28 under the three EG levels, with 37 SNPs in the low EG, 40 in the medium EG, and 41 in the high EG (Table 2). BTA12 stood out by presenting a substantial number of significant SNPs in all environments, particularly in the medium (n = 24) and high (n = 24) EGs, followed by the low EG (n = 22). BTA19 also showed an increased number of SNPs under more challenging environmental conditions: 5 under low EG, 11 under medium EG, and 12 under high EG.

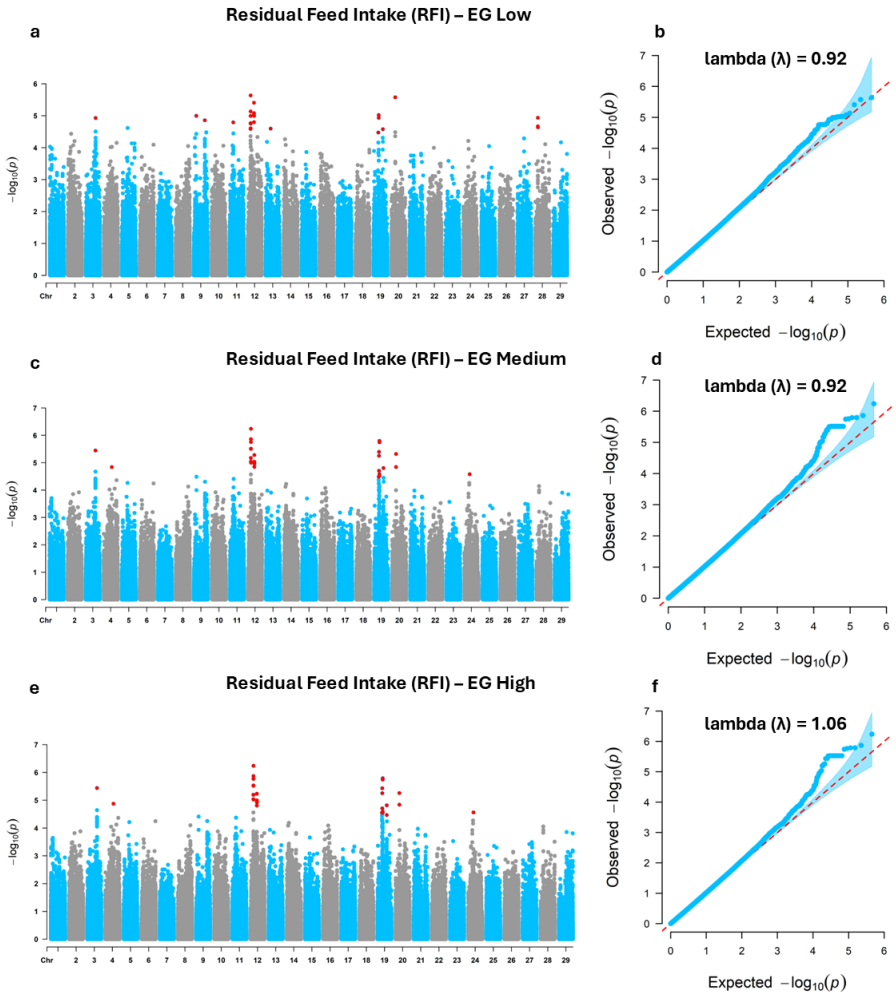

Figure 1. Manhattan and quantile-quantile (QQ) plots of genome-wide association study results for residual feed intake (RFI) in Nellore cattle. (a) Manhattan plot and (b)

QQ plot for the Low environmental gradient (EG); (c) Manhattan plot and (d) QQ plot for the Medium EG; (e) Manhattan plot and (f) QQ plot for the High EG; Low (THI 66), Medium (THI 74), and High (THI 81).

Table 2. Distribution of significant single nucleotide polymorphisms (SNPs) by chromosome (BTA) across low, medium, and high environmental gradients for residual feed intake (RFI) in Nellore cattle.

	Environment Gradient		
Significant SNP	Low	Medium	High
BTA 3	1	1	1
BTA 4	-	1	1
BTA 9	2	-	-
BTA 11	1	-	-
BTA 12	22	24	24
BTA 13	2	-	-
BTA 19	5	11	12
BTA 20	1	2	2
BTA 24	-	1	1
BTA 28	3	-	-
Total number of significant SNPs	37	40	41

Low (THI 66), Medium (THI 74), and High (THI 81).

For DMI (Figure 2, Table 3), 136 significant SNPs were detected, distributed across chromosomes BTA2, BTA4, BTA5, BTA6, BTA10, BTA11, BTA14, BTA16, BTA19, BTA20, BTA21, BTA22, BTA24, and BTA29, with 40 SNPs identified under the low EG, 124 under the medium EG, and 85 under the high EG (Table 3). BTA6 exhibited the highest number of significant SNPs (n = 110) under the medium EG, followed by the high EG (n = 71) and the low EG (n = 15). Additionally, BTA10, BTA11, BTA14, and BTA29 also stood out due to changes in significant SNPs across different gradients.

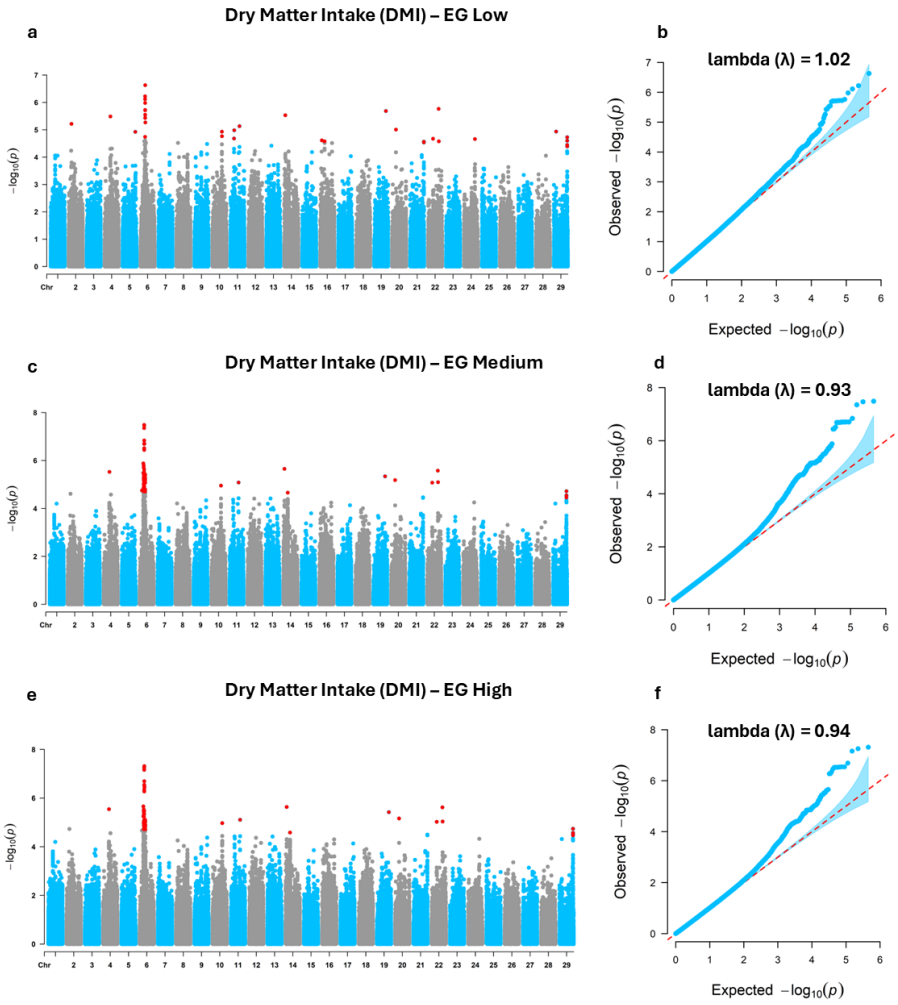

Figure 2. Manhattan and quantile-quantile (QQ) plots of genome-wide association study results for dry matter intake (DMI) in Nellore cattle. (a) Manhattan plot and (b) QQ plot for the Low environmental gradient (EG); (c) Manhattan plot and (d) QQ plot for the Medium EG; (e) Manhattan plot and (f) QQ plot for the High EG; Low (THI 66), Medium (THI 74), and High (THI 81).

Table 3. Distribution of significant single nucleotide polymorphisms by chromosome (BTA) across low, medium, and high environmental gradients for dry matter intake (DMI) in Nellore cattle.

	Environment Gradient		
Significant SNP	Low	Medium	High
BTA 2	1	-	-
BTA 4	1	1	1
BTA 5	1	-	-
BTA 6	15	110	71
BTA 10	2	1	1
BTA 11	3	1	1
BTA 14	1	2	2
BTA 16	2	-	-
BTA 19	1	1	1
BTA 20	1	1	1
BTA 21	2	-	-
BTA 22	3	3	3
BTA 24	2	-	-
BTA 29	5	4	4
Total number of significant SNPs	40	124	85

Low (THI 66), Medium (THI 74), and High (THI 81).

6.3.2. Specific and shared distribution of significant SNPs across the EGs

The overlap of significant SNPs across the different EG for feed efficiency traits was analyzed using Venn diagrams (Figure 3). For RFI (Figure 3a, Supplementary Table S1), 27 SNPs were found to be shared among all three environments (low, medium, and high EG), indicating genomic regions associated with RFI regardless of environmental variation. However, a considerable number of significant SNPs were unique to specific EGs, such as 10 SNPs (BTA9: 2, BTA11: 1, BTA12: 1, BTA13: 2, BTA19: 1, and BTA28: 3) exclusive to the low EG, and 1 SNP (BTA19) exclusive to the high EG (Supplementary Table S2). Detailed information on the significant SNPs for each EG, including chromosome, position, allele frequency, proportion of additive genetic variance explained, and effects are provided in Additional File 1: Tables S3 (low EG), S4 (medium EG), and S5 (high EG).

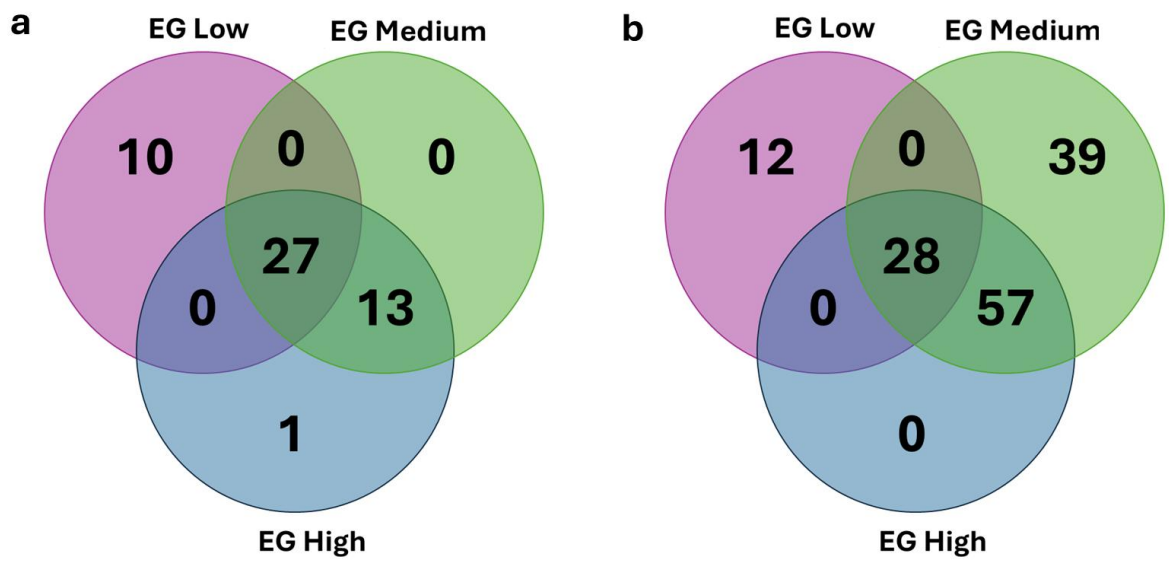

Figure 3. Venn diagram of significant single nucleotide polymorphisms, highlighting those that are specific and shared among the environmental gradients (EG): Low (THI 66), Medium (THI 74), and High (THI 81) for residual feed intake (RFI) (a) and dry matter intake (DMI) (b) in Nellore cattle.

For DMI (Figure 3b, Supplementary Table S6), 28 SNPs were shared across all three EG levels, while 12 SNPs (BTA2: 1, BTA5: 1, BTA10: 1, BTA11: 2, BTA16: 2, BTA21: 2, BTA24: 2, and BTA29: 1) were exclusive to the lowest THI group (Supplementary Table S7). In the medium EG, 39 exclusive SNPs were identified, all located on BTA6 (Supplementary Table S7). No SNPs were exclusive to the high EG, but 57 markers identified under this condition were shared with the medium EG. Detailed information on the significant SNPs per EG, including chromosome, position, allele frequency, proportion of additive genetic variance explained, and effect are provided in Additional File 1: Supplementary Tables S8 (low EG), S9 (medium EG), and S10 (high EG).

6.3.3. SNP effects across environmental gradients

Figure 4 illustrates the variation in the effects of significant SNPs across low (THI 66), medium (THI 74), and high (THI 81) EG for RFI (panel a) and DMI (panel b) in Nellore cattle. For both traits, a similar pattern is observed, a more pronounced change in SNP effects between the low and medium EGs, followed by a slight fluctuation between the medium and high EGs.

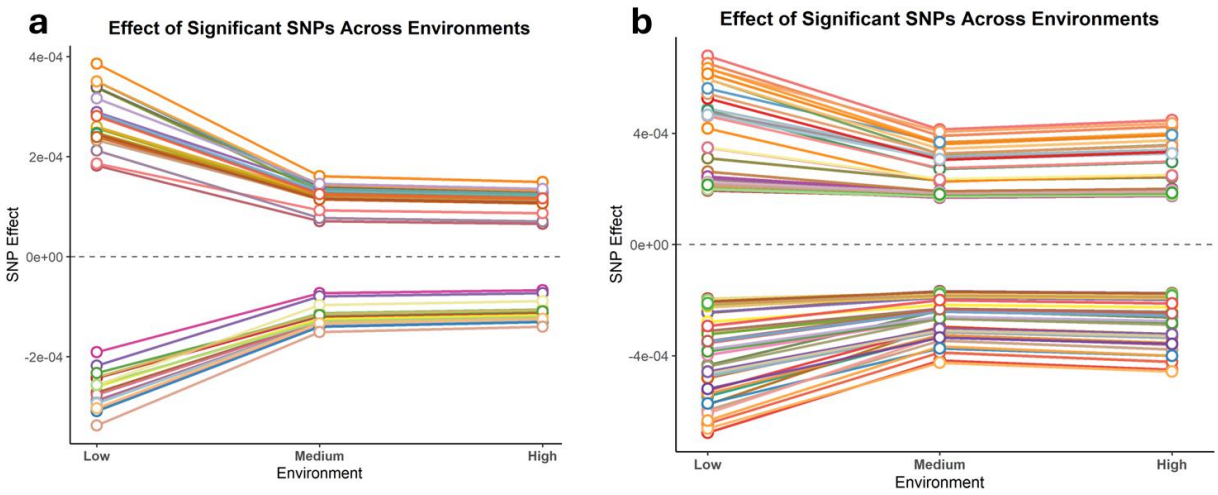

Figure 4. Effect of significant single nucleotide polymorphisms among the environmental gradients (EG): Low (THI 66), Medium (THI 74), and High (THI 81) for residual feed intake (RFI) (a) and dry matter intake (DMI) (b) in Nellore cattle.

6.3.4. Candidate genes identified under different thermal conditions for RFI and DMI

Candidate gene annotation was carried out for RFI and DMI under three distinct thermal conditions: low (THI 66), medium (THI 74), and high (THI 81). The results are illustrated in the Venn diagram presented in Figure 5, which summarizes the exclusive and shared genes across the different EG. Additional information, including chromosomal position, associated significant SNPs, gene boundaries (start and end positions), and functional classification (gene biotype), is available in Supplementary Tables S11 (EG Low), S12 (EG Medium), and S13 (EG High) for RFI, and S14 (EG Low), S15 (EG Medium), and S16 (EG High) for DMI.

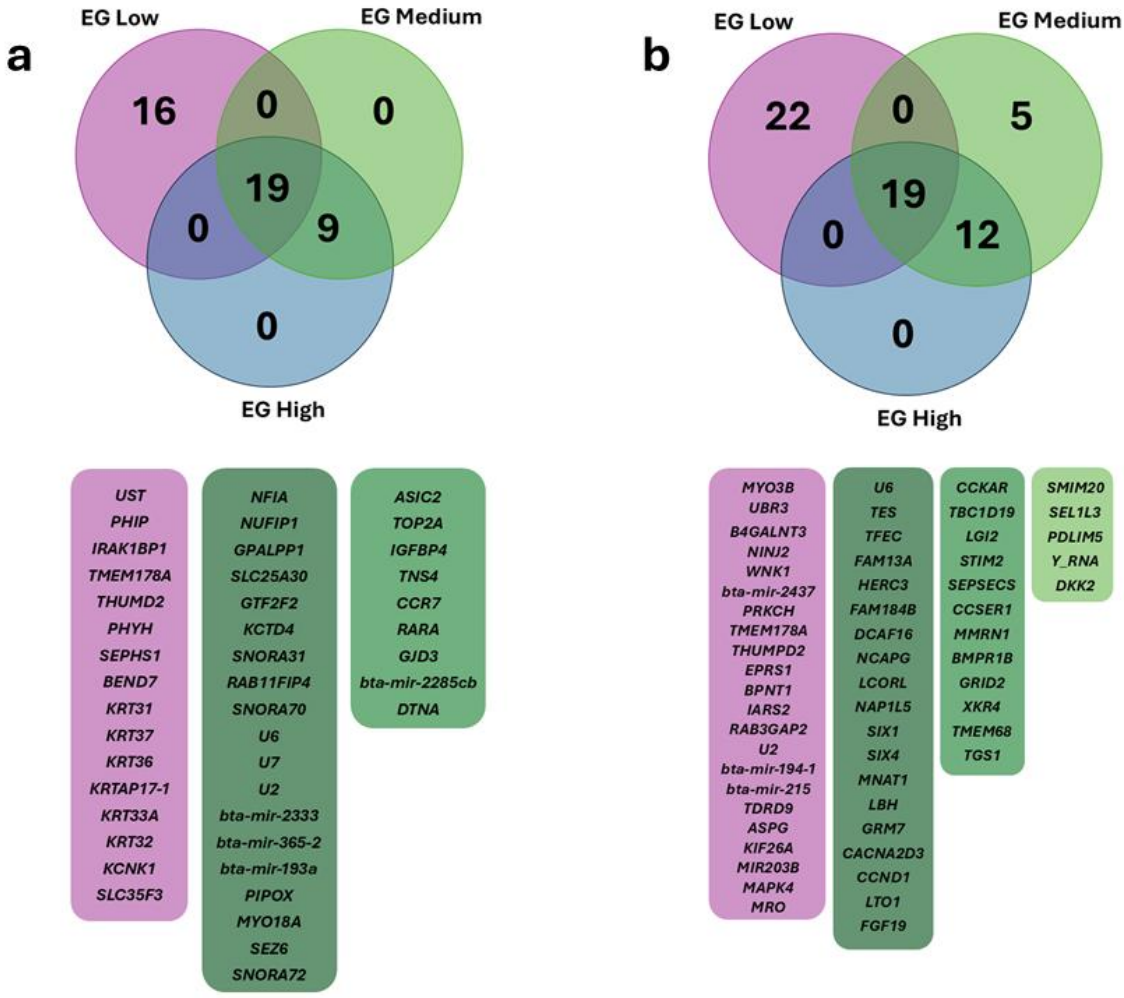

Figure 5. Venn diagram of genes associated with significant single nucleotide polymorphisms, highlighting those that are specific and shared among the environmental gradients (EG): Low (THI 66), Medium (THI 74), and High (THI 81) for residual feed intake (RFI) (a) and dry matter intake (DMI) (b) in Nellore cattle.

Nineteen genes were found to be commonly associated with RFI across the three EG levels, suggesting robust genetic effects independent of environmental variation (Figure 5a). Under low heat conditions (THI <66), 16 candidate genes were identified as specifically associated with this environment (Figure 5a). No genes were uniquely associated with RFI in either medium (THI = 74) or high (THI = 81) heat stress environments (Figure 5a). Nine genes were commonly identified under both moderate (THI = 74) and high (THI = 81) heat stress conditions. For DMI, a total of 58 candidate genes were annotated across the three thermal conditions (Figure 5b), with 22 genes exclusively associated with the EG Low and 5 with the EG Medium. 12 genes were

both identified in the EG Medium and EG High, while 19 genes were commonly detected across all three EG. No genes were exclusive to the High EG.

6.3.5. Functional network analysis for RFI across EG

The analysis of interaction networks of candidate genes associated with RFI in Nellore cattle in the low EG (THI = 66) displayed a high density of functional connections and the formation of well-defined clusters (Figure 6a). A particularly prominent cluster involved members of the keratin gene family (*KRT31*, *KRT32*, *KRT33A*, *KRT36*), which exhibited strong interconnectivity. Another relevant cluster includes *BEN Domain Containing 7* (*BEND7*) and *PHYH*, which appear centrally connected in the network and are functionally associated with the keratin group. Additional co-expression relationships were observed between *SLC35F3* and *KCNK1*, and between *IRAK1BP1* and *PHIP.*

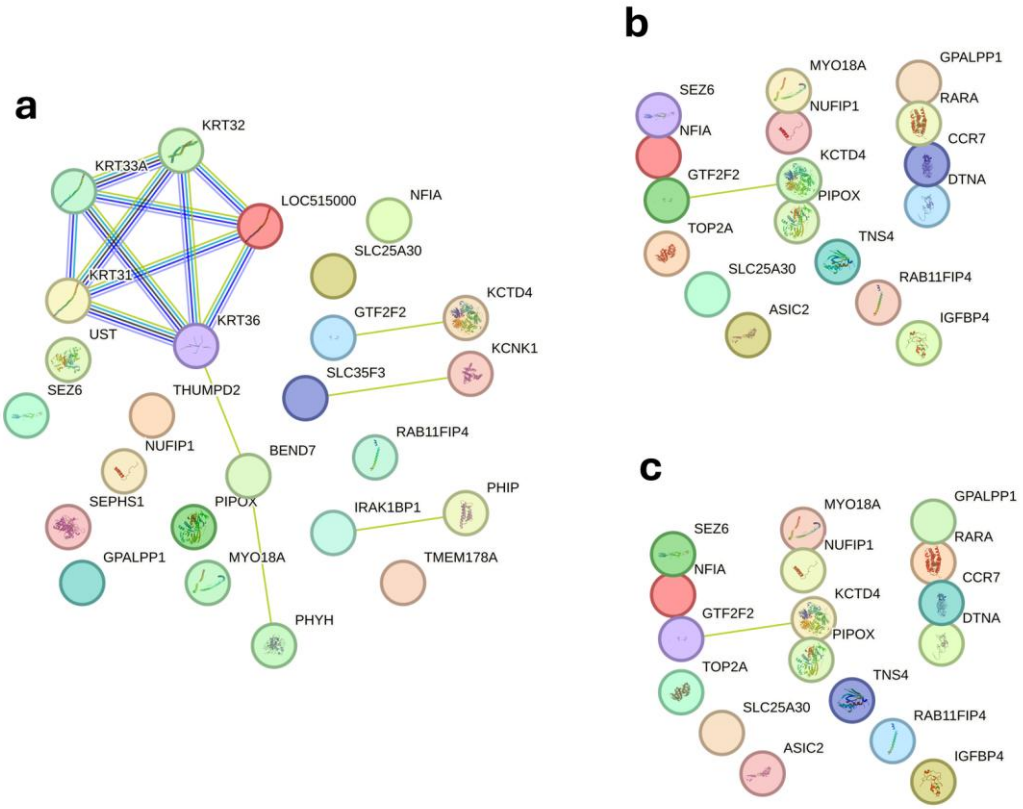

Figure 6. Functional network of genes mapping significant single-nucleotide polymorphisms for residual feed intake (RFI) at the low (EG = THI 66; a), medium (EG = THI 74; b) and high (EG = THI 81; c) environmental gradient in Nellore cattle. Each

node represents a gene, while the lines connecting the nodes indicate known functional interactions or associations between these genes. The different colors of the nodes and lines indicate distinct types of interactions or classifications of biological functions, based on the network analysis.

The functional network observed under moderate heat stress (THI 74) was relatively sparse and decreased density of functional modules (Figure 6b). A central interaction involves *General Transcription Factor IIF Subunit 2* (*GTF2F2*) and *Potassium Channel Tetramerization Domain Containing 4* (*KCTD4*). The *PIPOX* gene was also located near the network center. Several new genes emerged in the network compared to low EG (THI 66), including *CCR7*, *RARA*, *Dystrobrevin Alpha* (*DTNA)*, *IGFBP4, Acid Sensing Ion Channel Subunit 2* (*ASIC2*), *DNA Topoisomerase II Alpha* (*TOP2A*) and *Tensin 4* (*TNS4*), most of which aggregated into small, lowly connected groups (Figure 6b), yet suggesting functional relevance.

The functional network under high heat stress conditions (THI 81) revealed a pattern similar to that observed under moderate heat stress (Figure 6c). Both networks exhibited sparse links, with a predominance of isolated genes or genes with few direct interactions, as well as the presence of a recurrent functional core.

6.3.6. Functional network analysis for DMI across EG

The analysis of interaction networks of candidate genes associated with DMI under low heat load conditions (THI = 66), exhibited high connectivity, dense formation of functional modules, and the presence of genes with central regulatory roles (Figure 7a). Among the main interaction groups, the module composed of *Non-SMC Condensin I Complex Subunit G* (*NCAPG*)*, Ligand Dependent Nuclear Receptor Corepressor Like* (*LCORL*)*, Family With Sequence Similarity 184 Member B* (*FAM184B*)*, DDB1 And CUL4 Associated Factor 16* (*DCAF16*)*, Family With Sequence Similarity 13 Member A* (*FAM13A*)*, HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 3* (*HERC3*) and *Nucleosome Assembly Protein 1 Like 5* (*NAP1L5*)*,* stood out as the largest cluster in the network. Others relevant functional cluster is composed by the genes *Fibroblast Growth Factor 19* (*FGF19*)**,** *Cyclin D1* (*CCND1*) and *MNAT1 Component of CDK Activating Kinase* (*MNAT1*), as well, the genes *SIX Homeobox 1* (*SIX1*) and *4* (*SIX4*), members of the SIX homeobox gene family, and the pair *ASPG–KIF26A*.

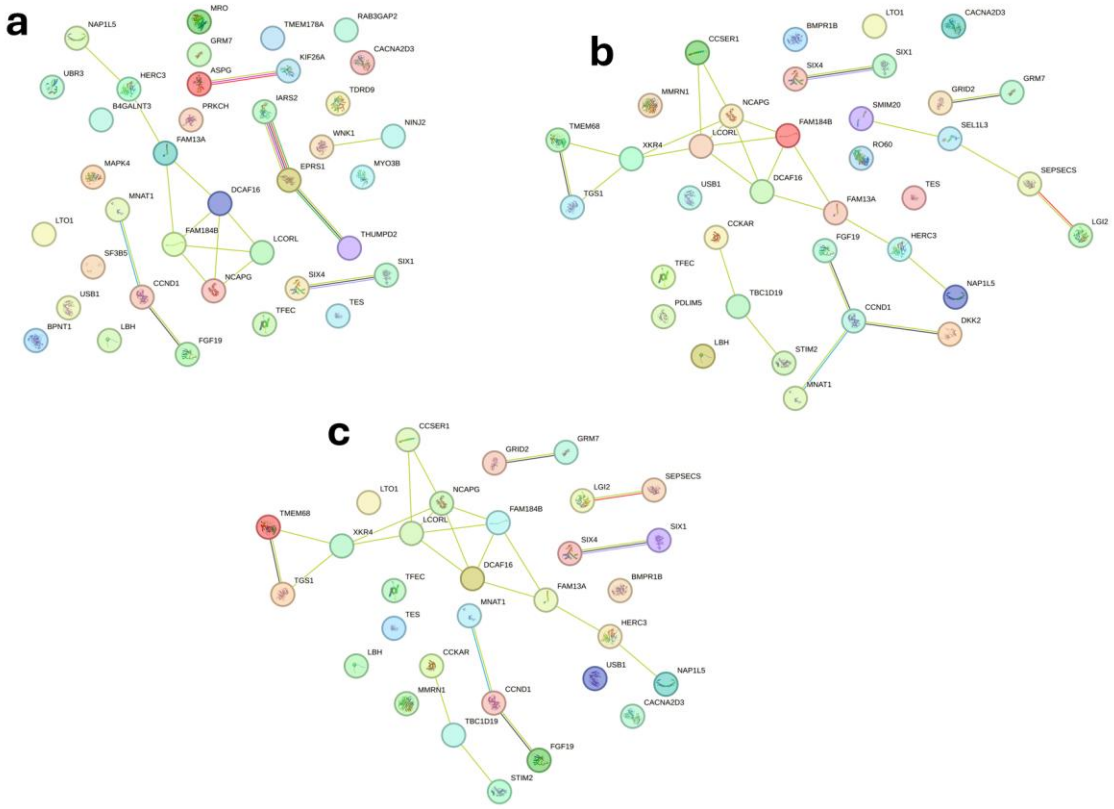

Figure 7. Functional network of mapping significant single nucleotide polymorphisms for dry matter intake (DMI) at the low (EG = THI 66; a), medium (EG = THI 74; b) and high (EG = THI 81; c) environmental gradient in Nellore cattle. Each node represents a gene, while the lines connecting the nodes indicate known functional interactions or associations between these genes. The different colors of the nodes and lines indicate distinct types of interactions or classifications of biological functions, based on the network analysis.

Under moderate heat stress (THI = 74), the network exhibits both conserved elements and notable changes in the composition and organization of the associated genes within the STRING network (Figure 7b). The major central cluster identified under low EG, involving the genes *NCAPG*, *LCORL*, *FAM184B*, *DCAF16*, *FAM13A*, *HERC3*, and *NAP1L5*, remains present. However, the genes *XKR4*, *Coiled-Coil Serine Rich Protein 1* (*CCSER1*), *Trimethylguanosine Synthase 1* (*TGS1*) and *Transmembrane Protein 68* (*TMEM68*) emerged as interacting partners within this cluster, forming a more complex network of gene interactions. Additional gene sets formed distinct clusters, including *CCKAR*, *TBC1 Domain Family Member 19*

(*TBC1D19*) and *Stromal Interaction Molecule 2* (*STIM2*) as well, the *SMIM20*, *SEL1L3*, *Sep (O-Phosphoserine) TRNA:Sec (Selenocysteine) TRNA Synthase* (*SEPSECS*), and *Leucine Rich Repeat LGI Family Member 2* (*LGI2*), which represents a connected cluster (Figure 7b).

The comparison of functional networks associated with DMI under moderate (THI = 74) and high (THI = 81) heat stress revealed a highly similar structural organization between the two environments (Figure 7c). Both networks exhibit strong connectivity among core genes, maintaining a functional nucleus that forms a robust and recurrent interactive axis.

6.3.7. Functional genomic enrichment for RFI across EG

The functional genomic enrichment of candidate genes associated with RFI under low heat load conditions (THI 66) revealed overrepresentation of metabolic processes, particularly those related to amino acid and organic acid metabolism (Table 4). Significant GO terms included, *proteinogenic amino acid metabolic process* (GO:0170039), *L-amino acid metabolic process* (GO:0170033), *organic acid catabolic process* (GO:0016054), and *carboxylic acid catabolic process* (GO:0046395). These terms were mainly driven by *SEPHS1*, *PIPOX*, and *PHYH*.

Table 4. Significant Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with residual feed intake (RFI) in the low environmental gradient (EG) in Nellore cattle.

GO/KEEG ID	Description	p-value	Gene ID
GO:0170039	Proteinogenic amino acid metabolic process	0.003	*SEPHS1, PIPOX*
GO:0170033	L-amino acid metabolic process	0.004	*SEPHS1, PIPOX*
GO:0050793	Regulation of developmental process	0.006	*PHIP, TMEM178A, SEZ6*
GO:0016054	Organic acid catabolic process	0.007	*PHYH, PIPOX*
GO:0046395	Carboxylic acid catabolic process	0.007	*PHYH, PIPOX*
GO:1901605	Alpha-amino acid metabolic process	0.008	*SEPHS1, PIPOX*
GO:0019752	Carboxylic acid metabolic process	0.009	*PHYH, SEPHS1, PIPOX*
GO:0043436	Oxoacid metabolic process	0.009	*PHYH, SEPHS1, PIPOX*
GO:0006082	Organic acid metabolic process	0.010	*PHYH, SEPHS1, PIPOX*
GO:0044282	Small molecule catabolic process	0.011	*PHYH, PIPOX*
bta04915	Estrogen signaling pathway	< 0.001	*KRT37, KRT36, KRT33A, KRT31, KRT32*
bta04146	Peroxisome	0.005	*PHYH, PIPOX*

At the pathway level, two KEGG pathways were significantly associated: the *estrogen signaling pathway* (bta04915) and *peroxisome pathway* (bta04146) (Table

4). The estrogen signaling pathway, involving keratin genes (*KRT31, KRT32, KRT33A, KRT36, KRT37*), while the peroxisome pathway, driven by *PHYH* and *PIPOX*. No significantly enriched biological processes and metabolic pathways were identified under medium and high heat stress conditions.

6.3.8. Functional genomic enrichment for DMI in the low EG

The functional analysis of candidate genes associated with DMI in Nellore cattle under low heat load conditions (THI 66) revealed the involvement of highly organized biological processes (Table 5). Among the significantly enriched terms, *regulation of cyclin-dependent protein kinase activity* (GO:0000079; GO:1904029) e *positive regulation of cell cycle* (GO:0045787), stood out, involving the genes *CCND1* and *MNAT1*. The *mitotic cell cycle process* (GO:1903047) also showed enrichment, with the involvement of *NCAPG*.

In parallel, biosynthetic processes were activated, including *tRNA metabolic process* (GO:0006399), *tRNA aminoacylation for protein translation* (GO:0006418), and *amino acid activation* (GO:0043038), associated with *IARS2*, *EPRS1*, and *THUMPD2* (Table 5). Protein phosphorylation regulation constituted another important functional axis, as evidenced by terms such as *regulation of phosphorylation* (GO:0042325), *regulation of protein kinase activity* (GO:0045859), *regulation of protein phosphorylation* (GO:0001932) and *positive regulation of protein phosphorylation* (GO:0001934). Genes such as *FGF19*, *CCND1*, and *MNAT1* emerged as central components within this group of processes. Other significantly enriched processes included *amino acid metabolic process* (GO:0006520), *regulation of transferase activity* (GO:0051338) and *monoatomic ion homeostasis* (GO:0050801), where *WNK1* stood out.

The KEGG pathway analysis also revealed the involvement of the *Aminoacyl-tRNA biosynthesis* (bta00970) and *Oxytocin signaling pathway* (bta04921), with genes *IARS2*, *EPRS1, CACNA2D3,* and *CCND1*.

Table 5. Significant Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with dry matter intake (DMI) in the low environmental gradient (EG) in Nellore cattle.

GO/KEEG ID	Description	p-value	Gene ID
GO:0000079	Regulation of cyclin-dependent protein serine/threonine kinase activity	0.002	*CCND1, MNAT1*
GO:1904029	Regulation of cyclin-dependent protein kinase activity	0.002	*CCND1, MNAT1*
GO:0006399	tRNA metabolic process	0.002	*THUMPD2, IARS2, EPRS1*
GO:0006418	tRNA aminoacylation for protein translation	0.002	*IARS2, EPRS1*
GO:0043039	tRNA aminoacylation	0.002	*IARS2, EPRS1*
GO:0043038	Amino acid activation	0.002	*IARS2, EPRS1*
GO:0045787	Positive regulation of cell cycle	0.002	*CCND1, MNAT1*
GO:0071900	Regulation of protein serine/threonine kinase activity	0.005	*CCND1, MNAT1*
GO:0001932	Regulation of protein phosphorylation	0.006	*FGF19, CCND1, MNAT1*
GO:0031399	Regulation of protein modification process	0.010	*FGF19, CCND1, MNAT1*
GO:0042325	Regulation of phosphorylation	0.010	*FGF19, CCND1, MNAT1*
GO:0045859	Regulation of protein kinase activity	0.014	*CCND1, MNAT1*
GO:0019220	Regulation of phosphate metabolic process	0.014	*FGF19, CCND1, MNAT1*
GO:0051174	Regulation of phosphorus metabolic process	0.014	*FGF19, CCND1, MNAT1*
GO:0034660	ncRNA metabolic process	0.018	*THUMPD2, IARS2, EPRS1*
GO:0043549	Regulation of kinase activity	0.025	*CCND1, MNAT1*
GO:0001934	Positive regulation of protein phosphorylation	0.027	*FGF19, MNAT1*
GO:0051338	Regulation of transferase activity	0.029	*CCND1, MNAT1*
GO:0031401	Positive regulation of protein modification process	0.034	*FGF19, MNAT1*
GO:0006520	Amino acid metabolic process	0.035	*IARS2, EPRS1*
GO:0050801	Monoatomic ion homeostasis	0.035	*WNK1, TMEM178A*
GO:0042327	Positive regulation of phosphorylation	0.041	*FGF19, MNAT1*
GO:0010562	Positive regulation of phosphorus metabolic process	0.043	*FGF19, MNAT1*
GO:0045937	Positive regulation of phosphate metabolic process	0.043	*FGF19, MNAT1*
GO:1903047	Mitotic cell cycle process	0.049	*CCND1, NCAPG*
bta00970	Aminoacyl-tRNA biosynthesis	0.006	*IARS2, EPRS1*
bta04921	Oxytocin signaling pathway	0.031	*CACNA2D3, CCND1*
bta05202	Transcriptional misregulation in cancer	0.048	*SIX1, SIX4*

6.3.9. Functional genomic enrichment for DMI in the medium EG

The functional analysis of genes associated with DMI in Nellore cattle under moderate heat stress conditions (THI 74) revealed the activation of complex biological processes (Table 6). The processes *regulation of cyclin-dependent protein kinase activity* (GO:0000079; GO:1904029), *positive regulation of cell cycle* (GO:0045787), and *mitotic cell cycle process* (GO:1903047) were strongly associated with the genes *CCND1* and *MNAT1*. Notably, an enrichment of terms associated with glutamatergic synaptic transmission and trans-synaptic signaling was observed, including *synaptic transmission, glutamatergic* (GO:0035249), *modulation of chemical synaptic transmission* (GO:0050804), and *regulation of trans-synaptic signaling* (GO:0099177), mediated by the genes *GRM7* and *GRID2*. In addition, activation of the pathways *response to growth factor* (GO:0070848) and *cellular response to growth factor stimulus* (GO:0071363), involving the genes *BMPR1B* and *FGF19*, remain relevant in the modulation of feed intake.

Several terms related to protein phosphorylation and modification were significant, including *positive regulation of phosphorylation* (GO:0042327), *positive regulation of protein modification process* (GO:0031401), and *positive regulation of kinase activity* (GO:0045859) mainly driven by *FGF19*, *CCND1*, and *MNAT1*. Finally, *RNA modification* (GO:0009451) and *regulation of transferase activity* (GO:0051338), were linked to genes such as *TGS1*, *SEPSECS*, and *MNAT1*. The enriched KEGG pathways include critical signaling cascades such as the *Wnt signaling pathway* (bta04310), *Hippo signaling pathway* (bta04390), *Oxytocin signaling pathway* (bta04921), and *Calcium signaling pathway* (bta04020), with key contributions from *CCKAR*, *FGF19*, and *STIM2* (Table 6).

Table 6. Significant Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with dry matter intake (DMI) in the medium environmental gradient (EG) in Nellore cattle.

GO/KEEG ID	Description	p-value	Gene ID
GO:0000079	Regulation of cyclin-dependent protein serine/threonine kinase activity	0.002	*CCND1, MNAT1*
GO:1904029	Regulation of cyclin-dependent protein kinase activity	0.002	*CCND1, MNAT1*
GO:0035249	Synaptic transmission, glutamatergic	0.002	*GRM7, GRID2*
GO:0045787	Positive regulation of cell cycle	0.002	*CCND1, MNAT1*
GO:0071900	Regulation of protein serine/threonine kinase activity	0.005	*CCND1, MNAT1*
GO:0001932	Regulation of protein phosphorylation	0.006	*FGF19, CCND1, MNAT1*
GO:0031399	Regulation of protein modification process	0.009	*FGF19, CCND1, MNAT1*
GO:0050804	Modulation of chemical synaptic transmission	0.009	*GRM7, GRID2*
GO:0099177	Regulation of trans-synaptic signaling	0.009	*GRM7, GRID2*
GO:0042325	Regulation of phosphorylation	0.009	*FGF19, CCND1, MNAT1*
GO:0045859	Regulation of protein kinase activity	0.012	*CCND1, MNAT1*
GO:0019220	Regulation of phosphate metabolic process	0.013	*FGF19, CCND1, MNAT1*
GO:0051174	Regulation of phosphorus metabolic process	0.013	*FGF19, CCND1, MNAT1*
GO:0009451	RNA modification	0.015	*TGS1, SEPSECS*
GO:0043549	Regulation of kinase activity	0.023	*CCND1, MNAT1*
GO:0070848	Response to growth factor	0.023	*BMPR1B, FGF19*
GO:0071363	Cellular response to growth factor stimulus	0.023	*BMPR1B, FGF19*
GO:0001934	Positive regulation of protein phosphorylation	0.025	*FGF19, MNAT1*
GO:0051338	Regulation of transferase activity	0.027	*CCND1, MNAT1*
GO:0007267	Cell-cell signaling	0.030	*GRM7, GRID2, DKK2*
GO:0031401	Positive regulation of protein modification process	0.032	*FGF19, MNAT1*
GO:0042327	Positive regulation of phosphorylation	0.038	*FGF19, MNAT1*
GO:0010562	Positive regulation of phosphorus metabolic process	0.040	*FGF19, MNAT1*
GO:0045937	Positive regulation of phosphate metabolic process	0.040	*FGF19, MNAT1*
GO:1903047	Mitotic cell cycle process	0.045	*CCND1, NCAPG*
bta04020	Calcium signaling pathway	0.007	*CCKAR, FGF19, STIM2*
bta04080	Neuroactive ligand-receptor interaction	0.022	*CCKAR, GRM7, GRID2*
bta04921	Oxytocin signaling pathway	0.024	*CACNA2D3, CCND1*
bta04390	Hippo signaling pathway	0.025	*BMPR1B, CCND1*
bta04310	Wnt signaling pathway	0.031	*CCND1, DKK2*
bta04081	Hormone signaling	0.049	*BMPR1B, CCKAR*

6.3.10. Functional genomic enrichment for DMI in the high EG

The functional analysis of genes associated with DMI in Nellore cattle under high heat stress (THI 81) revealed a strong overrepresentation of processes related to cell cycle regulation, protein phosphorylation, and synaptic signaling, similar to the patterns observed under moderate stress, but with higher levels of statistical significance (Table 7). A distinct set of functional processes was identified that were absent under moderate stress, most notably the enrichment of terms related to synaptic signaling, such as *chemical synaptic transmission* (GO:0007268), *anterograde trans-synaptic signaling* (GO:0098916), *trans-synaptic signaling* (GO:0099537), and *synaptic signaling* (GO:0099536), mediated by the genes *GRM7* and *GRID2*. In addition, the term *regulation of cell cycle* (GO:0051726) was uniquely detected under high THI. In contrast, the *Wnt signaling pathway* (bta04310), which was present under moderate THI conditions and associated with the genes *CCND1* and *DKK2*, was no longer detected under high heat stress (Table 7).

Table 7. Significant Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with dry matter intake (DMI) in the high environmental gradient (EG) in Nellore cattle.

GO/KEEG ID	Description	p-value	Gene ID
GO:0000079	Regulation of cyclin-dependent protein serine/threonine kinase activity	0.001	*CCND1, MNAT1*
GO:1904029	Regulation of cyclin-dependent protein kinase activity	0.001	*CCND1, MNAT1*
GO:0035249	Synaptic transmission, glutamatergic	0.001	*GRM7, GRID2*
GO:0045787	Positive regulation of cell cycle	0.002	*CCND1, MNAT1*
GO:0071900	Regulation of protein serine/threonine kinase activity	0.003	*CCND1, MNAT1*
GO:0001932	Regulation of protein phosphorylation	0.004	*FGF19, CCND1, MNAT1*
GO:0031399	Regulation of protein modification process	0.006	*FGF19, CCND1, MNAT1*
GO:0042325	Regulation of phosphorylation	0.006	*FGF19, CCND1, MNAT1*
GO:0050804	Modulation of chemical synaptic transmission	0.007	*GRM7, GRID2*
GO:0099177	Regulation of trans-synaptic signaling	0.007	*GRM7, GRID2*
GO:0019220	Regulation of phosphate metabolic process	0.009	*FGF19, CCND1, MNAT1*
GO:0051174	Regulation of phosphorus metabolic process	0.009	*FGF19, CCND1, MNAT1*
GO:0045859	Regulation of protein kinase activity	0.009	*CCND1, MNAT1*
GO:0009451	RNA modification	0.011	*TGS1, SEPSECS*
GO:0043549	Regulation of kinase activity	0.017	*CCND1, MNAT1*
GO:0070848	Response to growth factor	0.017	*BMPR1B, FGF19*
GO:0071363	Cellular response to growth factor stimulus	0.017	*BMPR1B, FGF19*
GO:0001934	Positive regulation of protein phosphorylation	0.019	*FGF19, MNAT1*
GO:0051338	Regulation of transferase activity	0.021	*CCND1, MNAT1*
GO:0031401	Positive regulation of protein modification process	0.024	*FGF19, MNAT1*
GO:0042327	Positive regulation of phosphorylation	0.029	*FGF19, MNAT1*
GO:0010562	Positive regulation of phosphorus metabolic process	0.031	*FGF19, MNAT1*
GO:0045937	Positive regulation of phosphate metabolic process	0.031	*FGF19, MNAT1*
GO:1903047	Mitotic cell cycle process	0.035	*CCND1, NCAPG*
GO:0007268	Chemical synaptic transmission	0.041	*GRM7, GRID2*
GO:0098916	Anterograde trans-synaptic signaling	0.041	*GRM7, GRID2*
GO:0099537	Trans-synaptic signaling	0.041	*GRM7, GRID2*
GO:0099536	Synaptic signaling	0.044	*GRM7, GRID2*
GO:0051726	Regulation of cell cycle	0.047	*CCND1, MNAT1*
bta04020	Calcium signaling pathway	0.006	*CCKAR, FGF19, STIM2*
bta04080	Neuroactive ligand-receptor interaction	0.018	*CCKAR, GRM7, GRID2*
bta04921	Oxytocin signaling pathway	0.021	*CACNA2D3, CCND1*
bta04390	Hippo signaling pathway	0.022	*BMPR1B, CCND1*
bta04081	Hormone signaling	0.043	*BMPR1B, CCKAR*

6.4. Discussion

6.4.1. Genomic Implications of Significant Markers Detected

The distribution of significant SNPs across different THI environments highlights the dynamic genetic regulation of feed efficiency traits in response to thermal load. BTA12 was consistently associated with RFI across all environmental conditions (Table 2), suggesting it may contain core regulatory regions influencing residual feed intake irrespective of thermal conditions. In contrast, BTA19 showed increased association under more severe heat stress, indicating potential environment-specific gene activation. This latter pattern is in line with the findings of Brunes et al. [49], who identified a genomic window on BTA19 (42.98 to 43.76 Mb) associated with RFI in Nellore cattle, located near the significant SNP detected in the present study (41.59 Mb), thereby reinforcing the relevance of this chromosomal region for the genetic regulation of feed efficiency in beef cattle. For DMI, the prominent role of BTA6, particularly under medium and high EGs (Table 3), suggests this chromosome may harbor key regulators involved in the physiological response to increased thermal load. The detection of multiple significant SNPs on BTA6, as well as additional associations on BTA10, BTA11, BTA14, and BTA29 under high THI levels, underscores the involvement of diverse genomic regions in feed intake regulation under challenging conditions. Notably, the significant SNP identified on BTA14 in this study (22.99 Mb) overlaps with the genomic windows reported by Brunes et al. [49] (22.29 to 22.98 Mb) and Mota et al. [50] (22.62 to 24.71 Mb), meaning that both previous studies converge on the same BTA14 segment highlighted here, thereby strengthening the evidence that this locus plays a central role in the genetic background of DMI in Nellore cattle. The identification of genomic regions with either stable or environment-specific effects provides valuable insights for designing targeted selection strategies. Such strategies could be tailored to improve feed efficiency while simultaneously enhancing resilience to climate variability, a crucial goal for sustainable beef production in tropical regions.

6.4.2. Insights into Specific and Shared SNPs across Thermal Environments

The overlap and exclusivity patterns of SNPs across the THI gradients provide insight into how thermal stress modulates the genetic architecture of feed efficiency traits (Figure 3). For RFI, the 27 SNPs shared across all three environments likely represent core genomic regions influencing this trait regardless of heat stress level.

Conversely, the SNPs exclusive to the low EG and the single SNP detected only under high EG suggest that certain loci exert environment-specific effects, which aligns with the presence of G×E interactions. For DMI, the clustering of 39 exclusive SNPs on BTA6 under medium EG reinforces the importance of this chromosome in regulating feed intake when animals are exposed to moderate thermal stress. The fact that no SNPs were exclusive to the high EG, yet many were shared between medium and high EG, suggests that as heat stress intensifies, the genetic regulation becomes more reliant on loci already active at intermediate levels of stress, rather than recruiting entirely new genomic regions. These findings highlight a nuanced genetic response to environmental challenges, where some loci are consistently important across environments, while others are “activated” only under specific thermal conditions. This dynamic profile is critical for developing genomic selection programs that aim to improve feed efficiency and resilience to climate stress, as it allows breeders to differentiate between stable and environment-sensitive genomic targets.

6.4.3. Environmental Modulation of SNP Effects

The pattern of SNP effect variation across THI gradients (Figure 4) suggests the presence of phenotypic plasticity, i.e., the ability of a genotype to alter its expression or effect in response to environmental changes [26, 51]. The greater variation in SNP effects between the low and medium EGs may reflect a transitional environmental phase (onset of moderate heat stress) in which environmentally sensitive loci begin to modulate their activity. In contrast, the relative similarity in SNP effects between the medium and high EGs suggests the involvement of more stable loci that maintain their effects even under extreme environmental conditions. In other words, phenotypic plasticity appears to be more evident in transitional environments (THI ≈ 74) than under extreme heat stress conditions (THI ≥ 81).

The reduction in additive genetic variance under higher heat stress conditions [19] may also be associated with the stabilization observed in genetic effects, suggesting that beyond a certain environmental threshold (THI ≈ 74), additive genetic effects become more consistent, even as environmental conditions worsen up to THI 81. This pattern may indicate a lower sensitivity to environmental fluctuations under more severe heat stress, as genetic mechanisms related to adaptation may have already been activated. This finding has important implications for genomic selection in tropical production systems, as it indicates greater genetic variability for heat

adaptation under intermediate stress conditions. In such environments, the stress level is sufficient to trigger detectable adaptive responses without masking the genetic variability among animals, making it particularly useful for identifying individuals that are genetically more resilient to heat stress.

6.4.4. Candidate genes identified under different thermal conditions for RFI

Among the nineteen genes found associated with RFI across the three EG levels suggest robust genetic effects independent of environmental variation (Figure 5a). The *Nuclear Factor IA* (*NFIA*, BTA3), a transcription factor implicated in lipid metabolism and adipocyte differentiation, may influence basal energy expenditure [52, 53]. *Pipecolic Acid And Sarcosine Oxidase* (*PIPOX*, BTA19) is involved in amino acid catabolism and nitrogen balance, reinforcing its relevance in maintaining cellular energetics [54]. *Myosin XVIIIA* (*MYO18A*, BTA19) is an unconventional myosin involved in maintaining myofiber integrity through cytoskeletal organization and Golgi function [55]. Disruption of *MYO18A* affects muscle morphology and intracellular trafficking, indicating its potential to influence basal energy expenditure and overall metabolic efficiency.

Under low heat load conditions (THI <66), the genes were associated with roles in key biological processes related to feed efficiency (Figure 5a). The detection of these genes suggests that milder thermal conditions may offer a more stable physiological baseline, reducing the environmental effects of stress-induced responses. Among these, *Pleckstrin Homology Domain Interacting Protein* (*PHIP,* BTA9), a key component of the insulin signaling through its interaction with IRS-1, may influence energy homeostasis and nutrient partitioning [56]. *Interleukin 1 Receptor Associated Kinase 1 Binding Protein 1* (*IRAK1BP1*, BTA9) modulates NF-κB signaling, and its association suggests a possible role of subclinical immune activation in energy expenditure [57]. *Transmembrane Protein 178A* (*TMEM178A,* BTA11), related to calcium signaling [58], and *Selenophosphate Synthetase 1* (*SEPHS1*, BTA13), involved in antioxidant defense via selenoprotein biosynthesis [59], may contribute to cellular homeostasis and oxidative balance, both important processes for metabolic efficiency under thermoneutral conditions.

In addition to the four genes described above, other candidates such as *Phytanoyl-CoA 2-Hidroxilase* (*PHYH*, BTA13), *Potassium Two Pore Domain Channel Subfamily K Member 1* (*KCNK1*, BTA28), and *Solute Carrier Family 35 Member F3*

(*SLC35F3*, BTA28) may also contribute to residual feed intake regulation under thermoneutral conditions (THI 66). *PHYH* is involved in lipid metabolism via peroxisomal oxidation [60]. Alterations in its function can affect lipid catabolism, thereby influencing basal energy expenditure and overall feed efficiency. *KCNK1* encodes a potassium channel potentially linked to energy homeostasis and cellular excitability [61, 62]. Changes in its activity may indirectly impact tissue-level energy efficiency, particularly in metabolically active tissues such as skeletal muscle and liver. Finally, *SLC35F3* participates in thiamine transport, essential for mitochondrial energy production. Genetic variation in this transporter may influence feed efficiency by modulating thiamine availability and, consequently, the efficiency of carbohydrate and energy metabolism [63, 64].

The absence of genes uniquely associated with RFI in either medium (THI = 74) or high (THI = 81) heat stress environments (Figure 5a), likely reflects the activation of shared regulatory mechanisms across stress levels. The genes commonly identified under both moderate (THI = 74) and high (THI = 81) heat stress conditions, confirming the presence of regulatory mechanisms that are consistently activated in response to thermal challenges (Figure 5a). The recurrence of these genes across environments may indicate the involvement of biological processes associated with adaptive response to heat stress, potentially contributing to the maintenance of metabolic stability under adverse conditions. Among these genes, *Insulin Like Growth Factor Binding Protein 4* (*IGFBP4*, BTA19) regulates IGF signaling and may influence growth and metabolic adaptation [65, 66]. *C-C Motif Chemokine Receptor 7* (*CCR7*, BTA19) is involved in immune cell trafficking [67], and this may reflect the energetic cost of immune system activation during heat stress. *Retinoic Acid Receptor Alpha* (*RARA*, BTA19) a nuclear receptor associated with lipid metabolism and adipogenesis [68], further supports the importance of metabolic regulation in feed efficiency under climatic stressful conditions.

Overall, the identification of both shared and environment-specific candidate genes associated with RFI highlights the complex interplay between genetic regulation and thermal stress. The presence of shared associations across all THI classes suggests a conserved genetic basis for feed efficiency, whereas the environment-specific signals particularly under low heat load indicate that milder conditions may enhance the detection of functionally relevant loci. These findings provide valuable insights into the genetic architecture of RFI under variable climatic scenarios and

support the development of genomic selection strategies targeting both metabolic efficiency and environmental resilience. For a better understanding of the genetic mechanisms underlying RFI variation within each EG, the functions of most identified genes, as well as their interactions and potential functional implications, are presented in detail on the network patterns section.

6.4.5. Candidate genes identified under different thermal environments for DMI

The findings suggest a dynamic genomic response to thermal stress, with both specific and conserved biological mechanisms regulating feed intake under varying degrees of heat stress. Nineteen genes were identified as commonly associated with DMI across all three EG, indicating the presence of constant mechanisms involved in the regulation of intake, independent of heat stress intensity (Figure 5b). The recurrence of these genes across diverse climatic conditions suggests a stable genomic influence on feed intake that may reflect core physiological mechanisms. Among these, *NCAPG* (BTA6) has been previously associated with growth traits and feed intake in cattle [69, 70]. This gene is involved in cell cycle regulation and it has been associated with growth rate and body size in several cattle breeds [71, 72]. *NCAPG* influences feed intake by modulating growth demands, where larger or faster-growing animals require more feed to meet their energy needs [73]. Thus, the association of *NCAPG* with DMI occurs indirectly, mediated by physiological processes related to growth and energy homeostasis. *LCORL* (BTA6) is a transcription factor associated with skeletal growth and body size in humans, horses, and cattle [74, 75]. *LCORL* has been linked to growth traits and feed efficiency in cattle, often acting in concert with *NCAPG* [76, 77]. Polymorphisms in the *LCORL* gene have been associated with variability in feed intake and gain, particularly in beef cattle (Angus, Hereford, Simmental, Limousin, Charolais, Gelbvieh and Red Angus) [70]. Its role in skeletal growth may be crucial for determining body size and the corresponding feed requirements, thereby influencing DMI. The *Calcium Voltage-Gated Channel Auxiliary Subunit Alpha2delta 3* (*CACNA2D3*, BTA22) may contribute to neuroregulatory control of feeding behavior, given the importance of calcium signaling in appetite regulation and neuronal excitability [78, 79]. Furthermore, *Glutamate Metabotropic Receptor 7* (*GRM7*, BTA22) may also participate in the neural regulation of feed intake through its role in synaptic signaling and behavioral responses to environmental stimuli [80].

Under low thermal conditions (THI 66), 22 genes were uniquely associated with DMI (Figure 5b). These genes likely reflect genetic mechanisms that are more detectable in thermoneutral conditions. Among these, *WNK Lysine Deficient Protein Kinase 1* (*WNK1*, BTA5) and *Ubiquitin Protein Ligase E3 Component N-recognin 3* (*UBR3*, BTA2), are of particular interest. *WNK1*, a kinase involved in ion transport and osmoregulation [81], could contribute to water and electrolyte balance, a factor closely related to feed intake. *UBR3*, a component of the E3 ubiquitin ligase complex, plays a role in protein turnover and cellular quality control [82, 83]. These pathways may influence metabolic efficiency and systemic adaptation under more physiologically stable conditions.

In contrast, five genes were exclusively identified under moderate heat stress conditions (THI 74), including *Small Integral Membrane Protein 20* (*SMIM20*, BTA6), *SEL1L Family Member 3* (*SEL1L3*, BTA6), *PDZ And LIM Domain 5* (*PDLIM5*, BTA6), *Ro60-Associated Y3* (*Y_RNA*, BTA6), and *Dickkopf WNT Signaling Pathway Inhibitor 2* (*DKK2*, BTA6). The limited number of unique associations observed in this EG may reflect a transitional physiological state, in which the onset of systemic stress responses begins to interfere with the genetic regulation of feed intake. However, despite their statistical association with DMI, the biological roles of these genes particularly in the context of heat stress adaptation and intake regulation in *Bos taurus indicus* cattle are not yet well characterized. Some of these genes lack direct functional links to thermal stress or metabolic processes in ruminants, underscoring the need for further functional annotation and gene expression studies to clarify their potential contributions under intermediate heat stress conditions.

Notably, no genes were exclusively associated with DMI under high heat stress conditions (Figure 5b). This lack of specific associations may be attributed to the systemic physiological disruptions caused by high thermal stress, which can reduce the expression of genetic effects associated with DMI regulation. In such environments, the organism response is likely dominated by heat stress response pathways aimed at preserving basic cellular and metabolic stability, rather than by mechanisms adjusted to feed intake modulation [84]. Additionally, the increased phenotypic variability and reduced genetic variability under more extreme conditions may further limit the detection of environment-specific genetic signals [19].

A subset of 12 genes was associated with DMI in medium (THI 74) and high (THI 81) EGs, suggesting the presence of biological mechanisms that are gradually

involved in response to increasing thermal challenge. Among these, *Cholecystokinin A Receptor* (*CCKAR*, BTA6) is particularly noteworthy due to its established role in satiety signaling and feed intake regulation through the cholecystokinin (*CCK*) pathway [85]. The *XK Related 4* (*XKR4*, BTA14) gene encodes a protein involved in apoptosis and membrane remodeling [86, 87]. *XKR4* is expressed in a wide range of tissues, including the nervous system and muscles [87]. Given that DMI influences muscle growth and energy balance, and that *XKR4* has been associated with feed intake and average daily gain [88, 89], its role in muscle-related processes may indicate functional relevance for energy metabolism under thermal stress conditions.

Overall, the identification of shared genes highlights the presence of genomic regions influencing DMI regardless of the heat stress level which the animals are being exposed to. These candidates may serve as valuable targets for future functional validation and for the development of breeding strategies aimed at improving feed efficiency across diverse environments. For a better understanding of the genetic mechanisms underlying DMI variation within each EG, the functions of most identified genes, as well as their interactions and potential functional implications, are presented in detail on the network patterns section.

6.4.6. RFI Network Patterns in the low EG

Before describing the environment-specific networks, it is important to note that our interpretation of the STRING graphs is qualitative and depends on the subset of genes that map to significant SNP windows at each environmental gradient. The underlying protein–protein interaction evidence in STRING is fixed and does not change with THI; what changes across EGs are the significant SNPs and, consequently, the genes included in each network. Thus, when only part of a functional module remains associated in a given environment, the genes that are no longer supported by significant SNPs are simply not displayed, and the interactions that relied on them disappear from the graph. Apparent gains, losses, or fragmentation of modules across environments should therefore be interpreted as reflecting differences in the set of associated genes captured at each EG, rather than true changes in protein–protein connectivity.

Under low heat load conditions, the RFI network exhibited a clearly structured architecture dominated by a keratin cluster (Figure 6a). These type I keratin isoforms are essential for epithelial integrity [90], and mutations in *KRT32* disrupt immune

homeostasis [91]. Although classically associated with skin, their coordinated activity may also influence epithelial renewal in metabolically relevant tissues, including the gastrointestinal epithelium. The interaction between *BEND7*, an epigenetic regulator associated with insulin metabolism [92], and *PHYH*, involved in peroxisomal α-oxidation [93], suggests an epigenetic-metabolic link within this cluster. Additional connections among *SLC35F3* (mitochondrial thiamine transport [63, 64]), *KCNK1* (membrane excitability [62, 94]), *IRAK1BP1* (Toll-like receptor-mediated inflammation [57, 95]), and *PHIP* (insulin signaling and energy balance [56, 96]) indicate coordinated regulation of mitochondrial metabolism, immune reactivity, and endocrine function.

In summary, the gene network associated with RFI under low thermal conditions (THI = 66) suggests that feed efficiency in this context is supported by the coordinated action of multiple biological processes. These include pathways related to epithelial integrity and tissue maintenance, metabolic activity, and epigenetic regulation. In addition, genes involved in mitochondrial function, immune signaling, and hormonal pathways reinforce the idea that more efficient animals are better able to balance energy production, inflammatory responses, and growth. Altogether, the observed network highlights the complex and integrated nature of the biological mechanisms contributing to feed efficiency, particularly under favorable environmental conditions.

6.4.7. RFI Network Patterns in the medium EG

The functional network associated with RFI under moderate heat stress (THI = 74) became markedly less integrated (Figure 6b). Central genes such as *GTF2F2*, involved in transcription initiation and stress-responsive inflammatory, hormonal, neurobehavioral, and epigenetic pathways [97–99], and *KCTD4*, associated with ionic homeostasis, mitochondrial dysfunction, inflammatory signaling, and hypothalamic–pituitary axis activity [100–102], indicate that transcriptional and intracellular signaling mechanisms gain prominence under these conditions. *PIPOX*, involved in lysine degradation and redox homeostasis [54], remained a consistent metabolic node.

Several new genes emerged, though with limited connectivity. These included *CCR7* (T-cell migration and inflammation resolution [103–105]), *RARA* (retinoic-acid signaling and metabolic regulation [68, 106–108]), *DTNA* (sarcolemmal structural integrity [109–111]), *IGFBP4* (IGF-mediated growth and adipogenesis [65, 112–114]), and *ASIC2*, which links pH sensing to metabolic and autonomic control [115–117].

Their dispersed configuration suggests that, under moderate heat stress, feed efficiency depends on multiple partially coordinated biological systems rather than on the cohesive metabolic–epithelial core observed at THI 66. This pattern indicates a more heterogeneous and potentially less efficient adaptive response, requiring greater physiological plasticity to sustain energy balance.

The moderate heat stress network suggests a possible decrease in functional integration among active genes in this context. This configuration may reflect more complex adaptive challenges imposed by intermediate thermal stress. The identified interactions indicate that feed efficiency under these conditions may rely on the coordinated activity of multiple biological systems, including transcriptional regulation, energy metabolism, inflammatory signaling, and neuroendocrine control. The emergence of new genes with limited connectivity but potential functional relevance points to a more heterogeneous and possibly less efficient adaptive response. These findings suggest that, under moderate thermal stress, maintaining homeostasis and bioenergetic efficiency may require greater physiological plasticity and the activation of compensatory pathways.

6.4.8. RFI Network Patterns in the high EG

The functional network under high heat stress conditions (THI 81, Figure 6c) revealed to be like that observed under moderate heat stress (THI 74). The observed structural convergence suggests the existence of conserved genetic mechanisms regulating feed efficiency, regardless of the severity of thermal challenge. The persistence of pathways associated with energy metabolism, transcriptional regulation, and cellular signaling supports the hypothesis that thermal stress adaptation occurs primarily through the modulation of pre-established essential functional routes, rather than through the activation of novel gene modules. This organization underscores the polygenic complexity and multifactorial nature of the mechanisms regulating feed efficiency in thermally challenging environments, potentially reflecting a state of physiological overload, with the activation of multiple pathways in response to environmentally induced cellular damage or dysfunction, which remain consistently active even under increased heat stress.

6.4.9. DMI Network Patterns in the low EG

Under low heat load conditions (THI = 66, Figure 7a), the main interaction module included *NCAPG* and *LCORL*, well-established GWAS candidates for body weight and DMI [69, 70, 118], together with *FAM184B* and *FAM13A*, genes involved in growth regulation, lipid metabolism, and energy homeostasis across species [119–124]. *HERC3*, an E3 ubiquitin ligase implicated in intracellular protein regulation and homeostasis [125, 126], was also part of this core. Collectively, these genes suggest coordinated control of somatic growth, metabolic efficiency, and protein turnover under favorable environmental conditions.

A second module included *FGF19*, *CCND1*, and *MNAT1*. *FGF19* plays central roles in hepatic metabolism, bile acid homeostasis, lipid regulation, and insulin-like signaling [127–129]. *CCND1* regulates the G1/S transition and cell proliferation [130–132], while *MNAT1* is part of the CDK-activating kinase complex and is responsive to oxidative and metabolic stress [133–136]. This axis indicates tight coupling between nutrient availability, proliferative activity, and metabolic state.

Additional genes included *SIX Homeobox 1* (*SIX1*) and *4* (*SIX4*), which regulate muscle differentiation and fiber-type programming [137–139], potentially influencing basal metabolic demand. The *Asparaginase* (*ASPG*) and *Kinesin Family Member 26A* (*KIF26A*) pair links nitrogen metabolism [140, 141] to gastrointestinal signaling and development [142], suggesting integration between amino acid utilization and digestive function. Overall, the network in favorable environmental conditions (THI 66) highlights strong functional connectivity among genes involved in growth, lipid metabolism, protein turnover, and muscular development, supporting a highly coordinated regulation of DMI in favorable thermal environments.

6.4.10. DMI Network Patterns in the medium EG

Under moderate heat stress (THI = 74, Figure 7b), the central cluster composed of *NCAPG*, *LCORL*, *FAM184B*, *DCAF16*, *FAM13A*, *HERC3*, and *NAP1L5* remained active, indicating a conserved regulatory core for growth and metabolism. However, new interacting partners emerged, including *XKR4*, *CCSER1*, *TGS1*, and *TMEM68*, suggesting remodeling of metabolic and stress-response pathways. *XKR4* has been associated with feed intake, growth, and endocrine modulation under environmental stress [89, 143, 144]. *TGS1* participates in RNA maturation, gluconeogenesis, and

inflammatory signaling [145–147], whereas *TMEM68* contributes to triacylglycerol synthesis and lipid homeostasis [148–150].

Genes linked to neuroendocrine and metabolic regulation also appeared. *CCKAR*, a key receptor in satiety, gastrointestinal motility, and glucose regulation, directly affects feed intake [151–156]. *STIM2* functions as a calcium sensor and stabilizer of intracellular Ca^{2+} signaling [157–159], connecting stress perception to metabolic adjustments. *SMIM20* encodes the precursor of the orexigenic neuropeptide Phoenixin, which stimulates feed intake and regulates appetite-related pathways [160–162]. *SEL1L3* and *SEPSECS* contribute to metabolic adaptation and oxidative stress protection through roles in energy balance and selenoprotein biosynthesis [163–168].

In summary, the functional network for DMI under moderate heat stress (THI 74) reveals a combination of conserved regulatory cores and newly emerging components, indicating both stability and adaptation in response to thermal challenges. While central clusters involved in growth and metabolism remain active, the incorporation of new genes related to satiety signaling, energy metabolism, and cellular homeostasis suggests a dynamic remodeling of regulatory pathways. These interactions reflect the activation of neuroendocrine and metabolic mechanisms aimed at maintaining feed intake and physiological balance under stress. The presence of genes involved in appetite regulation, lipid metabolism, oxidative stress response, and calcium signaling points to a multifaceted adaptive strategy. Overall, the network suggests that under moderate thermal stress, feed intake efficiency is supported by the integration of central and peripheral signals, helping to sustain metabolic function and promote resilience in challenging environmental conditions.

6.4.11. DMI Network Patterns in the high EG

The convergence between moderate (THI = 74) and high (THI = 81, Figure 7c) heat stress suggests the existence of shared genetic mechanisms that consistently regulate feed intake regardless of the severity of thermal stress. The persistence of connections among genes involved in transcriptional regulation, hormonal signaling, cell proliferation, and energy metabolism supports the hypothesis that the adaptive response to extreme heat stress occurs through functional adjustments in already established pathways. Moreover, the stability of the network indicates that the genetic control of DMI in Nellore cattle is highly resilient, potentially reflecting a conserved

regulatory system aimed at maintaining feed intake even under adverse environmental conditions. Collectively, these results support the hypothesis that functional coordination among DMI associated genes represents a key component of metabolic adaptation to heat stress.

6.4.12. RFI Enriched Pathways across EG

Under low thermal conditions (THI 66), the enrichment profile for RFI indicates that feed efficiency is strongly linked to amino acid and organic acid metabolism (Table 4). The overrepresentation of pathways involved in proteinogenic and L-amino acid turnover, mainly driven by *SEPHS1*, *PIPOX* and *PHYH*, suggests that efficient nitrogen recycling and oxidative catabolism of small molecules are key mechanisms through which animals minimize residual feed intake under thermally mild conditions. This pattern is consistent with a metabolic configuration that favors precise matching between nutrient supply and energy demands.

The KEGG enrichment for the estrogen signaling pathway, involving several keratin genes, points to potential interactions between hormonal regulation, tissue turnover and metabolic efficiency. In parallel, the peroxisome pathway, driven by *PHYH* and *PIPOX*, highlights the importance of peroxisomal oxidative metabolism and lipid catabolism in shaping variation in RFI. Together, these findings suggest that, when heat load is low or absent, animals with superior feed efficiency tend to rely on coordinated control of amino acid degradation, organic acid catabolism and hormone-mediated regulation of energy metabolism.

Although the number of significant SNPs was similar across the different environmental gradients (37 in the Low EG, 40 in the Medium EG, and 41 in the High EG), significantly enriched biological processes and metabolic pathways were identified only under the low heat load conditions. This result indicates that the number of associated SNPs was not a limiting factor for the detection of functional enrichment. A possible explanation is that, under moderate and severe heat stress, the genetic background of feed efficiency becomes more diffuse, possibly due to a greater functional dispersion of the associated genes, as observed in the functional gene networks (THI 74 and THI 81). In these environments, the identified SNPs may be linked to biologically diverse functions, lacking convergence into specific pathways [169]. Furthermore, more intense heat stress may induce the activation of nonspecific or redundant genetic responses, involving multiple compensatory mechanisms that

reduce the functional cohesion among the mapped genes [169]. It is also important to consider the reduction in trait heritability and the increase in residual variance [19], factors that compromise the statistical consistency of the detected loci. Additionally, the increased environmental variability under moderate to severe heat stress may further reduce the statistical power required to identify genomic regions associated with the genetic variation in RFI.

6.4.13. DMI Enriched Pathways in the low EG

Under low heat load (THI 66, Table 5), the functional profile associated with DMI revealed a predominantly anabolic molecular signature, suggesting that cattle express feed intake variation largely through pathways that support cellular growth and metabolic stability. The enrichment of cell-cycle regulators such as *CCND1*, *MNAT1*, and *NCAPG* indicates active tissue turnover in metabolically relevant organs, consistent with greater digestive and absorptive capacity when thermal constraints are minimal. The strong signal for tRNA metabolism and aminoacylation, driven by *IARS2*, *EPRS1*, and *THUMPD2*, points to increased translational demand and enhanced protein synthesis machinery. This pattern is compatible with animals sustaining higher rates of structural and enzymatic protein production, which may contribute to differences in feed utilization efficiency under favorable conditions.

Finally, enrichment for phosphorylation-dependent signaling, involving *FGF19*, *CCND1*, and *MNAT1*, highlights the role of intracellular signaling networks in coordinating nutrient sensing, hormonal responses, and metabolic homeostasis. Together, these pathways depict a coherent anabolic framework through which genetic variation in DMI is most effectively expressed when environmental stress is minimal. Such mechanisms may help explain why superior feed efficiency phenotypes tend to manifest more strongly under thermally mild conditions.

6.4.14. DMI Enriched Pathways in the medium EG

Under moderate heat stress (THI 74), the functional profile associated with DMI revealed a combination of anabolic signaling and neuroendocrine regulation, indicating that feed intake at this intermediate environmental level depends on both cellular growth processes and central modulation of appetite (Table 6). As observed under low heat load, enrichment for cell-cycle regulators such as *CCND1* and *MNAT1*

suggests that tissue renewal and basal anabolic activity remain important components of DMI variation even when animals experience moderate thermal challenge.

A key difference at this EG was the strong signal for glutamatergic synaptic transmission, driven by *GRM7* and *GRID2*, which points to a more prominent involvement of central neuroendocrine pathways in the control of feed intake. This enrichment is consistent with increased reliance on neural and metabolic integration when animals begin to experience thermal strain. In parallel, pathways associated with growth-factor signaling, particularly those involving *FGF19* and *BMPR1B*, indicate continued coordination between hormonal regulation, energy balance and digestive function.

The additional enrichment of phosphorylation-dependent signaling and calcium-mediated pathways (e.g., involving *CCKAR*, *FGF19* and *STIM2*) further supports the idea that feed intake under moderate heat stress is governed by a tightly regulated intracellular communication network. Together, these results suggest that, at THI 74, DMI reflects a shift from a predominantly anabolic configuration (as observed under THI 66) toward a more complex, multifactorial regulatory system, integrating cellular signaling, neuroendocrine communication and adaptive responses to environmental stress.

6.4.15. DMI Enriched Pathways in the high EG

Under high heat stress (THI 81), the enrichment profile for DMI showed the recurrence and amplification of similar biological themes with moderate stress levels, suggesting a progressive recruitment of adaptive mechanisms in response to increasing environmental challenges (Table 7). Although cell-cycle and phosphorylation processes remained present, as previously observed under low and medium heat load, the most distinctive feature at this EG was the intensified enrichment for synaptic signaling, particularly glutamatergic and trans-synaptic communication mediated by *GRM7* and *GRID2*. This pattern indicates that, under severe thermal stress, feed intake becomes increasingly governed by central neural circuits involved in appetite regulation, behavioral modulation, and the integration of metabolic stress cues.

Another notable feature was the unique enrichment of pathways associated with cell-cycle checkpoint regulation, suggesting heightened control of mitotic progression, potentially reflecting cellular responses to oxidative stress and heat-

induced damage. At the same time, the disappearance of pathways linked to Wnt signaling, which were detected under moderate heat stress, may indicate suppression of growth and cell renewal programs in favor of short-term adaptive responses that prioritize survival and systemic homeostasis. This shift, from anabolic maintenance under mild conditions to neuroendocrine compensation under severe heat load, highlights a progressive reorganization of physiological mechanisms as environmental stress intensifies.

Collectively, the results suggest that DMI regulation in Nellore cattle at high THI relies on a multilayered adaptive network, where neural signaling, hormonal coordination, and stress-response pathways become increasingly dominant as growth-related signaling is down-regulated. These findings underscore the relevance of GxE interactions for feed efficiency traits and reinforce the need to consider thermal variability when interpreting genomic mechanisms and designing breeding strategies for tropical production systems.

6.5. Challenges and Future Directions

Although stratifying thermal conditions into discrete THI levels (66, 74, and 81) allowed for a structured assessment of G×E interactions, this approach presents inherent limitations. Heat stress is a dynamic and temporally variable phenomenon, often characterized by marked diurnal fluctuations in ambient temperature, relative humidity, and solar radiation. In the present study, we used average THI values and phenotypic means per feeding trial to classify environmental conditions, which, while facilitating G×E modeling, may mask short-term thermal effects on feed efficiency traits. Studies have shown that THI at specific hours of the day, particularly during peak heat, can significantly alter metabolic responses and feeding behavior [170], with cattle typically reducing intake during hotter periods and compensating later in cooler hours. This temporal plasticity, however, is not captured when using averaged environmental and phenotypic data.

As highlighted by Silva Neto et al. [19], future studies should prioritize the collection of longitudinal data to identify critical time windows during which heat stress exerts the greatest impact on DMI and RFI, to characterize individual adaptation patterns and feeding strategies in response to thermal stress, and to enable the modeling of phenotypic variation based on real-time environmental fluctuations rather than static period-based averages. The integration of continuous phenotypic and

environmental data into G×E GWAS frameworks holds promise for improving the detection of environment-sensitive genomic regions, refining the estimation of SNP effects under variable thermal conditions, and ultimately enhancing the accuracy of genomic evaluations for selecting animals with greater resilience and metabolic stability in tropical production systems facing intensifying climate challenges.

6.6. Conclusions

The genetic control of feed intake and feed efficiency in Nellore cattle is not only complex and polygenic but also sensitive to thermal stress conditions. The variation in genomic associations and gene network organization across different levels of heat stress reinforces the multifactorial nature of adaptation to tropical environments. In summary, the genetic networks appeared more integrated under low THI conditions, reflecting a more stable architecture when animals were not exposed to heat stress, whereas at higher THI levels additional loci became associated with the traits, suggesting that heat stress may reshape the genetic architecture by activating stress-related regions and reducing overall network integration. The identification of both environment-specific and recurrent candidate genes, together with the distinct functional patterns observed between environments, provides useful insights for refining genetic improvement strategies aimed at sustaining animal performance under increasing thermal stress.

6.7. Supplementary files

The supplementary file(s) supporting the analyses presented in this chapter are available online and can be accessed through the link provided in the published version of the corresponding paper: https://doi.org/10.1038/s41598-025-33952-1

6.8. Availability of data and materials

The data analyzed in this study were obtained from the National Association of Breeders and Researchers (ANCP). The phenotypic and genotypic information was provided to the authors for academic research purposes only. The following restrictions apply: the dataset is not publicly available and its use requires formal authorization. Requests to access these datasets should be directed to Dr. João Carlos G. Giffoni Filho, President of ANCP (email: presidencia@ancp.org.br).

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CHAPTER 7: FINAL CONSIDERATIONS, PRACTICAL APPLICATIONS, AND FUTURE DIRECTIONS FOR RESEARCH

7.1. Overview and core contributions of this thesis

This doctoral thesis provided an integrated, quantitative, and genomic perspective on feed efficiency in Nellore cattle raised under tropical production systems, with particular emphasis on how genotype-by-environment interactions (G×E) shape the genetic expression and genomic architecture of dry matter intake (DMI) and residual feed intake (RFI). By combining five complementary studies, including reaction norm models across environmental gradients, a comprehensive review of G×E methodologies and applications, and environment-sensitive genome-wide association approaches, this thesis advanced the understanding of how environmental heterogeneity and heat stress affect the predictability of genetic merit for feed efficiency-related traits in *Bos taurus indicus* populations. Collectively, these studies addressed key gaps in the literature for Nellore cattle under tropical conditions and generated evidence with direct relevance to breeding programs increasingly reliant on genomic selection and required to adapt to future scenarios of climate change intensification.

7.2. Synthesis of major scientific findings

7.2.1. G×E in beef and dairy cattle

Chapter 2 provided a broad synthesis of the literature on G×E in beef and dairy cattle, summarizing the main statistical methodologies and discussing practical and conceptual challenges in translating G×E evidence into decision-making in breeding programs. Across 116 studies (60 in beef cattle and 56 in dairy cattle), the review demonstrated that G×E is highly prevalent in production systems, as 83.62% of studies reported genetic correlations across environmental gradients below 0.80, indicating biologically meaningful reranking and context-dependent genetic expression. In addition to discussing reaction norm approaches, multi-trait models by environment, and genomic implementations, the chapter highlighted that the increasing availability of large-scale phenotypic and genotypic data creates opportunities to more precisely quantify environmental sensitivity and select for robustness. Emerging perspectives, such as nutrigenomics and nutrigenetics, were also discussed as promising avenues to connect genotype–phenotype mechanisms under nutritional and climatic variability.

Taken together, this review positioned the findings of the present thesis within the broader body of evidence and reinforced that G×E is not an exception, but rather a central component of the genetic architecture of complex traits with major implications for sustainability.

7.2.2. Feed efficiency traits are influenced by G×E of relevant magnitude

Chapter 3 provided robust evidence that G×E influences feed efficiency indicator traits in Nellore cattle, leading to meaningful changes in genetic parameters and reranking of selection candidates across environmental conditions. Estimated breeding values (EBVs) for both RFI and DMI were sensitive to environmental variation, particularly when environments were highly divergent, as reflected by wide differences in average daily gain (ADG) across feeding trials. Importantly, the genetic association between RFI and DMI decreased under less restrictive (more favorable) environments, indicating that the expected correlated response between these traits becomes weaker as management conditions improve. Consequently, selection decisions based solely on one of these traits may not translate consistently across production scenarios, reinforcing the need to explicitly account for environmental heterogeneity when defining breeding objectives for feed efficiency. Furthermore, the results suggested that selection conducted in trials close to the recommended performance level (ADG ≈ 1 kg/day) is expected to produce only minor changes in animal ranking, whereas selection based on trials with ADG far from this level increases reranking, likely because nutritional differences can mask genetic potential and bias genetic evaluations for progeny raised under distinct conditions.

7.2.3. Heat stress compromises the genetic expression of feed efficiency

In Chapter 5, consistent evidence demonstrated that heat stress has a substantial impact on feed efficiency traits in Nellore cattle when evaluated along a temperature–humidity index (THI)-based environmental gradient. DMI tended to be lower under more severe heat stress conditions, accompanied by reduced feed efficiency performance, indicating both behavioral constraints on intake and physiological limitations under challenging environments. In addition, the genetic expression of DMI and RFI varied across the THI gradient, with heritability estimates ranging from 0.22 to 0.39 for DMI and from 0.08 to 0.28 for RFI, indicating that additive genetic variance becomes less pronounced as heat stress increases. Evidence of

G×E was more evident at higher THI levels, particularly above 76, when genetic correlations for the same trait across environments dropped below 0.80 and were accompanied by increased reranking of sires. Moreover, although Nellore is widely recognized as a heat-adapted breed, these findings suggest that a subset of the population displays high sensitivity to thermal fluctuations, which may lead to reduced productive performance and poorer feed efficiency under intensified heat load. Collectively, these results reinforce that conventional models may mask important sources of variability and that genetic plasticity should be explicitly considered to sustain genetic progress in tropical environments under warming trends.

7.2.4. The genomic architecture of feed efficiency is environment-dependent

Beyond the estimation of genetic parameters, this thesis advanced the field by establishing a direct connection between G×E and the underlying genomic architecture, highlighting genomic regions potentially involved in both baseline phenotypic expression and differential genotypic responses to environmental variation. In Chapter 4, a genome-wide association analysis based on *single-step* reaction norm models identified genomic windows explaining more than 1% of the additive genetic variance for both the intercept component (overall genetic level) and the slope component (environmental sensitivity). Candidate genes mapped to these regions showed functional enrichment related to energy balance, insulin/leptin signaling, glucose and lipid metabolism, digestion, feeding behavior, and heat stress response, supporting the concept that efficiency and resilience share overlapping yet dynamic biological mechanisms.

In Chapter 6, environment-specific ssGWAS results were evaluated under three levels of thermal load (THI 66, THI 74, and THI 81), revealing marked changes in the number and distribution of significant SNPs and distinct association patterns across THI classes. Additionally, gene network analyses provided strong evidence of environment-dependent functional rearrangement, with more pronounced alterations observed for RFI. Under higher THI levels, the RFI-associated network exhibited lower connectivity and reduced strongly interactive modules, suggesting that heat stress compromises the integration of key biological processes regulating this phenotype. This pattern supports the interpretation that RFI is particularly sensitive to thermal challenge, potentially because it depends on finely coordinated metabolic and regulatory mechanisms that become less synchronized under intense heat stress.

7.3. Future directions for research

The findings of this thesis reinforce that feed efficiency in Nellore cattle, particularly under tropical conditions, should be treated as a complex phenotype strongly shaped by environmental context. The dynamic and multifactorial nature of this trait indicates that further scientific and applied progress will depend on incorporating new layers of information, improving phenotyping resolution, and developing predictive tools aligned with real-world production environments. Future research should therefore prioritize the expanded use of longitudinal and high-frequency phenotypes, shifting from period-averaged measures toward records that represent feed efficiency as a temporally structured process. In tropical systems, heat stress frequently manifests intermittently, with daily and intra-daily fluctuations that simultaneously affect intake, feeding behavior, and energy balance. Thus, daily (or intra-daily) intake records, combined with intra-daily THI oscillations and behavioral indicators (e.g., visit frequency, bout duration, and temporal distribution of intake), can capture adaptive responses, quantify individual compensation patterns, and provide a more biologically realistic characterization of resilience as a measurable component of efficiency.

In parallel, the development of resilience indices integrating feed efficiency with productive performance, fertility, and thermotolerance represents a critical step toward sustainable breeding goals in tropical environments. Because long-term genetic gains depend on coordinated biological processes, indices combining DMI and RFI with fertility measures under heat stress and phenotypic/physiological indicators of adaptation (e.g., body temperature, respiration rate, and coat characteristics) may align selection more closely with the ultimate production objective: consistent and predictable performance under environmental variability. In this context, reaction norm-derived metrics such as slope and stability across gradients can be incorporated as direct robustness components, enabling selection of animals that are both efficient and less sensitive to increasing thermal stress.

Additionally, while progress in breeding has traditionally been driven by increasing genetic and genomic information, the results of this thesis highlight that the quality and resolution of environmental information may be equally decisive for maximizing accuracy under G×E. Therefore, investment in more complete and biologically informative environmental descriptors is warranted, including not only THI, but also solar radiation, wind speed, nutritional management, shade availability,

stocking density, and system-specific conditions. A stronger integration of phenotypic, genetic, and environmental data is expected to enhance connectedness across herds and improve the representativeness of target environments within training populations.

Finally, a decisive pathway to consolidate and validate environment-specific genomic findings is the integration of multi-omics and systems biology, enabling the transition from statistical associations to biologically plausible mechanisms. Combining transcriptomics, metabolomics, epigenomics, and the rumen microbiome can link genomic regions identified under different heat stress levels to physiological and regulatory changes related to energy metabolism, stress response, and nutrient utilization efficiency. This progress is expected to strengthen causal understanding of the underlying processes and increase the biological utility of candidate regions, ultimately enhancing the development of selection and management strategies aimed at sustainable feed efficiency in tropical environments.