UNIVERSITÀ
DEGLI STUDI
FIRENZE

PhD in
AGRICULTURAL AND ENVIRONMENTAL SCIENCES

CYCLE XXXVIII

Genomic Evaluations Aimed at Increasing Beef Cattle Sustainability and Resilience

Academic Discipline (SSD) AGRI-09/A

Doctoral Candidate
Dr. Simone Callegaro

Supervisor
Prof. Riccardo Bozzi

Co-Supervisor
Prof. Francesco Tiezzi

Coordinator
Prof. Carlo Viti

2022/2025

"As the dynamism of natural systems teaches us, resilience is not about clinging to stability but adapting and persisting in the face of change"

Ralph Waldo Emerson

"The bamboo that bends is stronger than the oak that resists."

Japanese Proverb

Genetic selection for resilience aims to breed cattle that resemble "bamboo rather than oak" able to adapt "bends" to climatic stressors and remain robust beyond optimal conditions.

Abstract

Beef cattle production plays a central role in sustainable food systems, yet it faces growing challenges from climate change, resource limitations, and societal expectations for welfare-oriented and environmentally responsible farming. Enhancing the genetic resilience, efficiency, and longevity of beef cattle is therefore essential to sustain productivity and animal welfare under increasingly variable conditions. This thesis developed quantitative and genomic approaches to improve the sustainability and resilience of Italian beef cattle, focusing on functional longevity, fertility, growth, and their interaction with environmental stressors. The research integrated linear and threshold single-step genomic best linear unbiased prediction models, genomic inbreeding analyses, multiple-trait models for genotype-by-environment interaction, and single-step genome-wide association studies.

The first study investigated functional longevity, expressed as stayability, in Italian Charolais and Limousine cattle using single-step genomic prediction under linear and threshold models. Heritability estimates were moderate (0.11–0.21), with higher values under threshold models, confirming that longevity can be improved through selection. Spearman rank correlations between early and late calvings were positive but declined with increasing parity, while re-ranking of genotyped sires suggested that stayability across calvings represents genetically distinct traits. Negative correlations between stayability and fertility traits (age at first calving and first calving interval) indicated that earlier and more fertile cows tend to remain productive longer. In Limousine cattle, positive genetic associations between stayability and body conformation traits reflected the contribution of morphological soundness to resilience and longevity, whereas some body traits in Charolais, such as rump convexity, were unfavorably associated with longevity.

The second study conducted a single-step genome-wide association analysis for longevity, fertility, and conformation traits in Limousine cattle, identifying genomic regions associated with immune regulation, reproductive efficiency, growth, and energy metabolism. The overlapping of different genes across longevity, fertility, and conformation highlights the polygenic nature of these complex traits. These candidate genes highlighted biological mechanisms underlying female productivity, adaptability, and overall resilience.

The third study evaluated inbreeding depression across growth, fertility, and survival traits using both pedigree- and genome-based coefficients. Significant phenotypic depression was observed for growth, fertility traits, and longevity. Genomic inbreeding explained a higher proportion of variance than pedigree-based estimates, underscoring the importance of managing both recent and ancient inbreeding to preserve genetic diversity in the studied population.

The fourth study assessed genotype-by-environment interaction for growth traits (average daily gain, weaning weight, and yearling weight) across different climatic environments in Limousine

cattle using a multiple-trait model. Results revealed genetic heterogeneity in environmental sensitivity, identifying genotypes capable of maintaining stable growth under heat stress conditions.

Overall, this thesis demonstrates that combining genomic prediction, association analyses, and environmental modelling provides an effective framework for identifying animals that are productive, resilient, and well adapted to diverse conditions. These findings provide scientific and practical foundations for future breeding programs that promote productivity, animal welfare, and long-term sustainability in modern beef production systems.

Outline

Abbreviations

Chapter 1

GHG: greenhouse gases

FAO: Food and Agriculture Organization of the United Nations

CO_2: carbon dioxide

CH_4: methane

N_2O: nitrous oxide

CFCs: chlorofluorocarbons

THI: temperature humidity index

EU: European Union

SDGs: Sustainable Development Goals

NDCs: nationally determined contributions

G × E: genotype-by-environment interactions

U.S.: United States

GS: genomic selection

GEBV**:** genomic estimated breeding value

SNP: single-nucleotide polymorphism

QTL**:** quantitative trait loci

EBV**:** estimated breeding value

ANACLI: Associazione Nazionale Allevatori Charolais e Limousine Italia

GWAS: genome-wide association studies

ssGBLUP: single-step genomic best linear unbiased prediction

ROH: runs of homozygosity

GRM: genomic relationship matrix

Chapter 2

ANACLI: Associazione Nazionale Allevatori Charolais e Limousine Italia

LIM: Limousine

CHA: Charolais

Chapter 3

ADG: average daily gain

WW: weaning weight

YW: yearling weight

G × E: genotype by environment interaction

Env: environmental conditions

MT: multi-trait models

THI: temperature humidity index

Temp: average daily temperature

RH: relative humidity

DP: dew point

maxRH_CLASS: class of maximum relative humidity

T_CLASS: class of temperature

DP_CLASS: class of dew point

BLUP: best linear unbiased prediction

GBLUP: genomic best linear unbiased prediction

h^2: heritability

h_i: herd-year effect

σ_a^2: additive genetic variance

Chapter 4

STAY: stay-ability

ssGBLUP: single-step genomic best linear unbiased prediction

ANACLI: Associazione Nazionale Allevatori Charolais e Limousine Italia

AFC: age at first calving

FCI: first calving interval

h^2: heritability

h_i: herd effect

h_{IH}^2: intra-herd heritability

Chapter 5

F_{PED}: pedigree-based inbreeding coefficients

SNP: single-nucleotide polymorphism

NCG: number of complete generations

CGE: complete generation equivalents

PCI: pedigree completeness index

BiW: birth weight

WW: weaning weight

YW: yearling weight

ADG: average daily gain

STAY: stayability

AFC: age at first calving

FCI: first calving interval

GRM: genomic relationship matrix

F_{GRM}: marker-by-marker

ROH: runs of homozygosity

F_{ROH}: segment-based approaches

Chapter 6

GWAS: Genome-wide association studies

QTL: quantitative trait loci

ssGWAS: single-step GWAS

STAY: stayability

ANACLI: National Italian Association of Limousine and Charolais Breeders

AFC: age at first calving

FCI: first calving interval

MAF: minor allele frequency

GEBV: genomic estimated breeding values

LD: linkage disequilibrium

h^2: heritability

σ_a^2: additive genetic variances

σ_h^2: herd variance

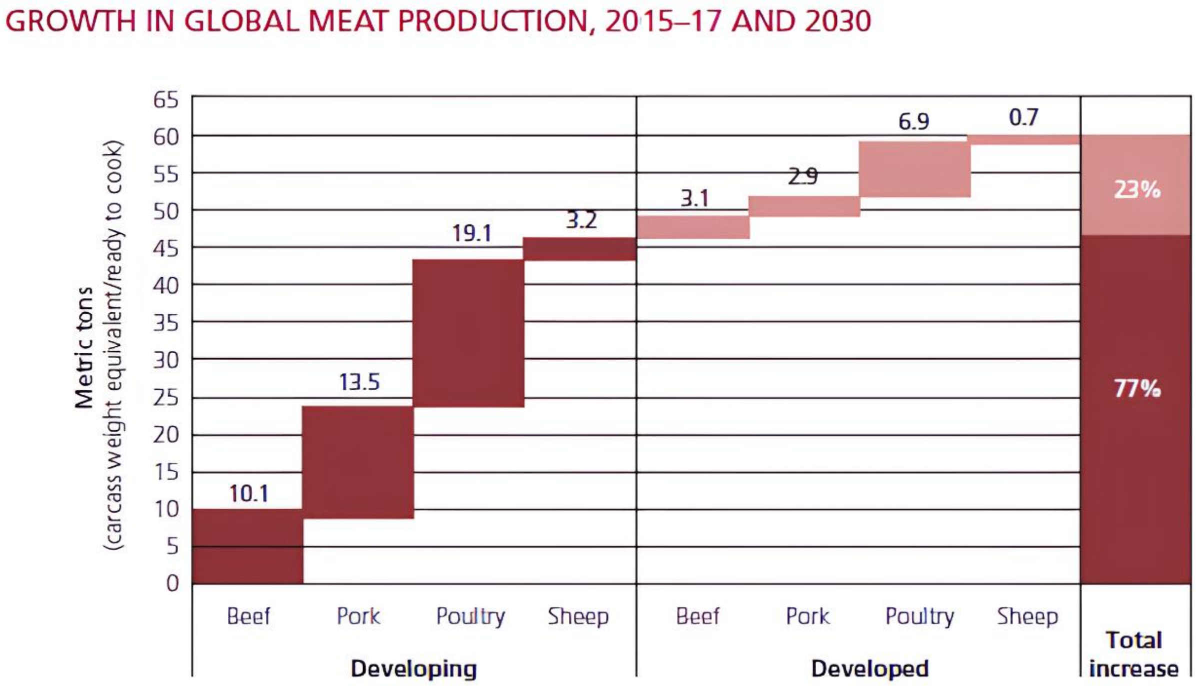
CHAPTER 1

1. General Introduction

1.1 Global challenges in the livestock sector: environmental impact, animal welfare, and the need for resilience

Livestock production plays a pivotal role in meeting global nutritional demands by supplying high-quality protein, employment opportunities, organic fertilizer, and draught power, thereby contributing to food security (Herrero et al., 2013). Ruminants are particularly essential for converting fibrous feed resources into edible products and mechanical energy through their symbiotic relationship with ruminal microbiota. According to the Food and Agriculture Organization of the United Nations (**FAO**), the livestock sector accounts for approximately 40% of total agricultural output and supports the livelihoods of about 1.3 billion people worldwide (Bonilla-Cedrez et al., 2023; Bilotto et al., 2024).

Livestock systems are commonly classified into three categories: (i) pastoral or grassland-based systems, covering extensive areas with low population density; (ii) mixed crop–livestock systems, integrating crop cultivation with animal husbandry; and (iii) intensive systems, typically located in peri-urban or urban areas (Herrero et al., 2013; Bilotto et al., 2024). Each system contributes significantly to local and national economies by providing milk, meat, hides, and other commodities. For example, beef production has grown annually since 2000 by approximately 3.7%, 2.3%, and 1.4% in Northern Africa, Central America, and Southeast Asia, respectively (FAOSTAT, 2023). Beyond economic relevance, livestock systems hold cultural and social significance, shaping landscapes and sustaining rural livelihoods even in industrialized nations (Sirimarco et al., 2023).

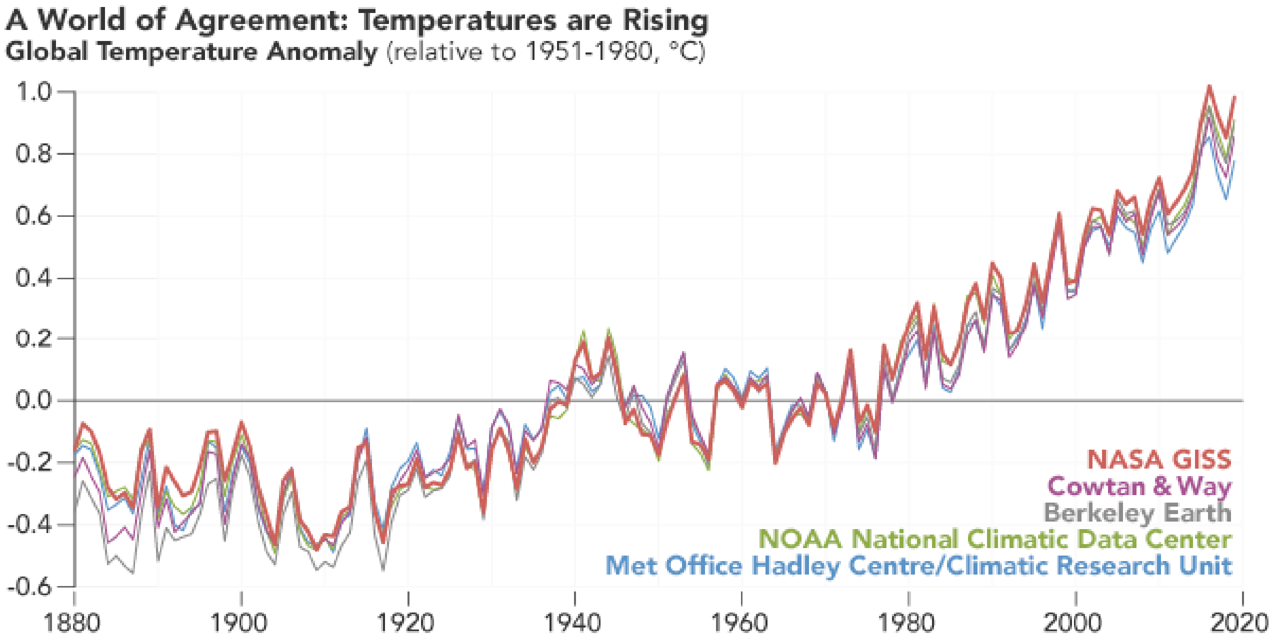


GROWTH IN GLOBAL MEAT PRODUCTION, 2015–17 AND 2030

Figure 1.1 "Projected Growth in Global Meat Production by Type and Development Status, 2015-17 to 2030". The figure illustrates projected increases in global meat production from the 2015–17 baseline to 2030, expressed in million tons carcass weight equivalent per year (FAO, 2018, *Transforming the Livestock Sector through the Sustainable Development Goals*). Growth is categorized by meat type (beef, pork, poultry, sheep) and country development status. The projections reveal substantial expansion, particularly in developing regions, underscoring the urgent need for efficient and sustainable livestock systems to meet rising global demand while minimizing environmental impacts and resource depletion. These trends highlight the necessity of implementing resilient production strategies addressing the three pillars of sustainability: environment, economy, and society.

Despite these benefits, livestock production faces growing scrutiny due to its environmental footprint. The sector is a major contributor to global greenhouse gas (**GHG**) emissions and is associated with biodiversity loss and land degradation (Gerber et al., 2013). Climate change, driven by rising atmospheric concentrations of carbon dioxide (**CO_2**), methane (**CH_4**), and nitrous oxide (**N_2O**), exacerbates the greenhouse effect, leading to progressive global warming.

a)



b)

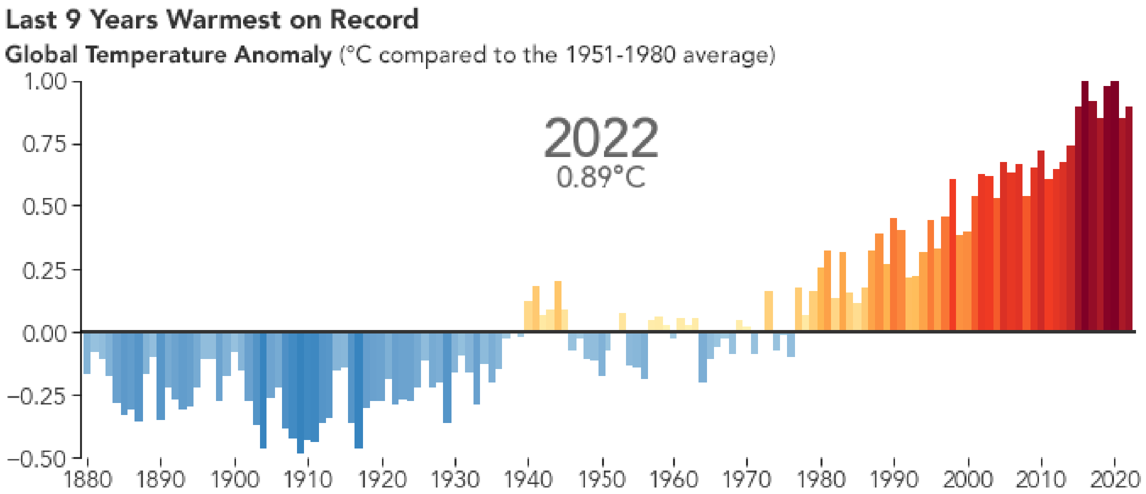


Figure 1.2 (a-b) "A World of Agreement: Temperatures are Rising". The panels present the observed rise in global mean surface temperature, reflecting consensus across multiple independent datasets. The continued upward trend in global temperatures is expected to increase both the frequency and intensity of heat stress events in livestock, with detrimental effects on animal health, fertility, and productivity. Concurrently, environmental consequences such as biodiversity loss, water scarcity, and ecosystem instability are anticipated to intensify, posing major challenges to the sustainability of agricultural systems worldwide. (https://earthobservatory.nasa.gov/world-of-change/global-temperatures).

Furthermore, the expected increase in global population to 9.4 billion by 2050, together with projected rises in meat, egg, and dairy consumption by 73% and 58%, respectively, will place additional pressure on land, water, and energy resources (Makkar, 2016). Addressing these interconnected challenges requires strategies that ensure environmental protection, economic sustainability, and social equity.

The complexity and uncertainty of climate change impacts underscore the urgent need for adaptation strategies that enhance resilience and sustainability in breeding populations. Effective adaptation depends on integrating technological innovations, improved management, and genetic selection strategies. Among these, breeding for resilience represents a long-term, cost-effective approach to improve farm efficiency, animal welfare, and overall sustainability while mitigating GHG emissions. Understanding these global challenges provides a foundation for assessing the specific effects of climate change on livestock health, productivity, and system viability.

1.2 Climate change: implications for animal health, production, and livestock system sustainability

Climate change and extreme weather events exert profound direct and indirect effects on livestock. Direct impacts include alterations in physiology, behavior, health, productivity, welfare, and mortality, while indirect effects arise from changes in feed and water availability, disease prevalence, and ecosystem dynamics (Faisal et al., 2021; Nardone et al., 2010; Freier et al., 2014; Rojas-Downing et al., 2017; Henry et al., 2018). The magnitude of these effects varies among species, regions, and production systems (Phelan et al., 2015), highlighting the need to improve animal welfare and resilience under changing climatic conditions.

Heat stress induces physiological and metabolic responses, including reduced feed intake, elevated respiration rate, and endocrine alterations, disrupting energy balance and homeostasis (Nardone et al., 2010; Baumgard et al., 2007). Consequences include negative energy balance, impaired hepatic function, metabolic dysfunction, and oxidative stress, ultimately affecting nutrient allocation for growth and lactation. Heat stress also predisposes cattle to ruminal acidosis due to decreased saliva production and buffering capacity (Kadzere et al., 2002), and increases summer mortality (Vitali et al., 2009).

The immune system is particularly sensitive to elevated temperatures, with heat waves impairing cellular immune function in dairy cattle (Lacetera et al., 2005). In females, heat stress disrupts ovarian activity and hormone secretion, reducing oocyte maturation, estradiol synthesis, and estrus expression (Rensis and Scaramuzzi, 2003). Consequently, reproductive performance declines in both sexes, with lower conception rates, compromised gamete quality, and reduced embryonic survival (Chebel et al., 2004; Hansen, 2007; Amundson et al., 2006; Nichi et al., 2006; Hansen, 2009; Ambrosini et al., 2016).

From a production standpoint, heat stress significantly reduces efficiency and profitability. Extreme heat waves in the United States and Europe have caused thousands of cattle deaths and financial losses in the billions (St-Pierre et al., 2003; Vitali et al., 2009). In beef cattle, elevated temperatures decrease dry matter intake, average daily gain, carcass weight, and meat quality, often resulting in darker meat with altered pH values due to compromised muscle metabolism (Kadim et al., 2004).

Overall, Prolonged exposure to heat and other stressors undermines welfare, productivity, and health, threatening long-term livestock sustainability. These impacts highlight the importance of enhancing adaptive capacity and resilience through targeted management and breeding programs. Integrating resilience traits into selection schemes is therefore critical for maintaining production efficiency and animal welfare under climate change.

1.3 Global strategies and policies driving sustainable livestock

In response to climate challenges and the need for resilient livestock systems, international and regional frameworks promote sustainable livestock development. Key initiatives are led by the FAO, the European Union (**EU**), and multistakeholder partnerships such as the Global Agenda for Sustainable Livestock. The FAO emphasizes the sector's role in achieving the Sustainable Development Goals (**SDGs**), particularly regarding food security, poverty alleviation, nutrition, and rural livelihoods, while mitigating environmental impacts and enhancing system resilience (FAO, 2018; FAO, 2020). Beef production is central in this context, providing high-quality protein, income diversification, draught power, and manure for soil fertilization, particularly in smallholder and mixed crop–livestock systems.

Beef production faces challenges in land and water use, biodiversity conservation, and GHG emissions (Gerber et al., 2013). Policies increasingly prioritize genetic improvement, optimized feeding, and adaptive management to enhance efficiency and reduce environmental footprints. Nationally Determined Contributions (**NDCs**) under the Paris Agreement further encourage low-emission, climate-adaptive production systems.

At the regional level, programs such as the EU Horizon Europe Work Program (2023–2025) establish priorities for biodiversity-friendly practices, nature-based climate adaptation, circular resource management, and governance frameworks fostering resilient, equitable food chains (European Commission, 2023). Complementary trade initiatives, such as UNECE meat standards, enhance transparency, product quality, and sustainability across global markets.

Recent high-level events, including the 2023 FAO Global Conference on Sustainable Livestock Transformation, reaffirm international commitment to mobilize science, policy, and finance toward sustainable livestock systems. The One Health framework, integrating animal, human, and environmental health, underscores the alignment of agricultural productivity with public health and ecosystem integrity.

Overall, these global and regional strategies aim to transform beef cattle production into a resource-efficient, climate-resilient, and socially inclusive system, establishing a foundation for breeding and management programs that enhance sustainability, productivity, and resilience, the core themes explored in this thesis.

1.4 Sustainability, efficiency and resilience in beef cattle production

Building on the global challenges discussed above, this section focuses on beef cattle production and examines how sustainability, efficiency, and resilience can be defined and strengthened to address environmental, economic, and societal pressures.

1.4.1 Conceptual foundations of sustainability, resilience, and robustness in livestock systems. Sustainability in livestock production represents a central challenge for animal science and agricultural policy, reflecting society's increasing concern with the long-term viability of food systems. Over recent decades, multiple frameworks have conceptualized sustainability as the integration of ecological, economic, and social dimensions that guide production decisions for both present and future generations (Gibon et al., 1999). Within livestock systems, sustainability entails maintaining productivity while conserving natural resources, protecting ecosystems, and supporting human well-being. Sustainable cattle production, therefore, combines efficient animal output with strong animal welfare, environmental stewardship, and herd resilience, ensuring stability under environmental and market fluctuations

Throughout their lifetimes, animals face diverse internal and external stressors. From both welfare and production perspectives, animals should be capable of coping with these challenges and recovering quickly when homeostasis is disrupted. Hence, resilience and robustness have become pivotal concepts (Hermesch and Dominik, 2014). Resilience encompasses responses not only to pathogens but also to environmental and social stressors (Russo et al., 2012; Wu et al., 2013). It refers to the capacity of animals to perceive, process, and adapt to environmental variation while maintaining functional stability. Adaptive responses operate at different time scales, from short-term homeostatic regulation to longer-term acclimatization (Colditz and Hine, 2016). For example, thermoregulatory responses in cattle include reduced feed intake, endocrine adjustments, and metabolic reprogramming to mitigate heat stress, often at the expense of productivity. Thus, resilience reflects an animal's ability to sustain performance, fertility, and health under variable or stressful conditions, an essential feature of sustainable beef production in the era of climate change.

The intensification of modern livestock systems, characterized by larger herd sizes and high production pressures, further emphasizes the need for resilient animals. Such animals require fewer interventions to remain healthy and productive, offering advantages for both animal welfare and farm profitability (Berghof et al., 2019). Genetic selection provides a key pathway to improve resilience, producing cumulative and heritable gains across generations that complement, rather than replace, management-based measures. Importantly, resilience and welfare are closely linked. The World Organisation for Animal Health (OIE, 2014) defines welfare as the animal's capacity to cope with its environment while maintaining health, comfort, nutrition, and freedom from pain, fear, or distress (Colditz and Hine, 2016). Breeding for resilience thus directly supports welfare and sustainability by favoring animals capable of thriving under a broad range of environmental and management conditions.

1.4.2 Defining resilience and robustness in livestock. Resilience has been applied across disciplines, from psychology (Vella and Pai, 2019) to ecology (Van Meerbeek et al., 2021), and more recently to livestock production (Scheffer et al., 2018). Despite its broad use, definitions in animal science remain variable. In this thesis, resilience is considered alongside related concepts such as robustness, resistance, and tolerance, as precise terminology is critical for accurate phenotyping and genetic evaluation.

Two key elements consistently characterize resilience: (i) the animal's response to a perturbation and (ii) its recovery capacity thereafter (Scheffer et al., 2018; Laghouaouta et al., 2024). A resilient individual withstands biological, environmental, or social disturbances, allowing temporary performance disruption but ensuring rapid restoration of a stable functional state (Berghof et al., 2019; Colditz and Hine, 2016). Perturbations may arise from pathogens, nutritional imbalances, management stress, transport, or climatic extremes (Cotticelli et al., 2024). Performance–time frameworks (Figure 1.3) visualize resilience through the depth and duration of performance decline and the rate of recovery.

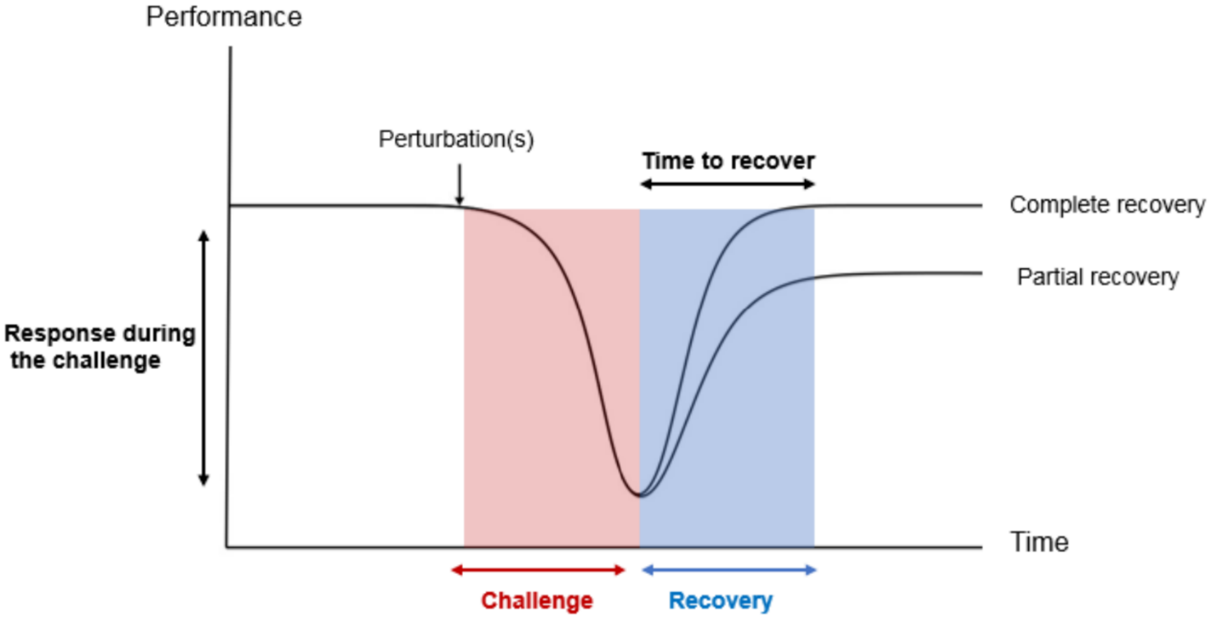


Figure 1.3 Dynamics of resilience after perturbation. The conceptual model illustrates animal response to a challenge, the decline in performance during exposure, and the recovery phase. Resilience is characterized by the speed and extent of recovery, which may result in complete or partial restoration of performance (Laghouaouta et al., 2024).

Disease resilience refers to recovery following specific infections, whereas general resilience encompasses the ability to maintain function under multiple, overlapping stressors (Colditz and Hine, 2016; Laghouaouta et al., 2024). Resilience can also be assessed at the herd or system level, representing collective adaptive capacity (Doeschl-Wilson et al., 2021). Robustness, though related, is a broader concept describing the capacity to maintain high productivity across variable environments while coping with persistent stressors (Knap, 2005; König and May, 2019). In this framework, resilience represents a component of robustness; all robust animals are resilient, but not all resilient animals are robust (Knap, 2005). Additional terms, resistance (ability to limit pathogen load) and tolerance (capacity to maintain function despite infection), further refine the resilience continuum (Berghof et al., 2019). Complementary quantitative genetics concepts such as plasticity, environmental sensitivity, and stability describe variation in individual responses to environmental perturbations. The biological basis of resilience includes a cascade from environmental perception to behavioral and physiological coping responses (Figure 1.4). Clarifying these relationships is essential for developing phenotypic indicators and implementing breeding strategies (Table 1.1).

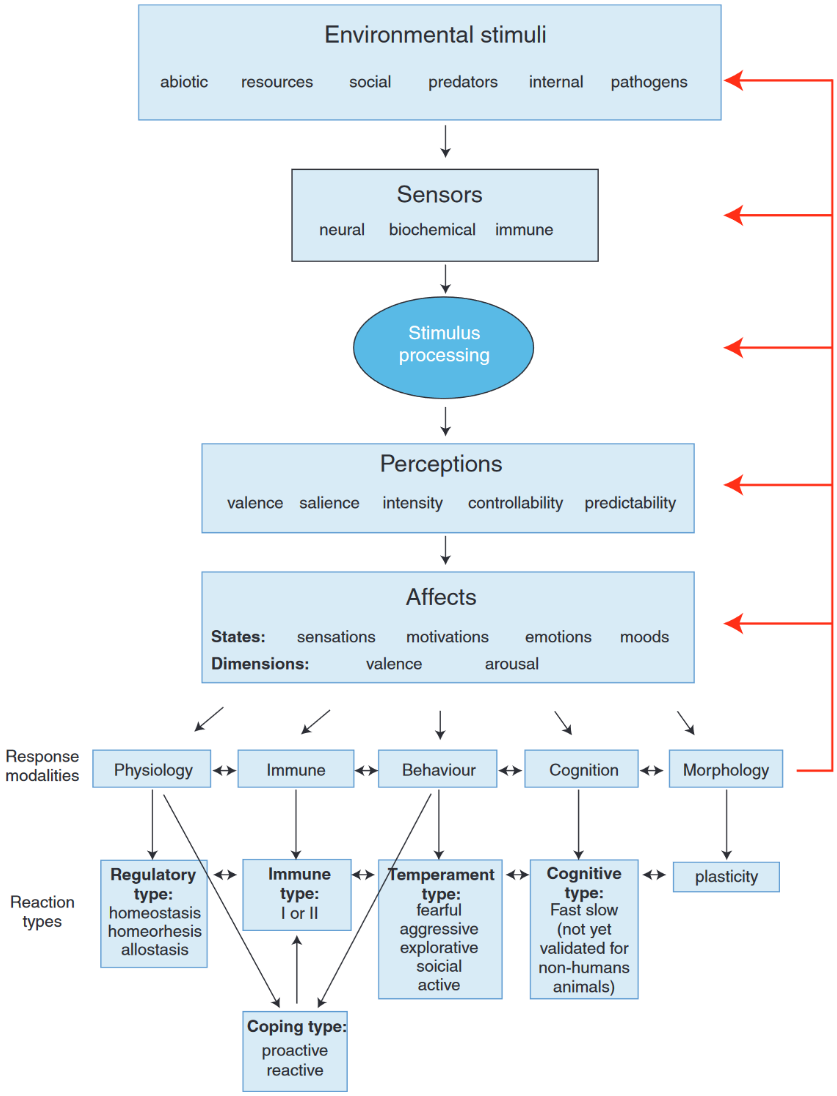


Figure 1.4 Pathways of animal responses to environmental stimuli. Schematic representation of how animals perceive and process environmental challenges through neural, biochemical, and immune sensors, leading to perceptions, affective states, and integrated responses at the physiological, behavioural, cognitive, and

morphological levels. These mechanisms collectively underpin resilience and robustness in livestock (Colditz and Hine, 2016).

Table 1.1 Key definitions of resilience-related and efficiency concepts in livestock science.

Concept	Definition	Key references
Resilience	The ability of an animal to cope with perturbations (biological, environmental, or social) and to rapidly recover normal functioning after a disturbance.	Scheffer et al., 2018; Colditz and Hine, 2016; Berghof et al., 2019
Robustness	Capacity to maintain high productivity and functional stability across a wide range of environments, including under persistent or cyclical stressors; incorporates resilience as a component.	Knap, 2005; König and May, 2019; Llonch et al., 2020
General efficiency/cost-output efficiency	Efficiency, in terms of cost per value-equivalent unit of output from a livestock production system, is influenced not only by the genetic potential for performance of the animals in the chosen breeding system, but also by environmental effects, by the management system, and by relative prices for inputs and outputs.	Wilkinson, 2011; FAO 2011, Livestock in food security
Resistance	Ability to limit or control pathogen loads upon infection.	Berghof et al., 2019
Tolerance	Capacity to minimize functional impairment or performance loss despite a pathogen load.	Berghof et al., 2019; Colditz and Hine, 2016
General resilience	Response capacity of an animal to a wide variety of perturbations (disease, heat stress, management, etc.) occurring during its productive life.	Colditz and Hine, 2016; Laghouaouta et al., 2024
Disease resilience	Specific capacity to respond and recover from infection by a given pathogen.	Colditz and Hine, 2016
Plasticity/ Environmental sensitivity	The degree to which an individual's phenotype changes in response to environmental variation.	Mulder et al., 2013; Tiezzi and Maltecca, 2022
Stability	Ability to maintain a consistent phenotype despite environmental or genetic perturbations.	Berghof et al., 2019

1.4.3 Approaches to quantifying animal resilience. The absence of a standardized definition has hampered the development of universally accepted resilience metrics. Because resilience is shaped by multiple, often antagonistic biological processes, its quantification requires integrative approaches

(Colditz and Hine, 2016). For example, immune activation demands substantial energy, potentially diverting resources from growth or reproduction (Broom and Kogut, 2018).

Resilience indicators are measurable phenotypes describing the magnitude of perturbations and recovery dynamics following biological or environmental stressors. These may include deviations in performance traits, immune parameters, or behavioral patterns (Taghipoor et al., 2023). Depending on the context, indicators can reflect disease-specific or general resilience. Accurate phenotyping remains fundamental. While traditional methods rely on post-perturbation deviations in productivity, modern precision livestock technologies now enable longitudinal monitoring of milk yield, body weight, and activity. Such high-frequency data facilitate dynamic resilience metrics at both individual and population levels, providing valuable inputs for selection.

The research presented in this thesis applies multiple quantitative and genomic approaches as proxies for resilience. Genotype-by-environment interaction (**G × E**) analyses for growth traits in Limousine cattle quantify environmental sensitivity and adaptive capacity. Functional longevity studies in Limousine and Charolais breeds evaluate long-term reproductive and productive performance. Inbreeding depression analyses assess the role of genetic diversity in maintaining resilience, while genome-wide association studies (**GWAS**) identify genomic regions contributing to stable performance under variable conditions. Collectively, these complementary approaches provide a comprehensive framework for quantifying and enhancing resilience in beef cattle.

1.4.4 Management strategies to enhance robustness, resilience, and efficiency in beef cattle. Beef cattle experience multiple stressors throughout their lives, including management procedures, transport, and handling, that can adversely affect welfare and performance (Colditz and Hine, 2016). Understanding these sources of stress enables the design of effective mitigation strategies. Persistent challenges such as inadequate housing, nutritional imbalances, or climatic extremes further constrain adaptive capacity. According to resource allocation theory, resilience may be limited when resources are preferentially directed toward production rather than coping functions. Short-term adaptation (resilience) supports recovery from acute stress, whereas long-term adaptation (robustness) depends on sustained physiological and genetic mechanisms (Colditz and Hine, 2016).

Integrating management optimization with selective breeding offers a synergistic approach to enhance resilience, efficiency, and robustness. Improvements in housing, nutrition, and precision monitoring reduce environmental stress and enable animals to fully express their genetic potential. Concurrently, selection for resilience-related traits such as consistent growth, fertility, and longevity reinforces herd-level stability across generations. This combined strategy supports production efficiency while safeguarding animal welfare, health, and long-term sustainability.

1.4.5 Breeding approaches for resilience, efficiency, and robustness in beef cattle. Modern breeding programs face the dual challenge of ensuring global food security while addressing climate change and societal expectations for ethical and sustainable production. Selective breeding, complemented by advances in management, nutrition, and precision technologies, has substantially improved livestock productivity over recent decades. However, historical emphasis on production traits alone has sometimes led to reduced fertility, diminished robustness, and greater susceptibility to environmental and health challenges (Knap, 2005; 2020).

Genetic selection has significantly enhanced growth, carcass quality, and feed efficiency in beef cattle, driving profitability (Rowan et al., 2024; Catrett and Rowan, 2024). Nevertheless, functional traits such as fertility and longevity, key determinants of herd efficiency, have often received less emphasis (Catrett and Rowan, 2024). These traits are crucial for reducing replacement costs and improving overall herd sustainability (Boyer et al., 2020). For example, selecting replacement heifers with superior genetic merit for fertility promotes both resilience and profitability. Early calving heifers tend to remain longer in the herd and produce more weaned calves throughout their lifetime (Cushman et al., 2013).

Balanced breeding objectives must therefore integrate resilience and robustness alongside productivity, particularly under heat stress and other environmental constraints (Berghof et al., 2019). Incorporating production, efficiency, health, and functional traits within selection indices supports long-term sustainability (Neeteson-van Nieuwenhoven et al., 2013; Miglior et al., 2017). Selection for resilient and robust animals enhances welfare, resource-use efficiency, and profitability while reducing reliance on antimicrobials (Mulder and Rashidi, 2017). Because trade-offs between resilience and productivity may occur, careful multi-trait evaluation is required to avoid economic losses and ensure genetic progress across all dimensions of sustainability.

Ultimately, sustainability, efficiency, resilience, and robustness in livestock systems depend on the integration of genetic, management, and technological strategies. Animals that are resilient, efficient, and robust form the cornerstone of productive, profitable, and ethically responsible beef production under changing climatic and societal conditions. The approaches described in this thesis demonstrate how these traits can be systematically incorporated into breeding goals to advance climate-resilient and socially responsible beef production systems.

1.5 Worldwide and context of the Italian beef cattle sector

1.5.1 Global situation of the beef sector. In 2025, global beef production is projected to remain stable at 61.6 million tons. Declines in output from the U.S., EU, and Argentina are expected to be offset by increases in Brazil, India, and Australia, with Brazil alone forecasted to reach a record 11.9

million tons (USDA-FAS, 2015). The global cattle population is estimated at nearly one billion head, concentrated primarily in India, Brazil, and China, and composed of *Bos taurus*, *Bos indicus*, and their crosses (USDA-FAS, 2015).

Beef exports are expected to rise slightly to 13.1 million tons in 2025, as increases from Brazil, India, and Australia compensate for declines in the U.S. Meanwhile, strong import demand from major markets such as China and the U.S. continues to shape global trade flows (USDA-FAS, 2015).

Although beef systems are often perceived as less efficient than monogastric livestock systems, this perception overlooks the unique ability of ruminants to convert grasslands and agro-industrial by-products into human-edible protein (O'Mara, 2012; Wilkinson, 2011). Efficiency assessments focusing solely on feedlot finishing phases underestimate the broader ecological and nutritional contributions of ruminant production systems, which utilize large volumes of non-human-edible feed resources.

Beef and dairy cattle account for approximately 70% of livestock-related GHG emissions, equivalent to roughly 6.3 Gt CO_2-eq annually, with beef cattle contributing substantially through enteric fermentation, manure management, and land-use change (Gerber et al., 2013). These figures underscore the urgent need for strategies to enhance productivity, feed efficiency, and environmental performance.

Despite their environmental footprint, ruminants play essential ecological and socio-economic roles, particularly in maintaining rural livelihoods and converting non-arable lands into valuable protein sources. Grasslands also act as major carbon reservoirs, with global carbon stocks estimated at ~343 Gt C, about 50% higher than forest carbon stocks (O'Mara, 2012; FAO, 2010).

Global beef production remains heterogeneous in production systems, genetic resources, and breeding goals. Many breeding objectives remain suboptimal, constrained by limited data on fertility, longevity, feed efficiency, meat quality, and health traits (Berry et al., 2016). Expanding accurate genetic evaluations and genomic selection across diverse production environments is therefore key to achieving sustainable and resilient global beef systems.

1.5.2 Italian beef sector. At the end of 2023, Italy's cattle population stood at just over 5.4 million head, of which approximately 2.3 million were raised primarily for beef production (ISMEA, 2023; 2024). These animals were distributed across 73 500–84 682 specialized farms, reflecting strong regional specialization (Figure 1.5a). National beef production reached ~671 000 tons, making Italy the fourth-largest producer in Europe, after France, Germany, and Spain. The agricultural phase of the beef supply chain was valued at ~€3.8 billion (5.3% of agricultural gross saleable production), while the industrial sector generated a turnover exceeding €6.3 billion (3.2% of the national agri-food industry) (ISMEA, 2023; 2024; Ruminantia, 2023).

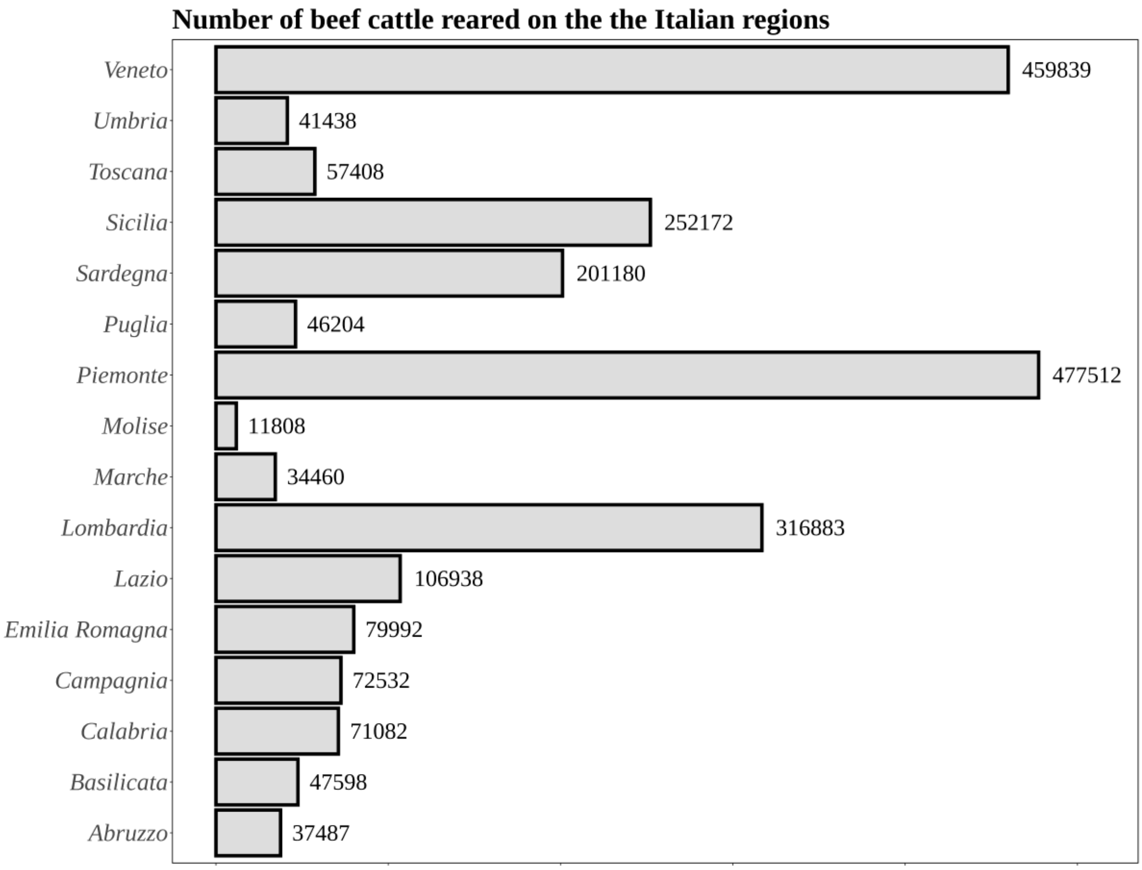
Between 2019 and 2023, both supply and demand experienced a structural contraction. Specialized beef farms declined by over 15,000 units, the total cattle population decreased slightly (from 5.56 to 5.4 million head), and national beef production dropped from 759 000 to 671 000 tons. Per capita beef consumption decreased from 17.2 to 16.1 kg, and the national self-sufficiency rate fell to a historical low of 40.3%, resulting in a trade deficit of €3.3 billion (ISMEA, 2024).

Imports continue to play a central role in meeting domestic demand. In 2023, imported beef consisted primarily of fresh meat (78%), followed by frozen meat (15%) and offal or processed products (7%). Live cattle imports were mainly for fattening (76%), with France accounting for 85% of live imports, reflecting the high degree of integration between the Italian and French beef sectors (Informatore Zootecnico, 2023).

Italy’s beef sector exhibits a diverse breed composition. Autochthonous breeds represent about one-quarter of the national herd, with Piemontese accounting for 14%. French breeds Limousine and Charolais contribute approximately 14% and 10%, respectively, while other breeds such as Chianina, Marchigiana, Sarda, Romagnola, and Maremmana each represent 1–3% of the national total (ISMEA, 2023; Figure 1.5b). Production systems vary regionally: intensive light and heavy veal systems dominate in northern and central Italy, whereas extensive heavy veal systems are prevalent in southern regions and the islands.

At the European level, total beef production slightly declined to 6.37 million tons in 2023, with Italy accounting for 11% of EU production. Prices in early 2024 decreased modestly by 1.2% compared with 2023 but remained 23% above the 2019–2021 average. Domestically, beef represents about 30% of fresh meat consumption by volume and 40% by value, with increasing consumption of young female beef observed over the past five years (FAO, 2025).

a)


Number of beef cattle reared on the the Italian regions
Veneto 459839
Umbria 41438
Toscana 57408
Sicilia 252172
Sardegna 201180
Puglia 46204
Piemonte 477512
Molise 11808
Marche 34460
Lombardia 316883
Lazio 106938
Emilia Romagna 79992
Campagnia 72532
Calabria 71082
Basilicata 47598
Abruzzo 37487

b)

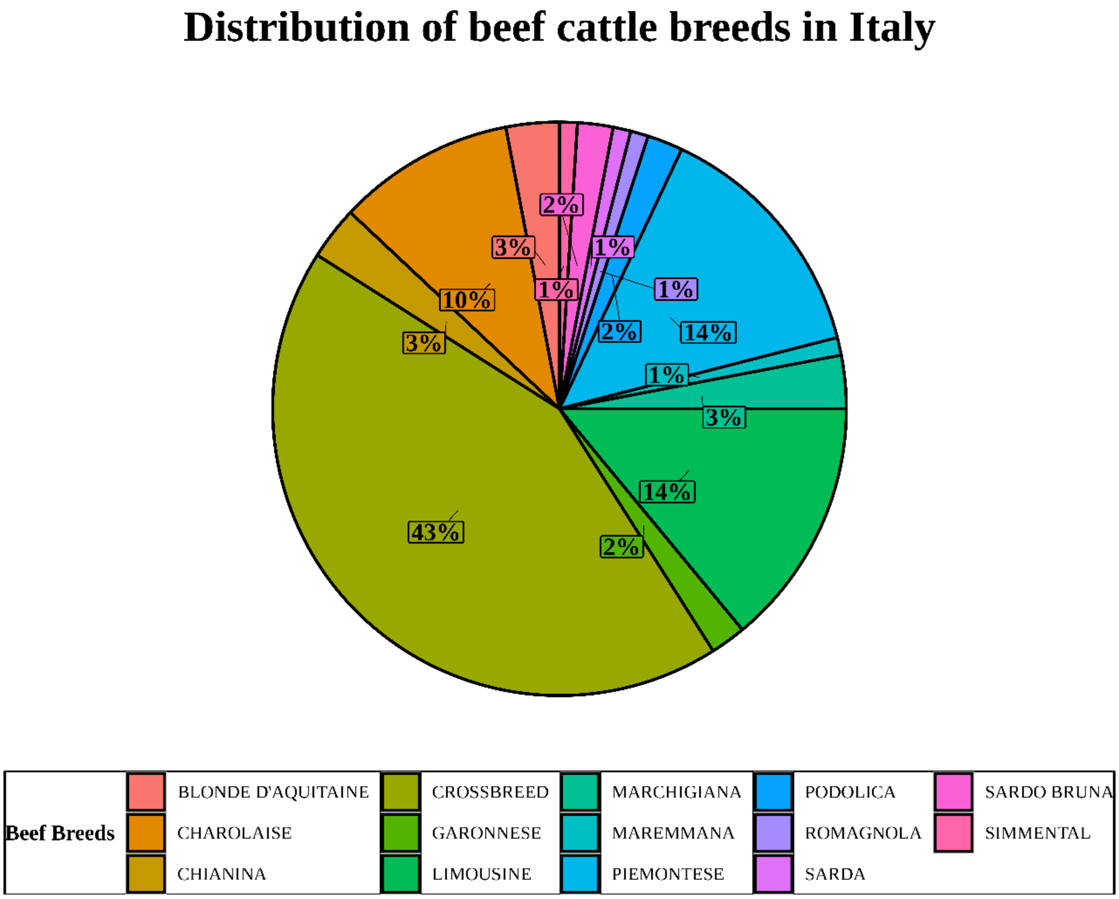

Distribution of beef cattle breeds in Italy
2%
3%
1%
1%
1%
10%
2%
14%
3%
1%
3%
14%
43%
2%
Beef Breeds
BLONDE D'AQUITAINE
CHAROLAISE
CHIANINA
CROSSBREED
GARONNESE
LIMOUSINE
MARCHIGIANA
MAREMMANA
PIEMONTESE
PODOLICA
ROMAGNOLA
SARDA
SARDO BRUNA
SIMMENTAL

Figure 1.5 (a-b) Distribution and breed composition of the Italian beef cattle sector. (a) Geographical distribution of specialized beef cattle farms in Italy, highlighting the concentration in northern regions (Veneto, Piemonte, Lombardia). (b) Breed composition of the national beef cattle herd, showing the proportion of autochthonous and French breeds (Limousine, Charolaise) as well as other minor breeds. Data from Tendenze dinamiche e recenti, Scheda di settore Bovino da carne, ISMEA, 2023. Carni - Carne bovina - Ismea Mercati (ISMEA, 2023).

Beyond market trends, genomic selection **(GS)** and systematic phenotyping offer substantial opportunities to modernize Italian breeding programs. Historical pedigree and phenotypic records—particularly for autochthonous and high-performance breeds provide a robust foundation for developing reference populations for genomic evaluations (Fabbri et al., 2024; Colombi et al., 2024b). National breed associations, consortia, and government programs such as the National Rural Development Plan **(PSRN)** are crucial in coordinating data sharing, standardizing phenotyping, and enabling large-scale genomic prediction.

Nevertheless, challenges persist, including the fragmented structure of the sector, small farm size, limited genotyping coverage, and high costs of advanced phenotyping (Biscarini et al., 2015; Billah et al., 2025). Furthermore, heterogeneous production environments and significant G × E interactions complicate breeding value estimation, underscoring the need for multi-environment models (Silva Neto et al., 2024; Callegaro et al., 2024).

Genetic improvement efforts for Limousine and Charolais cattle have been strengthened through collaborations with the Associazione Nazionale Allevatori Charolais e Limousine Italia **(ANACLI)** and initiatives under PSRN, which integrate genomic and phenotypic information to support breeding goals. Current strategic priorities include the development of genomic indexes for reproductive efficiency, heat stress tolerance, and reduced environmental impact, alongside monitoring genetic diversity and inbreeding control. Together, these actions aim to select cattle that are productive, efficient, and resilient, well adapted to Italy's diverse production conditions, thereby contributing to the sustainability and competitiveness of the national beef sector.

1.6 Genomic evaluations in beef cattle: opportunities and limitations

1.6.1 Benefits of genomic selection. GS is transforming beef cattle breeding by enabling earlier and more accurate prediction of genomic estimated breeding values **(GEBV)** for traits related to productivity, resilience, and sustainability. Dense single-nucleotide polymorphism (**SNP**) panels can increase prediction accuracy from approximately 0.5, based on pedigree alone, to over 0.8. Correspondingly, GEBVs can achieve reliabilities of 70–90%, compared with 45–70% for conventional pedigree-based indexes (Meuwissen et al., 2001; VanRaden, 2008).

GS has been implemented in some beef populations, such as Angus in the USA. Very large genomic reference populations are currently available, with more than 1.8 million genotyped animals integrated into the World Angus Evaluation (Lourenco et al., 2015; American Angus Association, 2024). Nonetheless, adoption remains slower and more heterogeneous than in the dairy sector. Prediction accuracies in beef typically range from 0.3 to 0.7, limited by smaller reference populations, low progeny testing, and reduced relatedness between reference and validation sets (Van Eenennaam et al., 2014). Multi-breed reference populations can partially mitigate these limitations, but gains are modest when target breeds are underrepresented (Akanno et al., 2014; Bolormaa et al., 2013; Meuwissen et al., 2016).

Genomic heterogeneity among breeds and crossbreds complicates GS. Differences between *Bos taurus* and *Bos indicus* influence allele substitution effects, and multi-breed evaluations often yield lower accuracy than single-breed analyses (McKay et al., 2008; Berry et al., 2016). Bayesian models and high-density SNP panels improve predictions by weighting markers according to estimated effects, enhancing the capture of quantitative trait loci **(QTLs)** in weakly related populations (Bolormaa et al., 2013; Khansefid et al., 2014).

Economic and logistical factors also influence GS adoption. Easily measurable traits, such as growth, are often selected traditionally, whereas feed efficiency and meat quality require costly phenotyping. Limited industry size restricts reference population scale and genotyping investment. Practical strategies to enhance GS efficiency include multi-breed reference populations, high-density or sequence-based analyses, selective genotyping, and single-step genomic best linear unbiased prediction (**ssGBLUP)** frameworks that integrate phenotypes, pedigrees, and genotypes of non-genotyped animals (Misztal et al., 2009; Legarra et al., 2014).

Beyond production traits, GS supports improvement in fertility, health, and resilience. For instance, GS can enhance resistance to infectious diseases, such as bovine respiratory disease and Johne’s disease, which are costly and challenging to phenotypes yet critical for welfare and profitability (Kasimanickam et al., 2025). Integration with reproductive technologies, such as embryo transfer, facilitates the broader dissemination of elite genetics (Van Eenennaam et al., 2014).

In Europe, the implementation of genomic evaluations is heterogeneous across countries and breeds and sometimes remain unofficial, though research and pilot programs are expanding. For example, Ireland planned official multi-breed genomic proofs in 2016 for over 100 000 animals, including sires and predominantly crossbred commercial cows (Irish Cattle Breeding Federation, 2016). In the UK, Limousine carcass trait evaluations are implemented using a one-step genomic approach by EGENEs (http://www.sruc.ac.uk/info/120275/egenes). France maintains unofficial genomic evaluations for Charolais, Limousine, and Blonde d’Aquitaine (Berry et al., 2016). In several countries (e.g. Germany, Nordic countries, Ireland, Czech Republic, Switzerland, and EBE partners), single-step genomic evaluations integrating SNP information are already operational or in pre-official use for beef cattle, whereas in other contexts genomics remains mainly confined to research projects or specific breeds (Bonifazi et al., 2022). These initiatives highlight the growing interest in GS despite the absence of formal national programs.

Future expansion of GS in beef cattle will depend on enlarging reference populations, leveraging high-density or sequence data, integrating advanced phenotyping, and providing economic incentives to overcome structural barriers. Continuous methodological improvements suggest that GS will increasingly drive genetic progress in beef cattle worldwide.

1.6.2 Advances in genomic research: progress in dairy versus limitations in beef. The adoption of GS has progressed faster in dairy cattle than in beef, primarily due to sector-specific constraints. Key challenges in beef include limited phenotyping of selection candidates, the prevalence of sex-limited traits, and widespread crossbreeding (Meuwissen et al., 2016). Many cow–calf systems rely on natural mating, with sires selected primarily based on phenotype or pedigree rather than reliable breeding values.

Limited animal identification and parentage recording in many countries restricts reference population development, reducing genomic prediction accuracy (Berry et al., 2016). Despite falling genotyping costs, structural and logistical barriers slow GS adoption in beef. Beef breeding programs are generally smaller, enroll fewer animals in structured evaluations, and are often linked to dairy systems through surplus calves or cull cows (Mohammaddiyeh et al., 2023). Consequently, beef associations and companies often have limited resources to support genotyping initiatives.

While genotyping elite sires is relatively cost-effective, genotyping of commercial and young animals typically falls on individual producers, limiting broader adoption (Mohammaddiyeh et al., 2023). Compared with dairy, beef farms adopt new technologies more conservatively due to narrower profit margins and lower perceived returns from genetic improvement. Key limiting factors for beef genomic evaluations include (Berry et al., 2016):

- multiple breeds and frequent crossbreeding,
- limited use of artificial insemination,
- relatively weak international genetic connectedness,
- low levels of systematic phenotyping,
- lower-margin business models.

Unlike the dairy sector, dominated by Holstein-Friesians, beef production involves a large diversity of breeds, including *Bos taurus*, *Bos indicus*, and composites such as Brangus and Bonsmara (McKay et al., 2008). Compared with dairy, beef systems are more heterogeneous, involving multiple breeds, frequent crossbreeding, and lower adoption of artificial insemination. These factors weaken genetic connectedness and reduce prediction accuracy (Berry et al., 2006; 2016). The accuracy of genomic predictions depends on relatedness between reference and candidate populations and is negatively affected if reference populations are unrepresentative (Daetwyler et al., 2008; Pszczola et al., 2012; Kachman et al., 2013; Saatchi et al., 2011). Artificial insemination in dairy cattle allows accumulation of accurate genetic information from influential sires, whereas natural mating predominates in beef (Berry and Kearney, 2011), reducing imputation accuracy and weakening connectedness. Large, effective reference populations are therefore more costly and difficult to establish (Daetwyler et al., 2008).

International initiatives such as INTERBULL (https://interbull.org) support multi-country dairy evaluations, whereas beef programs like INTERBEEF (https://www.icar.org/group/working-group-interbeef/) and BREEDPLAN (https://breedplan.une.edu.au/) involve fewer breeds and countries and exclude certain traits. Comprehensive phenotyping is also less available in beef, especially in commercial herds with multi-sire matings and incomplete sire identification (Berry et al., 2016; Daetwyler et al., 2008). Economic constraints further limit genotyping investment compared with dairy, where AI companies or research funding support reference populations (Weigel et al., 2012; Berry et al., 2016).

1.7 Genomic applications, analysis, and future perspectives in beef cattle selection

1.7.1 Impacts of genomic tools on improving growth, fertility, survival, and adaptation traits linked to sustainability. Sustainability-related traits in beef cattle, such as health, fertility, robustness, product quality, and resistance to genetic disorders, are difficult to improve due to polygenic architecture, low heritability, and measurement challenges (Harlizius et al., 2004). Traditional breeding has largely focused on productivity traits, leaving cow-centric traits like health, longevity, and resilience underrepresented. The advent of genomics has opened avenues to incorporate these low-heritability and complex traits into breeding goals (Catrett and Rowan, 2024).

GS enables accurate prediction of low-heritability traits, allowing early identification of animals with superior resilience (Johnsson, 2023; Mueller and Van Eenennaam, 2022). SNPs, abundant and cost-effective genotypes, are ideal for high-throughput evaluations. Next-generation sequencing and imputation provide dense genomic data at a population scale, increasing prediction accuracy (Hossein-Zadeh, 2024).

GWAS and multi-omics approaches (transcriptomics, metabolomics, epigenomics) help identify QTLs and candidate genes associated with feed efficiency, growth, fertility, and adaptation traits (Raza et al., 2022; Tan et al., 2023; Georges et al., 2019). These tools, combined with marker-assisted selection and QTL mapping, allow targeted incorporation of favorable alleles, though identification of causal genes remains challenging due to epistasis, pleiotropy, and variable penetrance (Carlborg et al., 2003).

Integration of genomics with reproductive and biotechnologies accelerates genetic gain while preserving diversity, enhancing climate adaptation, disease resistance, and efficiency. Microbiome data can further improve the prediction of resilience traits, promoting sustainable and climate-resilient beef systems. Collectively, these genomic tools, integrated with structured breeding objectives, support simultaneous improvements in productivity, welfare, and environmental sustainability.

1.7.2 Genomic selection strategies for improving cow fertility and longevity. Fertility and longevity are key determinants of profitability and sustainability in beef cattle systems. A cow's ability to remain productive across multiple parities substantially influences the economic efficiency of cow–calf operations (Boyer et al., 2020; Catrett and Rowan, 2024). Longevity reduces replacement costs, extends productive life, and maximizes returns on heifer development, while fertility ensures regular calving and sustained output.

Although fertility is influenced by management and environmental factors, measurable genetic variation allows improvement through selection (Fernandez-Novo et al., 2020; Diskin and Kenny, 2016). Stayability, a composite trait encompassing reproductive efficiency, structural soundness, udder quality, temperament, and overall productivity, serves as a key selection criterion. Selecting stayability enhances fertility and functional traits that support long-term survival (Catrett and Rowan, 2024).

Reproductive inefficiency remains a major source of economic loss; in the U.S., pregnancy failure in beef females incurs approximately USD 2.8 billion annually (Moorey and Biase, 2020; Mercadante et al., 2020). Selecting heifers with superior genetic merit for fertility traits is critical, as early calving heifers tend to remain longer in the herd and wean greater total calf weight over their lifetime (Akanno et al., 2015; Kertz et al., 2023). Their selection is therefore fundamental for the long-term profitability of cow–calf operations. Studies have shown that heifers calving earlier in the breeding season remain longer in the herd and wean more total calf weight over their lifetime, demonstrating the cumulative value of early reproductive success (Cushman et al., 2013).

Genetic improvement for fertility and longevity is challenging due to low heritability, late expression, and polygenic control with small additive and non-additive effects (Berry et al., 2014; Ma et al., 2019). However, sufficient additive genetic variance exists to allow improvement through breeding strategies (Berry et al., 2014; Ma et al., 2019). Genomics and multi-omics technologies, including high-density SNP genotyping, transcriptomics, proteomics, and metabolomics, have enhanced the identification of genetic determinants, biomarkers, and functional candidate genes, improving prediction accuracy for these complex traits (Holland and McGowan, 2018; McGettigan et al., 2016; Olasege et al., 2021). These allowed earlier and more reliable identification of superior animals without waiting for lifetime performance records due to various complex factors underlying fertility and related traits (Han and Peñagaricano, 2016; Wathes et al., 2014). These integrative approaches are helping to overcome the limitations of conventional selection and enable more effective improvement of reproductive efficiency in beef cattle.

1.7.3 GWAS and QTL approaches to uncover the genetic basis of sustainability-related traits in beef cattle. GWAS are fundamental for identifying QTL underlying economically important traits (Chen et al., 2022). While historically focused on dairy breeds due to large, genotyped populations, GWAS is now increasingly used in beef populations for low-heritability or difficult-to-measure traits like fertility, longevity, and functional conformation (Speidel et al., 2018; Pedrosa et al., 2023; Silva et al., 2024). Traits such as reproductive efficiency, longevity, and conformation directly affect replacement costs, culling rates, and lifetime productivity, making them central to herd sustainability (Silva et al., 2024; Callegaro et al., 2024). Incorporating these traits into GWAS reveals genetic mechanisms and supports multi-trait selection strategies that improve herd efficiency and economic performance.

GWAS detects pleiotropic regions and candidate genes, supporting multi-trait selection strategies. Functional genomics approaches, including SNP haplotype panels and expressed sequence tags, improve QTL characterization. Medium- or high-density SNP panels increase the likelihood of capturing causal variants via linkage disequilibrium (Goddard and Hayes, 2009). Detection methods include single-SNP linear mixed models and haplotype-based approaches (Zhou and Stephens, 2012; Dashab et al., 2012). Breed-specific variability exists, and joint analyses of multiple breeds enhance detection power while controlling for population structure (Toosi et al., 2018; Hassanine et al., 2025). Additional methods have also been proposed, including sparse or hybrid models such as the Bayesian Sparse Linear Mixed Model, which combine linear mixed models and sparse regression to capture both polygenic background and loci with large effects (Zhou et al., 2013), as well as post-GWAS and multi-omics approaches that integrate transcriptomic or other functional data to identify gene- or pathway-level associations (e.g. transcriptome-wide association study) (Ghoreishifar et al., 2024).

Molecular techniques have revealed numerous genes affecting quantitative traits and markers associated with QTL combined with traditional breeding, offering potential to accelerate genetic gains (Tambasco et al., 2003; Hassanine et al., 2025). Genome annotation further facilitates the interpretation of GWAS results and the identification of biologically relevant genes (Stein, 2001; Gao et al., 2024). Collectively, these genomic tools provide a solid basis for breeding strategies aimed at improving productivity, resilience, and sustainability.

1.7.4 Genomic applications for managing inbreeding in beef cattle: challenges and perspectives for sustainable production. The adoption of GS in beef breeding accelerates genetic progress but can also increase homozygosity, raise the risk of inbreeding depression, and threaten long-term population fitness. Evidence from dairy cattle demonstrates that the widespread adoption of GS has accelerated inbreeding rates in breeds such as Holstein, and similar risks exist in beef populations. For example, (Makanjuola et al., 2020) reported that genomic inbreeding increased more rapidly after the implementation of GS compared with the pre-genomic era.

Balancing rapid genetic gain with preservation of genetic diversity is critical. Genomic approaches such as genomic relationship matrix (**GRM**) and runs of homozygosity (**ROH**) analyses now enable precise quantification of genomic inbreeding and the detection of deleterious haplotypes, providing more accurate estimates than traditional pedigree-based coefficients. ROH directly measures realized autozygosity and offers valuable insight into both recent and ancient inbreeding events (Ceballos et al., 2018).

Strategies to mitigate inbreeding include optimal contribution selection, genomic mating plans, and multi-trait selection indices with inbreeding constraints (Clark et al., 2013). Integration with reproductive technologies (embryo transfer, in vitro fertilization, genomic mate allocation) can maintain selection intensity while reducing homozygosity. However, intensive use of such technologies without careful management may inadvertently accelerate diversity loss (Doublet et al., 2020).

Future programs will increasingly rely on genomic evaluations to monitor inbreeding and maintain adaptive potential. Emerging technologies, including genome editing and systems genomics, offer opportunities to reduce deleterious effects while preserving breed integrity (Mueller and Van Eenennaam, 2022). Future programs will increasingly rely on genomic evaluations to monitor inbreeding and maintain adaptive potential. Emerging technologies, including genome editing and systems genomics, offer opportunities to reduce deleterious effects while preserving breed integrity (Clark et al., 2013).

1.7.5 Genotype-by-environment interactions as drivers of efficiency and resilience in beef cattle. G × E occurs when an animal's genetic merit depends on its environment, or when environmental factors affect performance differently according to genotype (Tiezzi and Maltecca, 2022). Understanding G × E is crucial for developing climate-resilient beef systems, particularly under variable or extreme conditions.

Genomic studies show that G × E influences growth, fertility, feed efficiency, and health. Candidate genes related to thermotolerance (HSP70, HSF1, DNAJC8, STING1), coat and skin structure, immune regulation, and reproduction have been identified (Zeng et al., 2023; Colombi et al., 2024a). Meta-gene enrichment analyses highlight pathways such as transcriptional regulation, keratin filament formation, and calcium signaling as key mechanisms of adaptation (Fang et al., 2021; Zhang et al., 2023; Kim et al., 2020).

Locally adapted and indigenous breeds serve as reservoirs of adaptive alleles conferring heat tolerance, disease resistance, and efficient resource use (Kambal et al., 2023; Colombi et al., 2024a). Incorporating these breeds into structured genomic programs can strengthen environmental and social sustainability while safeguarding animal welfare and genetic diversity.

Distinguishing macro-environmental plasticity (response to predictable factors) from micro-environmental plasticity (response to random variation) enables selection for animals that are both adaptable and stable (Tiezzi and Maltecca, 2022). Multiple-trait models (**MTM**) and random regression models (**RRM**) estimate breeding values conditional on environmental covariates (Schaeffer, 2004; Tiezzi and Maltecca, 2022). Studies demonstrate that temperature humidity index (**THI**), altitude, and management systems modulate genetic potential for growth, reproduction, and health. For example, in American Red Angus, genes associated with feed efficiency and growth (PRKG1, DNAJC12, SIRT1) show environment-specific effects across ecoregion (Smith et al., 2022). Evidence from global studies also shows that breeds adapted to extreme environments often display superior feed efficiency with low-quality forages, parasite resistance, and greater responsiveness to environmental stimuli, traits that are increasingly valuable in the context of climate change. Methods such as reproducing kernel Hilbert space (**RKHS**) regression model complex environmental interactions by simultaneously incorporating multiple covariates with high predictive accuracy (Gianola and Van Kaam, 2008; Tiezzi and Maltecca, 2022). Such approaches are particularly valuable in beef systems where temperature, humidity, altitude, and nutritional availability interact to shape performance.

Successful integration of G × E into breeding programs requires robust experimental designs with balanced data and strong genetic connectedness across environments to ensure accurate estimation of variance components and breeding values (Tiezzi and Maltecca, 2022). The adoption of precision livestock technologies enhances environmental characterization, enabling more targeted selection for adaptation, resilience, and production efficiency.

1.8 Objectives and significance of the study

This thesis aims to unravel how genetics and genomics can be connected to breed beef cows that are not only productive but also resilient and efficient in the face of climate change and shifting production conditions. The main objective is to explore the genetic and genomic bases of resilience and efficiency in beef cattle, with a particular focus on the Italian Limousine and Charolais populations. Modern beef systems must deliver high-quality food while coping with heat stress, fluctuating feed resources, and growing societal demands for sustainability and animal welfare. In the context of climate change, increasing environmental variability, and evolving societal expectations, enhancing the ability of animals to maintain productive, reproductive, and health performance under diverse and challenging conditions has become a key priority in livestock breeding. Improving resilience and efficiency is essential not only to secure long-term productivity and profitability, but also to reduce the environmental footprint of beef production and to safeguard animal welfare and genetic diversity.

To address these global challenges, this research adopts a multifaceted approach that integrates quantitative genetics and genomic tools. Four complementary studies were developed to dissect different but interconnected themes of resilience and efficiency. The first study evaluates G × E for growth traits in Limousine cattle, providing insight into environmental sensitivity and adaptive capacity across climatic gradients. The second paper estimates genetic parameters for functional longevity in Italian Charolais and Limousine breeds, capturing the ability of cows to remain productive over multiple parities and production cycles. The third explores inbreeding depression in Limousine cattle, using both pedigree and genomic information to show how loss of genetic diversity affects growth, fertility, and survival, and thus herd and animal robustness. Finally, the fourth paper employs a single-step genome-wide association approach to identify genomic regions and candidate genes underlying female productivity, including longevity, fertility, and conformation traits.

Together, these studies form an integrated framework to quantify resilience-related traits and to evaluate their genetic architecture. By integrating their findings, the thesis provides practical knowledge and methodological strategies to support breeding programs that aim for animals capable of thriving in variable environments while reducing costs and environmental impacts that balance production efficiency with long-term sustainability. The outcomes contribute to national and international strategies aimed at improving the adaptability, profitability, and environmental sustainability of beef cattle herds. Beyond their scientific contribution, these results support breeding strategies that enhance the resilience and competitiveness of the Italian beef sector while contributing to global efforts toward climate-smart livestock production.

References

Akanno, E.C., Plastow, G., Fitzsimmons, C., Miller, S.P., Baron, V., Ominski, K., Basarab, J.A., 2015. Genome-wide association for heifer reproduction and calf performance traits in beef cattle. Genome 58, 549–557. doi:10.1139/gen-2015-0031

Akanno, E.C., Plastow, G., Woodward, B.W., Bauck, S., Okut, H., Wu, X.-L., Sun, C., Aalhus, J.L., Moore, S.S., Miller, S.P., Wang, Z., Basarab, J.A., 2014. Reliability of molecular breeding values for Warner-Bratzler shear force and carcass traits of beef cattle – An independent validation study1. Journal of Animal Science 92, 2896–2904. doi:10.2527/jas.2013-7374

Ambrosini, D.P., Malhado, C.H.M., Filho, R.M., Cardoso, F.F., Carneiro, P.L.S., 2016. Genotype × environment interactions in reproductive traits of Nellore cattle in northeastern Brazil. Tropical Animal Health and Production 48, 1401–1407. doi:10.1007/s11250-016-1105-7

Amundson, J.L., Mader, T.L., Rasby, R.J., Hu, Q.S., 2006. Environmental effects on pregnancy rate in beef cattle1. Journal of Animal Science 84, 3415–3420. doi:10.2527/jas.2005-611

Baes, C., Marras, G., Fleming, A., Malchiodi, F., & Miglior, F., 2018. Genetics, genomics and beyond: What to expect from new technologies in dairy cattle. WCDS Advances in Dairy. Technology, 30, 237–248.

Baumgard, L.H., Wheelock, J.B., O'Brien, M., Shwartz, G., Zimbelman, R.B., VanBaale, M.J., Collier, R.J., Rhoads, M.L., Rhoads, R.P., 2007. The Differential Effects of Heat Stress vs. Underfeeding on Production and Post-Absorptive Nutrient Partitioning. 22nd Annual Southwest Nutrition & Management Conference, February 22-23, 2007, Tempe, AZ.

Berghof, T.V.L., Poppe, M., Mulder, H.A., 2019. Opportunities to Improve Resilience in Animal Breeding Programs. Frontiers in Genetics 9, 692. doi:10.3389/fgene.2018.00692

Berry, D.P., Garcia, J.F., Garrick, D.J., 2016. Development and implementation of genomic predictions in beef cattle. Animal Frontiers 6, 32–38. doi:10.2527/af.2016-0005

Berry, D.P., Kearney, J.F., 2011. Imputation of genotypes from low- to high-density genotyping platforms and implications for genomic selection. Animal 5, 1162–1169. doi:10.1017/S1751731111000309

Berry, D.P., Madalena, F.E., Cromie, A.R., Amer, P.R., 2006. Cumulative discounted expressions of dairy and beef traits in cattle production systems. Livestock Science 99, 159–174. doi:10.1016/j.livprodsci.2005.06.006

Berry, D.P., Wall, E., Pryce, J.E., 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8, 105–121. doi:10.1017/S1751731114000743

Billah, M., Bermann, M., Hollifield, M.K., Tsuruta, S., Chen, C.Y., Psota, E., Holl, J., Misztal, I., Lourenco, D., 2025. Review: Genomic selection in the era of phenotyping based on digital images. animal 19, 101486. doi:10.1016/j.animal.2025.101486

Bilotto, F., Harrison, M.T., Vibart, R., Mackay, A., Christie-Whitehead, K.M., Ferreira, C.S.S., Cottrell, R.S., Forster, D., Chang, J., 2024. Towards resilient, inclusive, sustainable livestock farming systems. Trends in Food Science & Technology 152, 104668. doi:10.1016/j.tifs.2024.104668

Biscarini, F., Nicolazzi, E.L., Stella, A., Boettcher, P.J., Gandini, G., 2015. Challenges and opportunities in genetic improvement of local livestock breeds. Frontiers in Genetics 6. doi:10.3389/fgene.2015.00033

Bolormaa, S., Pryce, J.E., Kemper, K., Savin, K., Hayes, B.J., Barendse, W., Zhang, Y., Reich, C.M., Mason, B.A., Bunch, R.J., Harrison, B.E., Reverter, A., Herd, R.M., Tier, B., Graser, H.-U., Goddard, M.E., 2013. Accuracy of prediction of genomic breeding values for residual feed intake and carcass and meat quality traits in Bos taurus, Bos indicus, and composite beef cattle1. Journal of Animal Science 91, 3088–3104. doi:10.2527/jas.2012-5827

Bonifazi, R., Calus, M.P.L., Ten Napel, J., Veerkamp, R.F., Michenet, A., Savoia, S., Cromie, A., Vandenplas, J., 2022. International single-step SNPBLUP beef cattle evaluations for Limousin weaning weight. Genetic Selection Evolution 4, 54:57. doi: 10.1186/s12711-022-00748-0.

Bonilla-Cedrez, C., Steward, P., Rosenstock, T.S., Thornton, P., Arango, J., Kropff, M., Ramirez-Villegas, J., 2023. Priority areas for investment in more sustainable and climate-resilient livestock systems. Nature Sustainability 6, 1279–1286. doi:10.1038/s41893-023-01161-1

Boyer, C.N., Griffith, A.P., DeLong, K.L., 2020. Reproductive Failure and Long-Term Profitability of Spring- and Fall-Calving Beef Cows. doi:10.22004/AG.ECON.298435

Broom, L.J., Kogut, M.H., 2018. Inflammation: friend or foe for animal production? Poultry Science 97, 510–514. doi:10.3382/ps/pex314

Callegaro, S., Tiezzi, F., Fabbri, M.C., Biffani, S., Bozzi, R., 2024. Evaluating genotype by environment interaction for growth traits in Limousine cattle. animal 18, 101344. doi:10.1016/j.animal.2024.101344

Callegaro, Simone, Tiezzi, F., Maltecca, C., Fabbri, M.C., Bozzi, R., 2024. Genetic parameters of functional longevity and associated traits in Italian Charolais and Limousine breeds. Journal of Animal Science 102, skae354. doi:10.1093/jas/skae354

Carlborg, Ö., Kerje, S., Schütz, K., Jacobsson, L., Jensen, P., Andersson, L., 2003. A Global Search Reveals Epistatic Interaction Between QTL for Early Growth in the Chicken. Genome Research 13, 413–421. doi:10.1101/gr.528003

Catrett, C.C., Rowan, T.N., 2024. GENETIC SELECTION FOR COW FERTILITY AND LONGEVITY.

Ceballos, F.C., Joshi, P.K., Clark, D.W., Ramsay, M., Wilson, J.F., 2018. Runs of homozygosity: windows into population history and trait architecture. Nature Reviews Genetics 19, 220–234. doi:10.1038/nrg.2017.109

Chebel, R.C., Santos, J.E.P., Reynolds, J.P., Cerri, R.L.A., Juchem, S.O., Overton, M., 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Animal Reproduction Science 84, 239–255. doi:10.1016/j.anireprosci.2003.12.012

Chen, S.-Y., Schenkel, F.S., Melo, A.L.P., Oliveira, H.R., Pedrosa, V.B., Araujo, A.C., Melka, M.G., Brito, L.F., 2022. Identifying pleiotropic variants and candidate genes for fertility and reproduction traits in Holstein cattle via association studies based on imputed whole-genome sequence genotypes. BMC Genomics 23, 331. doi:10.1186/s12864-022-08555-z

Clark, S.A., Kinghorn, B.P., Hickey, J.M., Van Der Werf, J.H., 2013. The effect of genomic information on optimal contribution selection in livestock breeding programs. Genetics Selection Evolution 45, 44. doi:10.1186/1297-9686-45-44

Colditz, I.G., Hine, B.C., 2016. Resilience in farm animals: biology, management, breeding and implications for animal welfare. Animal Production Science 56, 1961. doi:10.1071/AN15297

Colombi, D., Perini, F., Bettini, S., Mastrangelo, S., Abeni, F., Conte, G., Marletta, D., Cassandro, M., Bernabucci, U., Ciampolini, R., Lasagna, E., 2024a. Genomic responses to climatic challenges in beef cattle: A review. Animal Genetics 55, 854–870. doi:10.1111/age.13474

Colombi, D., Rovelli, G., Luigi-Sierra, M.G., Ceccobelli, S., Guan, D., Perini, F., Sbarra, F., Quaglia, A., Sarti, F.M., Pasquini, M., Amills, M., Lasagna, E., 2024b. Population structure and identification of genomic regions associated with productive traits in five Italian beef cattle breeds. Scientific Reports 14, 8529. doi:10.1038/s41598-024-59269-z

Cotticelli, A., Bifulco, G., Pividori, I., Matera, R., Verde, M.T., Santinello, M., Prandi, A., Peric, T., 2024. Assessing cortisol concentration in different matrices: predictive potential and relationship with production levels, lactation stage and parity in dairy buffaloes. Italian Journal of Animal Science 23, 802–812. doi:10.1080/1828051X.2024.2354502

Cushman, R.A., Kill, L.K., Funston, R.N., Mousel, E.M., Perry, G.A., 2013. Heifer calving date positively influences calf weaning weights through six parturitions1. Journal of Animal Science 91, 4486–4491. doi:10.2527/jas.2013-6465

Daetwyler, H.D., Villanueva, B., Woolliams, J.A., 2008. Accuracy of Predicting the Genetic Risk of Disease Using a Genome-Wide Approach. PLoS ONE 3, e3395. doi:10.1371/journal.pone.0003395

Dashab, G.R., Kadri, N.K., Shariati, M.M., Sahana, G., 2012. Comparison of linear mixed model analysis and genealogy-based haplotype clustering with a Bayesian approach for association mapping in a pedigreed population. BMC Proceedings 6, S4. doi:10.1186/1753-6561-6-S2-S4

Dellar, M., Topp, C.F.E., Banos, G., Wall, E., 2018. A meta-analysis on the effects of climate change on the yield and quality of European pastures. Agriculture, Ecosystems & Environment 265, 413–420. doi:10.1016/j.agee.2018.06.029

Diskin, M.G., Kenny, D.A., 2016. Managing the reproductive performance of beef cows. Theriogenology 86, 379–387. doi:10.1016/j.theriogenology.2016.04.052

Doeschl-Wilson, A., Knap, P.W., Opriessnig, T., More, S.J., 2021. Review: Livestock disease resilience: from individual to herd level. Animal 15, 100286. doi:10.1016/j.animal.2021.100286

Doublet, A.-C., Restoux, G., Fritz, S., Balberini, L., Fayolle, G., Hozé, C., Laloë, D., Croiseau, P., 2020. Intensified Use of Reproductive Technologies and Reduced Dimensions of Breeding Schemes Put Genetic Diversity at Risk in Dairy Cattle Breeds. Animals 10, 1903. doi:10.3390/ani10101903

Dumont, B., Andueza, D., Niderkorn, V., Lüscher, A., Porqueddu, C., Picon-Cochard, C., 2015. A meta-analysis of climate change effects on forage quality in grasslands: specificities of mountain and Mediterranean areas. Grass and Forage Science 70, 239–254. doi:10.1111/gfs.12169

Esrafili Taze Kand Mohammaddiyeh, M., Rafat, S.A., Shodja, J., Javanmard, A., Esfandyari, H., 2023. Selective genotyping to implement genomic selection in beef cattle breeding. Frontiers in Genetics 14, 1083106. doi:10.3389/fgene.2023.1083106

European Commission. 2023. Horizon Europe Work Programme 2023–2024, Cluster 6: Food, Bioeconomy, Natural Resources, Agriculture and Environment. Brussels.

Fabbri, M.C., Tiezzi, F., Crovetti, A., Maltecca, C., Bozzi, R., 2024. Investigation of cosmopolitan and local Italian beef cattle breeds uncover common patterns of heterozygosity. animal 18, 101142. doi:10.1016/j.animal.2024.101142

Faisal, M., Abbas, A., Xia, C., Haseeb Raza, M., Akhtar, S., Arslan Ajmal, M., Mushtaq, Z., Cai, Y., 2021. Assessing small livestock herders’ adaptation to climate variability and its impact on livestock losses and poverty. Climate Risk Management 34, 100358. doi:10.1016/j.crm.2021.100358

Fang, H., Kang, L., Abbas, Z., Hu, L., Chen, Y., Tan, X., Wang, Y., Xu, Q., 2021. Identification of key Genes and Pathways Associated With Thermal Stress in Peripheral Blood Mononuclear Cells of Holstein Dairy Cattle. Frontiers in Genetics 12, 662080. doi:10.3389/fgene.2021.662080

FAO. 2010. Challenges and opportunities for carbon sequestration in grassland systems. A technical report on grassland management and climate change mitigation. Food and Agricultural Organization, Rome.

FAO. 2020. The State of Food and Agriculture 2020. Overcoming water challenges in agriculture. Rome.

FAO 2018. Transforming the livestock sector through the Sustainable Development Goals. European Commission. 2019. The European Green Deal. Brussels.

Felton, A.J., Shriver, R.K., Stemkovski, M., Bradford, J.B., Suding, K.N., Adler, P.B., 2022. Climate disequilibrium dominates uncertainty in long-term projections of primary productivity. Ecology Letters 25, 2688–2698. doi:10.1111/ele.14132

Fernandez-Novo, A., Pérez-Garnelo, S.S., Villagrá, A., Pérez-Villalobos, N., Astiz, S., 2020. The Effect of Stress on Reproduction and Reproductive Technologies in Beef Cattle—A Review. Animals 10, 2096. doi:10.3390/ani10112096

Freier, K., Finckh, M., Schneider, U., 2014. Adaptation to New Climate by an Old Strategy? Modeling Sedentary and Mobile Pastoralism in Semi-Arid Morocco. Land 3, 917–940. doi:10.3390/land3030917

Gao, Z., Lu, Y., Chong, Y., Li, M., Hong, J., Wu, J., Wu, D., Xi, D., Deng, W., 2024. Beef Cattle Genome Project: Advances in Genome Sequencing, Assembly, and Functional Genes Discovery. International Journal of Molecular Sciences 25, 7147. doi:10.3390/ijms25137147

Georges, M., Charlier, C., Hayes, B., 2019. Harnessing genomic information for livestock improvement. Nature Reviews Genetics 20, 135–156. doi:10.1038/s41576-018-0082-2

Gerber, P.J., Hristov, A.N., Henderson, B., Makkar, H., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan, A.T., Yang, W.Z., Tricarico, J.M., Kebreab, E., Waghorn, G., Dijkstra, J., Oosting, S., 2013. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. Animal 7, 220–234. doi:10.1017/S1751731113000876

Ghavi Hossein-Zadeh, N., 2024. An overview of recent technological developments in bovine genomics. Veterinary and Animal Science 25, 100382. doi:10.1016/j.vas.2024.100382

Ghoreishifar, M., Macleod, I.M., Chamberlain, A.J., Liu, Z., Lopdell, T.J., Littlejohn, M.D., Xiang, R., Pryce, J.E., Goddard, M.E., 2025 An integrative approach to prioritize candidate causal genes for complex traits in cattle. PLoS Genetics 30, 21:1011492. doi: 10.1371/journal.pgen.1011492.

Gianola, D., Van Kaam, J.B.C.H.M., 2008. Reproducing Kernel Hilbert Spaces Regression Methods for Genomic Assisted Prediction of Quantitative Traits. Genetics 178, 2289–2303. doi:10.1534/genetics.107.084285

Gibon, A., Sibbald, A.R., Thomas, C. 1999. Improved sustainability in livestock systems, a challenge for animal production science. Livestock Production Science, 61, 107-110. doi:10.1016/S0301-6226(99)00060-3

Goddard, M.E., Hayes, B.J., 2009. Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nature Reviews Genetics 10, 381–391. doi:10.1038/nrg2575

Han, Y., Peñagaricano, F., 2016. Unravelling the genomic architecture of bull fertility in Holstein cattle. BMC Genetics 17, 143. doi:10.1186/s12863-016-0454-6

Hansen, P.J., 2009. Effects of heat stress on mammalian reproduction. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 3341–3350. doi:10.1098/rstb.2009.0131

Hansen, P.J., 2007. Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. Theriogenology 68, S242–S249. doi:10.1016/j.theriogenology.2007.04.008

Harlizius, B., Wijk, R.V., Merks, J.W.M., 2004. Genomics for food safety and sustainable animal production. Journal of Biotechnology 113, 33–42. doi:10.1016/j.jbiotec.2004.03.021

Hassanine, N.N.A.M., Saleh, A.A., Essa, M.O.A., Adam, S.Y., Mohai Ud Din, R., Rehman, S.U., Ali, R., Husien, H.M., Wang, M., 2025. Candidate Genes, Markers, Signatures of Selection, and Quantitative Trait Loci (QTLs) and Their Association with Economic Traits in Livestock: Genomic Insights and Selection. International Journal of Molecular Sciences 26, 7688. doi:10.3390/ijms26167688

Henry, B.K., Eckard, R.J., Beauchemin, K.A., 2018. Review: Adaptation of ruminant livestock production systems to climate changes. Animal 12, s445–s456. doi:10.1017/S1751731118001301

Herrero, M., Havlík, P., Valin, H., Notenbaert, A., Rufino, M.C., Thornton, P.K., Blümmel, M., Weiss, F., Grace, D., Obersteiner, M., 2013. Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. Proceedings of the National Academy of Sciences 110, 20888–20893. doi:10.1073/pnas.1308149110

Holland, M.K., McGowan, M., 2018. Manipulation of fertility to enhance productivity of cattle. The Biochemist 40, 20–25. doi:10.1042/BIO04003020

Johnsson, M., 2023. Genomics in animal breeding from the perspectives of matrices and molecules. Hereditas 160, 20. doi:10.1186/s41065-023-00285-w

Kachman, S.D., Spangler, M.L., Bennett, G.L., Hanford, K.J., Kuehn, L.A., Snelling, W.M., Thallman, R.M., Saatchi, M., Garrick, D.J., Schnabel, R.D., Taylor, J.F., Pollak, E.J., 2013. Comparison of molecular breeding values based on within- and across-breed training in beef cattle. Genetics Selection Evolution 45, 30. doi:10.1186/1297-9686-45-30

Kadim, I.T., Mahgoub, O., Al-Ajmi, D.S., Al-Maqbaly, R.S., Al-Mugheiry, S.M., Bartolome, D.Y., 2004. The influence of season on quality characteristics of hot-boned beef m. longissimus thoracis. Meat Science 66, 831–836. doi:10.1016/j.meatsci.2003.08.001

Kadzere, C.T., Murphy, M.R., Silanikove, N., Maltz, E., 2002. Heat stress in lactating dairy cows: a review. Livestock Production Science 77, 59–91. doi:10.1016/S0301-6226(01)00330-X

Kambal, S., Tijjani, A., Ibrahim, S.A.E., Ahmed, M.A., Mwacharo, J.M., Hanotte, O., 2023. Candidate signatures of positive selection for environmental adaptation in indigenous African cattle: A review. Animal Genetics 54, 689–708. doi:10.1111/age.13353

Kasimanickam, R., Ferreira, J.C.P., Kastelic, J., Kasimanickam, V., 2025. Application of Genomic Selection in Beef Cattle Disease Prevention. Animals 15, 277. doi:10.3390/ani15020277

Kertz, N.C., Banerjee, P., Dyce, P.W., Diniz, W.J.S., 2023. Harnessing Genomics and Transcriptomics Approaches to Improve Female Fertility in Beef Cattle—A Review. Animals 13, 3284. doi:10.3390/ani13203284

Khansefid, M., Pryce, J.E., Bolormaa, S., Miller, S.P., Wang, Z., Li, C., Goddard, M.E., 2014. Estimation of genomic breeding values for residual feed intake in a multibreed cattle population1. Journal of Animal Science 92, 3270–3283. doi:10.2527/jas.2014-7375

Kim, E., Joo, S., Kim, D., Gu, B.-H., Park, D., Rahman, M., Son, J., Park, B., Kim, S., Hur, T.-Y., Kim, M., 2020. Common and Differential Dynamics of the Function of Peripheral Blood Mononuclear Cells between Holstein and Jersey Cows in Heat-Stress Environment. Animals 11, 19. doi:10.3390/ani11010019

Knap, P.W., 2020. The scientific development that we need in the animal breeding industry. Journal of Animal Breeding and Genetics 137, 343–344. doi:10.1111/jbg.12485

Knap, P.W., 2005. Breeding robust pigs. Australian Journal of Experimental Agriculture 45, 763. doi:10.1071/EA05041

König, S., May, K., 2019. Invited review: Phenotyping strategies and quantitative-genetic background of resistance, tolerance and resilience associated traits in dairy cattle. Animal 13, 897–908. doi:10.1017/S1751731118003208

Lacetera, N., Bernabucci, U., Scalia, D., Ronchi, B., Kuzminsky, G., Nardone, A., 2005. Lymphocyte functions in dairy cows in hot environment. International Journal of Biometeorology 50, 105–110. doi:10.1007/s00484-005-0273-3

Laghouaouta, H., Fraile, L.J., Pena, R.N., 2024. Selection for Resilience in Livestock Production Systems. International Journal of Molecular Sciences 25, 13109. doi:10.3390/ijms252313109

Legarra, A., Christensen, O.F., Aguilar, I., Misztal, I., 2014. Single Step, a general approach for genomic selection. Livestock Science 166, 54–65. doi:10.1016/j.livsci.2014.04.029

Llonch, P., Hoffmann, G., Bodas, R., Mirbach, D., Verwer, C., Haskell, M.J., 2020. Opinion paper: Measuring livestock robustness and resilience: are we on the right track? Animal 14, 667–669. doi:10.1017/S1751731119003306

Lourenco, D.A.L., Tsuruta, S., Fragomeni, B.O., Masuda, Y., Aguilar, I., Legarra, A., Bertrand, J.K., Amen, T.S., Wang, L., Moser, D.W., Misztal, I., 2015. Genetic evaluation using single-step genomic best linear unbiased predictor in American Angus1. Journal of Animal Science 93, 2653–2662. doi:10.2527/jas.2014-8836

Ma, L., Cole, J.B., Da, Y., VanRaden, P.M., 2019. Symposium review: Genetics, genome-wide association study, and genetic improvement of dairy fertility traits. Journal of Dairy Science 102, 3735–3743. doi:10.3168/jds.2018-15269

Makanjuola, B.O., Miglior, F., Abdalla, E.A., Maltecca, C., Schenkel, F.S., Baes, C.F., 2020. Effect of genomic selection on rate of inbreeding and coancestry and effective population size of Holstein and Jersey cattle populations. Journal of Dairy Science 103, 5183–5199. doi:10.3168/jds.2019-18013

Makkar, H.P.S., 2016. Animal nutrition in a 360-degree view and a framework for future R&D work: towards sustainable livestock production. Animal Production Science 56, 1561. doi:10.1071/AN15265

McGettigan, P.A., Browne, J.A., Carrington, S.D., Crowe, M.A., Fair, T., Forde, N., Loftus, B.J., Lohan, A., Lonergan, P., Pluta, K., Mamo, S., Murphy, A., Roche, J., Walsh, S.W., Creevey, C.J., Earley, B., Keady, S., Kenny, D.A., Matthews, D., McCabe, M., Morris, D., O'Loughlin, A., Waters, S., Diskin, M.G., Evans, A.C.O., 2016. Fertility and genomics: comparison of gene expression in contrasting reproductive tissues of female cattle. Reproduction, Fertility and Development 28, 11. doi:10.1071/RD15354

McKay, S.D., Schnabel, R.D., Murdoch, B.M., Matukumalli, L.K., Aerts, J., Coppieters, W., Crews, D., Neto, E.D., Gill, C.A., Gao, C., Mannen, H., Wang, Z., Van Tassell, C.P., Williams, J.L., Taylor, J.F., Moore, S.S., 2008. An assessment of population structure in eight breeds of cattle using a whole genome SNP panel. BMC Genetics 9, 37. doi:10.1186/1471-2156-9-37

Mercadante, V.R.G., Dias, N.W., Timlin, C.L., Pancini, S., 2020. 375 Economic consequences of pregnancy loss in beef cattle. Journal of Animal Science 98, 124. doi: 10.1093/jas/skaa278.226

Meuwissen, T., Hayes, B., Goddard, M., 2016. Genomic selection: A paradigm shift in animal breeding. Animal Frontiers 6, 6–14. doi:10.2527/af.2016-0002

Meuwissen, T.H.E., Hayes, B.J., Goddard, M.E., 2001. Prediction of Total Genetic Value Using Genome-Wide Dense Marker Maps. Genetics 157, 1819–1829. doi:10.1093/genetics/157.4.1819

Miglior, F., Fleming, A., Malchiodi, F., Brito, L.F., Martin, P., Baes, C.F., 2017. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. Journal of Dairy Science 100, 10251–10271. doi:10.3168/jds.2017-12968

Misztal, I., Legarra, A., Aguilar, I., 2009. Computing procedures for genetic evaluation including phenotypic, full pedigree, and genomic information. Journal of Dairy Science 92, 4648–4655. doi:10.3168/jds.2009-2064

Moorey, S.E., Biase, F.H., 2020. Beef heifer fertility: importance of management practices and technological advancements. Journal of Animal Science and Biotechnology 11, 97. doi:10.1186/s40104-020-00503-9

Mueller, M.L., Van Eenennaam, A.L., 2022. Synergistic power of genomic selection, assisted reproductive technologies, and gene editing to drive genetic improvement of cattle. CABI Agriculture and Bioscience 3, 13. doi:10.1186/s43170-022-00080-z

Mulder, H.A., Rashidi, H., 2017. Selection on resilience improves disease resistance and tolerance to infections. Journal of Animal Science 95, 3346. doi:10.2527/jas2017.1479

Mulder, H.A., Rönnegård, L., Fikse, W.F., Veerkamp, R.F., Strandberg, E., 2013. Estimation of genetic variance for macro- and micro-environmental sensitivity using double hierarchical generalized linear models. Genetics Selection Evolution 45, 23. doi:10.1186/1297-9686-45-23

Nardone, A., Ronchi, B., Lacetera, N., Bernabucci, U., 2006. Climatic Effects on Productive Traits in Livestock. Veterinary Research Communications 30, 75–81. doi:10.1007/s11259-006-0016-x

Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M.S., Bernabucci, U., 2010. Effects of climate changes on animal production and sustainability of livestock systems. Livestock Science 130, 57–69. doi:10.1016/j.livsci.2010.02.011

Neeteson-van Nieuwenhoven, A.-M., Knap, P., Avendaño, S., 2013. The role of sustainable commercial pig and poultry breeding for food security. Animal Frontiers 3, 52–57. doi:10.2527/af.2013-0008

Nichi, M., Bols, P.E.J., Züge, R.M., Barnabe, V.H., Goovaerts, I.G.F., Barnabe, R.C., Cortada, C.N.M., 2006. Seasonal variation in semen quality in Bos indicus and Bos taurus bulls raised under tropical conditions. Theriogenology 66, 822–828. doi:10.1016/j.theriogenology.2006.01.056

Olasege, B.S., Tahir, M.S., Gouveia, G.C., Kour, J., Porto-Neto, L.R., Hayes, B.J., Fortes, M.R.S., 2021. Genetic parameter estimates for male and female fertility traits using genomic data to improve fertility in Australian beef cattle. Animal Production Science 61, 1863–1872. doi:10.1071/AN21097

O’Mara, F.P., 2012. The role of grasslands in food security and climate change. Annals of Botany 110, 1263–1270. doi:10.1093/aob/mcs209

Pedrosa, V.B., Boerman, J.P., Gloria, L.S., Chen, S.-Y., Montes, M.E., Doucette, J.S., Brito, L.F., 2023. Genomic-based genetic parameters for milkability traits derived from automatic milking systems in North American Holstein cattle. Journal of Dairy Science 106, 2613–2629. doi:10.3168/jds.2022-22515

Phelan, D.C., Harrison, M.T., Kemmerer, E.P., Parsons, D., 2015. Management opportunities for boosting productivity of cool-temperate dairy farms under climate change. Agricultural Systems 138, 46–54. doi:10.1016/j.agsy.2015.05.005

Phocas, F., Belloc, C., Bidanel, J., Delaby, L., Dourmad, J.Y., Dumont, B., Ezanno, P., Fortun-Lamothe, L., Foucras, G., Frappat, B., González-García, E., Hazard, D., Larzul, C., Lubac, S., Mignon-Grasteau, S., Moreno, C.R., Tixier-Boichard, M., Brochard, M., 2016. Review: Towards the agroecological management of ruminants, pigs and poultry through the development of sustainable breeding programmes: I-selection goals and criteria. Animal 10, 1749–1759. doi:10.1017/S1751731116000926

Pszczola, M., Strabel, T., Mulder, H.A., Calus, M.P.L., 2012. Reliability of direct genomic values for animals with different relationships within and to the reference population. Journal of Dairy Science 95, 389–400. doi:10.3168/jds.2011-4338

Purfield, D.C., Bradley, D.G., Evans, R.D., Kearney, F.J., Berry, D.P., 2015. Genome-wide association study for calving performance using high-density genotypes in dairy and beef cattle. Genetics Selection Evolution 47, 47. doi:10.1186/s12711-015-0126-4

Raza, S.H.A., Hassanin, A.A., Pant, S.D., Bing, S., Sitohy, M.Z., Abdelnour, S.A., Alotaibi, M.A., Al-Hazani, T.M., Abd El-Aziz, A.H., Cheng, G., Zan, L., 2022. Potentials, prospects and applications of genome editing technologies in livestock production. Saudi Journal of Biological Sciences 29, 1928–1935. doi:10.1016/j.sjbs.2021.11.037

Rensis, F.D., Scaramuzzi, R.J., 2003. Heat stress and seasonal effects on reproduction in the dairy cow—a review. Theriogenology 60, 1139–1151. doi:10.1016/S0093-691X(03)00126-2

Rojas-Downing, M.M., Nejadhashemi, A.P., Harrigan, T., Woznicki, S.A., 2017. Climate change and livestock: Impacts, adaptation, and mitigation. Climate Risk Management 16, 145–163. doi:10.1016/j.crm.2017.02.001

Rowan, T.N., Durbin, H.J., Seabury, C.M., Schnabel, R.D., Decker, J.E., 2020. Powerful detection of polygenic selection and evidence of environmental adaptation in US beef cattle. doi:10.1101/2020.03.11.988121

Rowan, T.N., Schnabel, R.D., Decker, J.E., 2024. Uncovering the architecture of selection in two Bos taurus cattle breeds. Evolutionary Applications 17, e13666. doi:10.1111/eva.13666

Russo, S.J., Murrough, J.W., Han, M.-H., Charney, D.S., Nestler, E.J., 2012. Neurobiology of resilience. Nature Neuroscience 15, 1475–1484. doi:10.1038/nn.3234

Saatchi, M., McClure, M.C., McKay, S.D., Rolf, M.M., Kim, J., Decker, J.E., Taxis, T.M., Chapple, R.H., Ramey, H.R., Northcutt, S.L., Bauck, S., Woodward, B., Dekkers, J.C., Fernando, R.L., Schnabel, R.D., Garrick, D.J., Taylor, J.F., 2011. Accuracies of genomic breeding values in American Angus beef cattle using K-means clustering for cross-validation. Genetics Selection Evolution 43, 40. doi:10.1186/1297-9686-43-40

Schaeffer, L.R., 2004. Application of random regression models in animal breeding. Livestock Production Science 86, 35–45. doi:10.1016/S0301-6226(03)00151-9

Schaeffer, L.R., 1994. Multiple-Country Comparison of Dairy Sires. Journal of Dairy Science 77, 2671–2678. doi:10.3168/jds.S0022-0302(94)77209-X

Scheffer, M., Bolhuis, J.E., Borsboom, D., Buchman, T.G., Gijzel, S.M.W., Goulson, D., Kammenga, J.E., Kemp, B., Van De Leemput, I.A., Levin, S., Martin, C.M., Melis, R.J.F., Van Nes, E.H., Romero, L.M., Olde Rikkert, M.G.M., 2018. Quantifying resilience of humans and other animals. Proceedings of the National Academy of Sciences 115, 11883–11890. doi:10.1073/pnas.1810630115

Silva, D.O., Fernandes Júnior, G.A., Fonseca, L.F.S., Mota, L.F.M., Bresolin, T., Carvalheiro, R., De Albuquerque, L.G., 2024a. Genome-wide association study for stayability at different calvings in Nellore beef cattle. BMC Genomics 25, 93. doi:10.1186/s12864-024-10020-y

Silva, D.O., Fernandes Júnior, G.A., Fonseca, L.F.S., Mota, L.F.M., Bresolin, T., Carvalheiro, R., De Albuquerque, L.G., 2024b. Genome-wide association study for stayability at different calvings in Nellore beef cattle. BMC Genomics 25, 93. doi:10.1186/s12864-024-10020-y

Silva Neto, J.B., Mota, L.F.M., Londoño-Gil, M., Schmidt, P.I., Rodrigues, G.R.D., Ligori, V.A., Arikawa, L.M., Magnabosco, C.U., Brito, L.F., Baldi, F., 2024. Genotype-by-environment interactions in beef and dairy cattle populations: A review of methodologies and perspectives on research and applications. Animal Genetics 55, 871–892. doi:10.1111/age.13483

Sirimarco, X., Villarino, S., Barral, M.P., Puricelli, M., Laterra, P., 2023. Transformation of tall-tussock grasslands and soil water dynamics in the Flooding Pampa. Science of The Total Environment 896, 165362. doi:10.1016/j.scitotenv.2023.165362

skaa278.226, n.d.

Smith, J.L., Wilson, M.L., Nilson, S.M., Rowan, T.N., Schnabel, R.D., Decker, J.E., Seabury, C.M., 2022. Genome-wide association and genotype by environment interactions for growth traits in U.S. Red Angus cattle. BMC Genomics 23, 517. doi:10.1186/s12864-022-08667-6

Speidel, S.E., Buckley, B.A., Boldt, R.J., Enns, R.M., Lee, J., Spangler, M.L., Thomas, M.G., 2018. Genome-wide association study of Stayability and Heifer Pregnancy in Red Angus cattle. Journal of Animal Science 96, 846–853. doi:10.1093/jas/sky041

Stein, L., 2001. Genome annotation: from sequence to biology. Nature Reviews Genetics 2, 493–503. doi:10.1038/35080529

St-Pierre, N.R., Cobanov, B., Schnitkey, G., 2003. Economic Losses from Heat Stress by US Livestock Industries. Journal of Dairy Science 86, E52–E77. doi:10.3168/jds.S0022-0302(03)74040-5

Taghipoor, M., Pastell, M., Martin, O., Nguyen Ba, H., Van Milgen, J., Doeschl-Wilson, A., Loncke, C., Friggens, N.C., Puillet, L., Muñoz-Tamayo, R., 2023. Animal board invited review: Quantification of resilience in farm animals. animal 17, 100925. doi:10.1016/j.animal.2023.100925

Tambasco, D.D., Paz, C.C.P., Tambasco-Studart, M., Pereira, A.P., Alencar, M.M., Freitas, A.R., Coutinho, L.L., Packer, I.U., Regitano, L.C.A., 2003. Candidate genes for growth traits in beef cattle crosses Bos taurus × Bos indicus. Journal of Animal Breeding and Genetics 120, 51–56. doi:10.1046/j.1439-0388.2003.00371.x

Tan, X., He, Z., Fahey, A.G., Zhao, G., Liu, R., Wen, J., 2023. Research progress and applications of genome-wide association study in farm animals. Animal Research and One Health 1, 56–77. doi:10.1002/aro2.14

Tiezzi, F., Maltecca, C., 2022. Genotype by Environment Interactions in Livestock Farming. In Encyclopedia of Sustainability Science and Technology (ed. Meyers, R.A.). Springer New York, New York, NY, pp. 1–21. doi:10.1007/978-1-4939-2493-6_1115-1

Toosi, A., Fernando, R.L., Dekkers, J.C.M., 2018. Genome-wide mapping of quantitative trait loci in admixed populations using mixed linear model and Bayesian multiple regression analysis. Genetics Selection Evolution 50, 32. doi:10.1186/s12711-018-0402-1

Van Eenennaam, A.L., Weigel, K.A., Young, A.E., Cleveland, M.A., Dekkers, J.C.M., 2014. Applied Animal Genomics: Results from the Field. Annual Review of Animal Biosciences 2, 105–139. doi:10.1146/annurev-animal-022513-114119

Van Meerbeek, K., Jucker, T., Svenning, J., 2021. Unifying the concepts of stability and resilience in ecology. Journal of Ecology 109, 3114–3132. doi:10.1111/1365-2745.13651

VanRaden, P.M., 2008. Efficient Methods to Compute Genomic Predictions. Journal of Dairy Science 91, 4414–4423. doi:10.3168/jds.2007-0980

Vella, S.-L., Pai, N., 2019. A theoretical review of psychological resilience: Defining resilience and resilience research over the decades. Archives of Medicine and Health Sciences 7, 233. doi:10.4103/amhs.amhs_119_19

Vitali, A., Segnalini, M., Bertocchi, L., Bernabucci, U., Nardone, A., Lacetera, N., 2009. Seasonal pattern of mortality and relationships between mortality and temperature-humidity index in dairy cows. Journal of Dairy Science 92, 3781–3790. doi:10.3168/jds.2009-2127

Wathes, D.C., Pollott, G.E., Johnson, K.F., Richardson, H., Cooke, J.S., 2014. Heifer fertility and carry over consequences for life time production in dairy and beef cattle. Animal 8, 91–104. doi:10.1017/S1751731114000755

Weigel, K.A., Hoffman, P.C., Herring, W., Lawlor, T.J., 2012. Potential gains in lifetime net merit from genomic testing of cows, heifers, and calves on commercial dairy farms. Journal of Dairy Science 95, 2215–2225. doi:10.3168/jds.2011-4877

Wilkinson, J.M., 2011. Re-defining efficiency of feed use by livestock. Animal 5, 1014–1022. doi:10.1017/S175173111100005X

Wolfenson, D., Roth, Z., Meidan, R., 2000. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Animal Reproduction Science 60–61, 535–547. doi:10.1016/S0378-4320(00)00102-0

Wu, G., Feder, A., Cohen, H., Kim, J.J., Calderon, S., Charney, D.S., Mathé, A.A., 2013. Understanding resilience. Frontiers in Behavioral Neuroscience 7. doi:10.3389/fnbeh.2013.00010

Zhang, M., Wu, D., Ahmed, Z., Liu, X., Chen, J., Ma, J., Wang, M., Liu, J., Zhang, J., Huang, B., Lei, C., 2023. The genetic secrets of adaptation: decoding the significance of the 30-bp insertion in the KRT77 gene for Chinese cattle. Animal Biotechnology, 34, 3847–3854. Doi:10.1080/10495398.2023.2232663

Zeng, L., Qu, K., Zhang, J., Huang, B., Lei, C., 2023. Genes related to heat tolerance in cattle—a review. Animal Biotechnology 34, 1840–1848. doi:10.1080/10495398.2022.2047995

Zhou, X., Stephens, M., 2012. Genome-wide efficient mixed-model analysis for association studies. Nature Genetics 44, 821–824. doi:10.1038/ng.2310

Zhou, X., Carbonetto, P., Stephens, M., 2013. Polygenic modeling with bayesian sparse linear mixed models. PLoS Genetics 9, 1003264. doi: 10.1371/journal.pgen.1003264.

Sitography

American Angus Association, 2024. Angus Sire Evaluation Report updates implemented in May. https://www.angus.org/media-center/press-releases/2024/05/angus-sire-evaluation-report-updates-implemented-in-may 05222024#:~:text=%E2%80%9CThis%20is%20not%20a%20new,over%201.8%20million%20genotyped%20animals.%E2%80%9D

Bernardelli M., Carni bovine - mercato segnato dall'incertezza, Informatore Zootecnico, 2023. Carni bovine, mercato segnato dall'incertezza - IZ Informatore Zootecnico (edagricole.it).

https://informatorezootecnico.edagricole.it/bovini-da-carne/carni-bovine-mercato-segnato-incertezza/

FAOSTAT. (2023). Statistical database. Food and Agriculture Organization of the United Nations, Rome. Retrieved fromhttps://www.fao.org/faostat/en/#home (Accessed 20 December 2023).

FAO 2011. Livestock in food security. https://www.fao.org/4/i2373e/i2373e00.htm

Hermesch S, Dominik S., 2014. Breeding Focus 2014 improving resilience. Animal Genetics and Breeding Unit, University of New England: Armidale.https://agbu.une.edu.au/PDFs/BFW2014/BF%202014%20chapter%201%20Hermesch.pdf

ISMEA, 2023. Carni - Carne bovina - Ismea Mercati. https://www.ismeamercati.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/12727

ISMEA, 2024. Ismea mercati - Analisi e studio filiere agroalimentari. https://www.ismeamercati.it/analisi-e-studio-filiere-agroalimentari

Ruminantia, 2023. https://archivio2023-2024.ruminantia.it/la-carne-bovina-prodotta-in-italia-non-basta-per-tutti/?utm_

UNECE. 2004. UNECE Standard for Bovine Meat – Carcases and Cuts. Geneva. https://unece.org/sites/default/files/2024-03/Bovine_326E_2004.pdf

USDA-FAS, 2015. https://www.fas.usda.gov/2015-food-progress-priority-countries-and-issues

CHAPTER 2

2. Material

2.1 Breeds description

The Charolais **(CHA)** and Limousine **(LIM)** are the two predominant beef cattle breeds of French origin, both widely used worldwide in purebred and crossbreeding systems due to their high meat yield, growth efficiency, and adaptability (Bouquet et al., 2011). Introduced into Italy during the mid-20th century, they now play a fundamental role in national beef production, serving as both sires and dams for purebred and commercial herds. Although they share a common French origin, the two breeds have diverged through distinct selection goals and limited gene flow (Bouquet et al., 2011). Over time, processes of genetic drift and breed-specific selection have generated genetic differentiation between populations, counterbalanced to some extent by gene exchange (Wang and Caballero, 1999). The diffusion of breeding animals from France fostered the development of genetically distinct but interconnected metapopulations, influenced by local breeding strategies and moderate gene flow (Bouquet et al., 2011; Wang and Caballero, 1999; Blackburn and Gollin, 2009).

The LIM breed, belonging to the ancient red *Brachyceros* cattle of Europe, originated in the Limousin and Marche regions of France. Initially a triple-purpose breed (milk, meat, and draft), it became progressively specialized for beef production from the late 19th century onward (Giorgetti et al., 1992). Similarly, the CHA breed arose in Burgundy, particularly in the Charolles and Nièvre regions, where it was historically used for both draft and meat production. The mechanization of agriculture favored its transformation into a specialized beef breed. Selective breeding in both populations produced robust, genetically consolidated cattle that have spread globally through the export of breeding animals and semen.

LIM cattle are medium-sized, with a wheat-colored coat lighter on the underbelly, head, and inner thighs. They exhibit a fine skeletal structure, well-distributed musculature, and strong limbs. Adult cows weigh 650–950 kg, while bulls range between 1 000–1 400 kg. The breed is characterized by ease of calving, good fertility, and longevity, producing carcasses with a dressing yield of 62–65% and lean meat with low cholesterol content. LIM beef is renowned for its tenderness, fine texture, and low cooking losses, attributes associated with favorable muscle fiber and collagen structures (Giorgetti et al., 1992). Pasture-based feeding enhances the nutritional profile, increasing unsaturated fatty acids, especially omega-3, and improving the omega-6/omega-3 ratio, resulting in meat of high sensory and dietary value (Giorgetti et al., 1992; Ruminantia 2023-2024).

CHA cattle are larger, with a white to cream coat, fine depigmented skin, and harmonious body proportions. Cows weigh between 700–1 200 kg and bulls between 1 000–1 700 kg. The breed shows remarkable growth potential, with average daily gains of up to 1.25 kg and slaughter yields exceeding 62% (Giorgetti et al., 1992). Comparative studies among CHA, LIM, and Chianina have demonstrated superior daily gains and feed efficiency for CHA, as well as a slightly higher hindquarter yield. CHA meat is lean yet well-marbled, tender, and flavorful, with a lighter color that enhances consumer preference. Its docility, grazing efficiency, and adaptability to both extensive and semi-extensive systems make it particularly suitable for crossbreeding with dairy or rustic breeds under diverse environmental conditions (Ruminantia 2023-2024).

Both breeds are now central to Italian beef production systems. The LIM is particularly suited to semi-extensive and organic systems due to its adaptability, maternal ability, and resilience under variable environmental conditions. CHA is more commonly used in intensive systems and crossbreeding programs, often as a terminal sire to enhance growth rate and carcass conformation, especially when crossed with local breeds. Overall, both CHA and LIM represent key genetic resources for high-quality meat production and the continued improvement of the Italian beef sector.

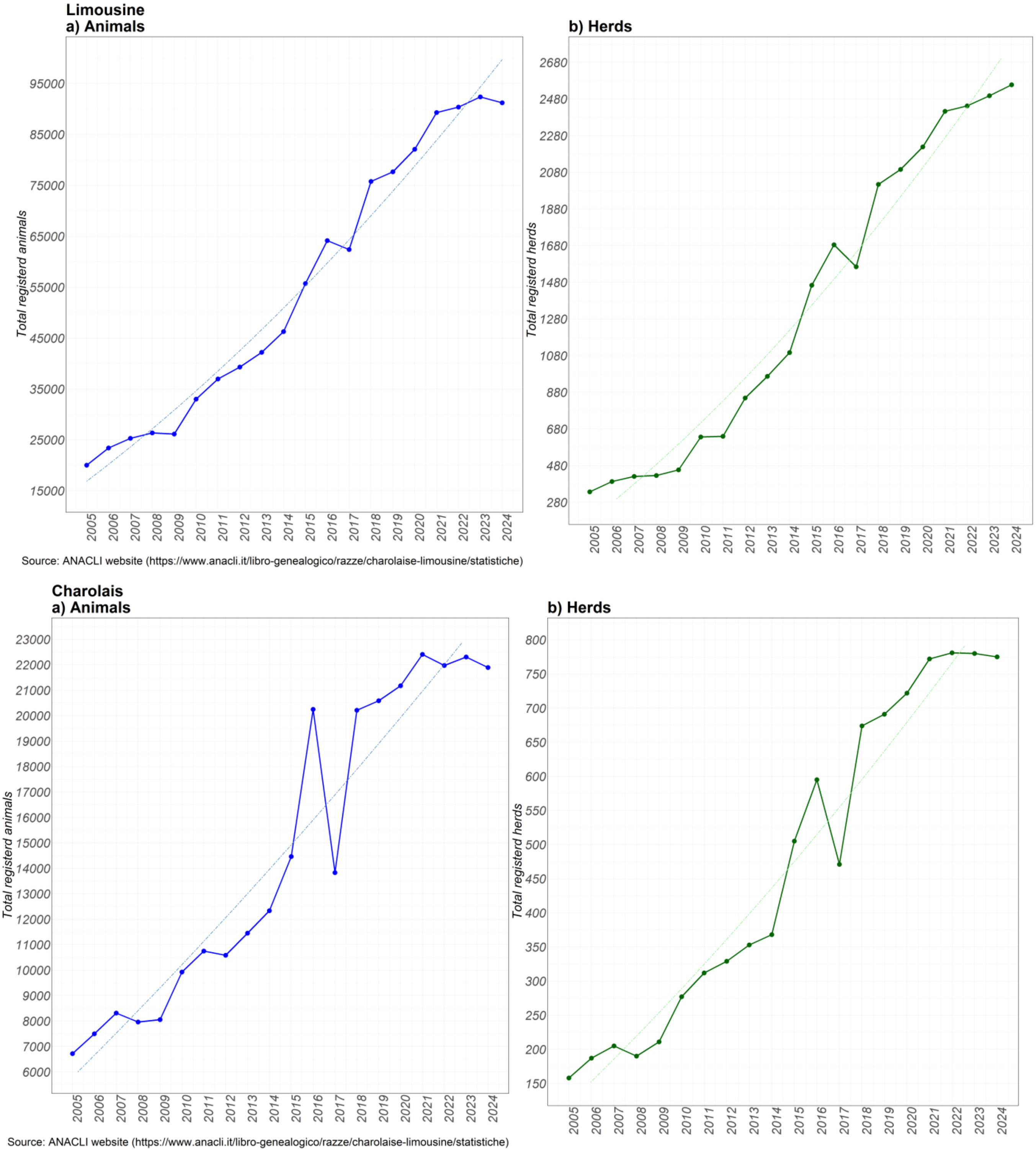
Figure 2.1 Italian Limousine cattle.

Figure 2.2 Italian Charolais cattle.

2.2 Population structure, herd distribution, and animals’ consistency

The Italian National Association of Breeders of Charolais and Limousine Cattle **(ANACLI)** manages the Herdbook for both breeds, established in 1999 by the Italian Ministry of Agricultural, Food, and Forestry Policies. Both populations have shown sustained growth over the past two decades, reflecting the increasing interest in specialized beef production across Italy.

Currently, the Italian LIM population comprises approximately 92 000 registered animals distributed across more than 2 600 farms, while the CHA population includes around 22 000 animals in approximately 775 herds (ANACLI, 2024). Both breeds have steadily expanded in recent years and now rank among the most economically important beef breeds in Italy. According to ANACLI records, the number of LIM herds increased from 337 in 2005 to 2 558 in 2024, representing more than a seven-fold expansion over 19 years (Figure 2.3). This increase has been especially pronounced since 2010, with an average annual rise exceeding 100 herds. The CHA population followed a similar but less marked trajectory, growing from 158 herds in 2005 to 775 in 2024, a nearly five-fold increase (Figure 2.3). Notably, the LIM herd growth rate was about 12% higher than that observed for CHA, resulting in LIM herds being more than three times as numerous as CHA herds by 2024.



Source: ANACLI website (https://www.anacli.it/libro-genealogico/razze/charolaise-limousine/statistiche)

Source: ANACLI website (https://www.anacli.it/libro-genealogico/razze/charolaise-limousine/statistiche)

Figure 2.3 Annual trend for Limousine and Charolais breeds, respectively, for total animals (a) and herds (b).

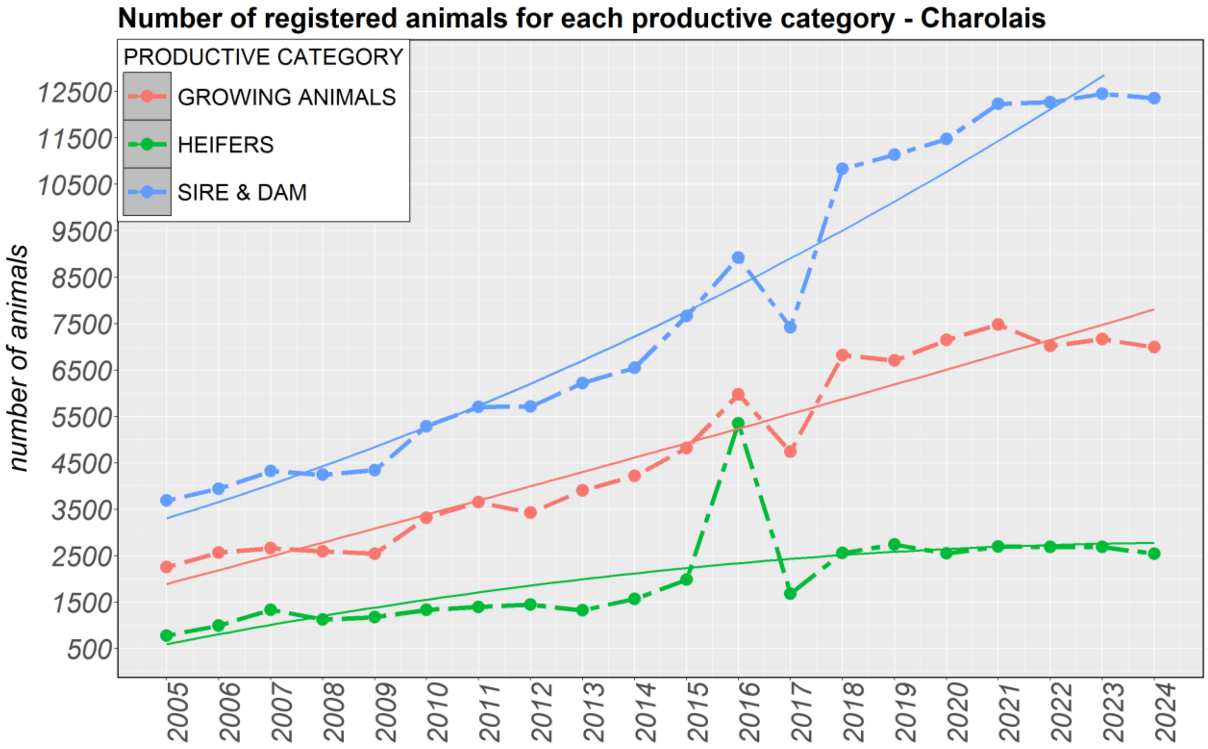
Within beef farms, registered animals are generally classified into three main categories:

1. Breeding animals (sires and dams) actively used for reproduction;
2. Growing animals, in the growth or finishing phase;
3. Heifers, young females not yet in reproductive service.

The analysis of registered animals by productive category revealed a steady upward trend in all groups for both breeds. The Sire & Dam category showed the most pronounced and consistent increase, reflecting the continuous expansion of the breeding nucleus. The Growing animals' category also exhibited a positive trend, indicating reinforcement of the replacement population. In contrast, the number of Heifers registered fluctuated more, particularly in CHA, where a temporary peak was observed around 2016. Overall, the upward trends across all categories underscore the demographic expansion and consolidation of both breeding populations (Figures 2.4a–b; ANACLI, 2024).

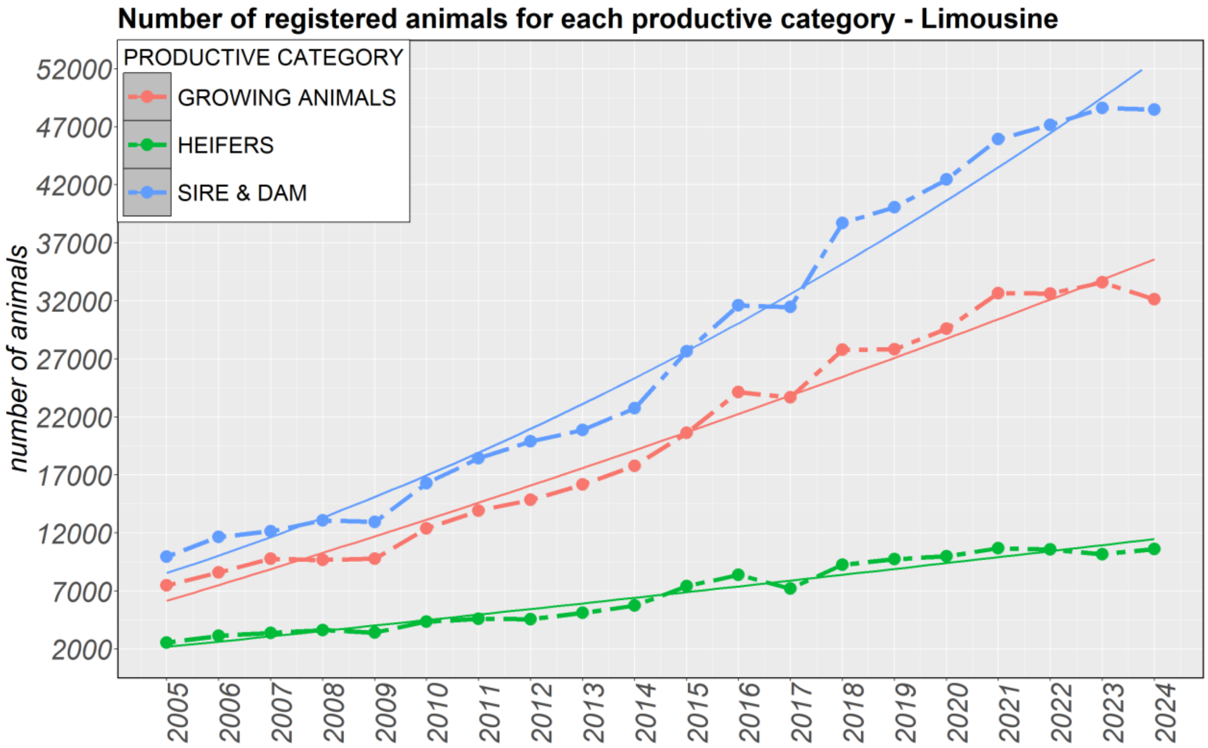
The composition of both populations by productive category also reflects the structure of breeding systems and production strategies. In CHA, the distribution across categories has evolved steadily since 2005 (Figure 2.4a), whereas the LIM population displays a similar pattern but with larger absolute numbers, mirroring its broader diffusion (Figure 2.4b).

a)



Source: ANACLI website (https://www.anacli.it/libro-genealogico/razze/charolaise-limousine/statistiche)

b)



Source: ANACLI website (https://www.anacli.it/libro-genealogico/razze/charolaise-limousine/statistiche)

Figure 2.4 (a-b) Number of registered animals for Charolais (a) and Limousine (n) for each productive category**.** The productive categories are breeding animals (sires and dams actively used for reproduction), growing animals (animals in the growth and finishing phase), and heifers (young females not yet entered reproductive service)**.**

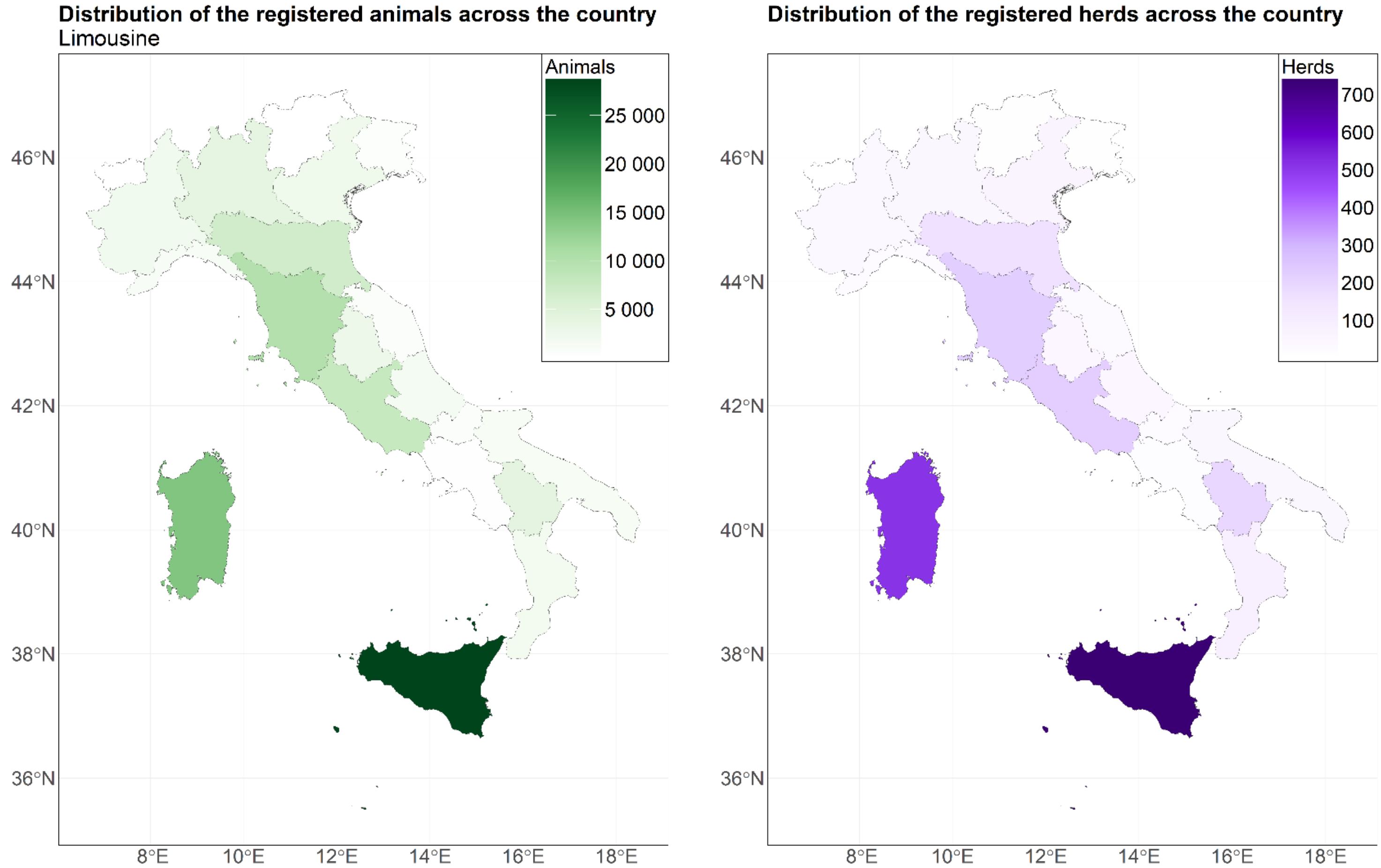
Geographically, the two breeds show distinct yet complementary distribution patterns across Italy, reflecting breed-specific adaptations and production system requirements (Figures 2.5a–b). LIM herds are widespread throughout the country, with the highest concentrations in Sicily, Sardinia, Tuscany, Emilia-Romagna, Lazio, and Basilicata (Figure 2.5a). CHA herds are mainly located in Sicily, Lazio, and Sardinia, where their density is greatest (Figure 2.5b). The prevalence of both breeds in southern regions and major islands reflects their suitability for extensive beef production systems traditionally predominant in these areas.

These spatial patterns also have genetic and production implications. Regional concentration helps genetic connectedness through shared artificial insemination sires and breeding stock exchanges, but may also lead to genotype-by-environment interactions if animals are predominantly evaluated under region-specific conditions, a topic further explored in Chapter 3. LIM broader geographic distribution provides opportunities for assessing adaptability across production systems but poses challenges for maintaining genetic connectedness between distant herds.

Italian beef systems cover a wide range of production intensities. Intensive systems, common in northern lowlands, involve housed animals and high feeding inputs. Semi-intensive systems, typical of central and southern regions, combine seasonal grazing with winter housing and moderate supplementation, balancing productivity and resource efficiency. Extensive systems, dominant in southern mountains and the inner areas of Sardinia, rely on rangeland grazing with minimal supplementation, prioritizing cost efficiency over maximum productivity.

The demographic growth of both breeds over the last two decades reflects not only the expansion of existing operations but also the entry of new producers, supported by European agricultural policies favoring extensive livestock systems, the rising demand for high-quality beef, and the recognition of cattle's role in maintaining marginal and pasture-based ecosystems.

a)



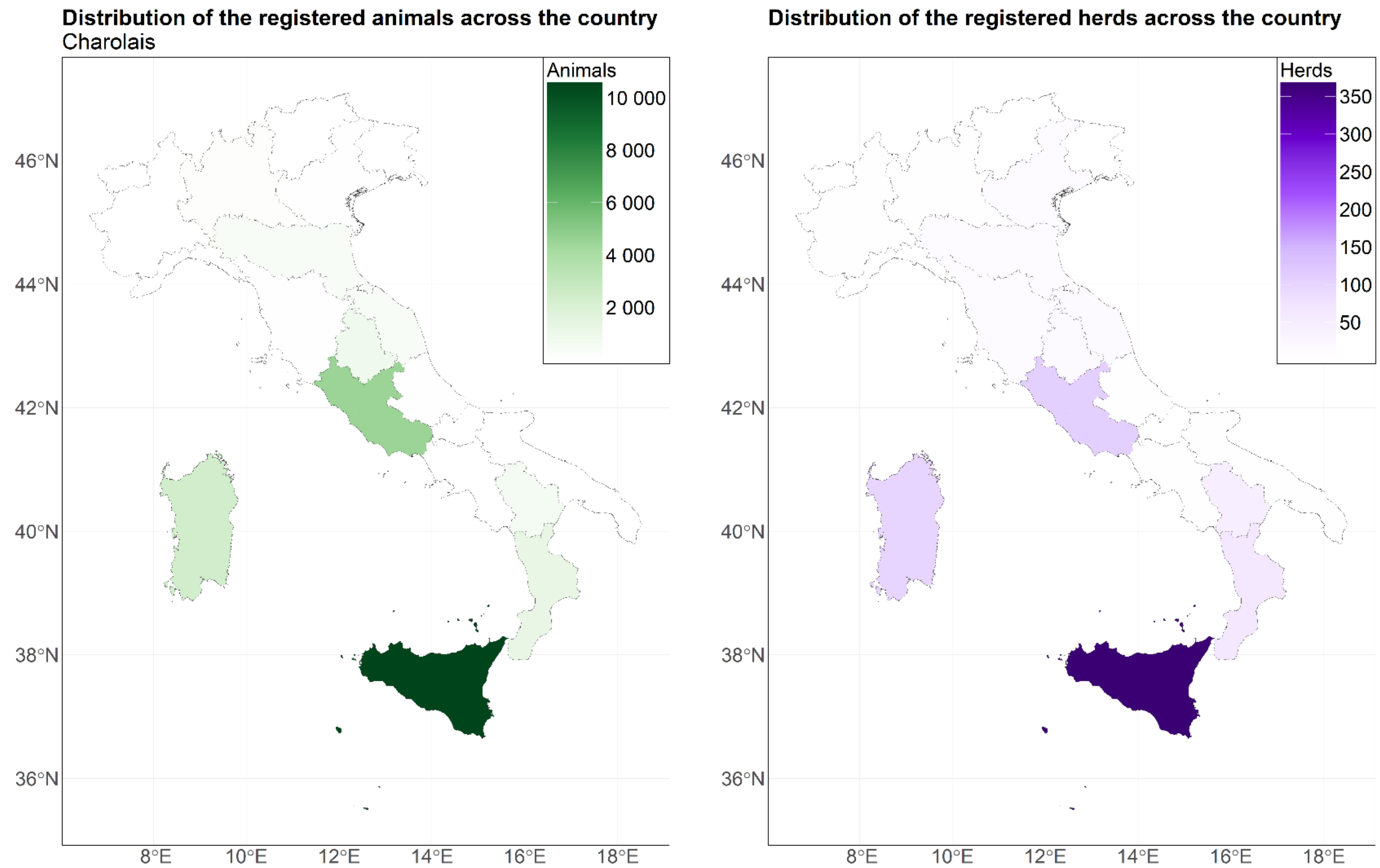
Distribution of the registered animals across the country
Limousine

Source: ANACLI website (https://www.anacli.it/libro-genealogico/razze/charolaise-limousine/statistiche)

Distribution of the registered herds across the country

Source: ANACLI website (https://www.anacli.it/libro-genealogico/razze/charolaise-limousine/statistiche)

b)


Distribution of the registered animals across the country
Charolais

Distribution of the registered herds across the country

Source: ANACLI website (https://www.anacli.it/libro-genealogico/razze/charolaise-limousine/statistiche)

Source: ANACLI website (https://www.anacli.it/libro-genealogico/razze/charolaise-limousine/statistiche)

Figure 2.5 (a-b) Geographical distribution in the Italian territory of registered animals and herds for Limousine (a) and Charolais (b), respectively.

References

Blackburn, H., Gollin, D., 2009. Animal genetic resource trade flows: The utilization of newly imported breeds and the gene flow of imported animals in the United States of America. Livestock Science 120, 240–247. doi:10.1016/j.livsci.2008.07.006

Bouquet, A., Venot, E., Laloë, D., Forabosco, F., Fogh, A., Pabiou, T., Moore, K., Eriksson, J.-Å., Renand, G., Phocas, F., 2011. Genetic structure of the European Charolais and Limousin cattle metapopulations using pedigree analyses. Journal of Animal Science 89, 1719–1730. doi:10.2527/jas.2010-3469

Giorgetti, A., Lucifero, M., Acciaioli, A., Sargentini, C., Martini, A., 1992. Production characteristics of Chianini, Charolais and Limousine calves. Zootec. Nutr. Anim 18, 84–85.

Wang, J., Caballero, A., 1999. Developments in predicting the effective size of subdivided populations. Heredity 82, 212–226. doi:10.1038/sj.hdy.6884670

Sitography

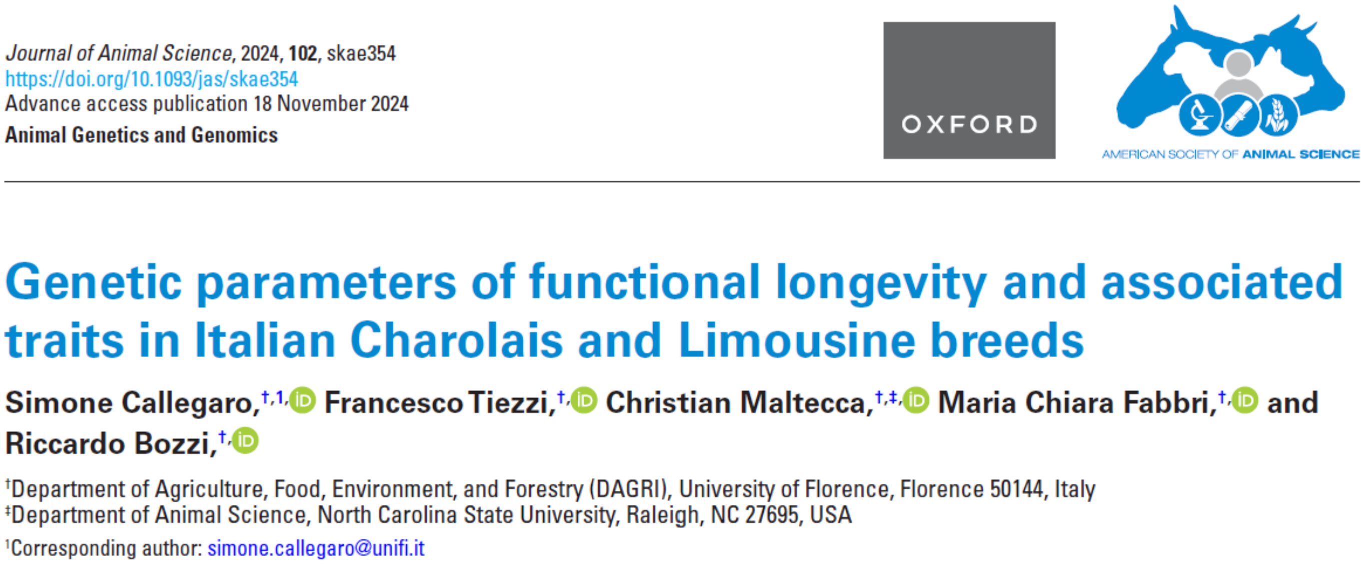
ANACLI, 2024. https://www.anacli.it/libro-genealogico/razze/charolaise-limousine/statistiche

Ruminantia, 2023-2024. https://archivio2023-2024.ruminantia.it/vi-raccontiamo-le-razze-la-charolaise/

Ruminantia, 2023-2024. https://archivio2023-2024.ruminantia.it/vi-raccontiamo-le-razze-la-limousine/#:~:text=La%20Limousine%20appartiene%20al%20gruppo,diventando%20la%20sua%20attitudine%20esclusiva.

CHAPTER 3

3. Genetic Parameters of Functional Longevity in Italian Charolais and Limousine Breeds



Journal of Animal Science, 2024, **102**, skae354
https://doi.org/10.1093/jas/skae354
Advance access publication 18 November 2024
Animal Genetics and Genomics

Genetic parameters of functional longevity and associated traits in Italian Charolais and Limousine breeds

Simone Callegaro,[†,1] **Francesco Tiezzi,**[†] **Christian Maltecca,**[†,‡] **Maria Chiara Fabbri,**[†] **and Riccardo Bozzi,**[†]

[†]Department of Agriculture, Food, Environment, and Forestry (DAGRI), University of Florence, Florence 50144, Italy
[‡]Department of Animal Science, North Carolina State University, Raleigh, NC 27695, USA
[1]Corresponding author: simone.callegaro@unifi.it

https://doi.org/10.1093/jas/skae354

Lay summary

Maximizing the longevity of cows is a crucial economic factor in cattle farms. A significant cost for farmers is maintaining heifers as replacements for older cows. Stay-ability is a trait that measures the likelihood of a cow remaining productive in the herd until a certain age. This trait reduces replacement costs and improves herd efficiency, increasing farm profitability. This study focuses on the genetic factors influencing longevity in Italian Charolais and Limousine beef cattle. We estimate the genetic components of stay-ability at different calvings using genomic information and explore its relationship with body conformation and fertility. This study investigates the longevity of Limousine and Charolais cattle to understand genetic and environmental factors affecting survival across calvings. By incorporating stay-ability into genetic evaluations, it is possible to develop more effective breeding strategies based on longer-lasting and more productive herds. We also examine intra-herd heritability, herd effects, and genetic correlations with fertility and conformation traits. Our results highlight breed-specific differences, suggesting the need for tailored breeding strategies to enhance cattle longevity.

Teaser text

Determine how genetic selection can improve the longevity and productivity of beef cattle herds. Our study explores the genetic background of functional longevity in Italian Charolais and Limousine breeds, aiming to reduce costs and enhance farm profitability through breeding strategies.

Abstract

This study aimed to estimate the genetic parameters of Stay-ability (**STAY**) at different calvings using a single-step genomic best linear unbiased prediction (**ssGBLUP**) approach, comparing Gaussian-linear and threshold models in Italian Charolais and Limousine beef cattle. It also examined the genetic relationship between STAY and other traits to identify potential indicators of longevity and assessed the impact of STAY selection on economically important traits. Stay-ability, a key trait for farm profitability, is defined as the probability of a cow surviving and remaining productive in the herd until a determined age. We evaluated STAY from the second to third calving and subsequent intervals (e.g., STAY23, STAY78), along with two fertility traits and several conformation traits. Data included 47 362 Limousine cows and 9 174 Charolais cows from 2 471 and 1 774 herds, respectively, born between 1977 and 2023. Analyses were performed fitting univariate threshold and Gaussian-linear animal models to estimate genetic parameters for STAY traits (STAY2 to STAY8) using ssGBLUP. Also, bivariate models were used to estimate genetic correlations between STAY and fertility and conformation traits. Heritabilities for STAY ranged from 0.13 to 0.11 and from 0.21 to 0.14 for Limousine, and from 0.14 to 0.11 and from 0.21 to 0.19 for Charolais, using Gaussian-linear and threshold models, respectively. Significant re-ranking of genotyped sires based on STAY traits was observed, particularly for more distant calvings (STAY8) compared to earlier ones (STAY3), indicating that STAY traits are genetically distinct. Genetic correlations were positive between STAY and conformation traits for Limousine. In Charolais, many traits were uncorrelated, but some conformation traits showed positive correlations, except for rump convexity, which had negative correlations with STAY. In conclusion, the heritability estimates of STAY suggests that genetic improvement for longevity in Limousine and Charolais herds is feasible. Selecting sires with consistently high genomic breeding values for STAY across early and late calvings highlights the importance of long-term longevity. Genetic correlations indicate that selection based on conformation traits could enhance herd survival by improving cow resilience for the Limousine. Instead for the Charolais some conformation traits showed positive correlations with STAY, while rump convexity had negative association, potentially affecting longevity.

Keywords: Beef cattle, functional longevity, genetic association, conformation traits, single step.

3.1 Introduction

In the majority of beef cattle production systems, the duration of a cow's productive life, the rate of calf production per cow over time, and the weight of calves at weaning are crucial economic aspects (Santana et al., 2013). A large part of the costs of beef cattle production are represented by the maintenance of heifers for replacement (Newman et al., 1992). Therefore, to reduce costs derived from replacement or culling, increasing herd longevity is one of the most effective strategies (Rizzo et al., 2015). A cattle farmer must ensure that replacements remain in the herd long enough to produce sufficient calves to offset the costs of their rearing and maintenance (Silva et al., 2024).

Stay-ability (**STAY**) is a crucial trait for on-farm profitability due to its association with specific costs and herd efficiency. Hudson and Van Vleck (1981) defined STAY as the likelihood of a cow surviving and producing in the herd until a specified age, provided that animals have the opportunity to reach that age. It serves as a measure of survival and is typically expressed as a binary trait ('yes' or 'no'; '1' or '0'), indicating whether animals remain in the herd or not, without the need for recording culling dates. Predicting the genetic value of STAY offers an opportunity to reduce costs and increase farm income. Selecting for high genetic values of STAY can reduce turnover rates and decrease replacement costs of the farm. This could improve average herd efficiency, by extending the productive lifespan of cows. Integrating this trait into genetic evaluations enables the identification of parents whose daughters are more likely to remain productive for extended periods in the herd (Van Melis et al., 2007; Jamrozik et al., 2013).

Threshold models are specifically designed for binary traits like STAY, but they may have limitations in terms of complexity and interpretability compared to linear mixed models (Gianola, 1982). In contrast, linear mixed models provide an alternative for analysing continuous and categorical traits, providing additional insights into genetic variation. By comparing these models is possible determining which approach provides a more accurate representation of STAY traits across different calvings. This comparison can help to identify the most suitable method for genetic evaluations in beef cattle and enhance the accuracy of genetic evaluation for longevity.

The primary challenge in STAY genetic evaluations stems from the time required for a female to exhibit the phenotype at a certain age, potentially leading to lower accuracies in young bulls (Hudson and Van Vleck, 1981; Silva et al., 2018). Longevity selection is further complicated by the trait's low heritability and the influence of environmental and management factors, along with genetics. Assessing traits like STAY within a multiple-trait model can reduce selection random errors and improve the accuracy on genetic parameter estimates for other traits (Pollak et al., 1984). Indeed, incorporating animals no longer in the herd into the analysis can reduce the number of records removed from the dataset (Pollak et al., 1984). Employing STAY within a multiple-trait approach could potentially enhance longevity in beef cattle. Furthermore, conformation traits may contribute to herd survival rates due to their correlation with longevity (Hu et al., 2021; Buonaiuto et al., 2023). These traits, which encompass aspects like body size, shape, and bone structure, contribute to individuals' overall health and resilience, thereby impacting the survival rates of the entire herd. Moreover, fertility traits can enhance average herd longevity, due to a low to moderate genetic association with longevity (Hu et al., 2021, Hu et al.,2023). Fertility can be used as indirect indicators to select cows with greater potential for early longevity selection (Hu et al., 2021). A study found that older heifers had a higher culling risk compared to those calving between 24 and 28 months, attributing late calvings to issues in herd management, fertility, health problems, and increased rearing costs (Sewalem et al., 2005). Understanding these relationships could improve breeding and management strategies aimed to increase herd longevity.

In dairy cattle, authors have observed positive genetic correlations among body traits and longevity and a strong relationship with functional herd life (Hu et al., 2021). In beef cattle, Forabosco et al. (2004) discovered that animals with higher muscle development were more likely to remain in the herd, indicating a phenotypic relationship between conformation and longevity. Body traits are routinely measured in Italian Charolais and Limousine herds and are part of the national breeding selection goals. However, to date, no studies have investigated the genetic association of conformation, fertility, and STAY traits in these breeds through a multiple-trait analysis.

Finally, despite numerous studies estimating heritability and genetic components in beef and dairy cattle, there is still a dearth of research on utilizing single-step genomic best linear unbiased prediction (**ssGBLUP**) approaches for consecutive calvings in beef cattle. Therefore, the objectives of this study were to: (1) estimate the genetic parameters of STAY at different calving using a ssGBLUP approach, both Gaussian-linear and threshold, on Italian Charolais and Limousine beef cattle; (2) to compare Gaussian-linear and threshold models for STAY at different calvings of Limousine and Charolais, determining which approach provides a more accurate estimation of the genetic parameters of STAY; (3) to estimate and to examine the genetic relationship between STAY and other traits, to identify potential indicators of longevity or to assess the impact of selection for STAY on other economically important traits and improve fertility on beef cattle.

3.2 Materials and methods

Phenotypic records, pedigree, and genomic information were provided by The National Italian Association of Limousine and Charolais Breeders (**ANACLI**) database; therefore, Animal Care and Use Committee approval was unnecessary.

In Italy, the National Herd Book currently lists 11 579 registered Charolais and 50 511 Limousine females, highlighting their significant role in the Italian beef industry. Originating from France, the Limousine and Charolais breeds are extensively exported to numerous countries worldwide, either in purebred form or as part of crossbreeding systems (Bouquet et al., 2011).

The initial dataset for Limousine and Charolais breeds in this study comprises 587 773 calving records involving 141 923 cows born between 1977 and 2023. These records originate from 3 387 registered herds located across the entire national territory.

3.2.1 Stay-ability traits

The trait STAY was chosen as a metric for assessing the longevity of animals, evaluated from the second to the eighth calving. By utilizing information on calving dates and order, seven cumulative STAY traits were assigned to each cow. These traits were defined as binary indicators of survival up to a specific calving order (e.g., survival up to the second calving, survival up to the third calving, and so on). Thus, each cow could have up to seven STAY records corresponding to survival up to the second through eighth calving (e.g., STAY2, STAY3, ..., STAY8). Consequently, each female could have up to seven different observations if she reached STAY8. For each parity, STAY was treated as a binary trait, where a value of '1' indicated cows with a recorded calving event and '0' indicated those without a calving record. Consequently, STAY (1 = success, 0 = failure) was assigned based on the presence of a calving date for each parity up to parity eight. The study also considered animals that remained in the herd from one calving to the next ‘STAY to STAY traits’ (traits STAY23, STAY34, …, STAY78), focusing solely on cows that survived from, for example, second to third calving. For instance, STAY23 measures survival from the second calving to the third, while STAY34 measures survival from the third calving to the fourth. Only cows that have reached the earlier calving event (e.g., second calving for STAY23) are included in these analyses. The inclusion of these traits in the model (e.g., STAY2 with STAY23, or STAY3 with STAY34), allowing for a more complete dataset by including cows that may not have reached the next calving.

Initial data editing involved keeping only females with age at first calving between 700 and 1,400 days of age. Cows with recorded calving events and with an appropriate interval before the following calving, allowing for the occurrence or absence of the subsequent calving event, were included in the analysis. Specifically, animals with any calving intervals shorter than 290 days or longer than 550 days were excluded from the dataset. Twins’ parities were considered as a single calving event. If the time elapsed between the last recorded calving of a cow and the date when the calving data was extracted was less than 550 days, any calving records that occurred after this period were classified as censored data. This means that these records were not fully available for analysis because the full calving history was incomplete by the time of data extraction. Censored records were treated as missing data in the model (Smith 1990; Stephen et al., 2023). Additionally, cows with missing sire and dam were removed. Supplementary Table S3.1 reported the percentage of censored records according to the total number of cows for all the STAY for the two breeds.

The final dataset included 47 362 cows, of which 38 188 were daughters of 6 194 sires belonging to 2 471 herds for Limousine, and 9 174 were daughters of 1 774 sires belonging to 712 herds for Charolaise respectively. Table 3.1 shows the number of records for all the STAY and the relative incidence of survival for the two breeds.

Table 3.1 Number of the cows and incidence to survival according to the number of records per each of the stayability (STAY) for Limousine and Charolaise.

Trait	Definition	Limousine			Charolais		
		N	N of cow survived	Incidence (survival %)	N^2	N of cow survived	Incidence (survival %)
STAY1	Stayability as a first parity = 1; failed = 0	38 188	38 188	100.00	9 174	9 174	100.00
STAY2	Stayability as a second parity = 1; failed = 0	33 209	25 526	66.84	8 071	5 557	60.57
STAY3	Stayability as a third parity = 1; failed = 0	30 105	18 764	49.14	7 519	3 913	42.65
STAY4	Stayability as a fourth parity = 1; failed = 0	27 978	14 381	37.66	7 078	2 820	30.74
STAY5	Stayability as a fifth parity = 1; failed = 0	26 546	11 257	29.48	6 774	2 041	22.25
STAY6	Stayability as a sixth parity = 1; failed = 0	25 372	8 838	23.14	6 550	1 467	16.00
STAY7	Stayability as a seventh parity = 1; failed = 0	24 429	6 890	18.04	6 381	1 042	11.36
STAY8	Stayability as a eight parity = 1; failed = 0	23 657	5 314	13.91	6 245	717	7.82

Abbreviations: N = Total number of cows, including both those that survived and were culled.

3.2.2 Fertility and conformation traits

Data on fertility traits were extracted from records of cows from the STAY dataset. Fertility traits considered were first calving interval (**FCI**, days) and age at first calving (**AFC**, days) and were treated as continuous responses. Values of FCI averaged 405.7 days (± 60.87 SD) and 401.50 (± 60.05 SD) for Limousine and Charolais, respectively. Values of AFC averaged 1 026 days with a SD of 143.69 and 1,026 days with a SD of 157.62 for Limousine and Charolais, respectively.

Conformation type traits in the two breeds are routinely measured once in the lifetime, typically around weaning and yearling (between 6 and 15 months of age). Conformation traits were evaluated for each animal by trained classifiers between 1990 and 2023 and were obtained by visual scores. Muscularity refers to the muscle development of the animal across several reference sites, including the shoulder, loin, rump, and hindquarter (Boligon et al., 2011). In this study we focused on eight traits related to conformation and muscularity traits. These traits assess muscular development, specifically the musculature of the thigh and back, as well as skeletal development, which measures proportionality. Additionally, traits like rump length and overall size capture general body development. Traits were assessed using scores ranging from one to ten, with ten representing the highest expression of the trait and one indicating the lowest expression. Conformation traits considered were: wither width, convexity and length of rump, length of dorsolumbar line, rear and back width, pelvic length, and development. The final dataset consisted of 32 119 and 7 537 measured cows for Limousine and Charolais, respectively. The number of records, the mean, and the definition per each conformation trait are reported in Supplementary Table S3.2.

3.2.3 Pedigree and genotypes

The raw pedigree files included 147 801 and 526 887 animals. For subsequent analyses, animals were traced back 8 generations for Charolais and 6 generations for Limousine, resulting in 13 222 and 35 418 animals, respectively.

In total, 276 and 71 animals were genotyped with a panel of 119 854 SNPs (GeneSeek GGP Bovine 150K; Illumina Inc., San Diego, California, USA) for Limousine and Charolais, respectively. 3 745, and 1 047 individuals were genotyped with a panel of 28 299 SNPs (GeneSeek GGP Bovine LD v3; Illumina Inc., San Diego, California, USA) for Limousine and Charolais, respectively. All SNPs were mapped according to ARS-UCD1.2 genome assembly (Rosen et al., 2020). After the mapping, 118 135 markers for GeneSeek GGP Bovine 150K and 27 654 markers for GeneSeek GGP Bovine LD v3 were considered. The two panels shared 13 984 SNPs. Animals genotyped with GeneSeek GGP Bovine LD v3 were imputed to GeneSeek GGP Bovine 150K, leveraging the common set of 13 984

SNPs shared between the two panels as a basis for alignment and enhancement. Subsequently, the 13 670 non-overlapping SNPs were imputed, and the resulting genotypes were integrated with the previously imputed set of 118 135 SNPs. This process led to a total of 131 805 imputed SNPs by the conclusion of the last step. FImpute v.3 (Sargolzaei et al., 2014) was utilized with default parameters for genotype imputation. The genotype imputation was performed separately for each breed, with genotypes for the Charolais and Limousine breeds being imputed independently.

Quality control (**QC**) and genotype data filtering were performed using PLINK v1.9 (Chang et al., 2015). Autosomal SNPs and individuals with more than 10% missing values, minor allele frequencies (MAF) lower than 1%, and a call rate lower than 90% were excluded during QC. After QC, 116 221 SNPs and 3 960 animals remained for Limousine, while 116 704 SNPs and 990 animals remained for Charolais. Table 3.2 summarizes the number of genotyped animals per category, including females with phenotype, sires, and dams.

Table 3.2 Number of genotyped animals for Charolaise and Limousine considering animals with phenotype, sires, and dams.

Breed	GeneSeek GGP Bovine LD v3			GeneSeek GGP Bovine 150K			Total
	Female with phenotype	Sires	Dams	Female with phenotype	Sires	Dams	
Charolais	504	274	182	10	52	6	1 028
Limousine	2 233	906	651	256	106	56	4 054

Abbreviations: Female with phenotype = are those individuals showing a phenotypic record in the dataset. They may appear as dams or other individuals; Sire = male without phenotype are those individuals not showing a phenotypic record in the dataset, but they appear as sires of individuals with phenotypes; Female = dame without phenotype are those individuals not showing a phenotypic record in the dataset, but they appear as dams of individuals with phenotypes.

3.2.4 Model computation and analysis

Univariate models. In our analysis, an underlying continuous variable termed liability was derived from the data based on the following assumption:

$$y = 0, \text{if } \lambda \leq \tau \text{ and } y = 1, \text{if } \lambda > \tau$$

where y represents the linear observation (0/1), λ denotes the liability, and τ stands for the specified threshold. Each observation of y assumes a value of 1 (indicating success in herd until the subsequent parity) when the liability surpasses τ, and 0 (reflecting failure in the herd on the subsequent parity) otherwise. The liability is assumed to follow a normal distribution with mean μ and covariance matrix $\mathbf{R} = \mathbf{I}\sigma_{\lambda}^{2}$, where σ_{λ}^{2} denotes the variance of the scale. Given that the mean and variance of the liability are non-identifiable, we set this parameter to $\sigma_e^2 = 1$ and $\tau = 0$. Consequently, there is no necessity for sampling the threshold value.

Stayability were analysed fitting a univariate threshold and Gaussian-linear animal model. Genetic parameters were estimated for STAY2 to STAY8 using the following models:

$$\text{MODEL 1: } y = \mathbf{X}_{\boldsymbol{\beta}} + \mathbf{Z}_{\mathbf{p}}p + \mathbf{Z}_{\mathbf{a}}a + e$$

$$\text{MODEL 2: } \lambda = \mathbf{X}_{\boldsymbol{\beta}} + \mathbf{Z}_{\mathbf{p}}p + \mathbf{Z}_{\mathbf{a}}a + e$$

where y is the linear vector of STAY traits (0/1) and λ the unobserved liability for threshold models; β was the vector associated to fixed effects of the year of calving (34 and 48 levels for Charolais and Limousine, respectively); p was the vector associated to the random effect of herd; a was the vector for the animal additive genetic effect; e was the vector associated to the random residual error; and $\mathbf{X}$, $\mathbf{Z}_{\mathbf{p}}$, $\mathbf{Z}_{\mathbf{a}}$, were the incidence matrices related to fixed, random, and additive genetic effect of the animal, respectively. The solutions for additive genetic effects were assumed as $a \sim N(0, \mathbf{H}\sigma_a^2)$, where **H** is the relationship matrix and σ_a^2 the additive genetic variance and the residual error values were assumed as $e \sim N(0, \mathbf{I}\sigma_e^2)$ where **I** is the identity matrix and σ_e^2 is their variance.

The SNPs information among animals was included in the form of genomic relationships matrix. Therefore, the **H** matrix was constructed by combining the pedigree relationship matrix (**A**) and a SNP derived genomic relationship matrix (**G**) (Legarra et al., 2014) and was estimated through ssGBLUP as described by Aguilar et al. 2010. The **G** matrix was built using the second method proposed by VanRaden (2008).

Multivariate models. Multivariate models enable the simultaneous analysis of several STAY traits as correlated traits and can be utilized for STAY to specific endpoints or within time intervals. Genetic correlations between the traits were estimated by using bivariate threshold models as follows:

$$\text{MODEL 3: } \begin{bmatrix}\lambda_1\\ \lambda_2\end{bmatrix} = \begin{bmatrix}\mathbf{X_1} & 0\\ 0 & \mathbf{X_2}\end{bmatrix}\begin{bmatrix}\beta_1\\ \beta_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z_{p1}} & 0\\ 0 & \mathbf{Z_{p2}}\end{bmatrix}\begin{bmatrix}p_1\\ p_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z_{a1}} & 0\\ 0 & \mathbf{Z_{a2}}\end{bmatrix}\begin{bmatrix}a_1\\ a_2\end{bmatrix} + \begin{bmatrix}e_1\\ e_2\end{bmatrix}$$

where λ_1 the unobserved liability for STAY observations (STAY2, ..., STAY8), and λ_2 was the unobserved liability for STAY23 until STAY78; fixed and random effects were the same as in the univariate analyses (model 1 and 2). Additionally, a bivariate model was applied also for fertility traits, and FCI, and AFC were treated as continuous variables using the same fixed and random effect. Censored records were treated as missing and not included in the analyses. In particular, the calving season for STAY23 refers to the period when a cow gives birth, marking the beginning of its second calving. This is the time frame during which the cow gives birth to its second calf and transitions into its third parity. As reported by Hardie et al. (2021) bivariate models were used to address possible selection bias on STAY23, as only cows that survived to their second parity had data for the STAY2 trait. Including STAY2 along with STAY23 in the analysis, it was possible to consider traits from all cows in the herd that fit the criteria reducing any bias that might occur using a non-random selection of cows that reached their third parity.

Genetic correlations among conformation and STAY traits were estimated with the following bivariate threshold model:

$$\text{MODEL 4:}\quad \begin{bmatrix}\lambda\\ \mathrm{y}\end{bmatrix} = \begin{bmatrix}\mathbf{X_1} & 0\\ 0 & \mathbf{X_2}\end{bmatrix}\begin{bmatrix}\beta_1\\ \beta_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z_{p1}} & 0\\ 0 & \mathbf{Z_{p2}}\end{bmatrix}\begin{bmatrix}\mathrm{p}_1\\ \mathrm{p}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z_{w1}} & 0\\ 0 & \mathbf{Z_{w2}}\end{bmatrix}\begin{bmatrix}0\\ \mathrm{w}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z_{a1}} & 0\\ 0 & \mathbf{Z_{a2}}\end{bmatrix}\begin{bmatrix}\mathrm{a}_1\\ \mathrm{a}_2\end{bmatrix} + \begin{bmatrix}\mathrm{e}_1\\ \mathrm{e}_2\end{bmatrix}$$

where λ the unobserved liability for STAY observations (STAY2, ..., STAY8), and y was the linear vector of the conformation trait; β_1 and β_2 were the vectors associated to fixed effects of the year of calving and age of the cow at the evaluation; p_1 and p_2 were the vectors associated to the random effect of herd; w_2 is the vector associated to the random effect of the code of the trained classifiers; a_1 and a_2 were the vectors for the animal additive genetic effect; e was the vector associated to the random residual error; and $\mathbf{X}$, $\mathbf{Z_w}$, $\mathbf{Z_a}$, were the incidence matrix related to fixed, random, and additive genetic effect of the animal, respectively. The random effect of the trained classifiers correlates and covaries only with the conformation traits, as only these traits are assessed by the classifiers.

In particular, the assumptions regarding the (co)variance structure in the bivariate models of additive genetics, the random effect of herd and trained classifiers, and residual effects were estimated using the following matrix notations:

$$\mathbf{G} = \begin{vmatrix}\sigma^2_{\mathrm{a1}} & \sigma_{\mathrm{a1a2}}\\ \sigma_{\mathrm{a2a1}} & \sigma^2_{\mathrm{a2}}\end{vmatrix}; \mathbf{P} = \begin{vmatrix}\sigma^2_{\mathrm{p1}} & \sigma_{\mathrm{p1p2}}\\ \sigma_{\mathrm{p2p1}} & \sigma^2_{\mathrm{p2}}\end{vmatrix}; \mathbf{W} = \begin{vmatrix}0 & 0\\ 0 & \sigma^2_{\mathrm{w2}}\end{vmatrix}; \mathbf{R} = \begin{vmatrix}\sigma^2_{\mathrm{e1}} & 0\\ 0 & \sigma^2_{\mathrm{e2}}\end{vmatrix}$$

Where **G**, was the matrix of additive genetic (co)variances σ^2_{a1}, σ_{a1a2}, σ^2_{a2} of traits one and two, **P**, and **W** were matrices of (co)variances of random effects σ^2_{p1}, σ_{p1p2}, σ^2_{p2} and σ^2_{w2}, respectively, of traits one and two, and **R** was the matrix of residual (co)variances σ^2_{e1}, σ^2_{e2} of traits one and two.

3.2.5 Genetic parameters

The (co)variance components were estimated using the Gibbs sampling algorithm implemented in the BLUPF90 family software (Aguilar et al., 2018). Threshold models were run using THRGIBBS1F90, and Gaussian-linear models were run using GIBBS3F90 programs. All analyses were run for 100 000 cycles with a burn-in of 50 000 samples and every 10th sample being stored, for a total of 5 000 samples used for subsequent inference. Convergence was assessed by visual inspection of trace plots and throughout Geweke's test using the package 'coda' (Plummer et al., 2006).

Heritability ($\mathbf{h^2}$), herd effect ($\mathbf{h_i}$), and intra-herd heritability ($\mathbf{h^2_{IH}}$) were expressed as follows:

$$\mathrm{h}^2 = \frac{\sigma^2_{\mathrm{a}}}{\sigma^2_{\mathrm{a}} + \sigma^2_{\mathrm{p}} + \sigma^2_{\mathrm{e}}}$$

$$h_i = \frac{\sigma_p^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

$$h_{IH}^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

where σ_a^2, σ_p^2, and σ_e^2, are the variance components related to additive genetic effect, random effect of the herd, and random residual error. Lower and upper limits of the 95% highest posterior probability distributions and the means for heritabilities were estimated from the Gibbs samples. Confidence intervals and posterior means were used as estimates and its attached standard error.

The study compared univariate analyses, incorporating both threshold and Gaussian-linear models, to examine the genetic relationship among STAY traits. To assess the possibility of re-ranking among genotyped sires, Spearman's rank correlations were estimated. Specifically, ssGEBV from univariate models for STAY2 through STAY8 were compared for both Charolais and Limousine breeds. Additionally, the study evaluated ssGEBVs for genotyped sires to verify their average genetic superiority and to determine whether any loss in expected progeny differences would occur if selection were based on STAY traits. For example, a reduction in the average genetic superiorities in STAY2 would be expected if the selection was based on STAY8.

The genetic correlation among traits in bivariate models was obtained as follows:

$$r_{gen} = \frac{\sigma_{a,xz}}{\sqrt{\sigma_{a,x}^2 \times \sigma_{a,z}^2}}$$

Where $\sigma_{a,xz}$ is the genetic covariance between the two considered traits in the bivariate analysis, and $\sigma_{a,x}^2$ and $\sigma_{a,z}^2$ are the additive genetic variances, respectively.

3.3 Results and discussion

Linear mixed models provide enhanced flexibility for modelling binary traits, even when the assumptions are violated. This preference is attributed to several factors, including the flexibility of linear mixed models in handling various types of data. They can handle both continuous and categorical predictors, making them suitable for a wide range of analyses. Furthermore, their ease of interpretation through understandable coefficients for fixed effects and their efficiency in providing unbiased estimates of parameters even when data violate assumptions (Breslow and Clayton, 1993). Despite this, both threshold and linear mixed models' approaches were assessed in this study to gain comprehensive insights into STAY traits in two different beef cattle breeds.

3.3.1 Incidence of survival

Table 3.1 shows the incidence of survival of STAY2 until STAY8 in the Limousine and Charolais. The cull rate of primiparous cows between the first and second STAY was 33% and 40% for Limousine and Charolais, respectively. The cull rate until STAY8 was 86% and 92% for Limousine and Charolaise, respectively. Culled animals were higher in early STAY than those between later STAY and relatively similar among breeds. On average, survival between parities was 6-7% lower in Charolais compared to Limousine.

The elevated culling rate observed in early STAY can be attributed to several factors. Firstly, primiparous cows often encounter reproductive challenges, such as calving difficulties or postpartum complications, which can lead to their removal from the herd. Additionally, some cows may exhibit inadequate maternal instincts or abilities, resulting in lower calf survival rates and subsequent culling. Furthermore, heifers that fail to initiate a first parity may be sold for meat purposes. Genetic selection also plays a role, as genetically superior females are retained in the herd, leading to the culling of less desirable individuals. These reproductive challenges, maternal abilities, and genetic selection pressures collectively contribute to the higher culling rate observed during early calving.

Martinez et al. (2005) investigated STAY traits from calving 2 to 6 in Hereford cows, while Silva et al. (2018) compared the same traits in Guzerá, Nelore, and Tabapuã cattle. Both studies found similar culling rates for early calving, consistent with the present study's findings.

3.3.2 Heritability

Estimates of heritability and their relative SE for STAY using threshold and Gaussian-linear ssGBLUP models are summarized in Figure 3.1. Generally, the heritability estimates using the Gaussian-linear model were low, ranging from 0.13 ± 0.01 for STAY2 to 0.11 ± 0.01 for STAY8 in Limousine cattle and from 0.14 ± 0.03 for STAY2 to 0.11 ± 0.02 for STAY8 in Charolais cattle. In contrast, heritability values were moderate when using the threshold model, ranging from 0.21 ± 0.02 for STAY2 to 0.14 ± 0.06 for STAY8 in Limousine and from 0.24 ± 0.04 for STAY2 to 0.19 ± 0.04 for STAY8 in Charolais. When considering the Gaussian-linear model, the trends for heritability were similar between the two breeds. However, the threshold model showed a slightly different trend in Limousine cattle, decreasing heritability with increasing parities.

Estimates of heritability using the Gaussian-linear model in our study are consistent with those obtained using linear random regression models for STAY in late calvings (0.12) (Jamrozik et al., 2013). Still, they are lower than the estimates for STAY in early calvings (0.38) reported for Canadian Simmental cattle (Jamrozik et al., 2013). For Guzerá, Nelore, and Tabapuã cattle, Silva et al. (2018) used a linear random regression model and, when transforming the heritabilities to the underlying normal scale, found estimates for the first calving number similar to our results but lower estimates for later calving numbers. In Hereford cattle, Martinez et al. (2005) used linear binomial models and found heritability estimates ranging from 0.18 ± 0.09 to 0.25 ± 0.07 for STAY to consecutive calving. These results were generally higher compared to our study.

Estimates of heritability using the threshold model in our study were, on average, slightly lower than the heritability estimate for STAY to consecutive calvings (0.24) on the underlying liability scale in Simmental cattle (Jamrozik et al., 2013). Santana et al. (2012) reported a heritability of 0.11 using a threshold model in Nelore cattle, which is similar to our estimates for STAY2 and STAY3 in Charolais and STAY7 in Limousine cattle. Our estimates were generally lower than the heritability values (0.25, 0.22, and 0.28) for STAY at specific ages in Nelore cattle provided by Van Melis et al. (2007). Martinez et al. (2005) used a threshold model in Hereford cattle and found heritability estimates from 0.29 ± 0.10 to 0.39 ± 0.11 for STAY to consecutive calving, the results from our study were generally lower in comparison.

In our analysis, as shown in Figure 3.1, we found that the heritability estimates obtained through a Gaussian-linear model were lower than those obtained through the threshold model, as also reported by Martinez et al. (2005). These moderate estimates of heritability for STAY imply the potential for a response to selection and genetic improvement through genetic selection. These findings underscore the utility of utilizing STAY as a selection criterion to enhance dam fertility. Supplementary Table S3.3 reported the numerical values of heritabilities with their respective HPDI. In addition, variance components and the corresponding HPDI considering threshold and Gaussian-linear model were reported in Supplementary Table S3.4.

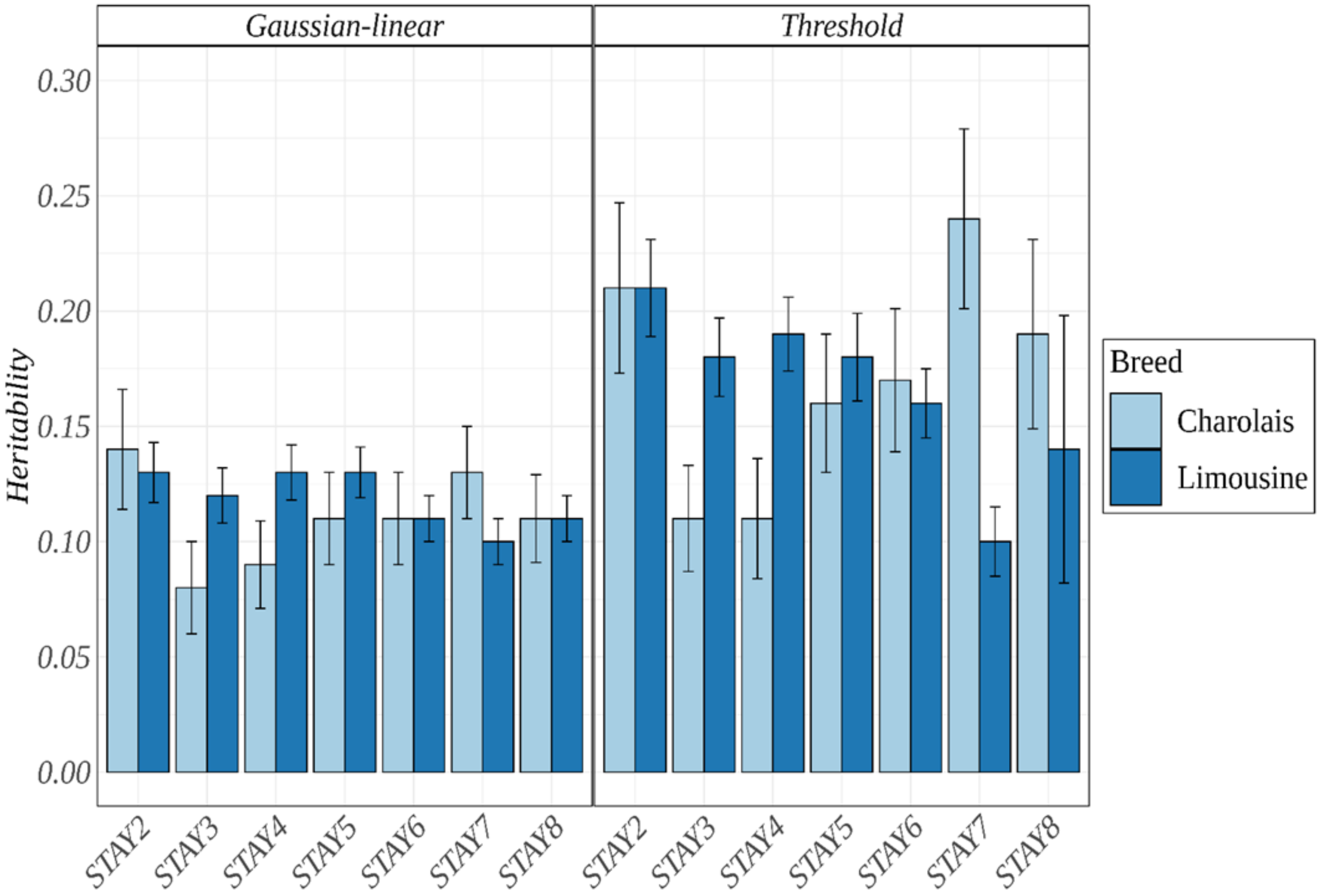


Figure 3.1 Heritability estimates with the relative SE across stay-ability (STAY) traits for Limousine and Charolais using a single-step GBLUP approach considering STAY (Gaussian-linear model) and liability of STAY (threshold model), respectively.

3.3.3 Intra-herd heritability and herd effect

In genetic analyses, estimation of herd effect and intra-herd heritability help to understand the balance between genetic and environmental influences within herd. Similar values between intra-herd heritability and overall heritability suggest that the herd genetic variability and environmental conditions are representative of the studied population. Conversely, large differences indicate significant environmental impacts, which could mask genetic variation. Higher herd effect estimates imply substantial environmental influence, with high variability in management practices, nutrition, and health care. This variability can slow genetic progress for the analysed trait.

Estimates of the herd effect and relative SE are reported in Supplementary Figure S3.1. Estimates were moderate for both Charolais and Limousine breeds. Using the Gaussian-linear model, herd effect values ranged from 0.25 ± 0.01 for STAY2 to 0.15 ± 0.01 for STAY8 in Limousine and from 0.20 ± 0.02 for STAY2 to 0.12 ± 0.01 for STAY8 in Charolais. Using the threshold model, herd effect values ranged from 0.34 ± 0.01 for STAY2 to 0.56 ± 0.09 for STAY8 in Limousine and from 0.30 ± 0.03 for STAY2 to 0.45 ± 0.04 for STAY8 in Charolais.

While intra-herd heritability focuses on genetic variation within a herd, the herd effect considers the impact of environmental factors common to all animals within the same group. These environmental factors include management practices, feeding regimes, housing conditions, disease exposure, and social interactions. The herd effect can significantly influence the observed phenotypic variation of traits within a herd, independent of genetic differences among individuals.

As reported Supplementary Figure 3.1, both models for both breeds exhibited an opposite trend: increasing for the Gaussian-linear model and decreasing for the threshold model. For both analyses, Limousine showed, on average, slightly higher values than Charolais of herd effect with significative differences in STAY2 using the Gaussian-linear model and STAY7 using the threshold model. The observed differences in herd effect estimates between the Charolais and Limousine breeds for STAY may be attributed to several factors. Genetic differences between the breeds could influence their respective responses to herd environments, with varying levels of resilience or susceptibility to environmental factors affecting longevity. Additionally, distinct management practices specific to herds, like feeding, housing, and herd management, could impact the two breeds differently. Environmental adaptability may also play a role, as one breed might be better suited to certain conditions or practices, thereby explaining the higher herd effect values observed in Limousine.

Estimates of intra-herd heritability were summarized in Supplementary Figure S3.2, showing lower to moderate estimations for Charolais and Limousine. Using the Gaussian-linear model, values ranged from 0.18 ± 0.02 for STAY2 to 0.13 ± 0.01 for STAY8 considering Limousine and 0.17 ± 0.03 for STAY2 to 0.12 ± 0.02 for STAY8 considering Charolais. Using the threshold model, intra-herd heritability showed values ranged from 0.32 ± 0.03 for STAY2 to 0.33 ± 0.04 for STAY8 and ranged from 0.30 ± 0.05 for STAY2 and 0.34 ± 0.06 for STAY8 for Limousine and Charolais, respectively. Intra-herd heritability refers to the proportion of the phenotypic variance of a trait attributable to genetic differences among individuals within a specific group or herd. The fraction of observed trait variability within a given population could be attributed to genetic variation among individuals within that population, excluding the effect of common environments shared by animals within the same group. Thus, intra-herd heritability provides an estimate of the heritability of a trait within a specific selection unit, such as a herd. In this study, the intra-herd heritability values were, on average, similar to the heritability, suggesting that the genetic potential for the trait within the herd is consistent with the population.

As reported in Supplementary Figure S3.2, both models for both breeds exhibited a trend comparable to that of heritabilities described in the previous section, with higher values detected using the threshold model. For both analyses, Limousine showed slightly higher average values compared to Charolais, with significant differences in STAY3, STAY4, and STAY5 using both Gaussian-linear and threshold models. Only for STAY7 did Charolais show significantly higher values compared to Limousine.

These differences between the two breeds may arise due to various factors. If one breed exhibits greater genetic variability for functional longevity compared to the other, a higher degree of intra-herd heritability may be observed in that breed. Disparities in rearing environments, management practices, and selection procedures between the two breeds might impact STAY and the estimation of intra-herd heritability. Differences in climate and nutrition could influence animal survival and fertility, thus affecting the estimation of trait intra-herd heritability. Additionally, if one breed is more susceptible to genetic-environmental interactions affecting functional longevity, a reduction in intra-herd heritability may be observed in that breed compared to the other. Numerical values for intra-herd heritabilities and herd effect with their respective HPDI are reported in Supplementary Table 3.3.

3.3.4 Re-ranking among genotyped sires

We investigated whether different models (threshold versus Gaussian-linear) would significantly re-rank genotyped sires, potentially impacting selection decisions based on ssGEBV for functional longevity. Only genotyped sires were considered for both Charolais and Limousine cattle.

Analysing regression coefficients and Spearman correlations with STAY2 as the predictor for sires ssGEBVs of STAY3 and STAY8 allow us to verify whether there would be loss in terms of expected progeny difference re-ranking of sires across different calvings if sire selection were based on early or late longevity. A reduction in the average genetic superiorities for sires in STAY2 would be expected if the selection were based on the more distant calving event, for example, STAY8. For the Limousine breed, the regression coefficients between STAY2 and STAY3 were 0.80 for the threshold model and 0.74 for the Gaussian-linear model, while between STAY2 and STAY8, the coefficients were 0.34 and 0.33, respectively. Similarly, for the Charolais breed, the regression coefficients between STAY2 and STAY3 were 0.86 for the threshold model and 0.83 for the Gaussian-linear model, and between STAY2 and STAY8, the coefficients were 0.21 and 0.32, respectively. In addition, to understand the extent of re-ranking between STAY2 with early (STAY3) and late (STAY8) calvings were analysed the top 100 sires based on their genomic values. By tracking their positions for STAY3 and STAY8, the changes in rankings were calculated, and the average re-ranking was determined. The average accuracy for sires in the Charolais breed was 0.24, 0.16, and 0.15 for STAY2, STAY3, and STAY8, respectively, using a Gaussian-linear model, and 0.45, 0.36, and 0.27 for STAY2, STAY3, and STAY8, respectively, using a threshold model. For the Limousine breed, the average accuracy for sires was 0.33, 0.29, and 0.22 for STAY2, STAY3, and STAY8, respectively, with a Gaussian-linear model, and 0.53, 0.51, and 0.39 for STAY2, STAY3, and STAY8, respectively, with a threshold model.

In both the Limousine and Charolais breeds, analyses using both Gaussian-linear and threshold models exhibited similar patterns (Figure 3.2), with a reduction in the regression coefficient between STAY2 and STAY8. Additionally, Spearman correlations among genotyped sires were assessed. For the Limousine breed, strong correlations between STAY2 and STAY3 were observed, with values of 0.70 for both the threshold and Gaussian-linear models. In contrast, the Charolais breed displayed moderate correlations, with values of 0.57 and 0.58 for the threshold and Gaussian-linear models, respectively. Lower correlations were found for both breeds when comparing STAY2 with STAY8. Specifically, Spearman's rank correlations were 0.24 and 0.20 for the Limousine breed using the threshold and Gaussian-linear models, respectively. For the Charolais breed, these values were 0.12 and 0.10 using the threshold and Gaussian-linear models, respectively.

The average re-ranking of sires between STAY2 and subsequent calvings revealed similar trends. In the Charolais breed, the average re-ranking was 26 positions for early (STAY3) and 32 for late (STAY8) calvings. For the Limousine breed, the average re-ranking was 21 positions for early (STAY3) and 31 for late (STAY8) calvings. These trends remained consistent across both models. Considering the regression coefficients from Figure 3.2 and the values from Spearman correlations among STAY traits, it appears that sires may be re-ranked across different parities, with more substantial re-ranking observed in more distant calvings. This potential re-ranking could lead to varying genetic gains. These findings suggest that STAY traits for different calvings represent distinct traits from a genomic perspective. Sires with high genomic values for STAY based on early calving may not necessarily maintain their performance in later calvings.

The reduction in the regression coefficient for STAY2 vs. STAY8 implies that selecting sires based on STAY8 might result in smaller improvements in STAY2 compared to selecting STAY3. This indicates that selecting sires for longevity would primarily result in genetic gains for STAY up to the second calving. The observed average re-ranking for Charolais and Limousine beef cattle among the top 100 sires indicates variability in sire performance between early and late calvings. This variability has significant implications for breeding strategies. Breeders should consider the potential for re-ranking when selecting sires based on early calving performance to ensure sustained genetic improvement across multiple calvings.

While assessments of STAY2 may be more predictive of early calving (STAY3), the relationship between STAY2 and STAY8 is lower, suggesting a weaker relationship between early and late STAY (Figure 3.2). More precisely, selecting sires based on STAY8 may result in smaller improvements in STAY2 than selecting sires based on STAY3. In this context, re-ranking sires based on STAY8 may prioritize those with consistently high longevity over the long term. Identifying sires with consistently high genomic values for STAY across both early and late calving emphasizes the importance of selecting sires based on their capacity to maintain superior STAY performance over time. This could contribute to the continual enhancement of functional longevity traits within the herd.

Jamrozik et al. (2013) reported a decreased genetic and phenotypic correlation among different STAY to calving in Canadian Simmental cattle. Authors found positive values, and the magnitude of correlations decreased with the increasing distance between parities, confirming that more distance STAY were not the same traits. In Nelore cattle, some authors analysed the regressions coefficient of the sires EBV for STAY at 5 and 6 years of age on the sires EBV for STAY at 7 years of age. They verified a reduction in the average genetic superiorities and expected progeny difference in STAY at 7 years of age if the selection was based on STAY at 5 and 6 years of age (Van Melis et al., 2007).

One of the objectives of this study was to test the potential of Gaussian-linear models to be used in place of threshold-liability models, for the genetic evaluation of functional longevity. While a threshold model is theoretically the optimal choice for the analysis of these groups of traits, the Gaussian model is considered more straightforward because it requires less computational effort and is easier to interpret. The results indicate that when genetic parameter estimates and sire rankings are similar between the two approaches, the Gaussian model may be preferable due to its simplicity and fewer assumptions. Therefore, if both models yield comparable results, selecting the simpler model is often advantageous. However, the final choice depends on specific analytical requirements and the context of application, with the threshold model remaining a valid option when it better captures the discrete nature of the trait.

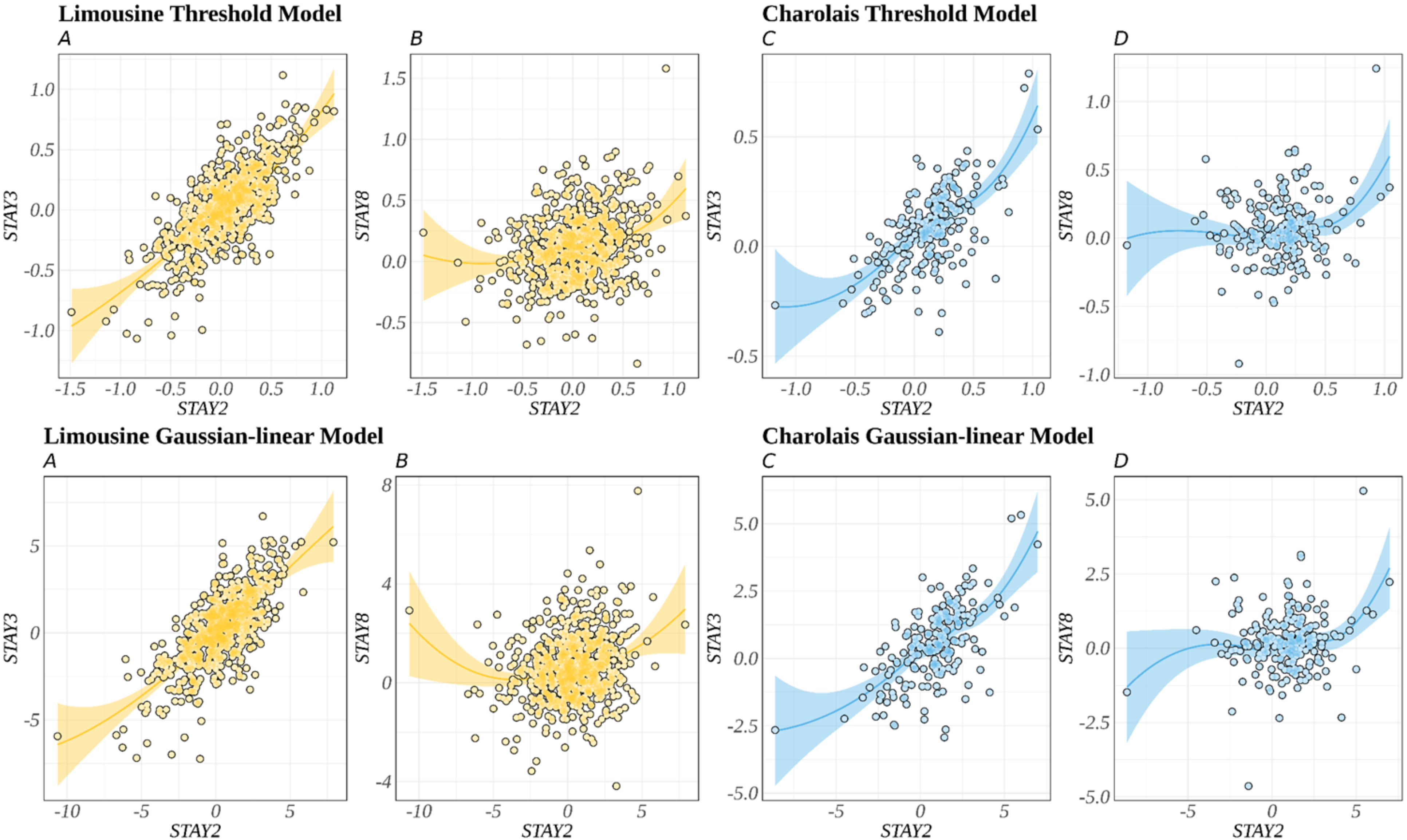


Figure 3.2 Regression coefficient for stay-ability (STAY) traits, more in specific between STAY2 with STAY3 and STAY8 among solutions for genotyped sires considering threshold and Gaussian-linear models for both breeds. (A, B) threshold and Gaussian-linear model for Limousine, respectively; (C, D) threshold and Gaussian-linear model for Charolais, respectively.

3.3.5 Genetic correlation

Genetic correlations were positive for all STAY traits for Charolais and Limousine breeds, ranging from moderate to high depending on the trait (Figure 3.3). Specifically, the correlations between STAY2 and STAY3 were 0.48 for Limousine and 0.23 for Charolais. For STAY3 and STAY4, the correlations were 0.75 for Limousine and 0.63 for Charolais. Between STAY4 and STAY5, the correlations were 0.56 for Limousine and 0.49 for Charolais. For STAY5 and STAY6, the correlations were 0.56 for Limousine and 0.37 for Charolais. The correlations between STAY6 and STAY7 were 0.39 for Limousine and 0.70 for Charolais. Finally, the correlations between STAY7 and STAY8 were 0.57 for Limousine and 0.68 for Charolais.

These solid and positive genetic correlations indicate a consistent genetic basis for these traits across different parities. A study on Holstein cows in US organic farms also reported strong and positive genetic correlations among STAY traits, yielding similar results to those in our study (Hardie et al., 2021).

Two fertility traits, AFC and FCI, were considered, and their genetic correlations with STAY, along with relative SE, are displayed in Figure 3.3. On average, these correlations were negative. For Limousine, AFC showed genetic correlations ranging from -0.07 for STAY2 to -0.26 for STAY8, with an increase in the negative correlation for later calvings. For FCI, moderate negative correlations were observed with STAY, with values ranging from -0.43 to -0.53 across different calvings. For Charolais, the genetic correlations for fertility traits were similar to those reported for Limousine. Age at first calving exhibited a similar trend, with an increasing negative genetic correlation across parities, except for STAY2, STAY3, and STAY4, where the correlations were not significantly different from zero. First calving interval showed strong and stable negative genetic correlations with STAY, ranging from -0.65 for STAY2 to -0.63 for STAY8.

Given the definition of STAY, negative correlations between STAY and AFC and FCI were expected. Animals that begin breeding earlier and have shorter intervals between calvings are more likely to achieve longevity. Buzanskas et al. (2010) analyzed the genetic association between STAY and reproductive traits in Canchim beef cattle (Charolais x Zebu) and found a negative genetic correlation of -0.63 ± 0.20 with AFC. Similarly, a study on the genetic association of reproductive traits with longevity in Nellore cattle reported negative genetic correlations with STAY (Rizzo et al., 2015). Fertility traits such as AFC and FCI provide potential indirect indicators that could be used in early selection for longevity in cattle breeding programs.

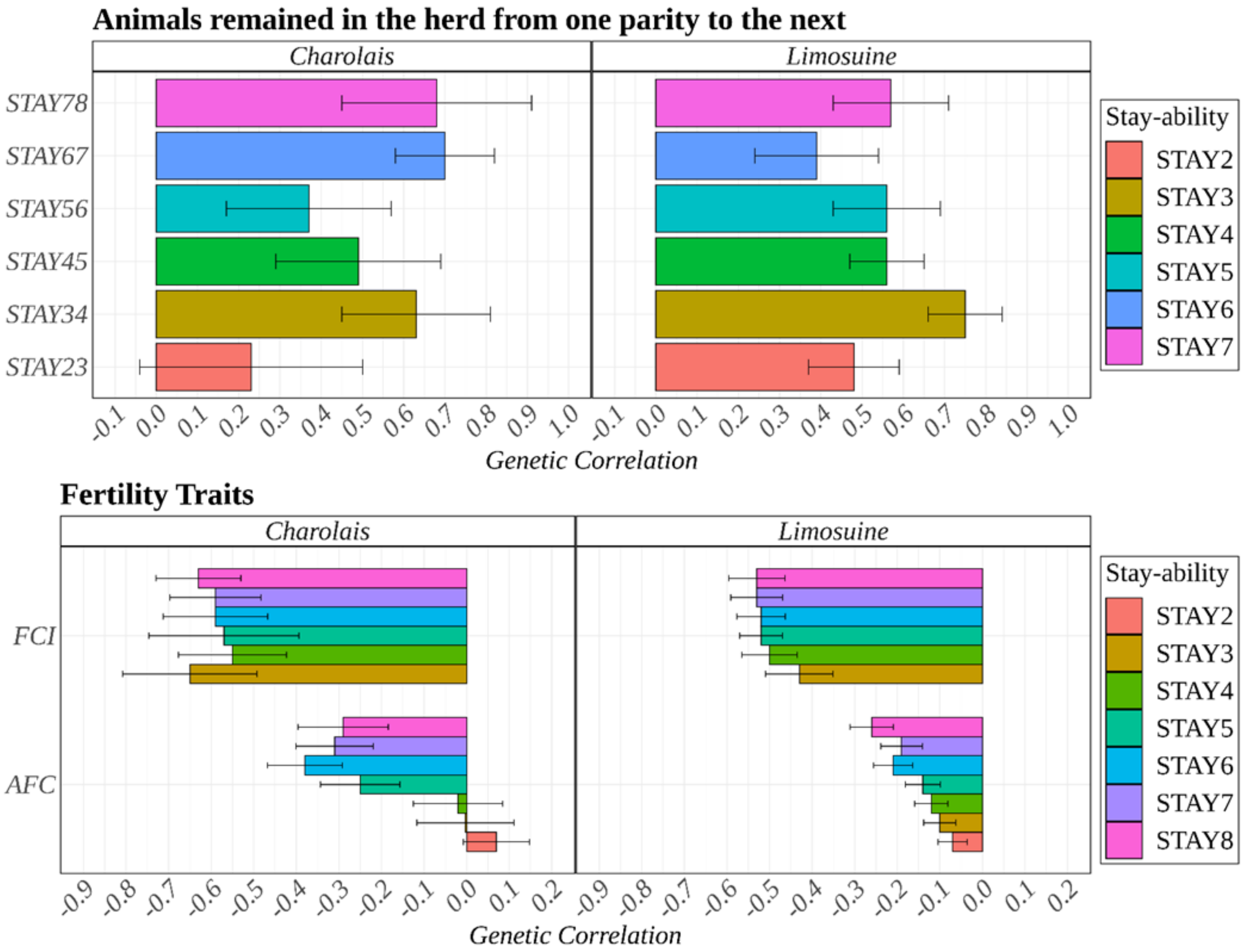


Figure 3.3 Genetic correlation between stay-ability (STAY) traits (STAY2 until STAY8) with fertility traits (FCI: first calving interval; AFC: age first calving) and animals remained in the herd from one parity to the next (STAY23 until STAY78), for Charolais and Limousine, respectively.

Figure 3.4 illustrates the genetic correlations between eight conformation traits and STAY traits for Limousine and Charolais. The analysis reveals notable differences between the two breeds in how these traits relate to cattle longevity from a genetic standpoint. For the Limousine breed, all correlations were positive and significantly different from zero. Mainly, traits such as pelvic length, dorsolumbar line length, development, and back width exhibited moderate genetic correlations with STAY traits, averaging above 0.20. This indicates that these specific conformation traits are likely to enhance the longevity of Limousine cattle due to their genetic influence.

In contrast, for the Charolais breed, many traits were essentially uncorrelated. This lack of significant correlation could be due to the fewer observations available for Charolais compared to Limousine cattle. Despite this, some conformation traits, such as pelvic length, dorsolumbar line length, and development, did show positive and significant correlations, mirroring the trends observed in Limousines. However, an exception was noted for rump convexity, which showed negative genetic correlations with STAY traits, suggesting that this particular trait may adversely affect the functional longevity of Charolais cattle. The observed differences in the relationship between longevity and conformation traits between Limousine and Charolais breeds could stem from various factors, including differences in herd sizes and genetic backgrounds. This variability highlights the importance of breed-specific strategies when selecting traits to improve cattle longevity.

Similarly to our results, many studies in the literature reported a positive genetic correlation between longevity and conformation linear type traits. Del Schneider et al. (2003) reported a strong relationship between feet and leg traits and functional herd life in Quebec Holsteins. The authors also noted a positive correlation between stature, size, and longevity, indicating that taller and larger cows have a better chance of survival. Additionally, Imbayarwo-Chikosi et al. (2018) and Sewalem et al. (2004) analysed various traits, including body height, chest width, loin strength, rump angle, rump width, foot angle, bone quality, rear leg, and conformation, exhibited positive genetic correlations with longevity. In Chianina cattle, a study on phenotypic correlation revealed a consistent trend showing increased longevity in cows with greater muscle development across all muscularity traits. More specifically, Chianina cows with a long body and a deep and broad chest had a higher probability of survival; this suggests that cows exhibiting higher muscle development are more likely to remain in the herd (Forabosco et al., 2004). Similar to our findings for Charolais, which revealed negative genetic correlations with rump convexity, Buenger et al. (2001) observed a decrease in functional herd life for cows exhibiting highly ascending rumps. In contrast to our results, a study conducted in the Pirenaica beef breed emphasized a negative correlation between longevity and conformation, probably because individuals with higher muscularity could present calving difficulties (Varona et al., 2012).

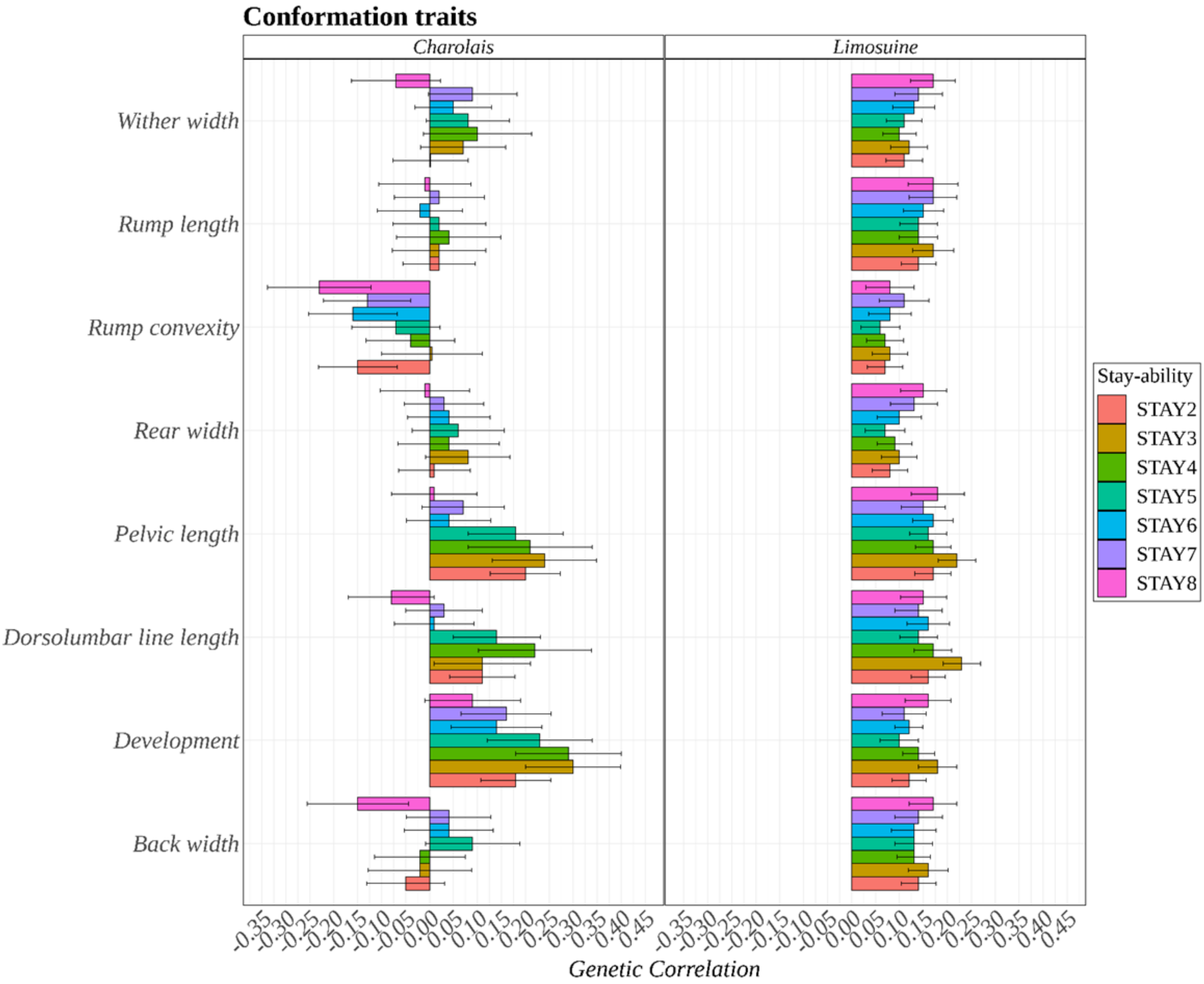


Figure 3.4 Genetic correlation between stay-ability (STAY) traits (STAY2 until STAY8) and ten conformation traits for Charolais and Limousine, respectively.

3.4 Conclusion

Longevity traits exhibited low to moderate heritability estimates using Gaussian-linear and threshold models. These heritability estimates for STAY suggest the potential for genetic improvement through selection. The moderate intra-herd heritability and herd effect estimation provide insights into the genetic and environmental factors influencing longevity within a herd. This knowledge enables breeders to enhance herd productivity and longevity through targeted breeding strategies. The regression coefficients, Spearman correlations, and average sire re-ranking observed in this study indicate significant re-ranking of sires between early and late calvings. Consequently, breeding strategies should account for these differences to ensure the selection of sires that maintain superior longevity traits across multiple calvings.

The strong positive genetic correlations for STAY across parities suggest that selecting for longevity traits can be effective in improving average herd longevity. However, the negative associations between STAY and fertility traits, such as age at first calving and first calving interval, can provide as indirect indicators to select cows with greater potential for early longevity selection. Cows that begin breeding earlier and have shorter calving intervals have a higher probability of remaining in the herd. For Limousine cattle, the positive correlations between conformation traits and functional longevity suggest that animals with higher conformation scores may have greater longevity, which can enhance herd productivity.

Overall, these results suggest that making selection decisions based on animal conformation could increase cows' robustness and resilience, thereby enhancing herd survival. Moreover, these findings emphasizing the selection for longevity could optimize productivity and sustainability in beef cattle farming. Further studies and continued investigation into the genomic background of these traits could improve cattle longevity and identify potential genomic regions associated with genes affecting STAY in Limousine and Charolais breeds.

3.5 Ethics approval

The data used in this study were part of a pre-existing dataset, so ethics approval was not needed.

3.6 Data and model availability statement

The datasets generated and/or analysed during the current study are not publicly available due owned by a third party, ANACLI, Associazione Nazionale degli Allevatori delle razze bovine Charolaise e Limousine Italiane (http://www.anacli.it/) but are available from the corresponding author on reasonable request.

3.7 Author ORCIDs

Simone Callegaro: https://orcid.org/0000-0002-9270-1845
Francesco Tiezzi: https://orcid.org/0000-0002-4358-9236
Christian Maltecca: https://orcid.org/0000-0002-9996-4680
Maria Chiara Fabbri: https://orcid.org/0000-0002-3224-745X
Riccardo Bozzi: https://orcid.org/0000-0001-8854-0834

3.8 Author contributions

Simone Callegaro: Conceptualization, methodology, software, formal analysis, writing – original draft
Francesco Tiezzi: Conceptualization, methodology, supervision, writing - review & editing
Christian Maltecca: Conceptualization, methodology, writing - review & editing
Maria Chiara Fabbri: Writing - review & editing
Riccardo Bozzi: Resources, supervision, funding acquisition, writing - review & editing

3.9 Declaration of interest

The authors declare no conflicts of interest.

3.10 Funding

This work was financially supported by Associazione Nazionale degli Allevatori delle razze bovine Charolaise e Limousine Italiane (Cup: J89H18000010005, URL: http://www.anacli.it/ibeef). Grant was received by RB. The funders had no role in study design and analysis, decision to publish, or manuscript preparation.

3.11 Literature cited

Aguilar, I., Misztal, I., Johnson, D.L., Legarra, A., Tsuruta, S., and Lawlor, T.J., 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. Journal of Dairy Science 93, 743–752. https://doi.org/10.3168/jds.2009-2730.

Aguilar, I., Tsuruta, S., Masuda, Y., Lourenco, D.A.L., Legarra, A., and Misztal, I., 2018. BLUPF90 suite of programs for animal breeding with focus on genomics. Journal of Animal Science 96, 341–342. https://doi.org/10.1093/jas/sky027.

Ahlman, T., Berglund, B., Rydhmer, L., and Strandberg, E., 2011. Culling reasons in organic and conventional dairy herds and genotype by environment interaction for longevity. Journal of Dairy Science 94, 1568–1575. https://doi.org/10.3168/jds.2010-3483.

Boligon, A.A., Mercadante, M.E.Z., and Albuquerque, L.G., 2011. Genetic associations of conformation, finishing precocity and muscling visual scores with mature weight in Nelore cattle. Livestock Science 135, 238–243. https://doi.org/10.1016/j.livsci.2010.07.011.

Bouquet, A., Venot, E., Laloë, D., Forabosco, F., Fogh, A., Pabiou, T., Moore, K., Eriksson, J.Å., Renand, G., and Phocas, F., 2011. Genetic structure of the European Charolais and Limousin cattle metapopulations using pedigree analyses. Journal of Animal Science 89, 1719–1730. https://doi.org/10.2527/jas.2010-3469.

Breslow, N.E., and Clayton, D.G., 1993. Approximate inference in generalized linear mixed models. Journal of the American Statistical Association 88, 9–25. https://doi.org/10.1080/01621459.1993.10594284.

Buenger, A., Ducrocq, V., and Swalve, H.H., 2001. Analysis of survival in dairy cows with supplementary data on type scores and housing systems from a region of Northwest Germany. Journal of Dairy Science 84, 1531–1541. https://doi.org/10.3168/jds.S0022-0302(01)70187-7.

Buonaiuto, G., Lopez-Villalobos, N., Costa, A., Niero, G., Degano, L., Mammi, L.M.E., Cavallini, D., Palmonari, A., Formigoni, A., and Visentin, G., 2023. Stayability in Simmental cattle as affected by muscularity and body condition score between calvings. Frontiers in Veterinary Science 10, 1141286. https://doi.org/10.3389/fvets.2023.1141286.

Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., and Lee, J.J., 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience 4, 7. https://doi.org/10.1186/s13742-015-0047-8.

Forabosco, F., Groen, A.F., Bozzi, R., Van Arendonk, J.A.M., Filippini, F., Boettcher, P., and Bijma, P., 2004. Phenotypic relationships between longevity, type traits, and production in Chianina beef cattle. Journal of Animal Science 82, 1572–1580. https://doi.org/10.2527/2004.8261572x.

Gianola, D., 1982. Theory and analysis of threshold characters. Journal of Animal Science 54, 1079–1096. https://doi.org/10.2527/jas1982.5451079x.

Hardie, L.C., Heins, B.J., and Dechow, C.D., 2021. Genetic parameters for stayability of Holsteins in US organic herds. Journal of Dairy Science 104, 4507–4515. https://doi.org/10.3168/jds.2020-19399.

Hu, H., Mu, T., Ma, Y., and Wang, X., 2021. Analysis of longevity traits in Holstein cattle: A review. Frontiers in Genetics 12, 695543. https://doi.org/10.3389/fgene.2021.695543.

Hu, H.H., Li, F., Mu, T., Han, L.Y., Feng, X.F., Ma, Y.F., Jiang, Y., Xue, X.S., Du, B.Q., Li, R.R., and Ma, Y., 2023. Genetic analysis of longevity and their associations with fertility traits in Holstein cattle. Animal 17, 100851. https://doi.org/10.1016/j.animal.2023.100851.

Hudson, G.F.S., and Van Vleck, L.D., 1981. Relationship between production and stayability in Holstein cattle. Journal of Dairy Science 64, 2246–2250. https://doi.org/10.3168/jds.S0022-0302(81)82836-6.

Imbayarwo-Chikosi, V.E., Ducrocq, V., Banga, C.B., Halimani, T.E., Van Wyk, J.B., Maiwashe, A., and Dzama, K., 2018. Impact of conformation traits on functional longevity in South African Holstein cattle. Animal Production Science 58, 481–489. https://doi.org/10.1071/AN16387.

Jamrozik, J., McGrath, S., Kemp, R.A., and Miller, S.P., 2013. Estimates of genetic parameters for stayability to consecutive calvings of Canadian Simmentals by random regression models. Journal of Animal Science 91, 3634–3643. https://doi.org/10.2527/jas.2012-6126.

Legarra, A., Christensen, O.F., Aguilar, I., and Misztal, I., 2014. Single step, a general approach for genomic selection. Livestock Science 166, 54–65. https://doi.org/10.1016/j.livsci.2014.04.029.

Martinez, G.E., Koch, R.M., Cundiff, L.V., Gregory, K.E., Kachman, S.D., and Van Vleck, L.D., 2005. Genetic parameters for stayability, stayability at calving, and stayability at weaning to specified ages for Hereford cows. Journal of Animal Science 83, 2033–2042. https://doi.org/10.2527/2005.8392033x.

Newman, S., Morris, C.A., Baker, R.L., and Nicoll, G.B., 1992. Genetic improvement of beef cattle in New Zealand: breeding objectives. Livestock Production Science 32, 111–130. https://doi.org/10.1016/S0301-6226(12)80031-5.

Plummer, M., Best, N., and Cowles, K., 2006. CODA: convergence diagnosis and output analysis for MCMC. R News 6, 7–11.

Pollak, E.J., Van Der Werf, J., and Quaas, R.L., 1984. Selection bias and multiple trait evaluation. Journal of Dairy Science 67, 1590–1595. https://doi.org/10.3168/jds.S0022-0302(84)81481-2.

Rizzo, E.C.A., Neto, F.R.A., Diaz, I.D.P.S., Dias, M.M., Costa, R.B., Ventura, H.T., Oliveira, H.N., and Falcão, A.J.S., 2015. Genetic association of productive and reproductive traits with stayability in Nellore cattle: analysis using Bayesian models. Genetics and Molecular Research 14, 14956–14966. https://doi.org/10.4238/2015.November.24.3.

Rosen, B.D., Bickhart, D.M., Schnabel, R.D., Koren, S., Elsik, C.G., Tseng, E., Rowan, T.N., Low, W.Y., Zimin, A., Couldrey, C., Hall, R., Li, W., Rhie, A., Ghurye, J., McKay, S.D., Thibaud-Nissen, F., Hoffman, J., Murdoch, B.M., Snelling, W.M., McDaneld, T.G., Hammond, J.A., Schwartz, J.C., Nandolo, W., Hagen, D.E., Dreischer, C., Schultheiss, S.J., Schroeder, S.G., Phillippy, A.M., Cole, J.B., Van Tassell, C.P., Liu, G., Smith, T.P.L., and Medrano, J.F., 2020. De novo assembly of the cattle reference genome with single-molecule sequencing. GigaScience 9, giaa021. https://doi.org/10.1093/gigascience/giaa021.

Santana, M.L., Eler, J.P., Ferraz, J.B.S., and Mattos, E.C., 2012. Genetic relationship between growth and reproductive traits in Nellore cattle. Animal 6, 565–570. https://doi.org/10.1017/S1751731111001856.

Santana, M.L., Eler, J.P., Bignardi, A.B., and Ferraz, J.B.S., 2013. Genetic associations among average annual productivity, growth traits, and stayability: a parallel between Nelore and composite beef cattle. Journal of Animal Science 91, 2566–2574. https://doi.org/10.2527/jas.2012-5856.

Sargolzaei, M., Chesnais, J.P., and Schenkel, F.S., 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics 15, 478. https://doi.org/10.1186/1471-2164-15-478.

Sewalem, A., Kistemaker, G.J., Miglior, F., and Van Doormaal, B.J., 2004. Analysis of the relationship between type traits and functional survival in Canadian Holsteins using a Weibull proportional hazards model. Journal of Dairy Science 87, 3938–3946. https://doi.org/10.3168/jds.S0022-0302(04)73533-X.

Sewalem, A., Kistemaker, G.J., Ducrocq, V., and Van Doormaal, B.J., 2005. Genetic analysis of herd life in Canadian dairy cattle on a lactation basis using a Weibull proportional hazards model. Journal of Dairy Science 88, 368–375. https://doi.org/10.3168/jds.S0022-0302(05)72696-5.

Silva, D.O., Santana, M.L., Ayres, D.R., Menezes, G.R.O., Silva, L.O.C., Nobre, P.R.C., and Pereira, R.J., 2018. Genetic parameters for stayability to consecutive calvings in Zebu cattle. Animal 12, 1807–1814. https://doi.org/10.1017/S1751731117003457.

Silva, D.O., Fernandes Júnior, G.A., Fonseca, L.F.S., Mota, L.F.M., Bresolin, T., Carvalheiro, R., and Albuquerque, L.G., 2024. Genome-wide association study for stayability at different calvings in Nellore beef cattle. BMC Genomics 25, 93. https://doi.org/10.1186/s12864-024-10020-y.

Smith, S.P., 1990. Survival, endurance and censored observations in animal breeding. In: Advances in Statistical Methods for Genetic Improvement of Livestock. Springer, Berlin, Heidelberg, pp. 344–360. https://doi.org/10.1007/978-3-642-74487-7_16.

Stephen, M.A., Burke, C.R., Pryce, J.E., Steele, N.M., Amer, P.R., Meier, S., Clair, C.V.C., and Garrick, D.J., 2023. Comparison of methods for deriving phenotypes from incomplete observation data with an application to age at puberty in dairy cattle. Journal of Animal Science and Biotechnology 14, 119. https://doi.org/10.1186/s40104-023-00921-5.

Van Melis, M.H., Eler, J.P., Oliveira, H.N., Rosa, G.J.M., Silva, J.A.V., Ferraz, J.B.S., and Pereira, E., 2007. Study of stayability in Nellore cows using a threshold model. Journal of Animal Science 85, 1780–1786. https://doi.org/10.2527/jas.2005-608.

VanRaden, P.M., 2008. Efficient methods to compute genomic predictions. Journal of Dairy Science 91, 4414–4423. https://doi.org/10.3168/jds.2007-0980.

Varona, L., Moreno, C., and Altarriba, J., 2012. Genetic correlation of longevity with growth, post-mortem, docility and some morphological traits in the Pirenaica beef cattle breed. Animal 6, 873–879. https://doi.org/10.1017/S1751731111002072.

3.12 Supplementary Material

3.12.1 Supplementary Table

Supplementary Table S3.1 Total number of cows used in the analyses and the percentage of censored data for each stayability (STAY) record, categorized by the number of records for Limousine and Charolais breeds.

Trait	Definition	Limousine		Charolais	
		N	Censored (%)	N	Censored (%)
STAY1	Stayability as a first parity = 1; failed = 0	38 188	0	9 174	0
STAY2	Stayability as a second parity = 1; failed = 0	33 209	13.04	8 071	12.02
STAY3	Stayability as a third parity = 1; failed = 0	30 105	21.17	7 519	18.04
STAY4	Stayability as a fourth parity = 1; failed = 0	27 978	26.74	7 078	22.85
STAY5	Stayability as a fifth parity = 1; failed = 0	26 546	30.49	6 774	26.16
STAY6	Stayability as a sixth parity = 1; failed = 0	25 372	33.56	6 550	28.60
STAY7	Stayability as a seventh parity = 1; failed = 0	24 429	36.03	6 381	30.45
STAY8	Stayability as a eight parity = 1; failed = 0	23 657	38.05	6 245	31.93

Abbreviations: STAY = Continuity in herd until the subsequent parity (Success = 1; Failure = 0); N = Total number of cows, including both those that survived and were culled (used in the genetic analyses), excluding censored data (which were treated as missing in the model)

Supplementary Table S3.2 Number of total conformation traits considered with the relative definition and related score for Limousine and Charolais.

Trait	Definition	N		Mean ± SD	
		Limousine	Charolais	Limousine	Charolais
Wither width	It is defined by the distance between the scapulae	32 119	7 537	5.86 ± 1.03	5.91 ± 0.92
Rump convexity	It is observed from the side, along with the width of the rear, and gives an idea of the development of the most valuable muscles of the thigh	32 119	7 537	6.04 ± 1.03	5.84 ± 1.07
Rump length	It is observed from the side and gives an idea of the length of the most valuable muscles of the thigh	32 119	7 537	5.82 ± 0.98	5.66 ± 1.01
Dorsolumbar line length	It measures the distance between the withers and the tip of the hips; in practice, it is measured by observing the distance between the front and rear legs, taking into account the development of the animal	32 119	7 537	6.51 ± 0.88	6.54 ± 0.84
Rear width	It is defined by the width of the thigh muscle, evaluated at mid-height	32 119	7 537	6.09 ± 1.04	6.10 ± 1.01
Back width	It defines the importance of the muscle just behind the scapulae	32 119	7 537	5.59 ± 1.12	5.50 ± 1.11
Pelvic length	It defines the length between the hips and the ischium and must be proportionate to the loin length	32 119	7 537	5.99 ± 0.89	6.01 ± 0.90
Development	It is defined by the size of the animal, which can be appreciated based on the height at the withers	32 119	7 537	6.05 ± 1.01	6.10 ± 0.93

Abbreviations: N = Total number of cows per each of the conformation traits considered from STAY2

Supplementary Table S3.3 Estimates of heritability, intra-herd heritability, and herd effect for stay-ability (STAY) traits for Limousine and Charolais using a single step GBLUP approach considering stayability (Gaussian-linear model) and liability of stayability (threshold model). Values within parentheses represent Highest Posterior Density Interval (HPDI).

Trait	Limousine						Charolais					
	Gaussian-linear model			Threshold model			Gaussian-linear model			Threshold model		
	h^2	h^2_{IH}	h_i	h^2	h^2_{IH}	h_i	h^2	h^2_{IH}	h_i	h^2	h^2_{IH}	h_i
STAY2	0.13 (0.11; 0.16)	0.18 (0.15; 0.21)	0.25 (0.23; 0.27)	0.21 (0.17; 0.24)	0.32 (0.26; 0.38)	0.34 (0.31; 0.37)	0.14 (0.10; 0.18)	0.17 (0.11; 0.22)	0.20 (0.16; 0.23)	0.21 (0.14; 0.27)	0.30 (0.20; 0.40)	0.30 (0.25; 0.36)
STAY3	0.12 (0.10; 0.14)	0.16 (0.13; 0.19)	0.24 (0.22; 0.26)	0.18 (0.15; 0.21)	0.30 (0.25; 0.35)	0.39 (0.36; 0.42)	0.08 (0.04; 0.13)	0.11 (0.06; 0.15)	0.23 (0.19; 0.26)	0.11 (0.06; 0.17)	0.19 (0.11; 0.26)	0.40 (0.34; 0.44)
STAY4	0.13 (0.10; 0.15)	0.17 (0.13; 0.19)	0.22 (0.20; 0.24)	0.19 (0.16; 0.22)	0.34 (0.29; 0.38)	0.43 (0.40; 0.46)	0.09 (0.04; 0.11)	0.11 (0.06; 0.15)	0.20 (0.17; 0.24)	0.11 (0.05; 0.15)	0.20 (0.13; 0.28)	0.40 (0.34; 0.45)
STAY5	0.13 (0.11; 0.15)	0.16 (0.13; 0.18)	0.20 (0.18; 0.21)	0.18 (0.15; 0.21)	0.33 (0.26; 0.39)	0.45 (0.41; 0.48)	0.11 (0.06; 0.13)	0.13 (0.09; 0.18)	0.20 (0.17; 0.24)	0.16 (0.10; 0.21)	0.27 (0.18; 0.36)	0.43 (0.36; 0.49)
STAY6	0.11 (0.09; 0.13)	0.13 (0.11; 0.15)	0.17 (0.15; 0.19)	0.16 (0.12; 0.19)	0.30 (0.26; 0.35)	0.45 (0.41; 0.49)	0.11 (0.07; 0.15)	0.14 (0.09; 0.18)	0.17 (0.15; 0.21)	0.17 (0.11; 0.23)	0.31 (0.22; 0.41)	0.45 (0.38; 0.53)
STAY7	0.10 (0.08; 0.12)	0.12 (0.10; 0.14)	0.16 (0.14; 0.17)	0.10 (0.08; 0.13)	0.23 (0.19; 0.28)	0.51 (0.45; 0.56)	0.13 (0.08; 0.16)	0.15 (0.11; 0.19)	0.14 (0.11; 0.17)	0.24 (0.17; 0.31)	0.40 (0.30; 0.50)	0.41 (0.33; 0.49)
STAY8	0.11 (0.10; 0.13)	0.13 (0.11; 0.15)	0.15 (0.13; 0.16)	0.14 (0.02; 0.20)	0.33 (0.26; 0.41)	0.56 (0.43; 0.91)	0.11 (0.07; 0.14)	0.12 (0.08; 0.16)	0.12 (0.10; 0.15)	0.19 (0.10; 0.26)	0.34 (0.18; 0.44)	0.45 (0.36; 0.54)

Abbreviations: h^2 = heritability; h^2_{IH} = intra-herd heritability; h_i= herd effect; STAY2 = stayability to parity 2; STAY3 = stayability to parity 3; STAY4 = stayability to parity 4; STAY5 = stayability to parity 5; STAY6 = stayability to parity 6; STAY7 = stayability to parity 7; STAY8 = stayability to parity 8.

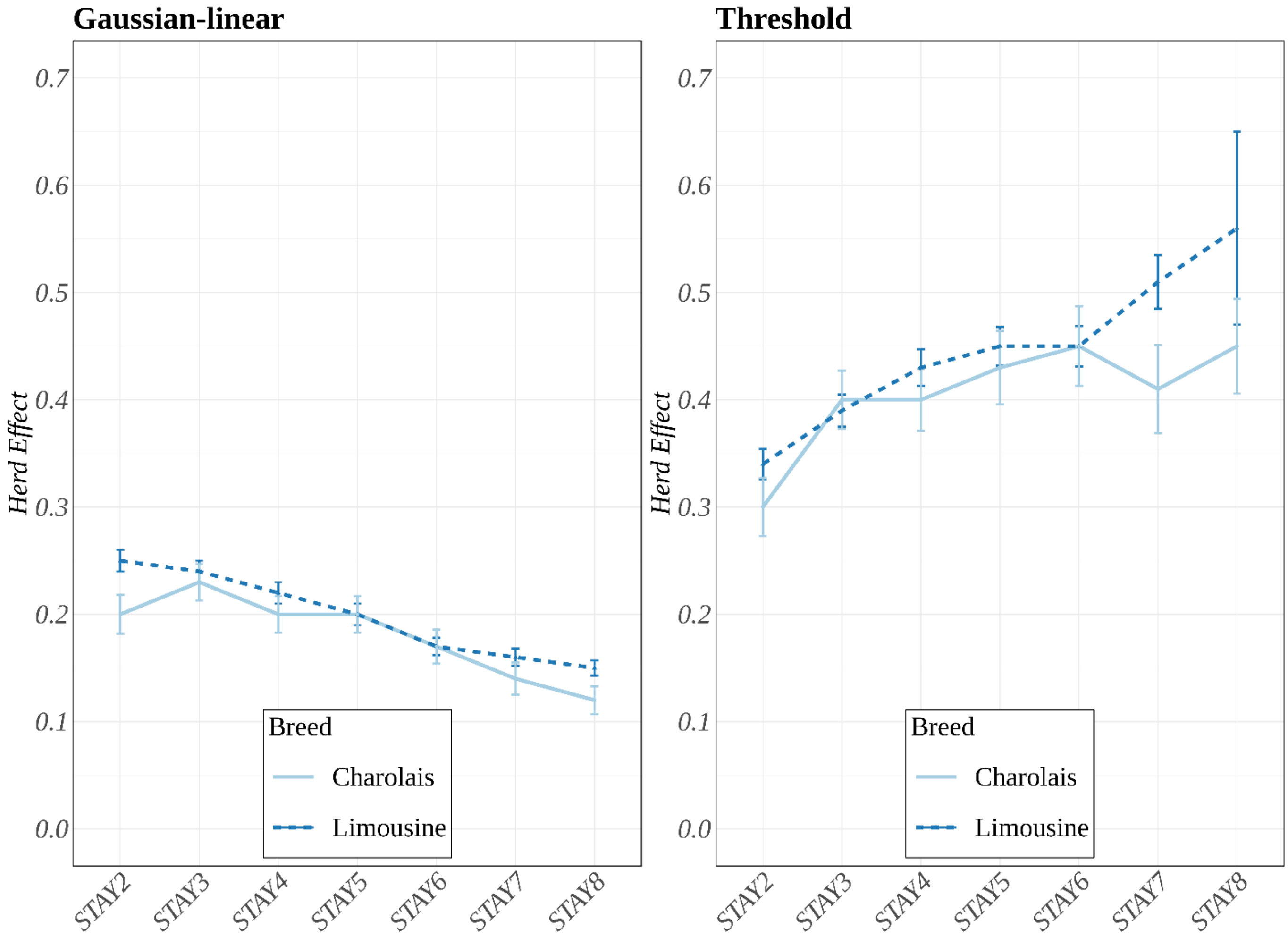
Supplementary Table S3.4 Estimates of variance components for STAY traits Limousine and Charolais using a single step GBLUP approach considering stayability (Gaussian-linear model) and liability of stayability (threshold model). Values within parentheses represent Highest Posterior Density Interval (HPDI).

Trait	Limousine				Charolais			
	Gaussian-linear model		Threshold model		Gaussian-linear model		Threshold model	
	σ_a^2	σ_h^2	σ_a^2	σ_h^2	σ_a^2	σ_h^2	σ_a^2	σ_h^2
STAY2	16.21 $^{(13.40; 19.39)}$	30.20 $^{(27.07; 32.96)}$	0.46 $^{(0.35; 0.56)}$	0.76 $^{(0.66; 0.86)}$	19.56 $^{(14.19; 25.11)}$	28.06 $^{(22.260 34.21)}$	0.43 $^{(0.23; 0.61)}$	0.63 $^{(0.48; 0.81)}$
STAY3	17.69 $^{(14.33; 21.13)}$	35.42 $^{(31.88; 38.97)}$	0.43 $^{(0.35; 0.52)}$	0.93 $^{(0.80; 1.04)}$	13.05 $^{(6.91; 19.89)}$	35.63 $^{(29.30; 42.88)}$	0.24 $^{(0.11; 0.38)}$	0.82 $^{(0.65; 1.01)}$
STAY4	18.81 $^{(15.74; 22.62)}$	32.16 $^{(28.97; 35.24)}$	0.49 $^{(0.37; 0.60)}$	1.11 $^{(0.96; 1.23)}$	10.89 $^{(5.92; 16.51)}$	28.96 $^{(23.80; 35.18)}$	0.22 $^{(0.09; 0.34)}$	0.81 $^{(0.63; 1.04)}$
STAY5	17.53 $^{(14.59; 20.47)}$	27.12 $^{(24.47; 29.92)}$	0.50 $^{(0.39; 0.61)}$	1.22 $^{(1.05; 1.42)}$	11.80 $^{(7.21; 15.78)}$	24.32 $^{(19.57; 29.20)}$	0.33 $^{(0.16; 0.48)}$	1.01 $^{(0.75; 1.27)}$
STAY6	13.54 $^{(11.25; 15.87)}$	21.34 $^{(18.99; 23.59)}$	0.41 $^{(0.31; 0.51)}$	1.14 $^{(0.97; 1.23)}$	10.97 $^{(7.23; 14.69)}$	17.37 $^{(13.95; 21.13)}$	0.42 $^{(0.22; 0.63)}$	1.17 $^{(0.85; 1.54)}$
STAY7	11.14 $^{(9.24; 13.25)}$	16.91 $^{(14.99; 18.93)}$	0.25 $^{(0.19; 0.32)}$	1.24 $^{(0.99; 1.50)}$	9.29 $^{(6.30; 12.70)}$	10.96 $^{(8.71; 13.50)}$	0.63 $^{(0.35; 0.97)}$	1.15 $^{(0.81; 1.6)}$
STAY8	10.61 $^{(8.92; 12.17)}$	13.63 $^{(12.21; 15.12)}$	0.45 $^{(0.29; 0.62)}$	2.98 $^{(0.76; 10.75)}$	5.92 $^{(3.78; 8.20)}$	7.09 $^{(5.48; 8.70)}$	0.45 $^{(0.24; 0.66)}$	1.20 $^{(0.78; 1.64)}$

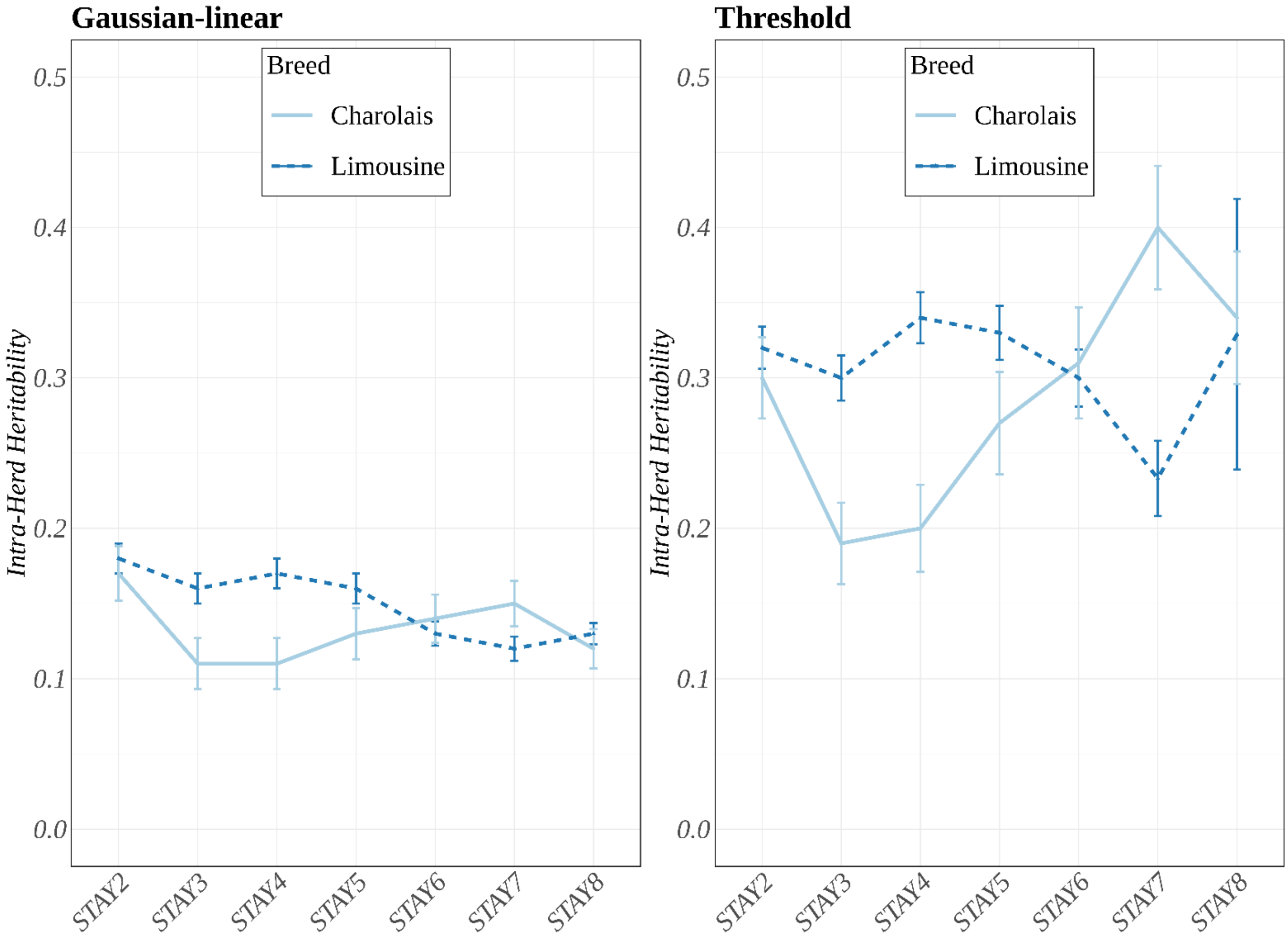
Abbreviations: σ_a^2 = additive genetic variance; σ_h^2= herd variance; STAY2 = stayability to parity 2; STAY3 = stayability to parity 3; STAY4 = stayability to parity 4; STAY5 = stayability to parity 5; STAY6 = stayability to parity 6; STAY7 = stayability to parity 7; STAY8 = stayability to parity 8.

3.13.2 Supplementary Figure

Supplementary Figure S3.1 Trends of herd effect with the relative SE for stay-ability (STAY) traits (STAY2 until STAY8) for Limousine and Charolais using a single step GBLUP approach considering STAY (Gaussian-linear model) and liability of STAY (threshold model), respectively.



Supplementary Figure S3.2 Trends of intra-herd heritability with the relative SE for stay-ability (STAY) traits (STAY2 until STAY8) for Limousine and Charolais using a single step GBLUP approach considering STAY (Gaussian-linear model) and liability of STAY (threshold model), respectively.



CHAPTER 4

4. Genomic insights into female productivity in Limousine cattle: a single-step genome-wide association on longevity, fertility, and conformation traits

Simone Callegaro[a], Christian Maltecca[a,b], Francesco Tiezzi[a], Maria Chiara Fabbri[a], Riccardo Bozzi[a]

[a] Department of Agriculture, Food, Environment, and Forestry (DAGRI), University of Florence, Florence, 50144, Italy

[b] Department of Animal Science, North Carolina State University, Raleigh, NC, 27695, USA

Corresponding author: simone.callegaro@unifi.it

Submitted to BMC Genomics journal on 6 October 2025

Abstract

Background: Improving female efficiency and resilience is a priority for beef cattle systems, where reproductive failure and early culling significantly affect sustainability and profitability. Longevity, fertility, and conformation traits provide complementary indicators of female performance, but their complex genetic architecture has limited progress in selection. This study aimed to investigate the genetic basis of these traits in the Italian Limousine population using a single-step genome-wide association, which integrates pedigree, phenotypic, and genomic information.

Results: The final dataset included 38,188 cows for longevity, 38,188 for fertility traits (age at first calving and first calving interval), and 32,316 for conformation traits, with 2,489 genotyped females entering the analyses. Heritability estimates were moderate for conformation (0.19–0.23) and for longevity at early parities (up to 0.21), lower for later parities (0.10–0.14), and moderate to low for fertility (0.18 for age at first calving, 0.14 for first calving interval). The single-step genome-wide association identified several genomic regions explaining more than 1% of the additive variance, a subset of which was validated by a bootstrapping test. Key candidate genes overlap among different traits included *KHDRBS2* (longevity and conformation), *CPEB4* (longevity, fertility, and conformation), *TOX* (longevity, age at first calving, and structural traits), and the *LAP3–NCAPG–LCORL* cluster (longevity and fertility). Functional annotation of overlapping quantitative trait loci highlighted pathways related to reproduction, health, and carcass quality, supporting pleiotropic effects across traits.

Conclusions: This study provides new insights into the genetic architecture of longevity, fertility, and conformation in Limousine cattle. The identification of pleiotropic loci and shared genomic regions supports the development of multi-trait genomic selection strategies. Targeting early expressed indicators such as age at first calving, longevity at early parities, and conformation could accelerate genetic progress, reduce replacement costs, and enhance the sustainability, resilience, and profitability of beef production systems.

Keywords: Longevity, fertility, animal conformation, genome-wide association study, beef cattle, pleiotropy

4.1 Background

Reproductive efficiency is a key driver of productivity and profitability in beef cattle systems, as it directly influences the number of animals available for both selection and harvest (Silva et al., 2024). One of the major economic challenges in these systems is the cost of raising replacement heifers, which can significantly impact overall profitability (Callegaro et al., 2024). Therefore, this aspect makes the reduction of involuntary culling one of the main priorities for sustainable herd management. In this context, longevity traits such as stayability (**STAY**) represent key breeding objectives. Animals with higher genetic merit for STAY contribute to reduce culling and turnover rates, lower replacement costs, and improved herd efficiency and profitability across multiple generations (Rizzo et al., 2015; Callegaro et al., 2024).

In parallel, fertility traits are essential for maintaining optimal reproductive performance and are genetically correlated with longevity, making them valuable indirect indicators for selecting cows with improved reproductive lifespan (Hu et al., 2021). Improving fertility not only increases reproductive efficiency but also extends herd longevity by reducing the risk of early culling due to reproductive failure. Finally, conformation traits, which reflect an animal's structural depth and physical development, could influence health, adaptability, and resilience. Traits such as pelvic dimensions, rump structure, and overall body proportionality affect calving ease, maternal capacity, mobility, and resilience to management and environmental stressors. These functional aspects of conformation ultimately influence survival rates and lifetime productivity, making them relevant indicators of both biological fitness and economic performance (Callegaro et al., 2024).

In Italy, selection programs for the Limousine breed have traditionally emphasized growth and muscular development traits, reflecting market demand for carcass quality. More recently, breeding goals have expanded to include functional traits such as fertility and longevity, driven by the need to enhance the sustainability, resilience, and profitability of beef cattle systems. This shift highlights the importance of investigating the genetic basis of reproductive performance, longevity, and conformation, specifically in the Limousine population.

The Limousine is a cosmopolitan beef cattle breed originally developed in France and exported worldwide. It is typically raised either as a purebred or as a terminal sire in local dairy crossbreeding systems (Bouquet et al., 2011). In Italy, the breed has a strong presence throughout the country, with higher concentrations in Central and Southern regions. According to the national herd book managed by the National Italian Association of Limousine and Charolais Breeders, approximately 100 000 animals are registered in Italy, including about 46 123 cows and 10 614 heifers, distributed across 2 558 officially recognized herds (https://www.anacli.it/libro-genealogico/razze/charolaise-limousine).

Taken together, longevity, fertility, and conformation traits provide complementary perspectives on female efficiency. Their integration into genomic studies offers an opportunity to improve the sustainability, resilience, and economic performance of beef cattle systems. Additionally, joint investigation of these traits is therefore crucial to understanding the biological mechanisms underlying productivity and to assessing more effective breeding strategies. Genetic improvement for complex traits such as STAY and fertility remains challenging due to their low to moderate heritability, late expression or sex limitation, as in the case of STAY, and the considerable influence of environmental and management factors (Jamrozik et al., 2013; Callegaro et al., 2024). Moreover, these traits often involve complex genetic architectures that further complicate selection.

Genome-wide association studies (**GWAS**) have been widely applied over the past two decades to identify quantitative trait loci (**QTL**) associated with economically important traits in cattle (Raven et al., 2014; Chen et al., 2022). Traditionally, these studies have focused on cosmopolitan dairy breeds, where the availability of large, genotyped populations facilitated the detection of genomic regions influencing complex traits. More recently, the increasing availability of genotypic data collected directly from female cattle has opened new opportunities for GWAS, particularly for traits with low heritability or those that are difficult to measure both in dairy and beef cattle (Speidel et al., 2018; Pedrosa et al., 2023; Bernini et al., 2024; Silva et al., 2024) such as reproductive performance, longevity, and functional traits. GWAS approaches typically require large numbers of genotyped animals, which are not always available in beef cattle populations where genotyping is still expanding. In parallel, new approaches have led to the development the single-step GWAS (**ssGWAS**) integrates pedigree, phenotypic, and genomic information within a single analytical framework (Wang et al., 2012). This approach has been widely applied in GWAS studies across livestock populations (Wang et al., 2012; Tiezzi et al., 2015; Aguilar et al., 2019; Silva et al., 2024).

Only a limited number of GWAS have previously been conducted to investigate the genetic basis of stayability in cattle, including general STAY (Engle et al., 2018, Teixeira et al., 2017) and calving-specific measures of STAY (Silva et al., 2024). Similarly, fertility traits have been explored in beef cattle through GWAS to identify genomic regions affecting reproductive performance (Keogh et al., 2021; Stegemiller et al., 2021) while conformation traits have been investigated as indicators of functionality and fitness (Yu et al., 2023; Colombi et al., 2024; Doyle et al., 2020a).

The aim of this study was to perform a ssGWAS analysis in Limousine cattle to identify genomic regions and candidate pathways associated with longevity, fertility, and conformation traits expressed throughout the productive lifespan of females. By leveraging integrated pedigree, phenotypic, and genomic data, the study seeks to provide new insights into the genetic architecture of these complex traits. The objective of this study was to identify genomic regions and genes shared across traits to uncover potential pleiotropic effects or biological mechanisms influencing multiple aspects of female efficiency and productivity. Achieving this goal may ultimately support the development of more efficient and targeted genomic selection strategies in beef cattle breeding.

4.2 Materials and methods

The data used in this study were obtained from the official database of the National Italian Association of Limousine and Charolais Breeders (**ANACLI**). As the study relied exclusively on pre-existing records, approval from an Animal Care and Use Committee was not required. Phenotypic, pedigree, and genomic information were retrieved from this association database.

4.2.1 Phenotypic database

Stayability and fertility traits. STAY was used as an indicator of female longevity and was evaluated from the second to the eighth calving to capture both early and late stages of reproductive life. STAY was defined as the probability of a cow remaining in the herd and producing up to a given parity, following the definition given by Hudson and Van Vleck. (Hudson and Van Vleck, 1981). For each parity, STAY was treated as a binary trait, where a value of "1" indicated that the cow had a calving record for that parity (i.e., survived to that parity), and "0" indicated no calving record (i.e., left the herd or failure). The initial dataset available comprised 460 731 calving records from 107 792 cows born between 1977 and 2023, coming from 3 228 registered herds. Fertility traits were extracted from cows included in the STAY dataset. The traits analysed were age at first calving (**AFC**, in days), and first calving interval (**FCI**, in days, defined as the number of days between the first and second calving), both treated as continuous variables. Mean values for FCI and AFC were 405.7 ± 60.87 days and 1 026 ± 143.69 days, respectively.

Data editing for STAY and fertility traits followed the procedure described in Callegaro et al. (2024). STAY was defined as a binary trait indicating whether a cow survived up to a given parity, conditional on calving in the previous one. Seven cumulative traits were derived (STAY2–STAY8), corresponding to survival from the second through the eighth calving. Data editing retained only cows with age at first calving between 700 and 1 400 days, and calving intervals within 290–550 days. Twin parities were treated as single events. Records with incomplete calving histories at the time of data extraction were classified as censored and treated as missing, while cows with no sire or dam information were removed. The final dataset included 38 188 cows, daughters of 4 900 sires, and raised in 2 121 herds. Additionally, descriptive statistics and rate of survival for each of the STAY are described in Callegaro et al. (2024), and reported in Table S4.1 in the Additional file 1.

Conformation-type traits. Conformation traits were assessed once in the lifetime of each animal, between 6 and 15 months of age, around either weaning or yearling. Evaluations were performed between 1990 and 2023 by trained classifiers using a standardized visual scoring system based on the ANACLI Technical Standards for the Limousine breed (ANACLI; https://www.anacli.it/public/NormeTecniche2015.pdf). Each trait was scored on a scale from 1 to 10, with 10 representing the highest expression and 1 the lowest. In this study, we focused on eight conformation traits related to muscularity and skeletal structure, especially those associated with the posterior region of the animal, which are economically relevant and included in national selection indices. The traits evaluated were wither width, rump convexity, rump length, dorsolumbar length, rear width, back width, pelvic length, and overall development. These traits assess muscular development, skeletal conformation, and body proportionality. Additionally, traits like rump length and overall size capture general body development. The final dataset included 32 316 measured cows. Detailed trait definitions, measurement protocols, and descriptive statistics are provided in Callegaro et al. 2024 and in Supplementary Table S4.2 (Additional File 1), which follows the ANACLI technical standards. Descriptive statistics for the Limousine population indicated moderate variation: wither width (5.86 ± 1.03), rump convexity (6.04 ± 1.03), rump length (5.82 ± 0.98), dorsolumbar line length (6.51 ± 0.88), rear width (6.09 ± 1.04), back width (5.59 ± 1.12), pelvic length (5.99 ± 0.89), and overall development (6.05 ± 1.01).

4.2.2 Pedigree and genotypic data

The initial pedigree dataset for the Limousine breed included records for 526 887 animals, comprising 15 025 sires and 123 701 dams. Pedigree records were traced back 6 generations to include all animals relevant for the subsequent ssGWAS. For these analyses, genotypic information was available for 4 054 Limousine animals, comprising 2 489 females with phenotypic records, 1 012 sires, and 707 dams without their own phenotypes.

At the population level, a set of 8 903 Limousine animals had been genotyped and was used for the imputation. Among these, 421 sires were genotyped with the GeneSeek GGP Bovine 150K panel (119 854 SNPs), while 8 482 animals were genotyped with the GeneSeek GGP Bovine LD v3 panel (28 299 SNPs), sharing 13 984 common SNPs. To increase marker density and harmonize datasets, all genotypes were imputed to the 150K panel using a multi-step pipeline. LD v3 genotypes were first imputed to the 150K panel, and then an additional 13 670 unique SNPs were integrated, resulting in a final dataset of 131 805 SNPs. Imputation was performed with FImpute v.3 using default parameters (Sargolzaei et al., 2014). Full details of the imputation strategy and pipeline are provided in Callegaro et al. (2024). Briefly, imputation accuracy was high, with a squared correlation (R^2) of 0.99 between observed and imputed allele frequencies. 94–98% of SNPs exhibiting a minor allele frequency (**MAF**) above than 0.05. Standard quality control metrics were applied, including removal of SNPs with MAF <0.01 and individuals with major genotype errors (>1% change). Masked-marker validation, removing a random subset of SNPs and then re-imputed using the same pipeline, confirmed consistent results (see Additional file 1, Table S4.3).

Additional quality control steps were applied to the imputed dataset. Individuals with a call rate <0.90, SNPs with MAF <0.05%, and a call rate <0.90 were removed. After these filtering procedures, 117 207 SNPs were retained for follow-up statistical and genome-wide association analyses.

4.2.3 Statistical analysis

The following statistical model was applied to fertility traits (AFC and FCI, modelled as a Gaussian trait) and to STAY (as a binary trait):

$$\lambda \text{ or } y = \mathbf{X}_{\boldsymbol{\beta}} + \mathbf{Z}_{\mathbf{p}}\mathrm{p} + \mathbf{Z}_{\mathbf{a}}\mathrm{a} + \mathrm{e},$$

where y represents the vector reporting the fertility phenotypes (AFC and FCI) and λ the unobserved liability for threshold models applied to STAY; β is the vector of fixed effects, specifically the year of calving (48 levels); p is the vector of the random effect of herd; a is the vector for the animal additive genetic effect; e is the vector containing the residual error. For conformation traits, the same model structure was used, with the addition of a fixed effect accounting for the classifier's code (i.e., the trainer evaluating the animals).

In both models, $\mathbf{X}$, $\mathbf{Z_p}$, $\mathbf{Z_a}$, are the incidence matrix related to fixed, random, and additive genetic effects, respectively. The additive genetic effect was assumed to follow a normal distribution $\mathrm{a} \sim N(0, \mathbf{H}\sigma_a^2)$, where **H** was the relationship matrix and σ_a^2 was the additive genetic variance. The herd effect was assumed to follow $\mathbf{p} \sim N(0, \mathbf{I}\sigma_\mathrm{h}^2)$, where **I** was an identity matrix and σ_h^2 was the herd variance. Residuals were assumed to follow $\mathrm{e} \sim N(0, \mathbf{I}\sigma_e^2)$, where **I** was the identity matrix and σ_e^2 was the residual variance.

Variance components and heritabilities were obtained through Gibbs Sampling using the software GIBBS3F90 and THRGIBBSF90 (Aguilar et al., 2018) for linear and threshold models, respectively. SNP effects were estimated using postGSF90 (Aguilar et al., 2014). A total of 100 000 iterations were performed, with the first 50 000 discarded as burn-in and a thinning interval of 10 retained samples. Variance components were estimated using single-trait animal models. Convergence was assessed by visual inspection of trace plots and Geweke's test.

4.2.4 Single-step genome-wide association study

The GWAS was performed using the single-step genomic BLUP approach (Aguilar et al., 2010) (Christensen and Lund, 2010). The **H** matrix combines the pedigree relationship matrix (**A**) and the genomic relationship matrix (**G**) (Legarra et al., 2009). The **G** matrix was computed using the second method of VanRaden (2008) in which marker contributions are weighted by their expected variance:

$$\mathbf{G} = \mathbf{ZDZ'}$$

where **Z** was the centered genotype matrix (i.e., genotypes corrected for allele frequency), and **D** was a diagonal matrix with elements $\mathbf{D}_{\mathrm{ii}} = 1/\mathrm{m}[2\mathrm{p}_\mathrm{i}(1 - \mathrm{p}_\mathrm{i})]$, representing the inverse of the expected marker variance, p_i is the allele frequency for the i_{th} SNP. The inverse of the **H** matrix (**H⁻¹**) was incorporated into the mixed model equations to solve the single-step genomic BLUP.

After obtaining genomic estimated breeding values (**GEBV**, â) for genotyped animals, SNP effects were back-solved using postGSF90, following the procedure in Wang et al. (2012). The SNP effect vector u was derived from:

$$\mathrm{var}\begin{bmatrix}\hat{\mathrm{a}}\\ \mathrm{u}\end{bmatrix} = \begin{bmatrix}\mathbf{ZDZ'} & \mathbf{ZD'}\\ \mathbf{DZ'} & \mathbf{D}\end{bmatrix}\sigma_\mathrm{u}^2$$

Individual marker effects were obtained by solving:

$$\mathrm{u} = \mathbf{DZ'G}^{-1}\,\hat{\mathrm{a}}$$

where u was the vector with SNP effects, **Z** was a matrix with genotypes corrected by allele frequency**,** **D** was a diagonal matrix containing the weights for each SNP marker, **G** was the genomic relationship matrix, and â was the vector of GEBV for genotyped animals.

A two-step reweighting procedure was applied to better capture the variance explained by each SNP. In the first step, marker variances were estimated using $2\mathrm{p}_\mathrm{i}(1 - \mathrm{p}_\mathrm{i})\mathrm{u}^2$, where p is the frequency of one of the 2 alleles. In the second iteration, a new weighted G matrix was constructed using the realized variances derived from the first round. This updated **D** matrix replaced expected variances with the observed SNP-specific variances. SNP effects were then re-estimated using the updated **G** matrix.

To identify genomic regions contributing significantly to trait variation, a 20-SNP moving window approach was applied to the estimated SNP effects across the genome. For downstream analysis, only windows explaining more than 1% of the total genetic variance were retained for gene annotation and QTL mapping. This method accounts for the fact that adjacent SNPs in strong linkage disequilibrium (**LD**) may jointly mark the same QTL. Sliding windows help to reduce statistical noise and to improve the detection of meaningful signals (Sun et al., 2011). Although Beissinger et al. (2015) suggested that 5- to 10-SNP windows may offer a favourable trade-off between power and false discovery rate, a 20-SNP window was adopted here to improve robustness across LD block sizes.

4.2.5 Significance threshold estimation via bootstrapping and candidate gene identification

Genomic windows explaining more than 1% of the total additive genetic variance were retained and tested for significance using a non-parametric bootstrap procedure with 100 replicates, following the strategy outlined by Howard et al. (2015) and Bergamaschi et al. (2020). To account for differences in SNP contributions across bootstrap replicates, we re-ran the single-step GWAS in a two-step procedure in which SNPs with null effects were replaced and marker weights were updated accordingly**.** For each replicate, the SNP effects in the candidate windows were replaced with values randomly sampled from the distribution of non-significant SNPs, based on their overall mean and standard deviation. These adjusted effect estimates were used to recalculate altered GEBV and corresponding sliding window variances, simulating a null distribution under no association. Although the software provides p-value for individual SNP effects, the bootstrap was implemented to derive empirical significance thresholds for the variance explained by 20-SNP sliding windows, which lack a direct p-value and require an approach more appropriate for polygenic traits.

For each window, a p-value was calculated as the proportion of bootstrap replicates in which the variance explained under the null distribution exceeded that of the observed data. Genomic windows that both surpassed the 1% variance threshold and were significant in the bootstrap analysis ($p < 0.05$) were retained for downstream functional annotation and visualization via Manhattan plots. Using the start and end coordinates of each window (Additional file 2, 3, and 4 for STAY, fertility, and conformation traits, respectively), gene annotations were retrieved from the Ensembl genome browser (Flicek et al., 2012) implemented via the biomaRt R package (http://www.bioconductor.org), using the Bos taurus ARS-UCD1.2 genome assembly as reference (Rosen et al., 2020).

QTL overlapping with the genomic windows identified by the ssGWAS were retrieved from the Animal QTL Database (https://www.animalgenome.org/cgi-bin/QTLdb/index), aligned to the ARS-UCD1.2 bovine genome assembly (version 1.2). Each QTL was assigned to one of six functional categories based on the associated trait description provided in the database: reproduction, milk, meat and carcass, production, health, and exterior. Trait to category assignment was performed by matching keywords and trait classifications in QTLdb. This approach allowed for functional summarization of thousands of QTLs and facilitated interpretation of biological relevance. For each trait, the proportion (in percentage) of QTLs in each functional category among all QTLs overlapping significant genomic windows was calculated, while the full list of individual QTLs identified for each trait (longevity, fertility, and conformation) and the relative abundance were provided in Additional file 1 (Figure S4.1 – S4.17).

4.3 Results

4.3.1 Genetic variation across traits

Estimates of variance components and heritability, along with their 95% highest posterior density intervals, are presented in Table 4.1.

Heritability estimates for conformation traits were moderate (0.19–0.23), consistent with their polygenic nature and shared biological basis in body structure and muscularity. The highest value was observed for development (0.23 ± 0.01), followed by rear width and pelvic length (0.22 ± 0.01), whereas rump convexity, rump length, and back width showed slightly lower values (~0.19 ± 0.01).

For longevity, from the second to the eighth calving (STAY2–STAY8), heritability declined with parity. STAY2 had the highest value (0.21 ± 0.02), followed by STAY3 (0.18 ± 0.02) and STAY4 (0.19 ± 0.03), while later parities such as STAY7 (0.10 ± 0.03) and STAY8 (0.14 ± 0.03) displayed the lowest estimates. This decline suggests stronger genetic determinism for early survival, with environmental and management factors becoming increasingly influential at later stages. Fertility traits showed moderate heritability for AFC (0.18 ± 0.01) but lower for FCI (0.14 ± 0.01), likely reflecting the greater sensitivity of the latter to environmental factors such as nutrition, health, and herd management.

Additive genetic variances (σ_{a}^2) mirrored heritability patterns: 0.15–0.21 for conformation traits, 0.25–0.50 for STAY, and higher scale-dependent values for fertility (388.08 for AFC; 368.62 for FCI, both expressed in days). Notably, herd variance (σ_{h}^2) expressed on the liability scale for STAY, tended to increase with parity and peaked at 2.98 in STAY8. This value indicates a substantial contribution of between-herd differences to longevity in older cows compared with earlier parities.

Although genetic correlations were not re-estimated in the present analysis, these traits were already evaluated in our previous study, which used the same dataset structure, the same animals, and the same trait definitions (Callegaro et al., 2024). In that work, AFC showed genetic correlations with STAY ranging from −0.07 (STAY2) to −0.26 (STAY8), while FCI exhibited moderate negative correlations (−0.43 to −0.53). Conversely, all conformation traits demonstrated positive and significant correlations with longevity, particularly pelvic length, dorsolumbar line length, development, and back width, with estimates exceeding 0.20. These established relationships provide quantitative support for interpreting shared genomic regions as putative pleiotropic signals.

Table 4.1. Variance components and heritability estimate for studied traits in Limousine cattle.

Trait	σ_a^2	σ_h^2	σ_e^2	h^2
Stayability				
STAY2	$0.46^{(0.35;\ 0.56)}$	$0.76^{(0.66;\ 0.86)}$	$1.01^{(0.97;\ 1.03)}$	$0.21^{(0.17;\ 0.24)}$
STAY3	$0.43^{(0.35;\ 0.52)}$	$0.93^{(0.80;\ 1.04)}$	$1.00^{(0.96;\ 1.03)}$	$0.18^{(0.15;\ 0.21)}$
STAY4	$0.49^{(0.37;\ 0.60)}$	$1.11^{(0.96;\ 1.23)}$	$1.01^{(0.97;\ 1.04)}$	$0.19^{(0.16;\ 0.22)}$
STAY5	$0.50^{(0.39;\ 0.61)}$	$1.22^{(1.05;\ 1.42)}$	$1.00^{(0.96;\ 1.04)}$	$0.18^{(0.15;\ 0.21)}$
STAY6	$0.41^{(0.31;\ 0.51)}$	$1.14^{(0.97;\ 1.23)}$	$1.02^{(0.99;\ 1.04)}$	$0.16^{(0.12;\ 0.19)}$
STAY7	$0.25^{(0.19;\ 0.32)}$	$1.24^{(0.99;\ 1.50)}$	$1.00^{(0.95;\ 1.06)}$	$0.10^{(0.08;\ 0.13)}$
STAY8	$0.45^{(0.29;\ 0.62)}$	$2.98^{(0.76;\ 10.75)}$	$0.99^{(0.93;\ 1.07)}$	$0.14^{(0.02;\ 0.20)}$
Fertility				
AFC	$388.08^{(327,60;\ 435,00)}$	$510.26^{(466.00;\ 556.50)}$	$1274.94^{(1232.00;\ 1320.00)}$	$0.18^{(0.16;\ 0.20)}$
FCI	$368.62^{(273.00;\ 453.20)}$	$538.19^{(472.00;\ 614.80)}$	$865.88^{(777.00;\ 956.00)}$	$0.14^{(0.07;\ 0.20)}$
Conformation				
Wither width	$0.18^{(0.16;\ 0.20)}$	$0.21^{(0.19;\ 0.23)}$	$0.48^{(0.47;\ 0.50)}$	$0.21^{(0.18;\ 0.23)}$
Rump convexity	$0.17^{(0.15;\ 0.19)}$	$0.22^{(0.20;\ 0.24)}$	$0.51^{(0.50;\ 0.53)}$	$0.19^{(0.17;\ 0.21)}$
Rump length	$0.16^{(0.14;\ 0.18)}$	$0.19^{(0.17;\ 0.21)}$	$0.48^{(0.47;\ 0.50)}$	$0.19^{(0.17;\ 0.21)}$
Dorsolumbar line length	$0.15^{(0.13;\ 0.16)}$	$0.16^{(0.14;\ 0.18)}$	$0.40^{(0.39;\ 0.41)}$	$0.21^{(0.18;\ 0.23)}$
Rear width	$0.20^{(0.17;\ 0.22)}$	$0.23^{(0.20;\ 0.25)}$	$0.48^{(0.46;\ 0.50)}$	$0.22^{(0.19;\ 0.24)}$
Back width	$0.18^{(0.16;\ 0.20)}$	$0.24^{(0.22;\ 0.27)}$	$0.51^{(0.50;\ 0.53)}$	$0.19^{(0.17;\ 0.21)}$
Pelvic length	$0.16^{(0.14;\ 0.17)}$	$0.17^{(0.15;\ 0.19)}$	$0.39^{(0.38;\ 0.41)}$	$0.22^{(0.19;\ 0.24)}$
Development	$0.21^{(0.19;\ 0.23)}$	$0.17^{(0.16;\ 0.19)}$	$0.51^{(0.49;\ 0.53)}$	$0.23^{(0.21;\ 0.25)}$

The 95% highest posterior density (HPD) intervals are reported in brackets. Abbreviations: σ_a^2 = additive genetic variance; σ_h^2 = herd variance; σ_e^2 = residual variance; h^2 = heritability; STAY2 = stayability2; STAY3 = stayability3, STAY4 = stayability4; STAY5 = stayability5; STAY6 = stayability6; STAY7 = stayability7; STAY8 = stayability8; AFC = age at first calving; FCI = first calving interval.

4.3.2 Genome-wide association results

The association analysis identified multiple genomic regions associated with longevity, fertility, and conformation traits (Figures 4.1–4.3; Additional file 1, Table S4.4). Manhattan plots show the proportion of additive genetic variance explained by 20-SNP sliding windows across the genome (Figures 1–3). Windows highlighted in red represent those that both exceeded the 1% variance threshold and achieved statistical significance after bootstrap test ($p < 0.05$). Only windows that remained statistically significant after bootstrapping were used for downstream functional annotation. Table S4.4 reports the number of windows exceeding 1% variance, total genome coverage by the top windows, and chromosomal locations.

For longevity traits, multiple regions showed consistent signals, including a dense cluster on chromosome 3 (52.26–52.27 Mb) for STAY2 (peak = 1.72%) and chromosome 14 (25.17–25.27 Mb) across STAY2, STAY3, and STAY7. STAY3 associations were detected on chromosomes 6, 14, 17, and 23, particularly chromosome 17 (1.38–2.04 Mb, peak = 1.97%) and chromosome 23 (0.39–1.36 Mb, multiple windows > 1.5%). STAY4 was associated with three distinct regions on chromosomes 6 (37.31–37.35 Mb), 8 (45.76–46.34 Mb), and 20 (5.86–5.92 Mb). STAY5 and STAY6 displayed strong overlapping peaks on chromosome 23 (1.01–1.80 Mb, peaks = 2.76% and 3.98%, respectively), while later parities (STAY7 and STAY8) and STAY6 shared a genomic region on chromosome 9 (93.28–94.00 Mb, highest peak = 4.07%). Later parity shared additional signals on chromosomes 3 (76.15–76.57 Mb). Other significant regions occurred on chromosomes 10, 20, and 23, highlighting both parity-specific and pleiotropic genomic influences.

For fertility traits, a cluster on chromosome 20 (5.85–5.96 Mb, peak = 3.49%) was strongly associated with AFC, overlapping with longevity regions (STAY4), with additional associations detected on chromosomes 2, 7, 12, and 14. FCI presented a broader distribution, with the top signal on chromosome 3 (52.63–52.73 Mb, > 2%), and additional extended clusters on chromosomes 6 (37.15–37.40 Mb, peak = 1.52%) and 8 (45.74–46.36 Mb, > 1.5%). Other significant regions were detected on chromosomes 14 and 24, reflecting the multifactorial genetic basis of fertility traits.

For conformation traits, rear width and back width both showed overlapping association signals on chromosome 23 (0.23–1.89 Mb, peaks of 2.76% and 1.78%, respectively), with additional associations on chromosomes 7, 5, and 24. Dorsolumbar line length was primarily associated with a peak on chromosome 23 (0.89–1.93 Mb, 2.63%), with further contributions from regions on chromosomes 2, 4, 14, and 17. Pelvic length showed notable signals on chromosome 9 (93.37–93.94 Mb, 2.75%) and chromosome 23 (0.68–1.41 Mb, 2.24%). Wither width was associated with a region on chromosome 20 (5.40–5.96 Mb, 1.85%), with other peaks on chromosomes 5 and 24.

Regions on chromosomes 20 (36.37–36.45 Mb) and 8 (5.40–5.52 Mb) contributed to variation in rump convexity, explaining up to 2.28% of the additive genetic variance. Variation in rump length was influenced by regions on chromosomes 4 (48.15–48.70 Mb), 12 (42.78–43.61 Mb), and 23 (0.97–1.37 Mb), with peak effects of up to 1.50%. Developmental variation was mainly associated with regions on chromosomes 7 (22.54–22.64 Mb) and 14 (24.77–24.81 Mb), with additional contributions from chromosomes 17, 20, and 23. Collectively, these significant regions highlight shared genetic components affecting conformation, fertility, and longevity traits.

Additional files 2, 3, and 4 provide detailed lists of significant windows with $p < 0.05$ for longevity, fertility, and conformation traits, respectively, including variance explained, physical positions, and chromosomes.

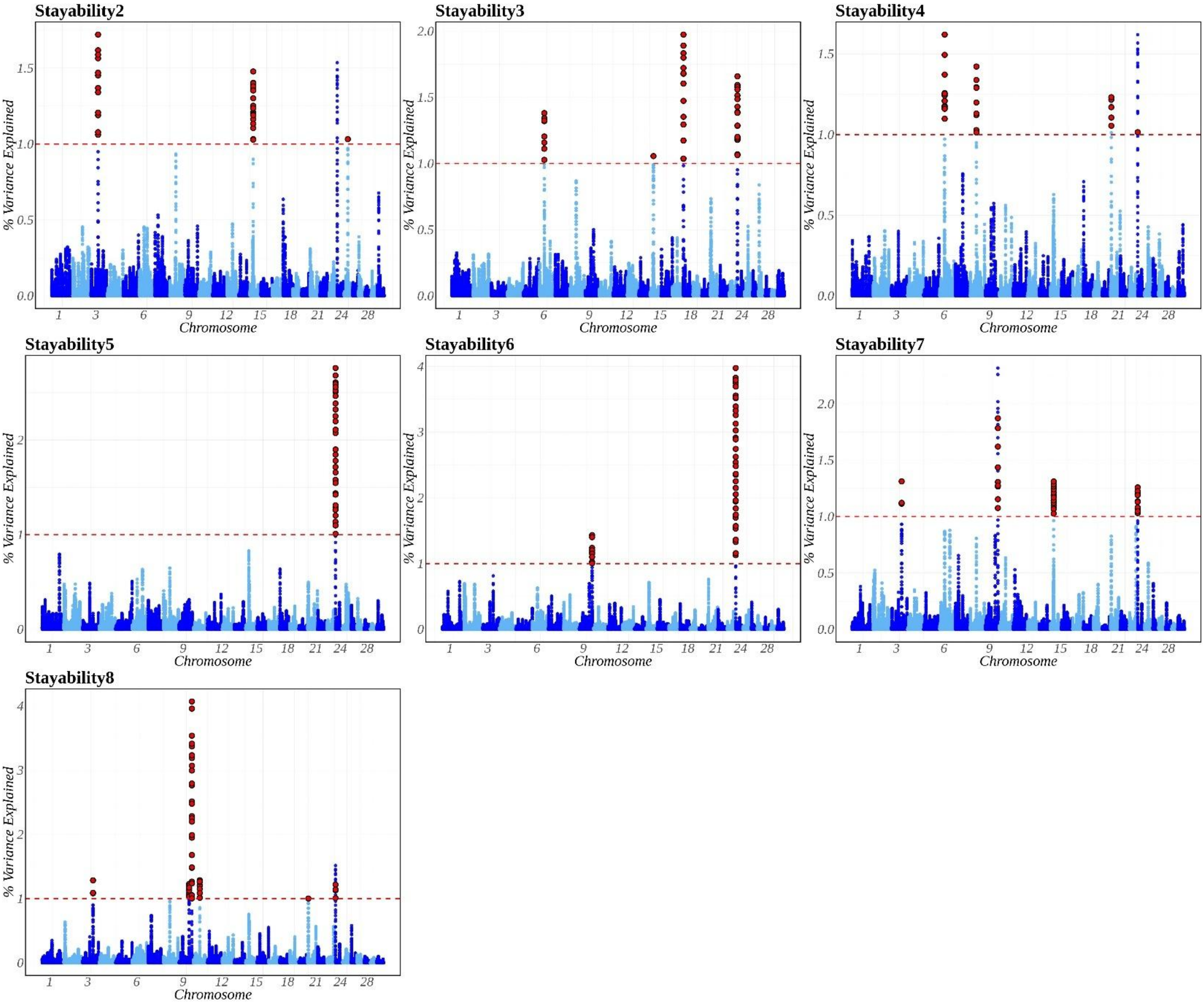


Figure 4.1 Title: Genetic variance explained by 20-SNP windows for stayability traits in Limousine cattle. Legend: Manhattan plot showing the proportion of additive genetic variance explained by consecutive 20-SNP moving windows for stayability (STAY) traits in Limousine cattle. Each point represents the percentage of genetic variance explained by a window across the genome.

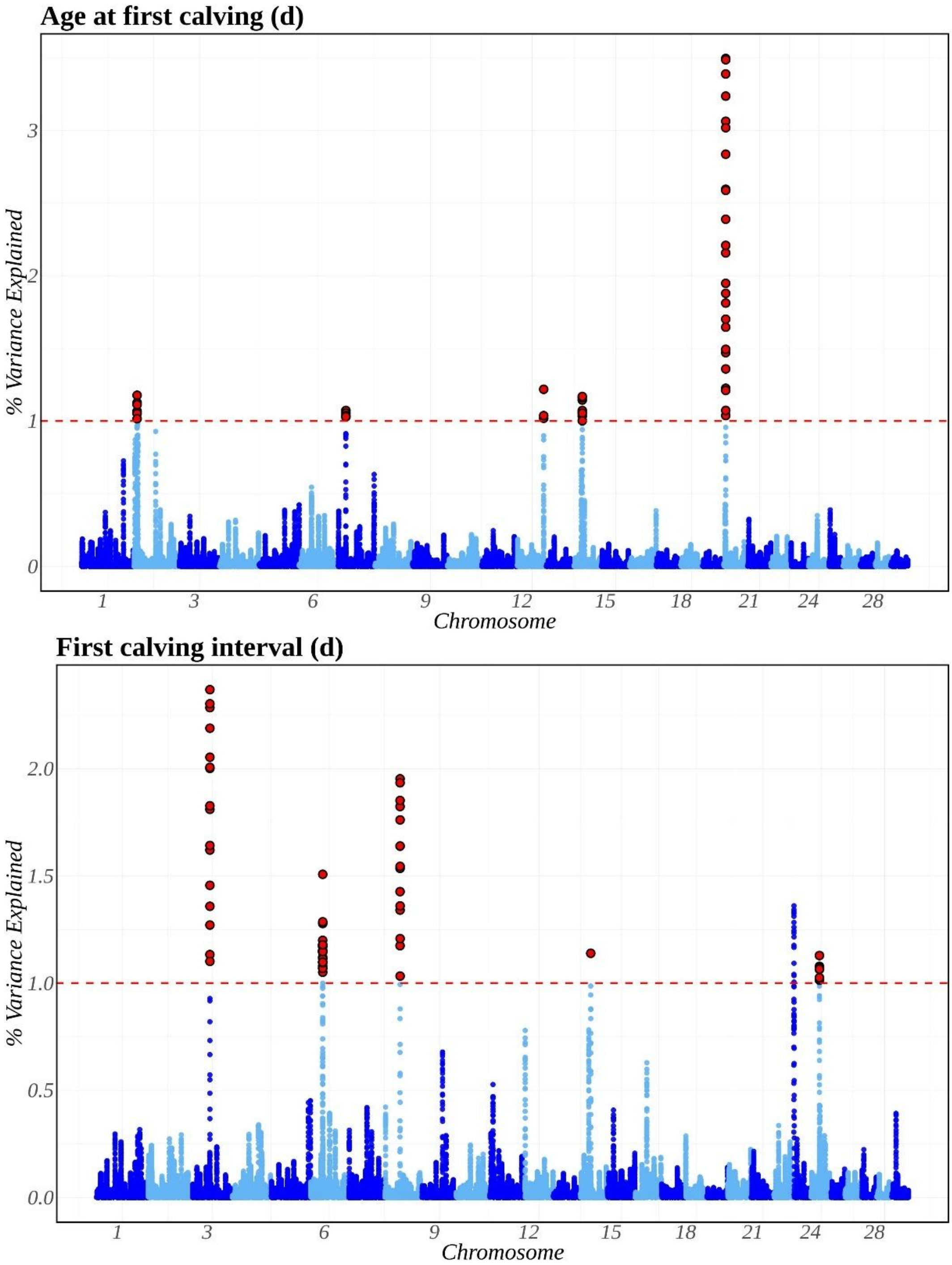


Figure 4.2 Title: Genetic variance explained by 20-SNP windows for fertility traits in Limousine cattle.

Legend: Manhattan plot showing the proportion of additive genetic variance explained by consecutive 20-SNP moving windows for fertility traits in Limousine cattle. Traits include age at first calving (AFC) and first calving interval (FCI).

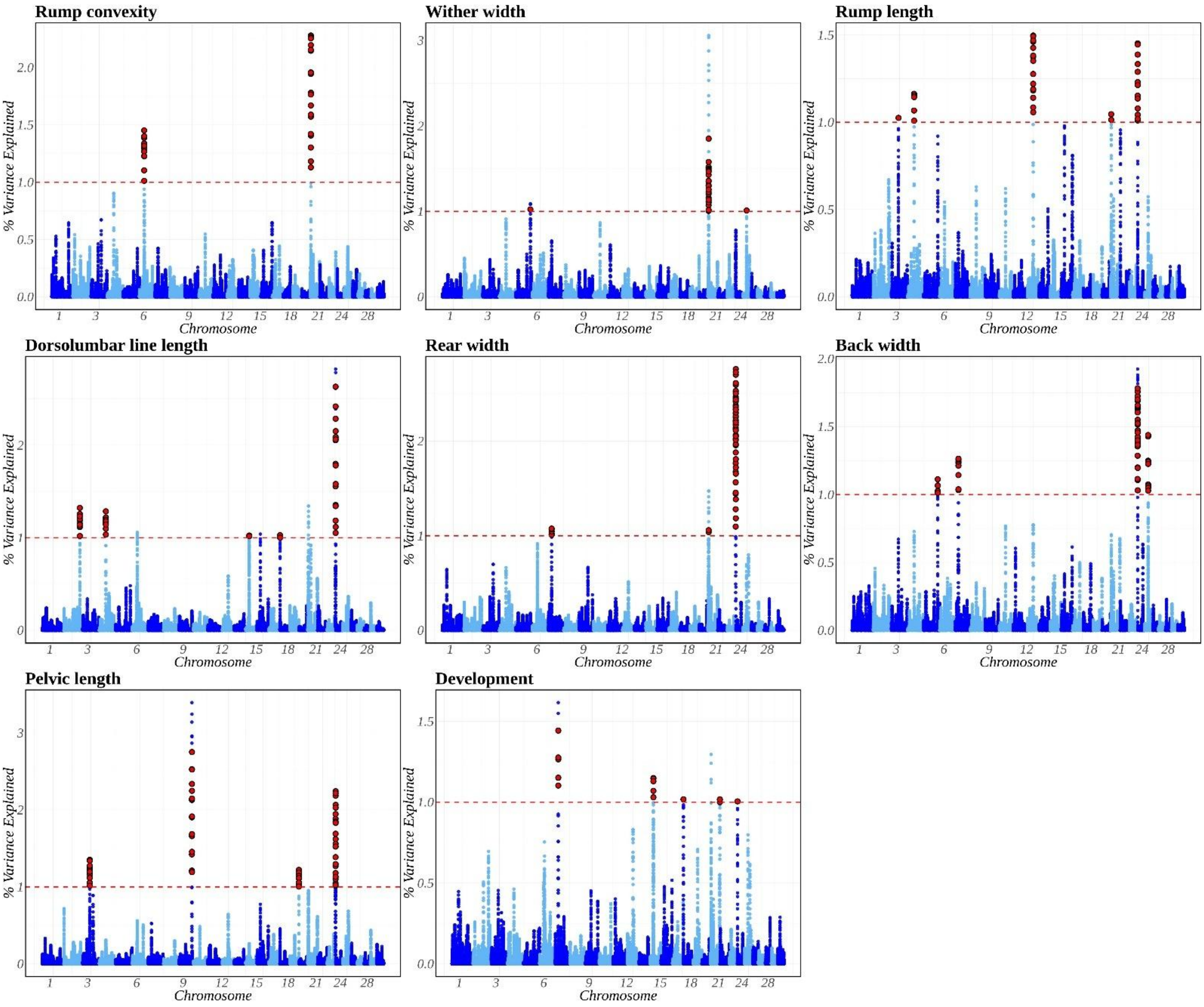


Figure 4.3 Title: Genetic variance explained by 20-SNP windows for conformation traits in Limousine cattle. Legend: Manhattan plot showing the proportion of additive genetic variance explained by consecutive 20-SNP moving windows for conformation traits in Limousine cattle.

4.3.3 Genes and QTLs identified

The most informative genomic windows from the ssGWAS revealed several annotated candidate genes associated with longevity, fertility (Table 4.2), and conformation traits (Table 4.3) in Limousine cattle. For each trait, the proportion of QTLs in each functional category (reproduction, milk, meat and carcass, health, production, and exterior) among all overlapping significant windows was calculated (Figures 4.4 and 4.5; detailed counts in Figures 4.1S–4.17S).

Longevity traits. Across parities, several loci recurred consistently, suggesting pleiotropic effects among multiple STAY traits. Notably, *KHDRBS2* (KH RNA Binding Domain Containing Signal Transduction, BTA23) was detected from STAY3 to STAY8, *TOX* (thymocyte selection-related HMG box protein, BTA14) in STAY2, STAY3, STAY7, and *CPEB4* (Cytoplasmic polyadenylation element binding protein 4, BTA20) was linked to STAY4 and STAY8. *ARID1B* (AT-rich Interaction Domain 1B) and *TMEM242* (Transmembrane Protein 242) on BTA9 were also recurrent in later parities (STAY6 and STAY8). The *LAP3–NCAPG–LCORL* cluster (Leucine Aminopeptidase 3, non-SMC condensin I complex subunit G, and Ligand Dependent Nuclear Receptor Corepressor Like, respectively) on BTA6 for STAY4 was also linked to fertility. Trait-specific signals for longevity traits were specifically reported in Table 2. Functional enrichment of longevity QTLs was dominated by reproduction (28–43%) and health (12–40%) categories, while milk- and exterior-related QTLs showed moderate contributions and increased influence in later parities. Across all STAY traits, different pleiotropic regions were identified with recurrent genes, supporting both parity-specific and shared genetic effects (see Additional file 1, Table S4.5).

Fertility traits. AFC variability was primarily associated with a region on BTA20 harboring *CPEB4* and *C20H5orf47* (Chromosome 20 Open Reading Frame 47), with additional signals detected on BTA2 and BTA7 (Table 2). Importantly, two genes showed shared effects between fertility and longevity, specifically *TOX* and *CPEB4*, both recurrent across AFC and multiple STAY traits. FCI also overlapped with longevity, involving *LAP3–NCAPG–LCORL* and *BARHL2* (BarH-like Homeobox 2). Across fertility traits, no genes were shared exclusively between AFC and FCI; different genes displayed pleiotropic effects between fertility and conformation, namely CSF2 (Colony Stimulating Factor 2), *IL3* (Interleukin 3), *ACSL6* (Acyl-CoA Synthetase Long-Chain Family Member 6), and *C20H5orf47*. Trait-specific associations for AFC and FCI are reported in Table 2. The functional distribution of fertility QTLs (Figure 4) reveals that QTLs for AFC were enriched for meat and carcass (34.6%), milk (24.5%), and reproduction (22.6%) functions, reflecting contributions from both developmental and reproductive pathways. In contrast, QTLs for FCI were dominated by health (40.7%) and production (39.4%) categories.

Conformation-type traits. Several loci displayed pleiotropic effects across multiple conformation traits, most notably *KHDRBS2* on BTA23, detected in six traits, and *CPEB4* on BTA20, shared among four traits. Additional recurrent genes included *TOX* and *ARID1B,* both also overlapping with longevity, and CSF2 together with *C20H5orf47*, which were shared between conformation and fertility. Several loci displayed pleiotropic effects across conformation traits (trait-specific associations), including genes also shared with fertility. In particular, *CSF2*, *IL3*, *ACSL6*, and *C20H5orf47* were detected in both conformation and AFC, while *KHDRBS2*, *CPEB4*, *TOX*, and *ARID1B* overlapped with longevity. Functionally, conformation QTLs (Figure 5) displayed distinct enrichment patterns across traits. Traits such as dorsolumbar line length and pelvic length were dominated by milk (26–38%) and reproduction (25–31%), whereas development and rump convexity showed higher proportions of meat-and-carcass (36–40%) and production categories, highlighting both structural and adaptive relevance.

Table 4.2 Candidate genomic regions and genes for stayability and fertility traits in Limousine cattle.

Trait	Chr	Start, bp	Stop, bp	Genes
Stayability				
STAY2	3	52253335	52531871	ZNF644, BARHL2
	14	24664833	25258596	CYP7A1, SDCBP, NSMAF, TOX
STAY3	6	35690805	36198478	TIGD2, FAM13A, HERC3
	14	24946881	25258596	TOX
	17	1510061	1847108	TLL1
	23	268730	961248	KHDRBS2
STAY4	6	37117688	37557145	LAP3, MED28, FAM184B, DCAF16, NCAPG, LCORL
	8	45449963	46053422	ENTREP1, APBA1, PTAR1, CFAP95
	20	5606065	5720066	CPEB4, C20H5orf47
	20	5750345	5930098	NSG2
	23	268730	961248	KHDRBS2
STAY5	23	268730	961248	KHDRBS2
STAY6	9	93404799	93843981	ARID1B
	23	268730	961248	KHDRBS2
STAY7	9	93404799	93967554	ARID1B, TMEM242
	14	24946881	25258596	TOX
	23	268730	961248	KHDRBS2
STAY8	9	73010914	73549046	HBS1L, MYB, AHI1
	9	93404799	93967554	ARID1B, TMEM242
	10	45661323	46512870	CSNK1G1, PPIB, SNX22, SNX1, CIAO2A, DAPK2, HERC1, FBXL22, USP3
	20	5606065	5678507	CPEB4

	23	268730	961248	KHDRBS2
Fertility				
AFC	2	8777042	9020446	TFPI, CALCRL
	7	22398938	22820845	CSF2, IL3, ACSL6, MEIKIN, FNIP1
	12	78857762	79290585	TPP2, METTL21C, TEX30, POGLUT2, BIVM, ERCC5, SLC10A2
	14	24587176	25258596	UBXN2B, CYP7A1, SDCBP, NSMAF, TOX
	20	5606065	5720066	CPEB4, C20H5orf47
	20	5750345	5930098	NSG2
FCI	3	52531871	52537696	BARHL2
	6	37117688	37557145	LAP3, MED28, FAM184B, DCAF16, NCAPG, LCORL
	8	45449963	46053422	ENTREP1, APBA1, PTAR1, CFAP95
	14	30827097	31488376	MYBL1, VCPIP1, SGK3, MCMDC2, TCF24, PPP1R42, COPS5, CSPP1, ARFGEF1
	24	25777696	26136865	DSG1, DSC2, DSC3

Abbreviations: Chr = chromosomes; STAY2 = stayability2; STAY3 = stayability3; STAY4 = stayability4; STAY5 = stayability5; STAY6 = stayability6; STAY7 = stayability7; STAY8 = stayability8; AFC = age at first calving; FCI = first calving interval.

Table 4.3 Candidate genomic regions and genes for conformation traits in Limousine cattle.

Trait	Chr	Start, bp	Stop, bp	Genes
Conformation				
Rump convexity	6	36052150	36672487	HERC3, PYURF, HERC5, HERC6, PPM1K, ABCG2, PKD2
	20	5606065	5678507	CPEB4
Rump length	3	52531871	52537696	BARHL2
	4	47988419	48589336	PIK3CG, PRKAR2B, HBP1, COG5, GPR22, DUS4L
	20	5606065	5678507	CPEB4
	23	268730	961248	KHDRBS2
Dorsolumbar line length	2	115737770	116276540	SLC19A3, SCYGR6, SCYGR8, CCL20, DAW1, SPHKAP
	4	47988419	48736608	PIK3CG, PRKAR2B, HBP1, COG5, GPR22, DUS4L, BCAP29, SLC26A4
	14	24946881	25258596	TOX
	17	1510061	1847108	TLL1
	23	268730	961248	KHDRBS2
Pelvic length	3	51979806	52348830	HFM1, ZNF644
	9	93404799	93967554	ARID1B, TMEM242
	18	65078931	65569228	ZSCAN4, ZNF814, ZNF135, ZNF329, ZNF274
	23	268730	961248	KHDRBS2
Development	7	22395790	22820845	CSF2, IL3, ACSL6, MEIKIN, FNIP1
	14	24587176	25258596	UBXN2B, CYP7A1, SDCBP, NSMAF, TOX
	17	1510061	1847108	TLL1
	20	71144803	71674393	TERT, SLC6A18, SLC6A19, SLC12A7, NKD2, TRIP13, BRD9, TPPP, CEP72, SLC9A3, EXOC3
	23	268730	961248	KHDRBS2

Wither width	5	105402375	105786234	GALNT8, NDUFA9, AKAP3, DYRK4, RAD51AP1, FERRY3, FGF6, FGF23, TIGAR, CCND2
	20	5606065	5720066	CPEB4, C20H5orf47
	20	5750345	5930098	NSG2
Rear width	7	22087256	22683900	SLC22A4, P4HA2, PDLIM4, CSF2, IL3, ACSL6, MEIKIN
	20	5606065	5720066	CPEB4, C20H5orf47
	20	5750345	5930098	NSG2
	23	268730	961248	KHDRBS2
Back width	5	105402375	105786234	GALNT8, NDUFA9, AKAP3, DYRK4, RAD51AP1, FERRY3, FGF6, FGF23, TIGAR, CCND2
	7	22398938	22683900	CSF2, IL3, ACSL6, MEIKIN
	23	268730	961248	KHDRBS2

Abbreviations: Chr = chromosomes.

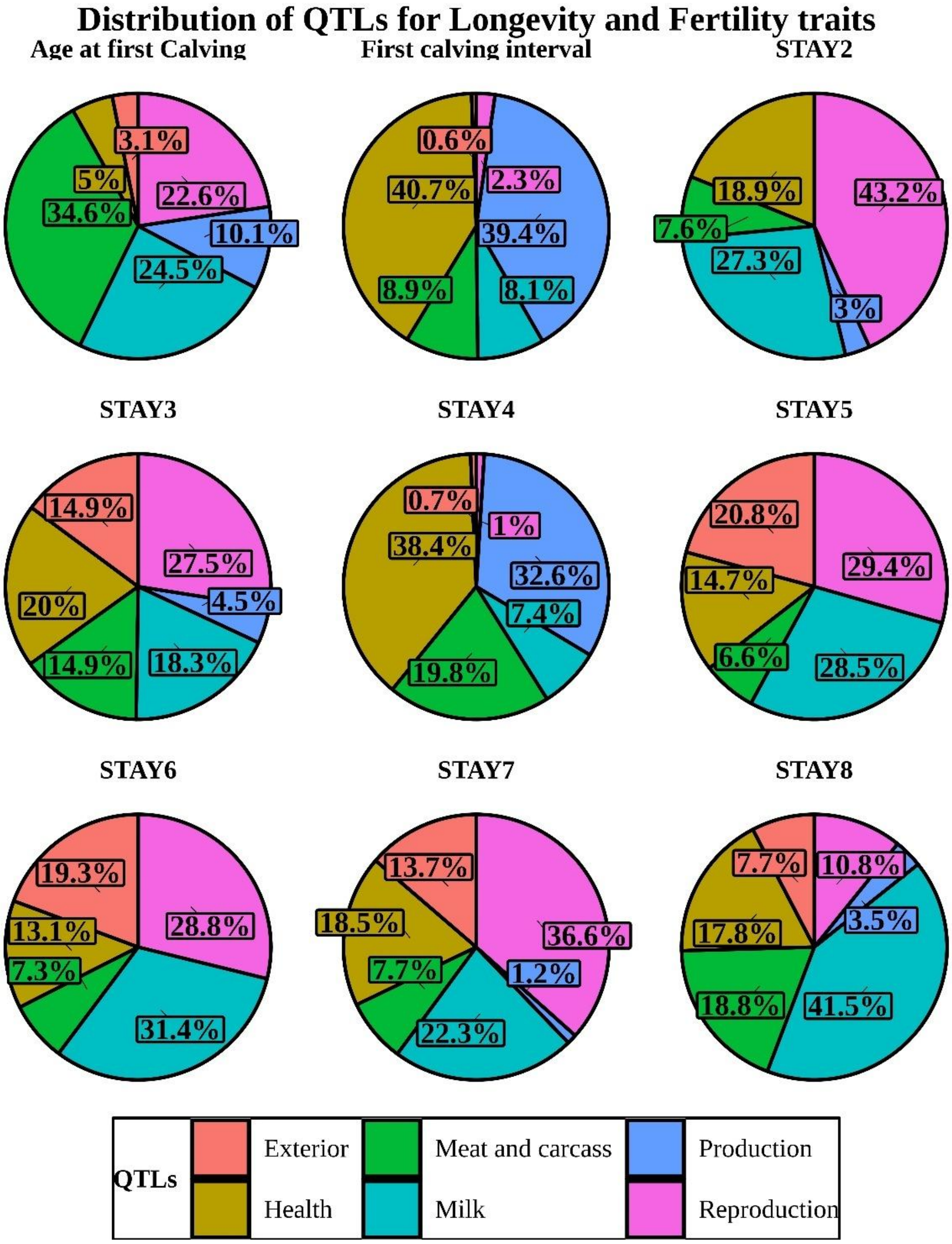


Figure 4.4 Title: Functional classification of QTLs for stayability and fertility traits.

Legend: Distribution of quantitative trait loci (QTLs) identified by ssGWAS across six biological functional categories for stayability (STAY) and fertility traits in Limousine cattle. QTLs were grouped into reproduction, milk, meat and carcass, health, production, and exterior traits. The figure shows the relative abundance (percentage) of genetic variants associated with each functional class.

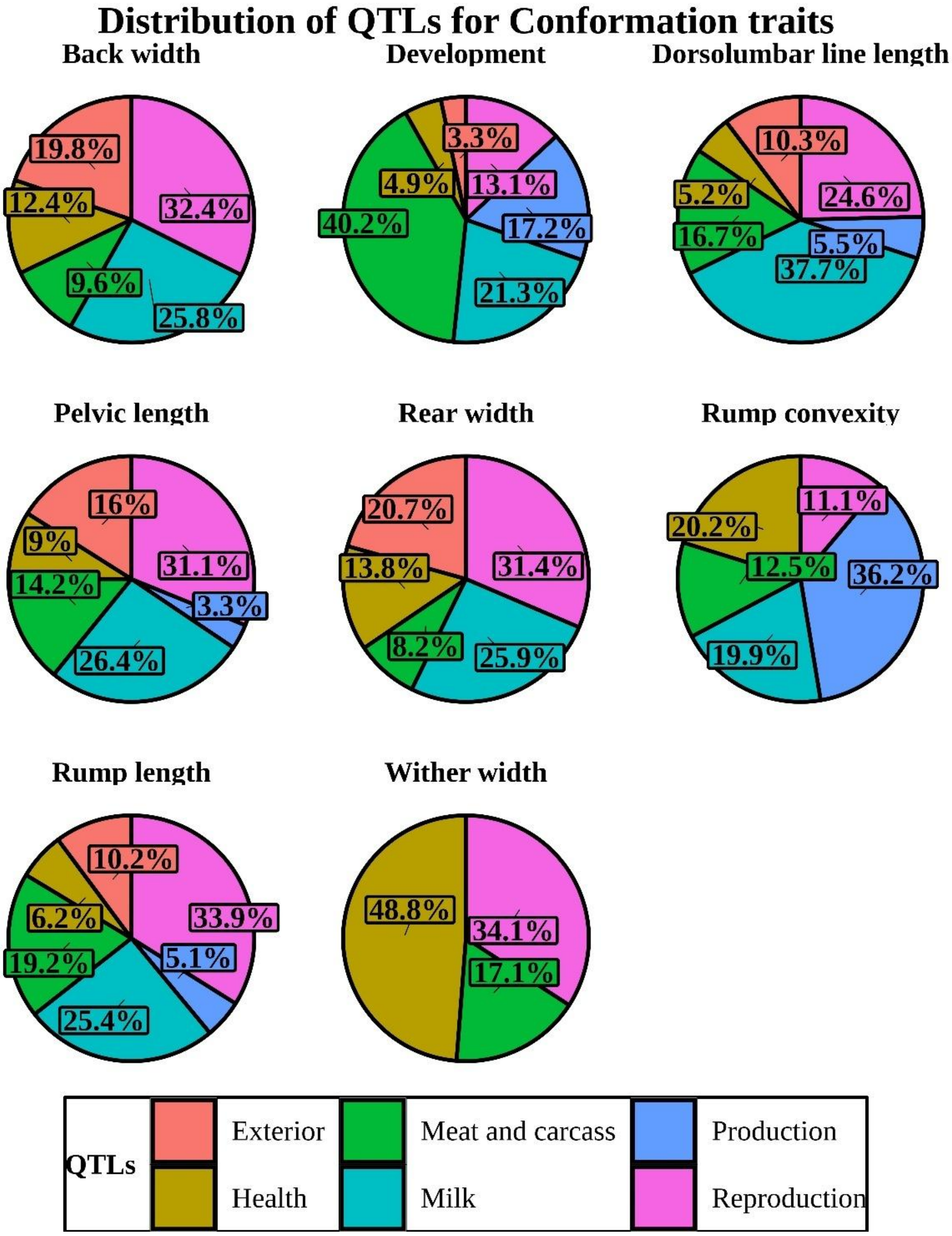


Figure 4.5 Title: Functional classification of QTLs for conformation traits. Legend: Distribution of quantitative trait loci (QTLs) identified by ssGWAS across six biological functional categories for conformation traits in Limousine cattle. QTLs were grouped into reproduction, milk, meat and carcass, health, production, and exterior traits. The figure shows the relative abundance (percentage) of genetic variants associated with each functional class.

4.3.4 Genes and QTL overlaps

Several key genes demonstrated pleiotropic effects, linking multiple traits and calvings. Notably, *KHDRBS2* and *CPEB4* were recurrent across both longevity and conformation traits, while *TOX* connected longevity, fertility, and structural traits. Shared loci between longevity and fertility included *LAP3*, *LCORL*, and *BARHL2*, indicating overlapping genetic control. These recurrent signals suggest that certain genomic regions contribute simultaneously to multiple aspects of female efficiency, including reproductive performance, structural development, and survival across parities. A graphical summary or table highlighting these overlaps could further aid interpretation and discussion of pleiotropic effects. A comprehensive overview of gene-trait overlaps is provided in detail in Additional file 1, Table S4.5, and Figure 4.6.

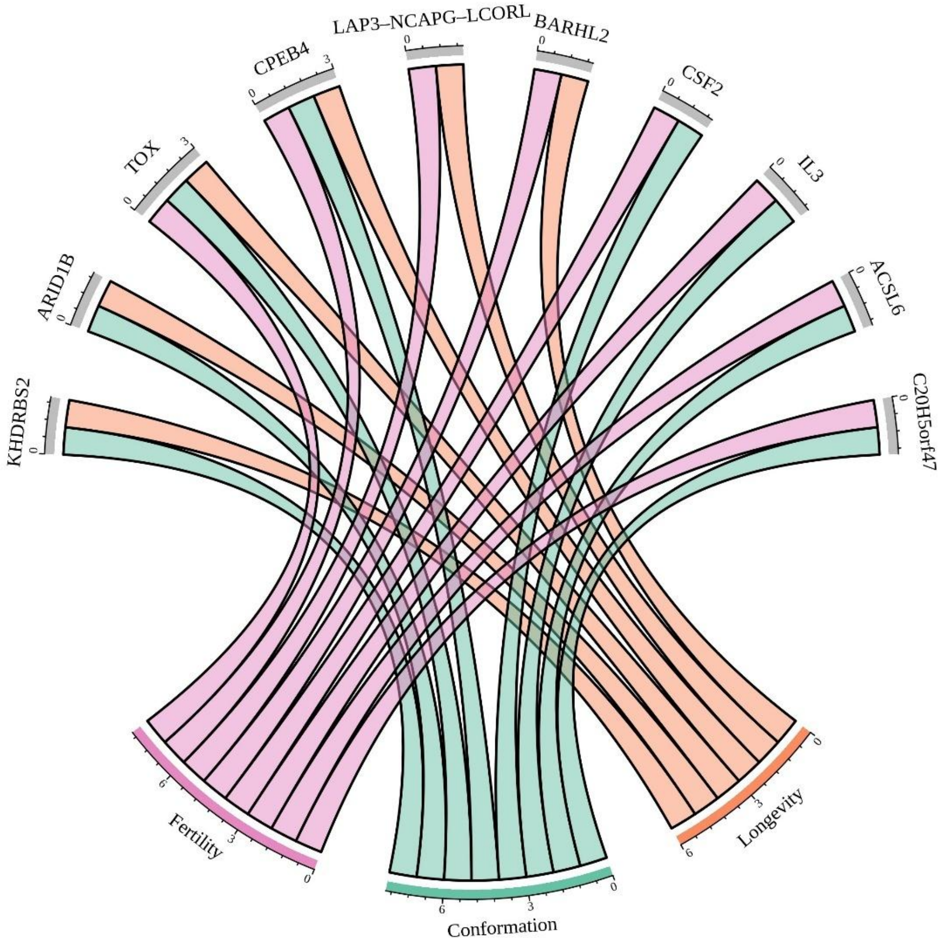


Figure 4.6 Title: Gene–trait overlaps for longevity, fertility, and conformation traits in Limousine cattle. Legend: Chord diagram showing pleiotropic genes identified by ssGWAS that are shared across longevity, fertility, and conformation traits in Limousine cattle. Coloured links represent overlaps between functional categories.

4.4 Discussion

4.4.1 Genetic variation across traits

Heritability estimates for conformation traits in this study were broadly consistent with those reported by Doyle et al. (2018) across multiple beef breeds, including Angus, Charolais, Hereford, Limousine, and Simmental. That study evaluated muscularity, skeletal development, and functional traits, with our estimates for muscular traits and pelvic length closely matching those for Limousine and Simmental. We found slightly lower values for some traits compared with Charolais, whereas Angus and Hereford generally showed lower heritabilities in Doyle et al. (2018), underlying potential breed-specific differences in genetic variation. Pelvic length showed consistent heritability across breeds, reinforcing its stability regardless of population differences.

For longevity, threshold model estimates for STAY were slightly lower than those reported in previous studies. Jamrozik et al. (2013) found a heritability of 0.24 for consecutive calvings in Simmental, while Martinez et al. (2005) reported 0.29 ± 0.10 to 0.39 ± 0.11 in Hereford across calvings. Such discrepancies may reflect differences in breed genetics, management practices, or data structure.

For fertility traits, heritability of AFC (0.18) aligned closely with the 0.17 reported for Holstein Friesian cattle by Berry et al. (2014), who also observed mean estimates of 0.09 ± 0.04 across beef and dairy breeds and even lower values for calving interval (0.03). These results confirm the strong environmental influence and multifactorial nature of fertility traits. In a previous study the Italian Limousine population, a higher heritability of 0.24 was reported (De Rezende et al., 2020b). Moreover, when genotype-by-environment interaction was modeled using a random regression approach, heritability values ranged from 0.02 to 0.15 along an environmental gradient (De Rezende et al., 2020a), further highlighting the sensitivity of AFC to environmental variability.

Variation in herd and residual variance components across conformation, longevity, and fertility likely reflect differences in culling decisions, herd management, and local production strategies. Several conformation traits are functionally linked to reproductive efficiency and longevity (Callegaro et al., 2024), and their moderate heritabilities suggest that selection for improved structural traits could yield indirect benefits for early survival and reproductive performance. For fertility traits, the greater residual variance reflects their sensitivity to non-genetic factors such as nutrition, housing, health management, and social stress.

Overall, the moderate heritability estimates observed for conformation, AFC, and early STAY indicate promising potential for genetic improvement through selection, supporting the use of STAY as a possible indicator for enhancing both longevity and fertility. Conversely, the lower heritabilities observed for late STAY and fertility traits highlight their susceptibility to environmental variation, underscoring the importance of integrated genetic and management strategies.

4.4.2 Genome-wide association results

This GWAS provided novel insights into the complex genetic architecture underlying longevity, fertility, and conformation traits in Limousine cattle. The widespread distribution of significant windows across multiple chromosomes supports a moderately polygenic model, where numerous loci with small to medium effects contribute to phenotypic variability.

For longevity (STAY2 to STAY8), the detection of both persistent and parity-specific regions suggests that different biological mechanisms might influence survival at distinct stages of a cow's productive life. A prominent cluster on chromosome 23 (0.23–1.89 Mb) was consistently associated with survival from early to late parities (STAY3–STAY8), while chromosome 9 (93.28–94.00 Mb) was specifically linked to later parities (STAY7 and STAY8). Recurrence of chromosome 14 signals in both early (STAY2, STAY3) and late (STAY7) parities indicates potential long-term genetic effects. The persistence of these regions suggests pleiotropic effects on adaptive and reproductive functions, possibly through pathways related to resilience and sustained productivity. Similar findings have been reported in other beef breeds, with overlapping genomic regions on chromosomes 6, 9, 20, and 23 associated with longevity (Teixeira et al., 2017; Speidel et al., 2018: Silva et al., 2024).

For fertility, AFC showed a major QTL on chromosome 20 (5.85–5.96 Mb), overlapping with a longevity region (STAY4) and potentially influencing reproductive maturity. These results align with previous associations between BTA20 and fertility traits such as days open, calving ease, and non-return rate in Holstein cattle and may reflect effects on puberty onset and first ovulation (Chen et al., 2022). In contrast, FCI showed a broader distribution of signals on chromosomes 3, 6, 8, 14, and 24, supporting a combination of shared and trait-specific genetic regulation.

Conformation traits exhibited polygenic architecture, with several chromosomes harboring moderate to high-effect regions. Chromosome 23 (0.27-0.96 Mb) emerged as a major region of interest, with overlapping associations for dorsolumbar line length, rump length, rear width, back width, pelvic length, and development. This consistent signal suggests the presence of a key genomic region potentially involved in skeletal and muscular development, with implications for both animal structure and functional traits like calving ease and animal longevity. Chromosome 20 harbored distinct loci for wither width and rump convexity, although at distinct physical locations (5.60–5.72 Mb and 5.75-5.93 Mb). Other traits, including rump length and development, showed more dispersed signals, consistent with their polygenic basis. These patterns agree with Doyle et al. (2020a) who reported extensive breed- and trait-specific QTL for muscularity across beef cattle populations, including key loci for wither width on chromosome 20 (5.40–5.96 Mb) and for traits on chromosome 23 (~0.23–1.93 Mb). Particularly, the major pleiotropic MSTN locus found by Doyle et al. (2020a) on chromosome 2 highlighted in Charolais and Limousine was not among the leading signals in our study, reflecting possible differences in trait definitions, statistical thresholds, or population structure.

Overall, these results highlight biologically relevant genomic regions that appear to influence reproductive lifespan and efficiency, robustness, and structural soundness. Additionally, overlap among conformation, longevity, and fertility suggests possible pleiotropy or tight linkage of genes affecting structural and functional performance. The identification of overlapping regions among different traits suggests opportunities for multi-trait genomic selection strategies aimed at simultaneously improving reproductive efficiency, functional conformation, and cow longevity in beef breeding programs.

4.4.3 Genes and QTLs identified

Stayability traits. The recurrent detection of *KHDRBS2* on BTA23 across almost all stayability traits (STAY3–STAY8) suggests a pleiotropic role in longevity. Previous studies in cattle reported associations with AFC (Colombian cattle breeds), pregnancy status (Brahman cows), economic and reproduction (Xiangxi cattle), supporting its involvement in reproductive performance and long-term productivity (De León et al., 2019; Reverter et al., 2016; Luo et al., 2022). In goats, *KHDRBS2* has been shown to influence fertility and fecundity traits, further supporting its involvement in reproductive success (Guo et al., 2018; Islam et al., 2020). Another recurrent region (BTA9: 93.40–93.96 Mb) harbored *ARID1B* and *TMEM242* (late-parity STAY6–STAY8), with *ARID1B's* known role in chromatin remodeling, cell cycle regulation, and tissue maintenance (Sim et al., 2015; 2014), supporting its potential impact on reproductive organ integrity and longevity.

The *TOX* on BTA14 (STAY2, STAY3, STAY7) encodes a transcription factor involved in cell cycle control and genomic stability (Han et al., 2022). De Camargo et al. (2015) detected four polymorphisms in the *TOX* gene associated with reproductive traits in Nellore heifers, specifically affecting early pregnancy probability, days to first calving, and AFC. Furthermore, Fortes et al. (2011) identified *TOX* and *NCOA2* as transcription factors active in the hypothalamus of Brahman cattle. These results suggested a key role of *TOX* in pubertal development through the regulation of genes that influence puberty onset. *CPEB4* (BTA20: 5.61–5.68 Mb), linked to STAY4 and STAY8, in a study on dairy heifers, appears among the genes linked to fertility and health in livestock, with significant roles in other mammals for the regulation of reproductive processes and longevity at the cellular level (Marrella et al., 2024). In addition, it was associated with growth traits in red Angus cattle and conception rate in Holstein heifers (Smith et al., 2022; Kiser et al., 2019).

Parity-specific loci support the contribution of distinct biological mechanisms at different stages of productive life. These include *ZNF644* (BTA3) for STAY2, potentially involved in epigenetic control (Bian et al., 2015). The *LAP3–NCAPG–LCORL* cluster (STAY4), previously associated with growth, stature, birth weight, and carcass weight on bovine (Majeres et al., 2024; Bouwman et al., 2018; Smith et al., 2022; Keogh et al., 2021). In addition, Doyle et al. (2020b) found that *NCAPG* and *LCORL* genes were also associated with all skeletal traits in the Limousine population. A study on Piedmontese cattle breeds found that *LAP3–NCAPG–LCORL* affected direct calving ease (Bongiorni et al., 2012). To further confirm the results, additional SNPs within these genes were genotyped and analyzed for association with direct calving ease, confirming the initial findings and highlighting *LAP3* as the most probable gene involved. *HERC1*, *FBXL22*, *DAPK2* (BTA10: 45.66-46.51) and *TIGD2*, *FAM13A*, *HERC3* (BTA6: 35.89-36.20) for STAY3 and STAY8, respectively. Specifically, *TIGD2* on East Adriatic sheep breeds was identified as a candidate in disease resistance and adaptation, and in cattle was associated with resistance to disease and bacterial infection (Lukic et al., 2023; Ghoreishifar et al., 2020), which can impact livestock longevity through resilience.

Overall, the QTL landscape confirms that longevity in Limousine cattle is governed primarily by reproduction- and health-related pathways, with increasing contributions from structural traits in later parities. The strong and consistent presence of reproduction-related QTLs across all parities highlights the central role of fertility in sustaining a long productive life. This pattern reflects the progressive relevance of fertility efficiency, disease resistance, and conformation and robustness for maintaining cows in the herd over time.

Fertility traits. Multiple shared loci connect fertility with both longevity and conformation. For AFC, the BTA20 region containing *CPEB4*, *NSG2*, and *C20H5orf47* showed overlaps with STAY traits, consistent with the regulatory function of CPEB4 in oocyte maturation and meiotic progression (Walker et al., 2022; Uzbekova et al., 2008). The TOX locus, shared between AFC and several STAY traits, supports its role in the timing of sexual maturity onset and earlier pubertal development, allowing heifers to lower AFC, thereby improving lifetime reproductive potential. AFC-specific associations on BTA7 included *CSF2* and *IL3*, which regulate early embryonic development and ovarian microenvironment (Amaral et al., 2022; Warma et al., 2020), and were also shared with conformation traits, suggesting pleiotropic effects linking reproductive regulation and structural development.

Considering FCI, overlapping regions included *BARHL2* (BTA3) and the *LAP3–NCAPG–LCORL* cluster (BTA6), supporting shared influences on growth, calving performance, and reproductive recovery across parities. The involvement of these growth-related genes in both longevity and fertility points to pleiotropy, suggesting that skeletal development may influence reproductive performance through their effects on calving difficulty and subsequent recovery. The trait-specific FCI region on BTA14 (30.83-31.49 Mb) harbored *MYBL1* and *CSPP1*. The first gene is a regulator of meiotic genes that are involved in multiple processes in spermatocytes and may be the candidate regulator for pachytene spermatocyte genes dysregulated in cattle (Bolcun-Filas et al., 2011; Zhang et al., 2023).

The second gene was found to be related to conception and pregnancy rate and productive life in Holstein cows (Cochran et al., 2013), candidate genes for longevity (Liu et al., 2021), and in five Swedish breeds was associated with reproduction (Ghoreishifar et al., 2020).

The contrasting QTL distributions for AFC and FCI reinforce their distinct genetic architecture. AFC was characterized by meat, milk, and reproduction-related QTLs, suggesting that growth and developmental pathways influence maturation and reproductive onset. In contrast, FCI showed strong enrichment for health and production QTLs, highlighting the role of metabolic resilience and environmental sensitivity in sustaining fertility across calvings.

Conformation-type traits. KHDRBS2 reappeared as a candidate for six traits (rump length, dorsolumbar line length, pelvic length, rear width, back width, and development), suggesting pleiotropic links between reproductive fitness and skeletal-muscular development. Its role in reproduction and longevity suggests it may influence body conformation features, like pelvic structure and rump morphology, that are critical for calving ease and maternal capacity. The BTA9 region containing *ARID1B*, shared with late parity stayability, was associated with pelvic length, supporting a role in tissue integrity and skeletal development. In this study, *CPEB4*, a gene also identified in association with fertility and longevity, emerged as a positional candidate for several conformation traits (wither width, rear width, rump length, and rump convexity). Its role in reproductive processes and rump morphology highlights a functional link between fertility and body conformation, with clear implications for productive lifespan in Limousine cattle. *C20H5orf47* was linked in beef cattle to rear and wither widths and has been associated with carcass merit traits **(**average backfat thickness) (Li et al., 2022), reinforcing the economic relevance of this locus. Another key gene, *TOX*, shared across stayability, AFC, and conformation (dorsolumbar line length, development), further supports coordinated regulation of structural growth and reproductive maturation, consistent with its reported effects on body weight (Calderón-Chagoya et al., 2023). Importantly, *CSF2*, *IL3*, and *ACSL6*, previously associated with AFC, were also detected in rear width, back width, and development, demonstrating pleiotropic links between immune regulation, ovarian function, and skeletal traits. In another studies, *ACSL6*, was associated with meat color in Nellore cattle (Marín-Garzón et al., 2021) weaning weight in sheep (Khazaei-Koohpar et al., 2024), dry matter intake, and mid-test body weight in Angus cattle (Saatchi et al., 2014).

Trait-specific regions, such as the BTA14 locus (*GALNT8*, *FGF6*, *FGF23*, *TIGAR*, *CCND2*) affecting wither width, align with previous associations with conformation in Brazilian Holstein cattle (Silva et al., 2024) and on different cattle breeds on body size, stature, and body conformation (Ghoreishifar et al., 2020; Fang et al., 2020). Another study suggested that *FGF6* has a possible effect on muscle mass in the carcass in young Charolais bull calves (Bernard et al., 2009) and for traits underlying feed efficiency in Holstein cows (Hardie et al., 2017). The BTA6 cluster (*PYURF*, *HERC5*, *HERC6*, *PPM1K*, *ABCG2*, *PKD2*) found in rump convexity contains genes previously linked to carcass quality and primal cuts in Nellore bulls and Hanwoo Cattle, respectively (Arikawa et al., 2024; Naserkheil et al., 2021). These findings highlight the importance of trait-specific genomic regions where conformation traits relate to meat yield and quality, suggesting that selection for conformation could also impact production efficiency, carcass merit, and overall economic value in beef cattle.

The distribution of QTLs across conformation traits reveals a complex genetic basis and supports their role as integrators of multiple biological functions. Milk- and reproduction-related QTLs in pelvic and rump regions underline their importance for calving ease and maternal capacity efficiency, while meat- and carcass-related QTLs in development highlight links with muscle mass and productivity. Health-related QTLs in wither width point to skeletal robustness and metabolic stability. Together, these patterns emphasize that conformation traits represent valuable targets for improving structural soundness, functionality, and long-term productive efficiency in beef cattle.

4.5 Conclusions

This study provides new insights into the genetic architecture of longevity, fertility, and conformation traits in Limousine cattle using ssGWAS. Heritability estimates confirmed moderate genetic control for conformation, age at first calving, and early stayability, highlighting the feasibility of genetic improvement through selection on early expressed traits, complemented by management strategies to support performance in later parities.

The identification of shared genomic regions and pleiotropic loci indicates that fertility, skeletal development, conformation, and productive lifespan are genetically interconnected. From a practical perspective and breeding strategies, targeting early indicators of performance, such as age at first calving, early stayability, and conformation traits, combined with effective management, could accelerate genetic progress, reduce replacement costs, and enhance both the efficiency and sustainability of beef cattle systems. Overall, these findings support the implementation of multi-trait genomic selection strategies that may simultaneously improve female productivity while maintaining balance among fitness, functionality, and structural soundness.

4.6 List of abbreviations

GWAS: Genome-wide association studies
QTL: quantitative trait loci

ssGWAS: single-step GWAS

STAY: stayability

ANACLI: National Italian Association of Limousine and Charolais Breeders

AFC: age at first calving

FCI: first calving interval

MAF: minor allele frequency

GEBV: genomic estimated breeding values

LD: linkage disequilibrium

4.7 Ethics approval and consent to participate

Not applicable.

4.8 Consent for publication

Not applicable.

4.9 Availability of data and materials

The datasets generated and analysed during the current study are not publicly available because they are owned by the National Association of Breeders of Limousine and Charolaise breeds (ANACLI, Roma, Italy; email: anacli@anacli.it) but are available from the corresponding author on reasonable request.

4.10 Competing interests

The authors declare that they have no competing interests.

4.11 Funding

This work was financially supported by Associazione Nazionale degli Allevatori delle razze bovine Charolaise e Limousine Italiane (Cup: J89H18000010005, URL: http://www.anacli.it/ibeef). RB received the grant. The funders had no role in study design and analysis, decision to publish, or manuscript preparation.

4.12 Authors' contributions

SC carried out the analysis and wrote the first draft of the manuscript. FT conceived the study. SC, FT, CM, MCF, and RB contributed to the study design and reviewed the draft manuscript. All authors have read and approved the final manuscript.

4.13 Acknowledgements

Not applicable.

4.14 Reference

Aguilar, I., Legarra, A., Cardoso, F., Masuda, Y., Lourenco, D., Misztal, I., 2019. Frequentist p-values for large-scale-single step genome-wide association, with an application to birth weight in American Angus cattle. Genetics Selection Evolution 51. doi:10.1186/s12711-019-0469-3

Aguilar, I., Misztal, I., Johnson, D.L., Legarra, A., Tsuruta, S., Lawlor, T.J., 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. Journal of Dairy Science 93, 743–752. doi:10.3168/jds.2009-2730

Aguilar, I., Misztal, I., Tsuruta, S., Legarra, A., Huiyu Wang, 2014. PREGSF90 – POSTGSF90: Computational Tools for the Implementation of Single-step Genomic Selection and Genome-wide Association with Ungenotyped Individuals in BLUPF90 Programs. doi:10.13140/2.1.4801.5045

Aguilar, I., Tsuruta, S., Masuda, Y., Lourenco, D.A.L., Legarra, A., Misztal, I., 2018. BLUPF90 suite of programs for animal breeding with focus on genomics.

Amaral, T.F., De Grazia, J.G.V., Martinhao, L.A.G., De Col, F., Siqueira, L.G.B., Viana, J.H.M., Hansen, P.J., 2022. Actions of CSF2 and DKK1 on bovine embryo development and pregnancy outcomes are affected by composition of embryo culture medium. Scientific Reports 12, 7503. doi:10.1038/s41598-022-11447-7

Arikawa, L.M., Mota, L.F.M., Schmidt, P.I., Frezarim, G.B., Fonseca, L.F.S., Magalhães, A.F.B., Silva, D.A., Carvalheiro, R., Chardulo, L.A.L., Albuquerque, L.G.D., 2024. Genome-wide scans identify biological and metabolic pathways regulating carcass and meat quality traits in beef cattle. Meat Science 209, 109402. doi:10.1016/j.meatsci.2023.109402

Barreto Amaral Teixeira, D., Alves Fernandes Júnior, G., Beraldo Dos Santos Silva, D., Bermal Costa, R., Takada, L., Gustavo Mansan Gordo, D., Bresolin, T., Carvalheiro, R., Baldi, F., Galvão De Albuquerque, L., 2017. Genomic analysis of stayability in Nellore cattle. PLOS ONE 12, e0179076. doi:10.1371/journal.pone.0179076

Beissinger, T.M., Rosa, G.J., Kaeppler, S.M., Gianola, D., De Leon, N., 2015. Defining window-boundaries for genomic analyses using smoothing spline techniques. Genetics Selection Evolution 47. doi:10.1186/s12711-015-0105-9

Bergamaschi, M., Maltecca, C., Fix, J., Schwab, C., Tiezzi, F., 2020. Genome-wide association study for carcass quality traits and growth in purebred and crossbred pigs1. Journal of Animal Science 98, skz360. doi:10.1093/jas/skz360

Bernard, C., Cassar-Malek, I., Renand, G., Hocquette, J.-F., 2009. Changes in muscle gene expression related to metabolism according to growth potential in young bulls. Meat Science 82, 205–212. doi:10.1016/j.meatsci.2009.01.012

Bernini, F., Mancin, E., Sartori, C., Mantovani, R., Vevey, M., Blanchet, V., Bagnato, A., Strillacci, M.G., 2024. Genome-wide association studies for milk production traits in two autochthonous Aosta cattle breeds. animal 18, 101322. doi:10.1016/j.animal.2024.101322

Berry, D.P., Wall, E., Pryce, J.E., 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8, 105–121. doi:10.1017/S1751731114000743

Bian, C., Chen, Q., Yu, X., 2015. The zinc finger proteins ZNF644 and WIZ regulate the G9a/GLP complex for gene repression. eLife 4, e05606. doi:10.7554/eLife.05606

Bolcun-Filas, E., Bannister, L.A., Barash, A., Schimenti, K.J., Hartford, S.A., Eppig, J.J., Handel, M.A., Shen, L., Schimenti, J.C., 2011. A-MYB (MYBL1) transcription factor is a master regulator of male meiosis. Development 138, 3319–3330. doi:10.1242/dev.067645

Bongiorni, S., Mancini, G., Chillemi, G., Pariset, L., Valentini, A., 2012. Identification of a Short Region on Chromosome 6 Affecting Direct Calving Ease in Piedmontese Cattle Breed. PLoS ONE 7, e50137. doi:10.1371/journal.pone.0050137

Bouquet, A., Venot, E., Laloë, D., Forabosco, F., Fogh, A., Pabiou, T., Moore, K., Eriksson, J.-Å., Renand, G., Phocas, F., 2011. Genetic structure of the European Charolais and Limousin cattle metapopulations using pedigree analyses. Journal of Animal Science 89, 1719–1730. doi:10.2527/jas.2010-3469

Bouwman, A.C., Daetwyler, H.D., Chamberlain, A.J., Ponce, C.H., Sargolzaei, M., Schenkel, F.S., Sahana, G., Govignon-Gion, A., Boitard, S., Dolezal, M., Pausch, H., Brøndum, R.F., Bowman, P.J., Thomsen, B., Guldbrandtsen, B., Lund, M.S., Servin, B., Garrick, D.J., Reecy, J., Vilkki, J., Bagnato, A., Wang, M., Hoff, J.L., Schnabel, R.D., Taylor, J.F., Vinkhuyzen, A.A.E., Panitz, F., Bendixen, C., Holm, L.-E., Gredler, B., Hozé, C., Boussaha, M., Sanchez, M.-P., Rocha, D., Capitan, A., Tribout, T., Barbat, A., Croiseau, P., Drögemüller, C., Jagannathan, V., Vander Jagt, C., Crowley, J.J., Bieber, A., Purfield, D.C., Berry, D.P., Emmerling, R., Götz, K.-U., Frischknecht, M., Russ, I., Sölkner, J., Van Tassell, C.P., Fries, R., Stothard, P., Veerkamp, R.F., Boichard, D., Goddard, M.E., Hayes, B.J., 2018. Meta-analysis of genome-wide association studies for cattle stature identifies common genes that regulate body size in mammals. Nature Genetics 50, 362–367. doi:10.1038/s41588-018-0056-5

Calderón-Chagoya, R., Vega-Murillo, V.E., García-Ruiz, A., Ríos-Utrera, Á., Martínez-Velázquez, G., Montaño-Bermúdez, M., 2023. Genome and chromosome wide association studies for growth traits in Simmental and Simbrah cattle. Animal Bioscience 36, 19–28. doi:10.5713/ab.21.0517

Callegaro, S., Tiezzi, F., Maltecca, C., Fabbri, M.C., Bozzi, R., 2024. Genetic parameters of functional longevity and associated traits in Italian Charolais and Limousine breeds. Journal of Animal Science 102, skae354. doi:10.1093/jas/skae354

Chen, S.-Y., Schenkel, F.S., Melo, A.L.P., Oliveira, H.R., Pedrosa, V.B., Araujo, A.C., Melka, M.G., Brito, L.F., 2022. Identifying pleiotropic variants and candidate genes for fertility and reproduction traits in Holstein cattle via association studies based on imputed whole-genome sequence genotypes. BMC Genomics 23, 331. doi:10.1186/s12864-022-08555-z

Christensen, O.F., Lund, M.S., 2010. Genomic prediction when some animals are not genotyped. Genetics Selection Evolution 42. doi:10.1186/1297-9686-42-2

Cochran, S.D., Cole, J.B., Null, D.J., Hansen, P.J., 2013. Discovery of single nucleotide polymorphisms in candidate genes associated with fertility and production traits in Holstein cattle. BMC Genetics 14, 49. doi:10.1186/1471-2156-14-49

Colombi, D., Rovelli, G., Luigi-Sierra, M.G., Ceccobelli, S., Guan, D., Perini, F., Sbarra, F., Quaglia, A., Sarti, F.M., Pasquini, M., Amills, M., Lasagna, E., 2024. Population structure and identification of genomic regions associated with productive traits in five Italian beef cattle breeds. Scientific Reports 14. doi:10.1038/s41598-024-59269-z

De Camargo, G.M.F., Costa, R.B., De Albuquerque, L.G., Regitano, L.C.A., Baldi, F., Tonhati, H., 2015. Polymorphisms in TOX and NCOA2 genes and their associations with reproductive traits in cattle. Reproduction, Fertility and Development 27, 523. doi:10.1071/RD13360

De León, C., Manrique, C., Martínez, R., Rocha, J.F., 2019. Research Article Genomic association study for adaptability traits in four Colombian cattle breeds. Genetics and Molecular Research 18. doi:10.4238/gmr18373

De Rezende, M.P.G., Malhado, C.H.M., Biffani, S., Carneiro, P.L.S., Carrillo, J.A., Bozzi, R., 2020a. Genotype-environment interaction for age at first calving in Limousine and Charolais cattle raised in Italy, employing reaction norm model. Livestock Science 232, 103912. doi:10.1016/j.livsci.2019.103912

De Rezende, M.P.G., Malhado, C.H.M., Biffani, S., Souza Carneiro, P.L., Bozzi, R., 2020b. Genetic diversity derived from pedigree information and estimation of genetic parameters for reproductive traits of Limousine and Charolais cattle raised in Italy. Italian Journal of Animal Science 19, 762–771. doi:10.1080/1828051X.2020.1778547

Doyle, J.L., Berry, D.P., Veerkamp, R.F., Carthy, T.R., Evans, R.D., Walsh, S.W., Purfield, D.C., 2020a. Genomic regions associated with muscularity in beef cattle differ in five contrasting cattle breeds. Genetics Selection Evolution 52. doi:10.1186/s12711-020-0523-1

Doyle, J.L., Berry, D.P., Veerkamp, R.F., Carthy, T.R., Walsh, S.W., Evans, R.D., Purfield, D.C., 2020b. Genomic Regions Associated With Skeletal Type Traits in Beef and Dairy Cattle Are Common to Regions Associated With Carcass Traits, Feed Intake and Calving Difficulty. Frontiers in Genetics 11, 20. doi:10.3389/fgene.2020.00020

Doyle, J.L., Berry, D.P., Walsh, S.W., Veerkamp, R.F., Evans, R.D., Carthy, T.R., 2018. Genetic covariance components within and among linear type traits differ among contrasting beef cattle breeds. Journal of Animal Science 96, 1628–1639. doi:10.1093/jas/sky076

Engle, B.N., Herring, A.D., Sawyer, J.E., Riley, D.G., Sanders, J.O., Gill, C.A., 2018. Genome-wide association study for stayability measures in Nellore–Angus crossbred cows1. Journal of Animal Science 96, 1205–1214. doi:10.1093/jas/sky067

Fang, L., Cai, W., Liu, S., Canela-Xandri, O., Gao, Y., Jiang, J., Rawlik, K., Li, B., Schroeder, S.G., Rosen, B.D., Li, C., Sonstegard, T.S., Alexander, L.J., Van Tassell, C.P., VanRaden, P.M., Cole, J.B., Yu, Y., Zhang, S., Tenesa, A., Ma, L., Liu, G.E., 2020. Comprehensive analyses of 723 transcriptomes enhance genetic and biological interpretations for complex traits in cattle. Genome Research 30, 790–801. doi:10.1101/gr.250704.119

Flicek, P., Ahmed, I., Amode, M.R., Barrell, D., Beal, K., Brent, S., Carvalho-Silva, D., Clapham, P., Coates, G., Fairley, S., Fitzgerald, S., Gil, L., García-Girón, C., Gordon, L., Hourlier, T., Hunt, S., Juettemann, T., Kähäri, A.K., Keenan, S., Komorowska, M., Kulesha, E., Longden, I., Maurel, T., McLaren, W.M., Muffato, M., Nag, R., Overduin, B., Pignatelli, M., Pritchard, B., Pritchard, E., Riat, H.S., Ritchie, G.R.S., Ruffier, M., Schuster, M., Sheppard, D., Sobral, D., Taylor, K., Thormann, A., Trevanion, S., White, S., Wilder, S.P., Aken, B.L., Birney, E., Cunningham, F., Dunham, I., Harrow, J., Herrero, J., Hubbard, T.J.P., Johnson, N., Kinsella, R., Parker, A., Spudich, G., Yates, A., Zadissa, A., Searle, S.M.J., 2012. Ensembl 2013. Nucleic Acids Research 41, D48–D55. doi:10.1093/nar/gks1236

Fortes, M.R.S., Reverter, A., Nagaraj, S.H., Zhang, Y., Jonsson, N.N., Barris, W., Lehnert, S., Boe-Hansen, G.B., Hawken, R.J., 2011. A single nucleotide polymorphism-derived regulatory gene network underlying puberty in 2 tropical breeds of beef cattle1. Journal of Animal Science 89, 1669–1683. doi:10.2527/jas.2010-3681

Ghoreishifar, S.M., Eriksson, S., Johansson, A.M., Khansefid, M., Moghaddaszadeh-Ahrabi, S., Parna, N., Davoudi, P., Javanmard, A., 2020. Signatures of selection reveal candidate genes involved in economic traits and cold acclimation in five Swedish cattle breeds. Genetics Selection Evolution 52, 52. doi:10.1186/s12711-020-00571-5

Guo, J., Tao, H., Li, P., Li, L., Zhong, T., Wang, L., Ma, J., Chen, X., Song, T., Zhang, H., 2018. Whole-genome sequencing reveals selection signatures associated with important traits in six goat breeds. Scientific Reports 8, 10405. doi:10.1038/s41598-018-28719-w

Han, J., Wan, M., Ma, Z., He, P., 2022. The TOX subfamily: all-round players in the immune system. Clinical and Experimental Immunology 208.

Hardie, L.C., VandeHaar, M.J., Tempelman, R.J., Weigel, K.A., Armentano, L.E., Wiggans, G.R., Veerkamp, R.F., De Haas, Y., Coffey, M.P., Connor, E.E., Hanigan, M.D., Staples, C., Wang, Z., Dekkers, J.C.M., Spurlock, D.M., 2017. The genetic and biological basis of feed efficiency in mid-lactation Holstein dairy cows. Journal of Dairy Science 100, 9061–9075. doi:10.3168/jds.2017-12604

Howard, J.T., Jiao, S., Tiezzi, F., Huang, Y., Gray, K.A., Maltecca, C., 2015. Genome-wide association study on legendre random regression coefficients for the growth and feed intake trajectory on Duroc Boars. BMC Genetics 16, 59. doi:10.1186/s12863-015-0218-8

Hu, H., Mu, T., Ma, Yanfen, Wang, X., Ma, Yun, 2021. Analysis of Longevity Traits in Holstein Cattle: A Review. Frontiers in Genetics 12, 695543. doi:10.3389/fgene.2021.695543

Hudson, G.F.S., Van Vleck, L.D., 1981. Relationship Between Production and Stayability in Holstein Cattle. Journal of Dairy Science 64, 2246–2250. doi:10.3168/jds.S0022-0302(81)82836-6

Islam, R., Liu, X., Gebreselassie, G., Abied, A., Ma, Q., Ma, Y., 2020. Genome-wide association analysis reveals the genetic locus for high reproduction trait in Chinese Arbas Cashmere goat. Genes & Genomics 42, 893–899. doi:10.1007/s13258-020-00937-5

Jamrozik, J., McGrath, S., Kemp, R.A., Miller, S.P., 2013. Estimates of genetic parameters for stayability to consecutive calvings of Canadian Simmentals by random regression models1. Journal of Animal Science 91, 3634–3643. doi:10.2527/jas.2012-6126

Keogh, K., Carthy, T.R., McClure, M.C., Waters, S.M., Kenny, D.A., 2021. Genome-wide association study of economically important traits in Charolais and Limousin beef cows. Animal 15, 100011. doi:10.1016/j.animal.2020.100011

Khazaei-Koohpar, H., Gholizadeh, M., Hafezian, S.H., Esmaeili-Fard, S.M., 2024. Weighted single-step genome-wide association study for direct and maternal genetic effects associated with birth and weaning weights in sheep. Scientific Reports 14, 13120. doi:10.1038/s41598-024-63974-0

Kiser, J.N., Keuter, E.M., Seabury, C.M., Neupane, M., Moraes, J.G.N., Dalton, J., Burns, G.W., Spencer, T.E., Neibergs, H.L., 2019. Validation of 46 loci associated with female fertility traits in cattle. BMC Genomics 20, 576. doi:10.1186/s12864-019-5935-3

Legarra, A., Aguilar, I., Misztal, I., 2009. A relationship matrix including full pedigree and genomic information. Journal of Dairy Science 92, 4656–4663. doi:10.3168/jds.2009-2061

Li, J., Wang, Y., Mukiibi, R., Karisa, B., Plastow, G.S., Li, C., 2022. Integrative analyses of genomic and metabolomic data reveal genetic mechanisms associated with carcass merit traits in beef cattle. Scientific Reports 12, 3389. doi:10.1038/s41598-022-06567-z

Liu, D., Chen, Z., Zhao, W., Guo, L., Sun, H., Zhu, K., Liu, G., Shen, X., Zhao, X., Wang, Q., Ma, P., Pan, Y., 2021. Genome-wide selection signatures detection in Shanghai Holstein cattle population identified genes related to adaption, health and reproduction traits. BMC Genomics 22, 747. doi:10.1186/s12864-021-08042-x

Lukic, B., Curik, I., Drzaic, I., Galić, V., Shihabi, M., Vostry, L., Cubric-Curik, V., 2023. Genomic signatures of selection, local adaptation and production type characterisation of East Adriatic sheep breeds. Journal of Animal Science and Biotechnology 14, 142. doi:10.1186/s40104-023-00936-y

Luo, X., Li, J., Xiao, C., Sun, L., Xiang, W., Chen, N., Lei, C., Lei, H., Long, Y., Long, T., Suolang, Q., Yi, K., 2022. Whole-Genome Resequencing of Xiangxi Cattle Identifies Genomic Diversity and Selection Signatures. Frontiers in Genetics 13, 816379. doi:10.3389/fgene.2022.816379

Majeres, L.E., Dilger, A.C., Shike, D.W., McCann, J.C., Beever, J.E., 2024. Defining a Haplotype Encompassing the LCORL-NCAPG Locus Associated with Increased Lean Growth in Beef Cattle. Genes 15, 576. doi:10.3390/genes15050576

Mariasegaram, M., Reverter, A., Barris, W., Lehnert, S.A., Dalrymple, B., Prayaga, K., 2010. Transcription profiling provides insights into gene pathways involved in horn and scurs development in cattle. BMC Genomics 11, 370. doi:10.1186/1471-2164-11-370

Marín-Garzón, N.A., Magalhães, A.F.B., Mota, L.F.M., Fonseca, L.F.S., Chardulo, L.A.L., Albuquerque, L.G., 2021. Genome-wide association study identified genomic regions and putative candidate genes affecting meat color traits in Nellore cattle. Meat Science 171, 108288. doi:10.1016/j.meatsci.2020.108288

Marrella, M.A., Schettini, G.P., Morozyuk, M., Walsh, A., Cockrum, R., Biase, F.H., 2024. Genome-wide association analyses in dairy heifers highlight genes overlapping with mouse and human fertility and human health traits. doi:10.1101/2024.12.19.629410

Martinez, G.E., Koch, R.M., Cundiff, L.V., Gregory, K.E., Kachman, S.D., Van Vleck, L.D., 2005. Genetic parameters for stayability, stayability at calving, and stayability at weaning to specified ages for Hereford cows1. Journal of Animal Science 83, 2033–2042. doi:10.2527/2005.8392033x

Naserkheil, M., Mehrban, H., Lee, D., Park, M.N., 2021. Genome-wide Association Study for Carcass Primal Cut Yields Using Single-step Bayesian Approach in Hanwoo Cattle. Frontiers in Genetics 12, 752424. doi:10.3389/fgene.2021.752424

Pedrosa, V.B., Boerman, J.P., Gloria, L.S., Chen, S.-Y., Montes, M.E., Doucette, J.S., Brito, L.F., 2023. Genomic-based genetic parameters for milkability traits derived from automatic milking systems in North American Holstein cattle. Journal of Dairy Science 106, 2613–2629. doi:10.3168/jds.2022-22515

Raven, L.-A., Cocks, B.G., Hayes, B.J., 2014. Multibreed genome wide association can improve precision of mapping causative variants underlying milk production in dairy cattle. BMC Genomics 15, 62. doi:10.1186/1471-2164-15-62

Reverter, A., Porto-Neto, L.R., Fortes, M.R.S., McCulloch, R., Lyons, R.E., Moore, S., Nicol, D., Henshall, J., Lehnert, S.A., 2016. Genomic analyses of tropical beef cattle fertility based on genotyping pools of Brahman cows with unknown pedigree1. Journal of Animal Science 94, 4096–4108. doi:10.2527/jas.2016-0675

Rizzo, E.C.A., Neto, F.R.A., Diaz, I.D.P.S., Dias, M.M., Costa, R.B., Ventura, H.T., Oliveira, H.N., Falcão, A.J.S., 2015. Genetic association of productive and reproductive traits with stayability in Nellore cattle: analysis using Bayesian models. Genetics and Molecular Research 14, 14956–14966. doi:10.4238/2015.November.24.3

Rosen, B.D., Bickhart, D.M., Schnabel, R.D., Koren, S., Elsik, C.G., Tseng, E., Rowan, T.N., Low, W.Y., Zimin, A., Couldrey, C., Hall, R., Li, W., Rhie, A., Ghurye, J., McKay, S.D., Thibaud-Nissen, F., Hoffman, J., Murdoch, B.M., Snelling, W.M., McDaneld, T.G., Hammond, J.A., Schwartz, J.C., Nandolo, W., Hagen, D.E., Dreischer, C., Schultheiss, S.J., Schroeder, S.G., Phillippy, A.M., Cole, J.B., Van Tassell, C.P., Liu, G., Smith, T.P.L., Medrano, J.F., 2020. *De novo* assembly of the cattle reference genome with single-molecule sequencing. GigaScience 9. doi:10.1093/gigascience/giaa021

Saatchi, M., Beever, J.E., Decker, J.E., Faulkner, D.B., Freetly, H.C., Hansen, S.L., Yampara-Iquise, H., Johnson, K.A., Kachman, S.D., Kerley, M.S., Kim, J., Loy, D.D., Marques, E., Neibergs, H.L., Pollak, E.J., Schnabel, R.D., Seabury, C.M., Shike, D.W., Snelling, W.M., Spangler, M.L., Weaber, R.L., Garrick, D.J., Taylor, J.F., 2014. QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. BMC Genomics 15, 1004. doi:10.1186/1471-2164-15-1004

Sargolzaei, M., Chesnais, J.P., Schenkel, F.S., 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics 15, 478. doi:10.1186/1471-2164-15-478

Silva, D.O., Fernandes Júnior, G.A., Fonseca, L.F.S., Mota, L.F.M., Bresolin, T., Carvalheiro, R., De Albuquerque, L.G., 2024. Genome-wide association study for stayability at different calvings in Nellore beef cattle. BMC Genomics 25, 93. doi:10.1186/s12864-024-10020-y

Silva, E.F.P., Gaia, R.C., Mulim, H.A., Pinto, L.F.B., Iung, L.H.S., Brito, L.F., Pedrosa, V.B., 2024. Genome-Wide Association Study of Conformation Traits in Brazilian Holstein Cattle. Animals 14, 2472. doi:10.3390/ani14172472

Sim, J.C.H., White, S.M., Fitzpatrick, E., Wilson, G.R., Gillies, G., Pope, K., Mountford, H.S., Torring, P.M., McKee, S., Vulto-van Silfhout, A.T., Jhangiani, S.N., Muzny, D.M., Leventer, R.J., Delatycki, M.B., Amor, D.J., Lockhart, P.J., 2014. Expanding the phenotypic spectrum of ARID1B-mediated disorders and identification of altered cell-cycle dynamics due to ARID1B haploinsufficiency. Orphanet Journal of Rare Diseases 9, 43. doi:10.1186/1750-1172-9-43

Sim, J.C.H., White, S.M., Lockhart, P.J., 2015. *ARID1B*-mediated disorders: Mutations and possible mechanisms. Intractable & Rare Diseases Research 4, 17–23. doi:10.5582/irdr.2014.01021

Smith, J.L., Wilson, M.L., Nilson, S.M., Rowan, T.N., Schnabel, R.D., Decker, J.E., Seabury, C.M., 2022. Genome-wide association and genotype by environment interactions for growth traits in U.S. Red Angus cattle. BMC Genomics 23, 517. doi:10.1186/s12864-022-08667-6

Speidel, S.E., Buckley, B.A., Boldt, R.J., Enns, R.M., Lee, J., Spangler, M.L., Thomas, M.G., 2018. Genome-wide association study of Stayability and Heifer Pregnancy in Red Angus cattle. Journal of Animal Science 96, 846–853. doi:10.1093/jas/sky041

Stegemiller, M.R., Murdoch, G.K., Rowan, T.N., Davenport, K.M., Becker, G.M., Hall, J.B., Murdoch, B.M., 2021. Genome-Wide Association Analyses of Fertility Traits in Beef Heifers. Genes 12, 217. doi:10.3390/genes12020217

Sun, X., Habier, D., Fernando, R.L., Garrick, D.J., Dekkers, J.C., 2011. Genomic breeding value prediction and QTL mapping of QTLMAS2010 data using Bayesian Methods. BMC Proceedings 5. doi:10.1186/1753-6561-5-s3-s13

Tiezzi, F., Parker-Gaddis, K.L., Cole, J.B., Clay, J.S., Maltecca, C., 2015. A Genome-Wide Association Study for Clinical Mastitis in First Parity US Holstein Cows Using Single-Step Approach and Genomic Matrix Re-Weighting Procedure. PLOS ONE 10, e0114919. doi:10.1371/journal.pone.0114919

Uzbekova, S., Arlot-Bonnemains, Y., Dupont, J., Dalbiès-Tran, R., Papillier, P., Pennetier, S., Thélie, A., Perreau, C., Mermillod, P., Prigent, C., Uzbekov, R., 2008. Spatio-Temporal Expression Patterns of Aurora Kinases A, B, and C and Cytoplasmic Polyadenylation-Element-Binding Protein in Bovine Oocytes During Meiotic Maturation1. Biology of Reproduction 78, 218–233. doi:10.1095/biolreprod.107.061036

VanRaden, P.M., 2008. Efficient Methods to Compute Genomic Predictions. Journal of Dairy Science 91, 4414–4423. doi:10.3168/jds.2007-0980

Walker, B.N., Nix, J., Wilson, C., Marrella, M.A., Speckhart, S.L., Wooldridge, L., Yen, C.-N., Bodmer, J.S., Kirkpatrick, L.T., Moorey, S.E., Gerrard, D.E., Ealy, A.D., Biase, F.H., 2022. Tight gene co-expression in BCB positive cattle oocytes and their surrounding cumulus cells. Reproductive Biology and Endocrinology 20, 119. doi:10.1186/s12958-022-00994-3

Wang, H., Misztal, I., Aguilar, I., Legarra, A., Muir, W.M., 2012. Genome-wide association mapping including phenotypes from relatives without genotypes. Genetics Research 94, 73–83. doi:10.1017/s0016672312000274

Warma, A., Descarreaux, M., Chorfi, Y., Dupras, R., Rémillard, R., Ndiaye, K., 2020. Interleukins' expression profile changes in granulosa cells of preovulatory follicles during the postpartum period in dairy cows. Cytokine: X 2, 100022. doi:10.1016/j.cytox.2020.100022

Yu, H., Yu, S., Guo, J., Cheng, G., Mei, C., Zan, L., 2023. Genome-Wide Association Study Reveals Novel Loci Associated with Body Conformation Traits in Qinchuan Cattle. Animals 13, 3628. doi:10.3390/ani13233628

Zhang, G.-W., Wang, L., Wu, J., Ye, Y., Zhao, J., Du, Y., Tu, Y., Luo, Z., Fu, S., Zuo, F., 2023. Evaluation of MYBL1 as the master regulator for pachytene spermatocyte genes dysregulated in interspecific hybrid dzo. Journal of Dairy Science 106, 4366–4379. doi:10.3168/jds.2022-22963

4.15 Supplementary Information

4.15.1 Additional file 1

Table S4.1 Number of cows and survival incidence by stayability records in Limousine cattle.

Trait	Definition	Limousine		
		N	N of cow survived	Incidence (survival %)
STAY1	Stayability as a first parity = 1; failed = 0	38,188	38,188	100.00
STAY2	Stayability as a second parity = 1; failed = 0	33,209	25,526	66.84
STAY3	Stayability as a third parity = 1; failed = 0	30,105	18,764	49.14
STAY4	Stayability as a fourth parity = 1; failed = 0	27,978	14,381	37.66
STAY5	Stayability as a fifth parity = 1; failed = 0	26,546	11,257	29.48
STAY6	Stayability as a sixth parity = 1; failed = 0	25,372	8,838	23.14
STAY7	Stayability as a seventh parity = 1; failed = 0	24,429	6,890	18.04
STAY8	Stayability as a eight parity = 1; failed = 0	23,657	5,314	13.91

Abbreviations: N = Total number of cows, including both those that survived and were culled

Table S4.2 Number of total conformational traits considered with the relative definition and related score.

Trait	Definition	N	Mean ± SD
Wither width	It is defined by the distance between the scapulae	32 316	5.86 ± 1.03
Rump convexity	It is observed from the side, along with the width of the rear, and gives an idea of the development of the most valuable muscles of the thigh	32 316	6.04 ± 1.03
Rump length	It is observed from the side and gives an idea of the length of the most valuable muscles of the thigh	32 316	5.82 ± 0.98
Dorsolumbar line length	It measures the distance between the withers and the tip of the hips; in practice, it is measured by observing the distance between the front and rear legs, taking into account the development of the animal	32 316	6.51 ± 0.88
Rear width	It is defined by the width of the thigh muscle, evaluated at mid-height	32 316	6.09 ± 1.04
Back width	It defines the importance of the muscle just behind the scapulae	32 316	5.59 ± 1.12
Pelvic length	It defines the length between the hips and the ischium and must be proportionate to the loin length	32 316	5.99 ± 0.89
Development	It is defined by the size of the animal, which can be appreciated based on the height at the withers	32 316	6.05 ± 1.01

N = Total number of cows per each of the morpho traits considered from STAY2

Table S4.3 Comparison of imputation accuracy between full imputation and masked-marker validation.

Quality metrics	Imputation (Full)	Imputation with Masked Markers
Square correlation (r^2)	0.99	0.98
Mendelian Errors (%)	0.42 %	0.75 %
SNP Retention (%)	98% successfully imputed	98% successfully imputed

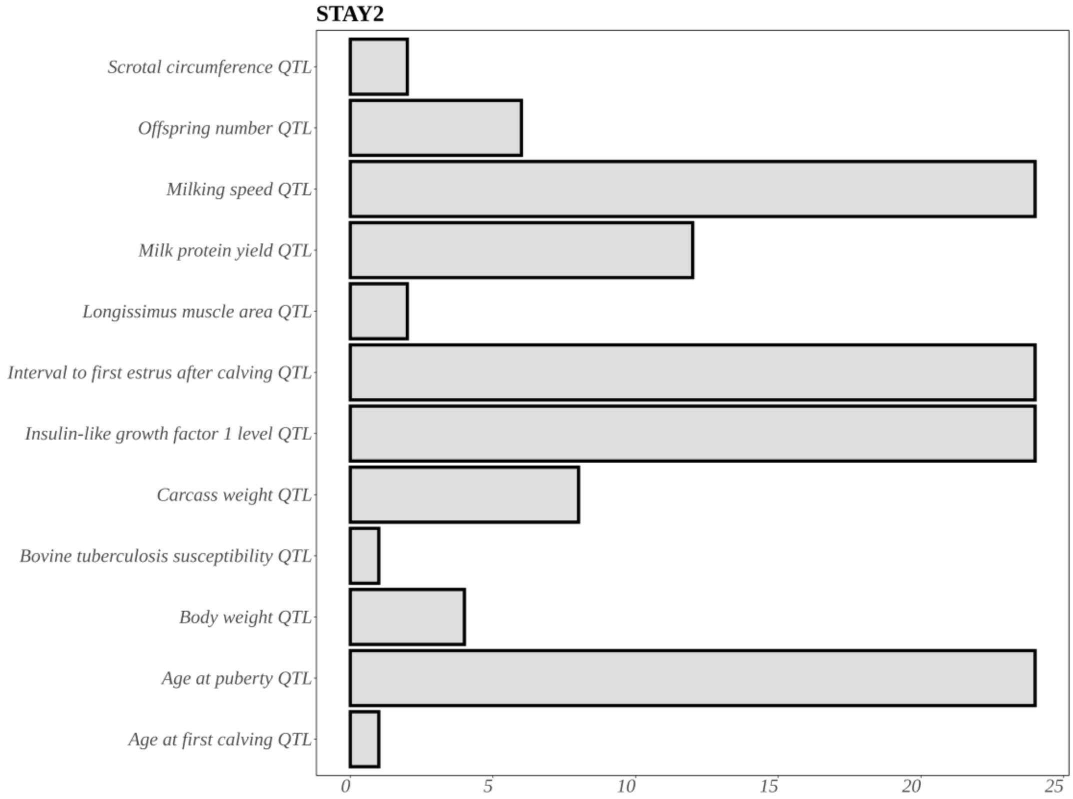
Table S4.4 Top genomic windows explaining variance > 1% for longevity, fertility, and conformation traits.

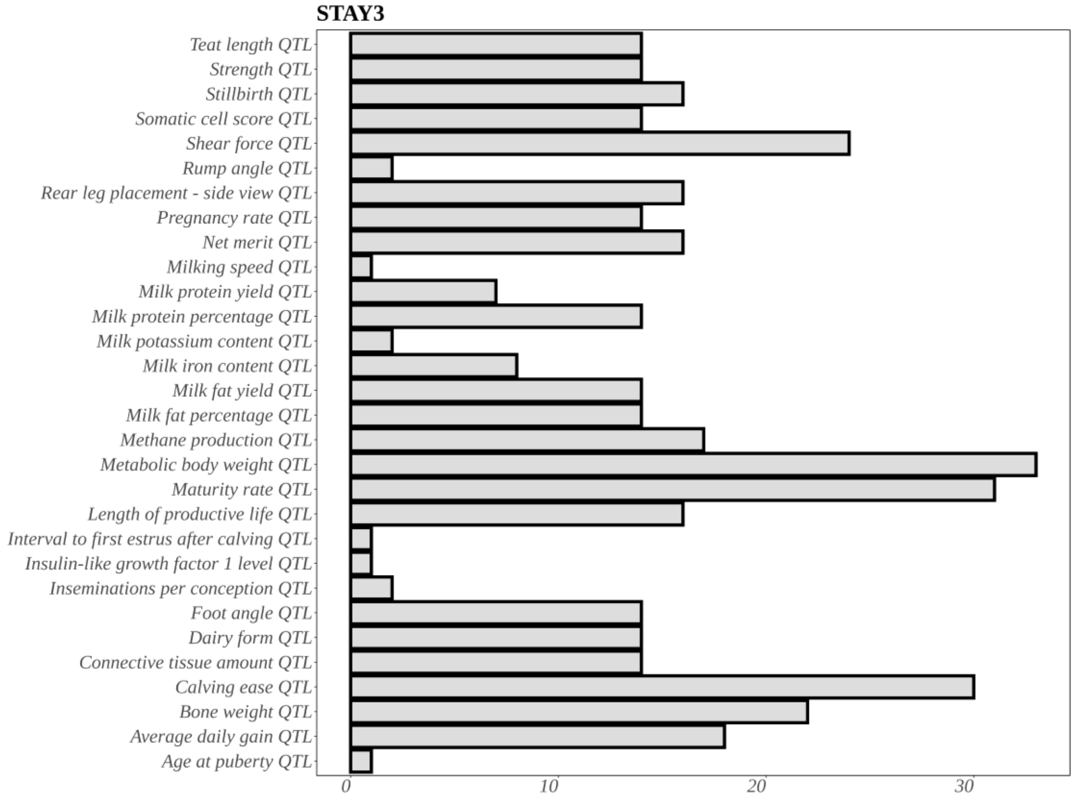
Trait	N	% on the variance covered in the whole genome by the top windows	Chr
Stayability			
STAY2	31	4.00%	3, 14, 24
STAY3	41	4.50%	6, 14, 17, 23
STAY4	28	3.70%	6, 8, 20, 23
STAY5	32	3.70%	23
STAY6	51	7.00%	9, 23
STAY7	44	4.20%	3, 9, 14, 23
STAY8	54	7.00%	3, 9, 10, 20, 23
Fertility			
AFC	47	4.60%	2, 7, 12, 14, 20
FCI	63	6.40%	3, 6, 8, 14, 24
Conformation			
Wither width	28	4.60%	5, 20, 24
Rump convexity	34	3.60%	6, 20
Rump length	37	3.00%	3, 4, 12, 20, 23
Dorsolumbar line length	46	6.20%	2, 4, 14, 17, 23
Rear width	61	9.00%	7, 20, 23
Back width	61	7.00%	5, 7, 23, 24
Pelvic length	61	8.00%	3, 9, 18, 23
Development	15	2.00%	7, 14, 17, 20, 23

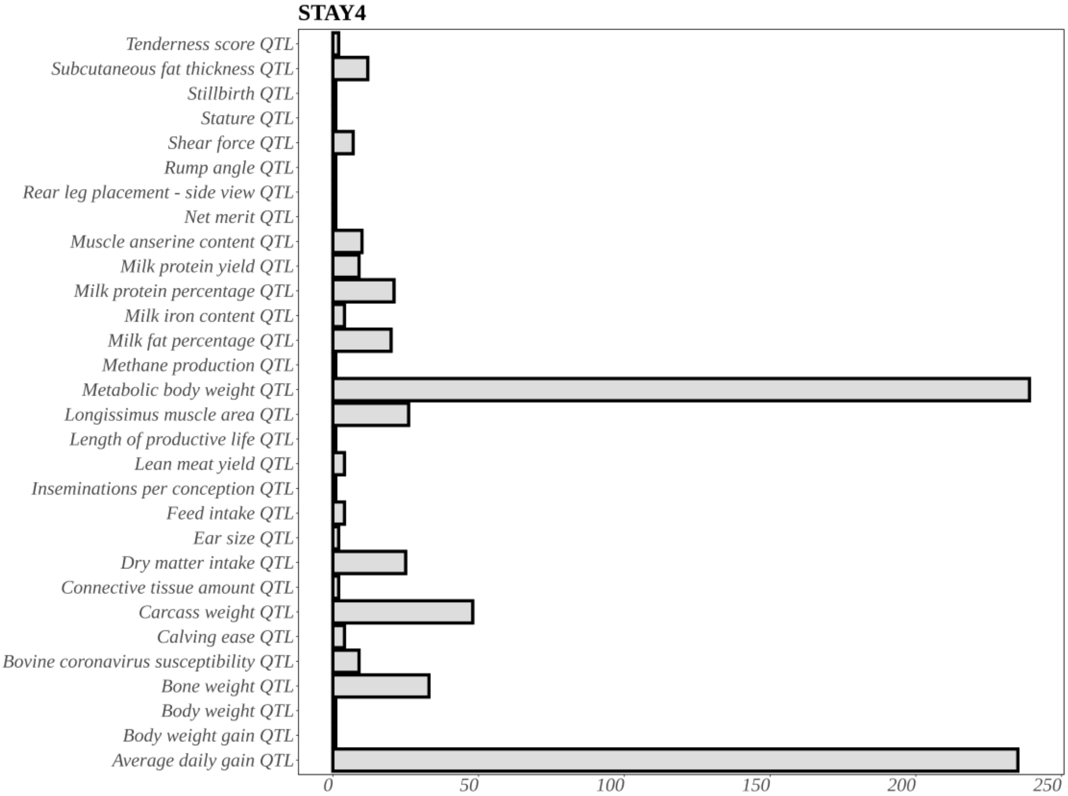
Table S4.5 Gene-trait overlaps for longevity, fertility, and conformation in Limousine cattle.

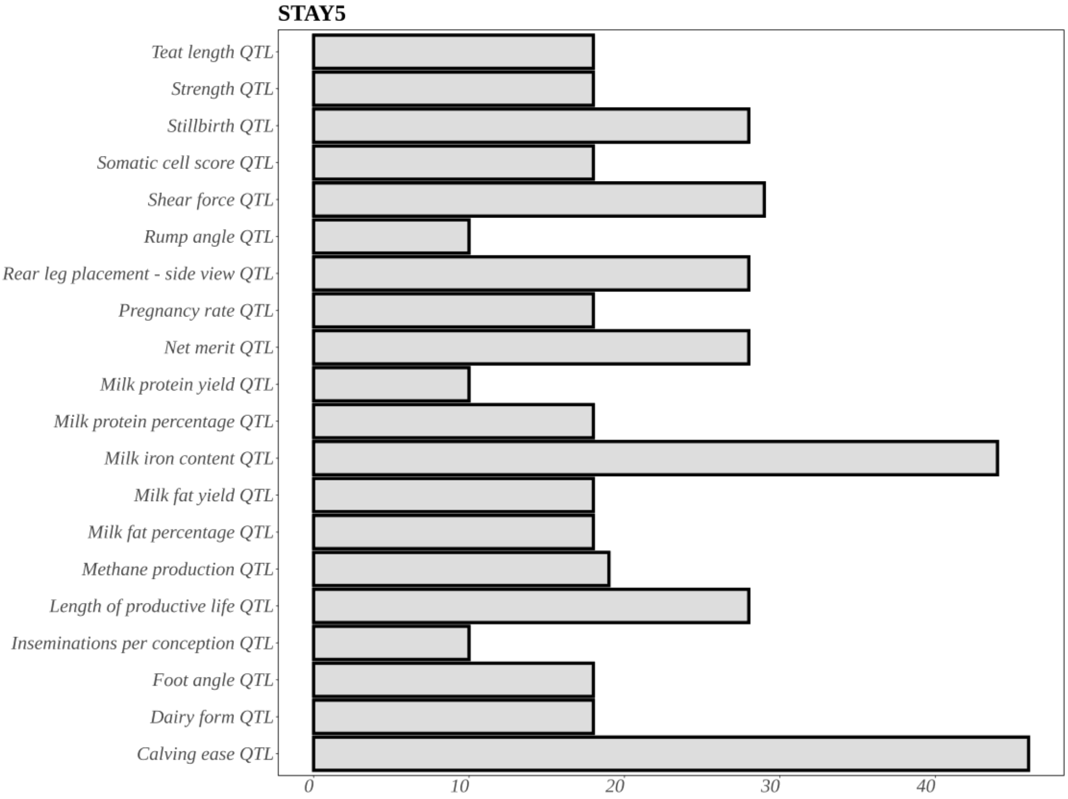
Gene		**Traits**									
	Chr	**STAY2**	**STAY3**	**STAY4**	**STAY5**	**STAY6**	**STAY7**	**STAY8**	**AFC**	**FCI**	**Conformation**
KHDRBS2	23	-	✓	✓	✓	✓	✓	✓	-	-	rump length, dorsolumbar line, pelvic length, rear width, back width, development
ARID1B	9	-	-	-	-	✓	-	✓	-	-	pelvic length
TMEM242	9	-	-	-	-	✓	-	✓	-	-	-
TOX	14	✓	✓	-	-	-	✓	-	✓	-	dorsolumbar line length
CPEB4	20	-	-	✓	-	-	-	✓	✓	-	rump convexity, rump length, rear width, wither width
LAP3–NCAPG–LCORL	6	-	-	✓	-	-	-	-	-	✓	-
BARHL2	3	✓	-	-	-	-	-	-	-	✓	-
CSF2	7	-	-	-	-	-	-	-	✓	-	rear width, back width, development
IL3	7	-	-	-	-	-	-	-	✓	-	rear width, back width, and development
ACSL6	7	-	-	-	-	-	-	-	✓	-	rear width, back width, and development
C20H5orf47	20	-	-	-	-	-	-	-	✓	-	rear width, wither width

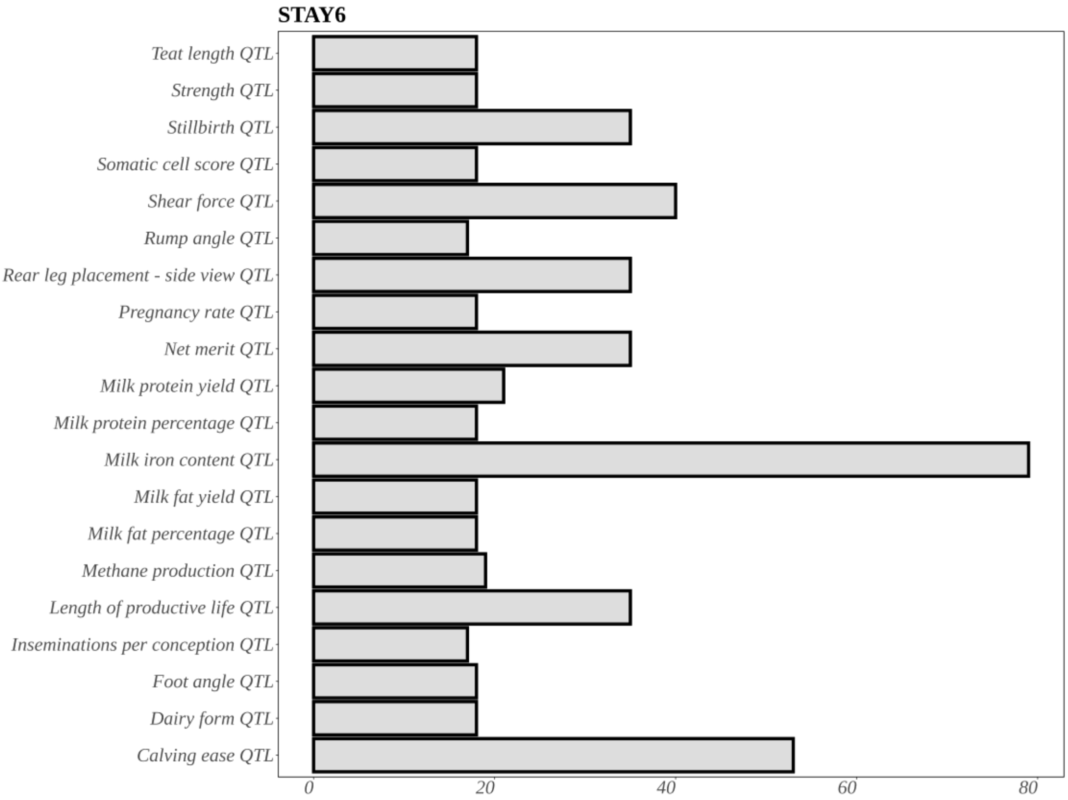
Figure S4.1 – S4.17 Detailed counts and individual quantitative traits loci (QTLs) for all traits.

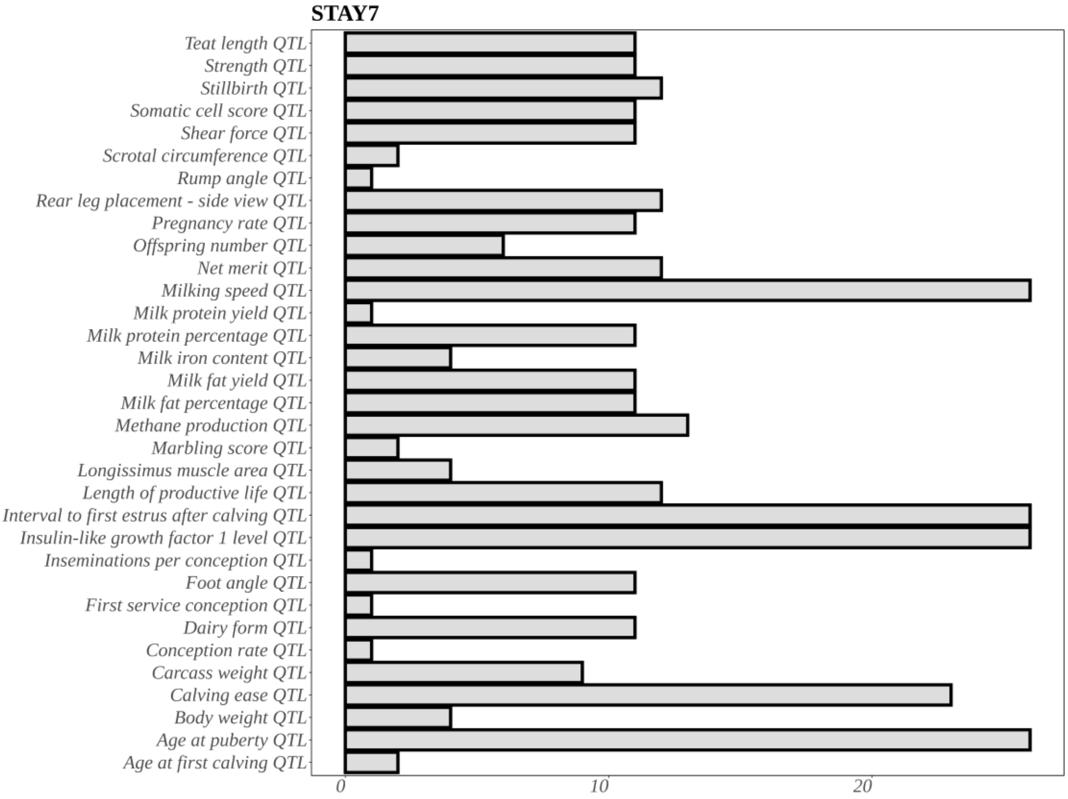


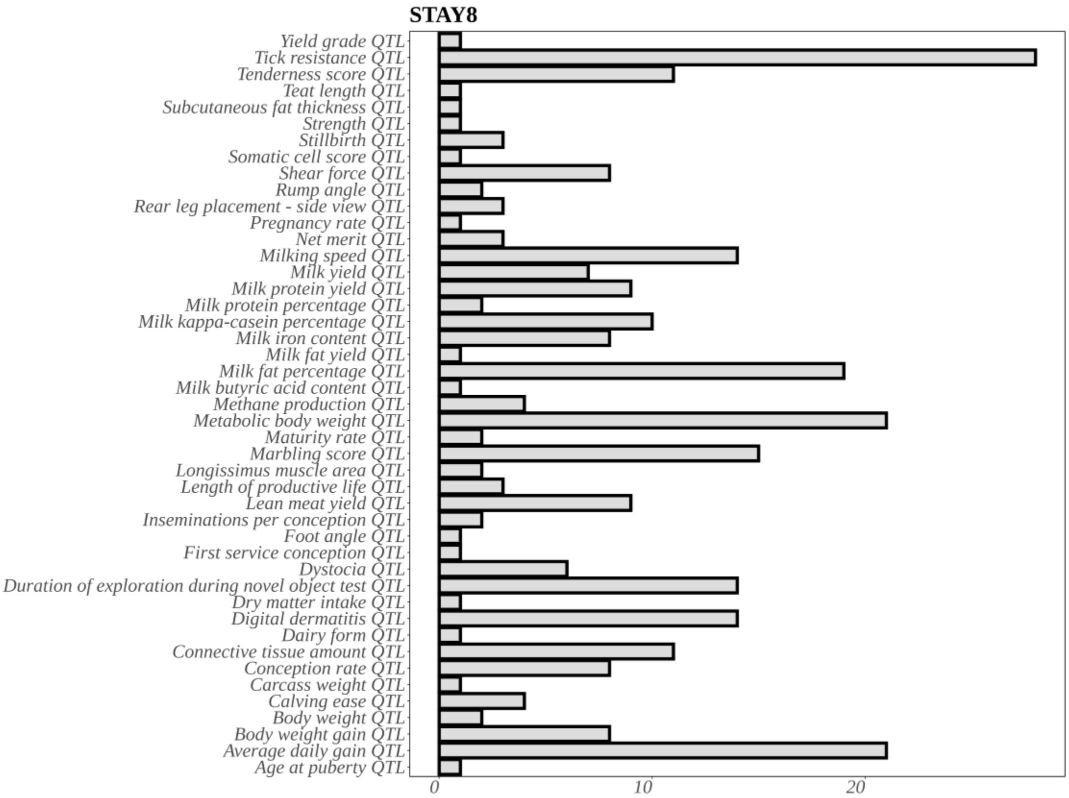


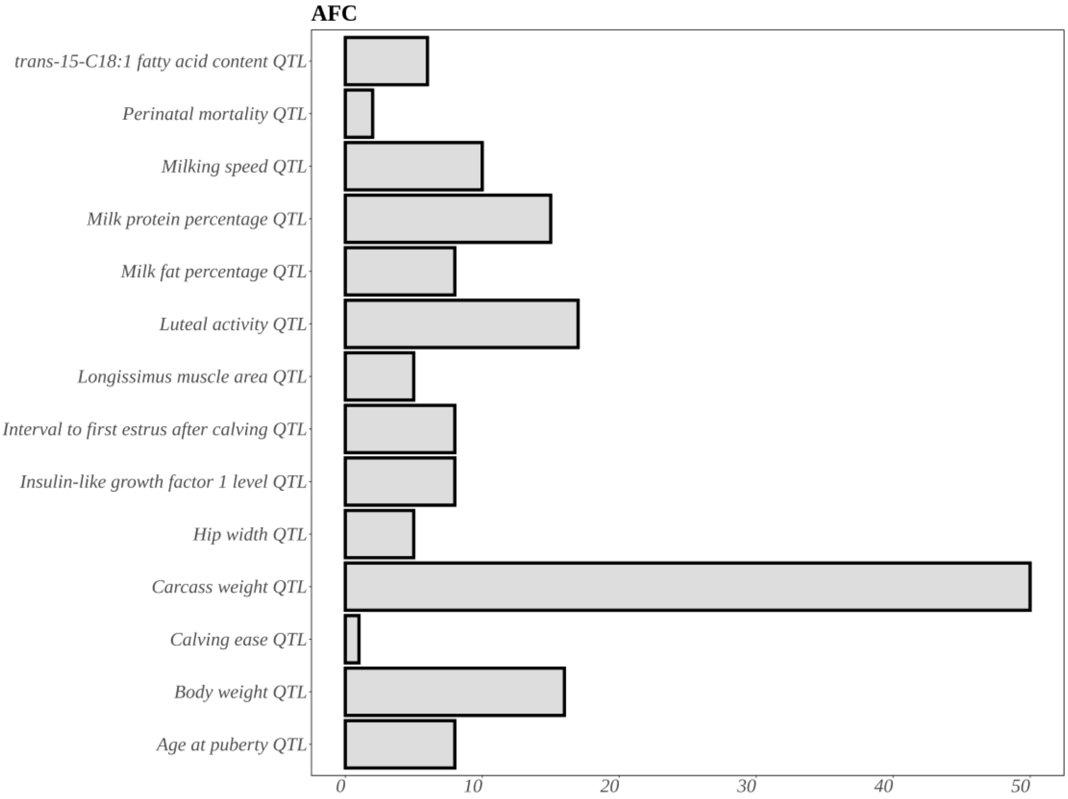

STAY4
Tenderness score QTL
Subcutaneous fat thickness QTL
Stillbirth QTL
Stature QTL
Shear force QTL
Rump angle QTL
Rear leg placement - side view QTL
Net merit QTL
Muscle anserine content QTL
Milk protein yield QTL
Milk protein percentage QTL
Milk iron content QTL
Milk fat percentage QTL
Methane production QTL
Metabolic body weight QTL
Longissimus muscle area QTL
Length of productive life QTL
Lean meat yield QTL
Inseminations per conception QTL
Feed intake QTL
Ear size QTL
Dry matter intake QTL
Connective tissue amount QTL
Carcass weight QTL
Calving ease QTL
Bovine coronavirus susceptibility QTL
Bone weight QTL
Body weight QTL
Body weight gain QTL
Average daily gain QTL
0
50
100
150
200
250

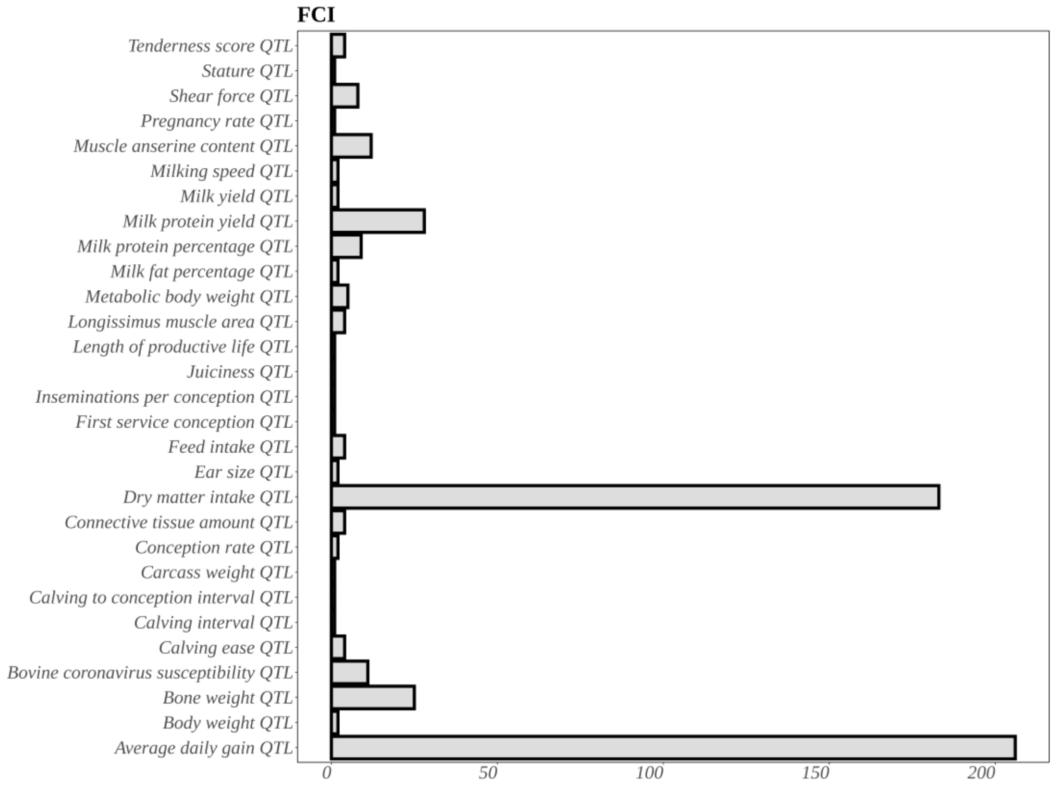

STAY5
Teat length QTL
Strength QTL
Stillbirth QTL
Somatic cell score QTL
Shear force QTL
Rump angle QTL
Rear leg placement - side view QTL
Pregnancy rate QTL
Net merit QTL
Milk protein yield QTL
Milk protein percentage QTL
Milk iron content QTL
Milk fat yield QTL
Milk fat percentage QTL
Methane production QTL
Length of productive life QTL
Inseminations per conception QTL
Foot angle QTL
Dairy form QTL
Calving ease QTL
0
10
20
30
40

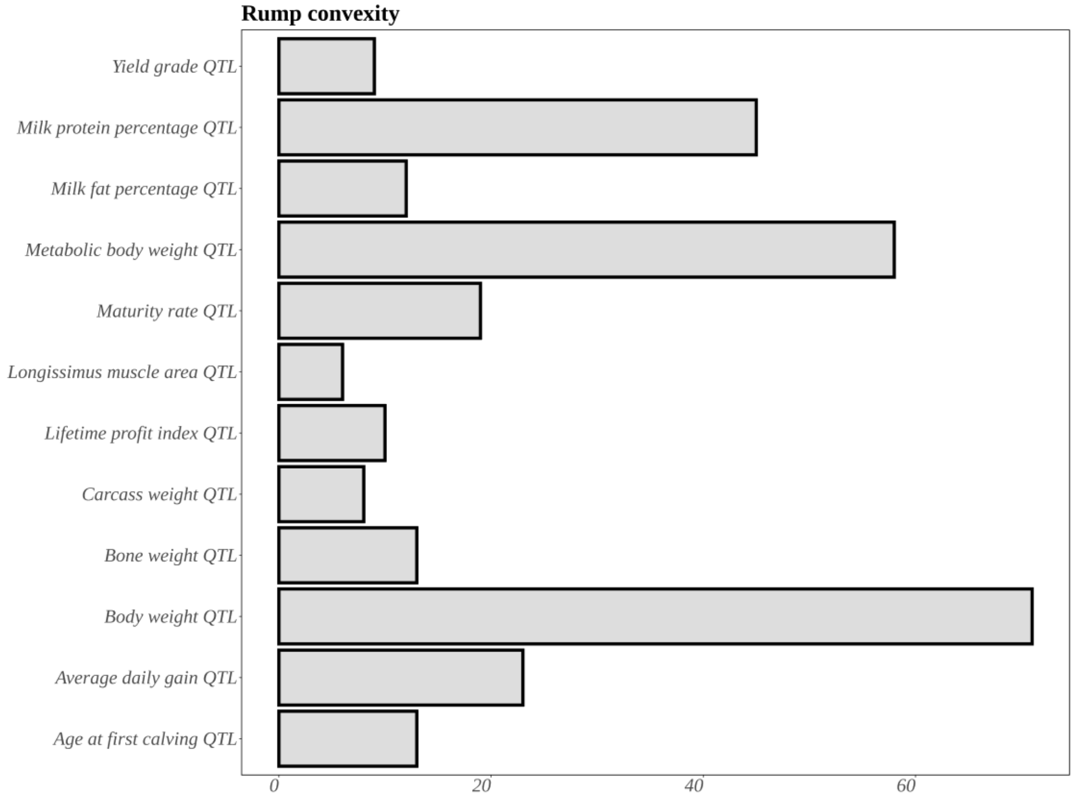

STAY6
Teat length QTL
Strength QTL
Stillbirth QTL
Somatic cell score QTL
Shear force QTL
Rump angle QTL
Rear leg placement - side view QTL
Pregnancy rate QTL
Net merit QTL
Milk protein yield QTL
Milk protein percentage QTL
Milk iron content QTL
Milk fat yield QTL
Milk fat percentage QTL
Methane production QTL
Length of productive life QTL
Inseminations per conception QTL
Foot angle QTL
Dairy form QTL
Calving ease QTL
0
20
40
60
80


STAY7
Teat length QTL
Strength QTL
Stillbirth QTL
Somatic cell score QTL
Shear force QTL
Scrotal circumference QTL
Rump angle QTL
Rear leg placement - side view QTL
Pregnancy rate QTL
Offspring number QTL
Net merit QTL
Milking speed QTL
Milk protein yield QTL
Milk protein percentage QTL
Milk iron content QTL
Milk fat yield QTL
Milk fat percentage QTL
Methane production QTL
Marbling score QTL
Longissimus muscle area QTL
Length of productive life QTL
Interval to first estrus after calving QTL
Insulin-like growth factor 1 level QTL
Inseminations per conception QTL
Foot angle QTL
First service conception QTL
Dairy form QTL
Conception rate QTL
Carcass weight QTL
Calving ease QTL
Body weight QTL
Age at puberty QTL
Age at first calving QTL
0
10
20

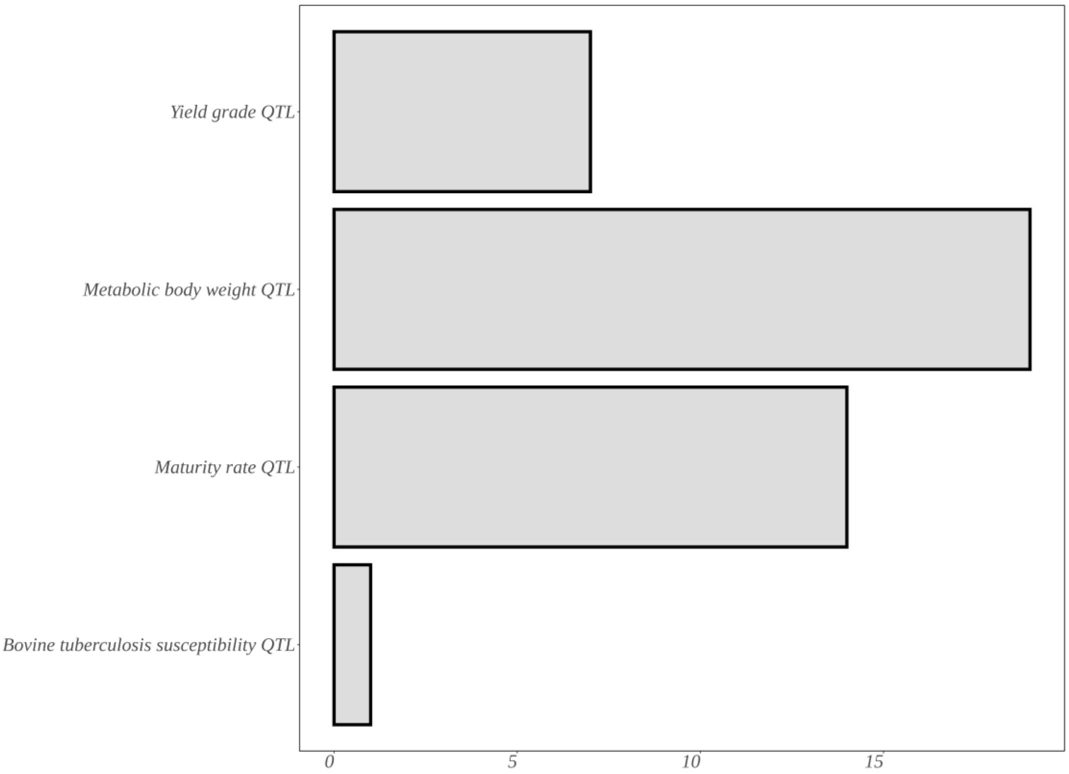

STAY8
Yield grade QTL
Tick resistance QTL
Tenderness score QTL
Teat length QTL
Subcutaneous fat thickness QTL
Strength QTL
Stillbirth QTL
Somatic cell score QTL
Shear force QTL
Rump angle QTL
Rear leg placement - side view QTL
Pregnancy rate QTL
Net merit QTL
Milking speed QTL
Milk yield QTL
Milk protein yield QTL
Milk protein percentage QTL
Milk kappa-casein percentage QTL
Milk iron content QTL
Milk fat yield QTL
Milk fat percentage QTL
Milk butyric acid content QTL
Methane production QTL
Metabolic body weight QTL
Maturity rate QTL
Marbling score QTL
Longissimus muscle area QTL
Length of productive life QTL
Lean meat yield QTL
Inseminations per conception QTL
Foot angle QTL
First service conception QTL
Dystocia QTL
Duration of exploration during novel object test QTL
Dry matter intake QTL
Digital dermatitis QTL
Dairy form QTL
Connective tissue amount QTL
Conception rate QTL
Carcass weight QTL
Calving ease QTL
Body weight QTL
Body weight gain QTL
Average daily gain QTL
Age at puberty QTL
0
10
20


AFC
trans-15-C18:1 fatty acid content QTL
Perinatal mortality QTL
Milking speed QTL
Milk protein percentage QTL
Milk fat percentage QTL
Luteal activity QTL
Longissimus muscle area QTL
Interval to first estrus after calving QTL
Insulin-like growth factor 1 level QTL
Hip width QTL
Carcass weight QTL
Calving ease QTL
Body weight QTL
Age at puberty QTL
0
10
20
30
40
50

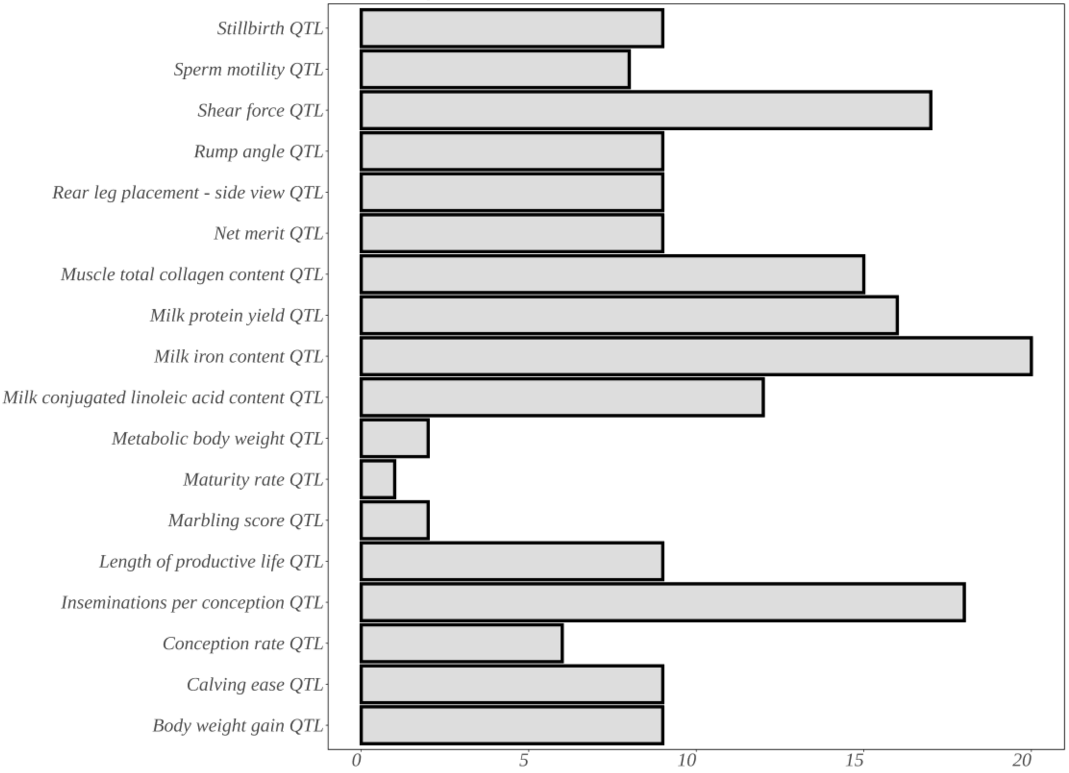

FCI

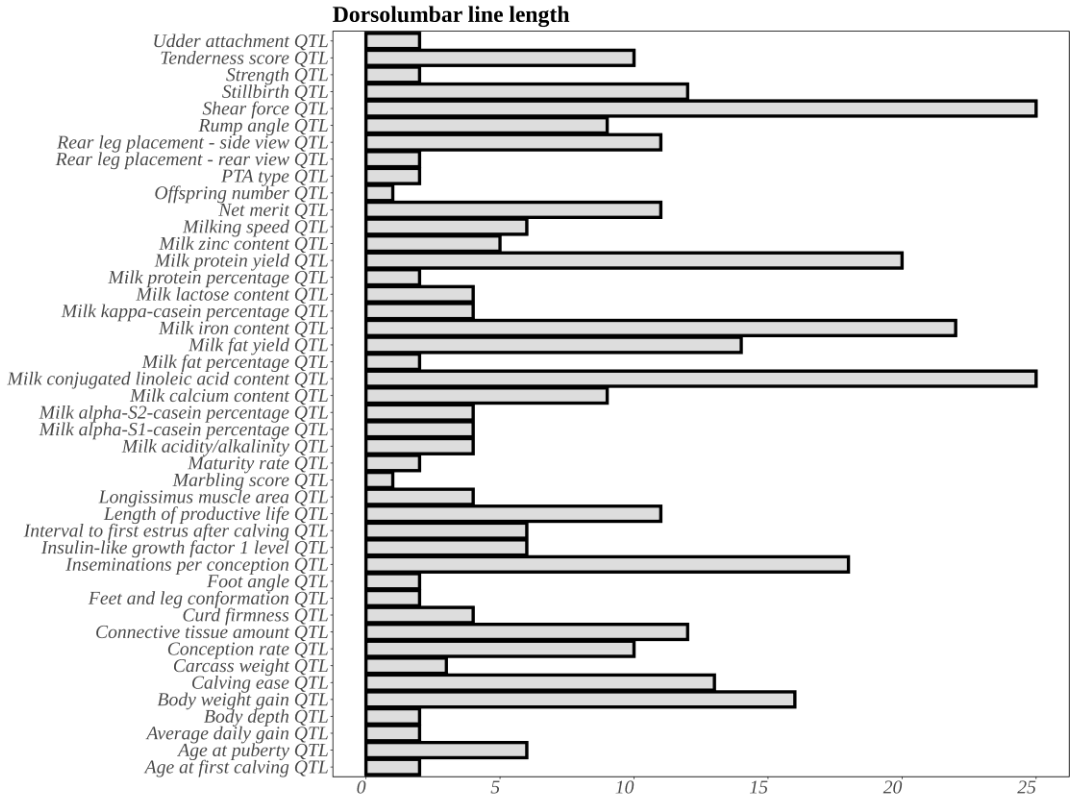

Rump convexity

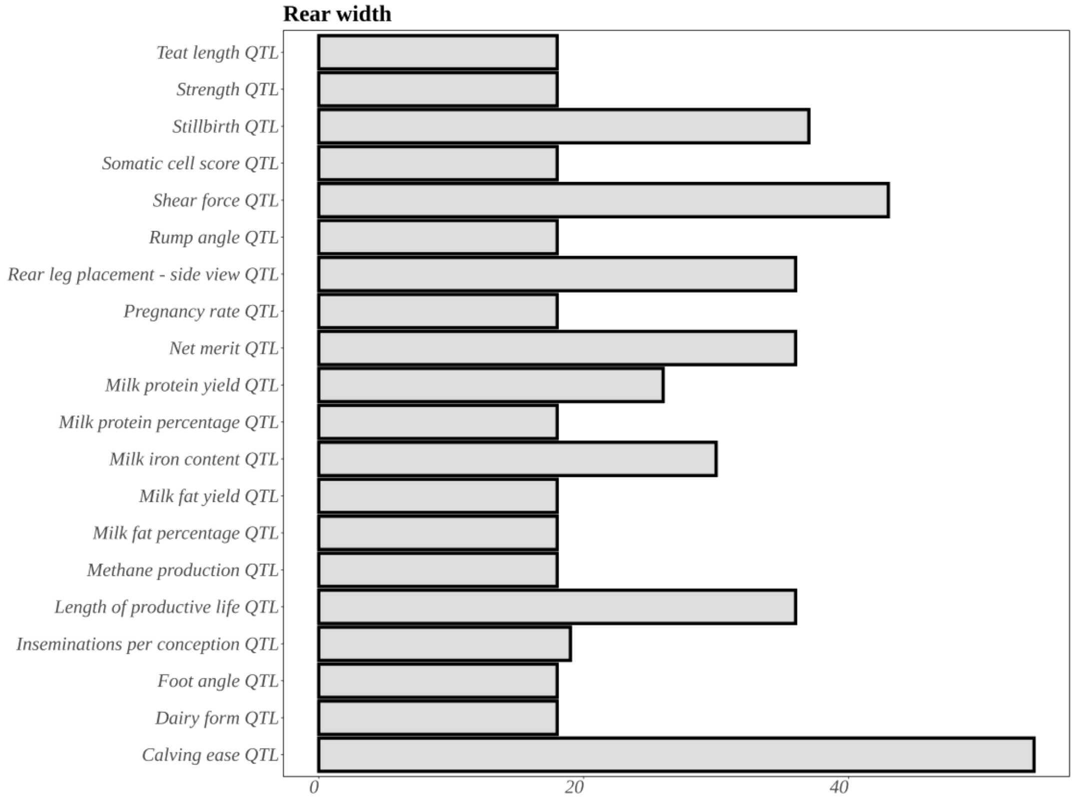
Wither width

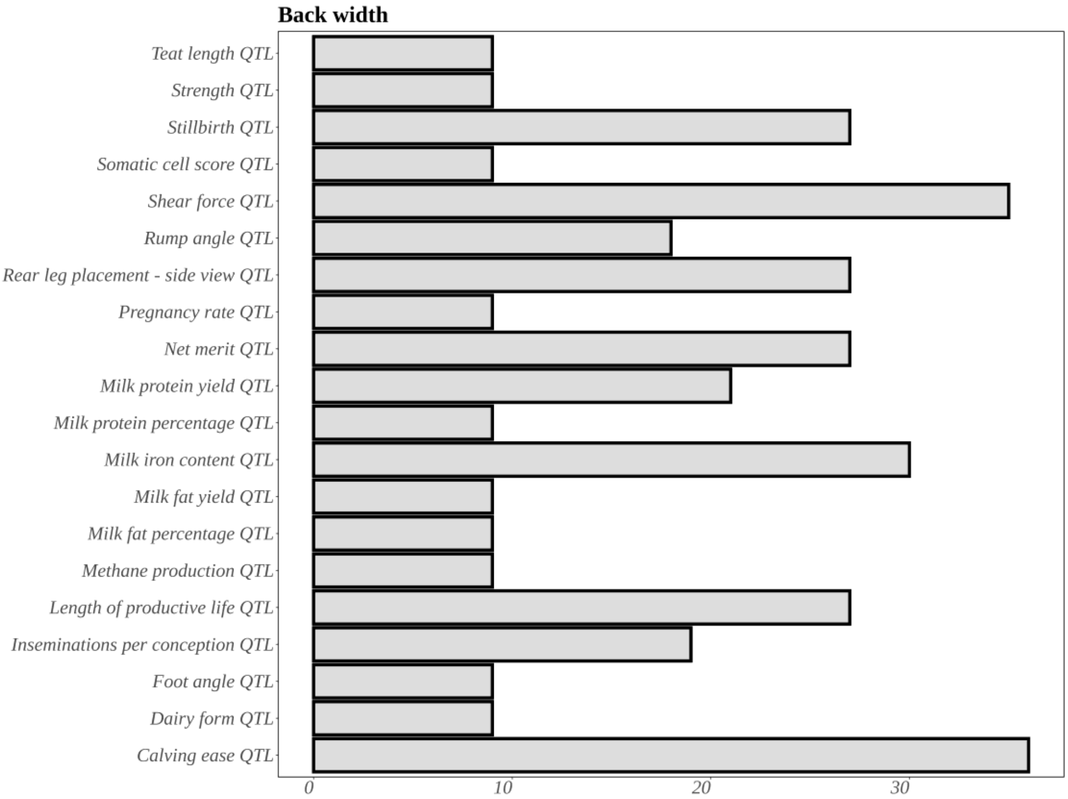


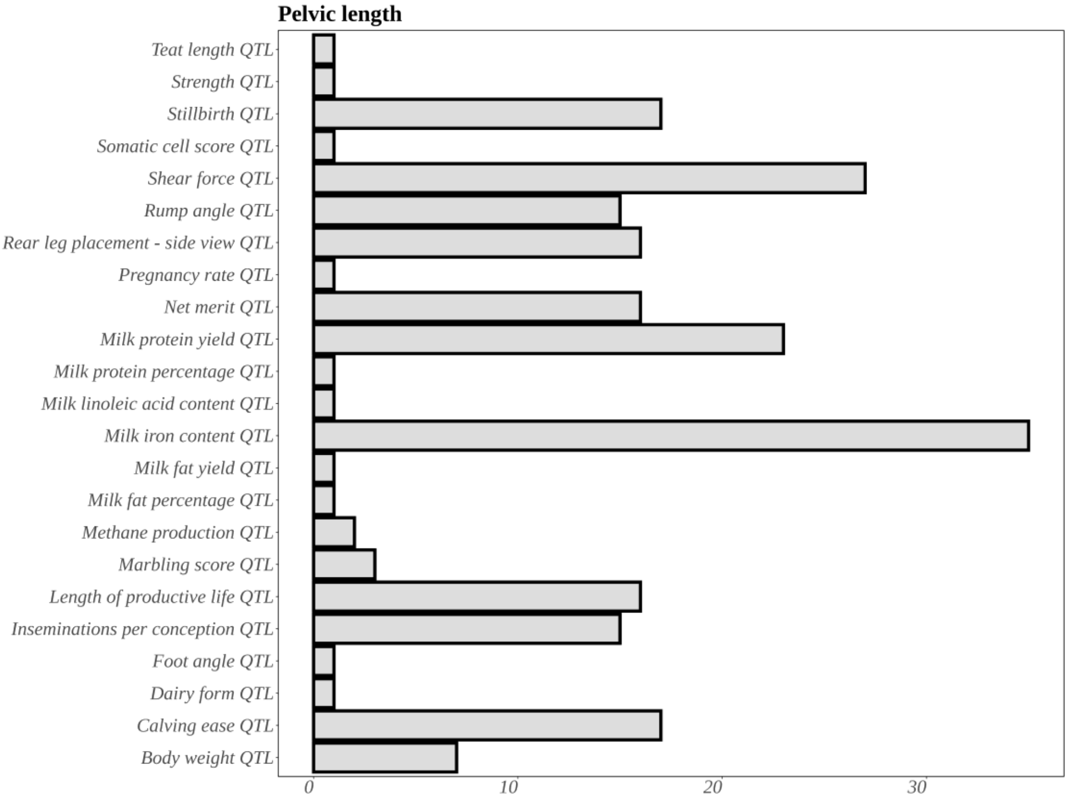
Rump length

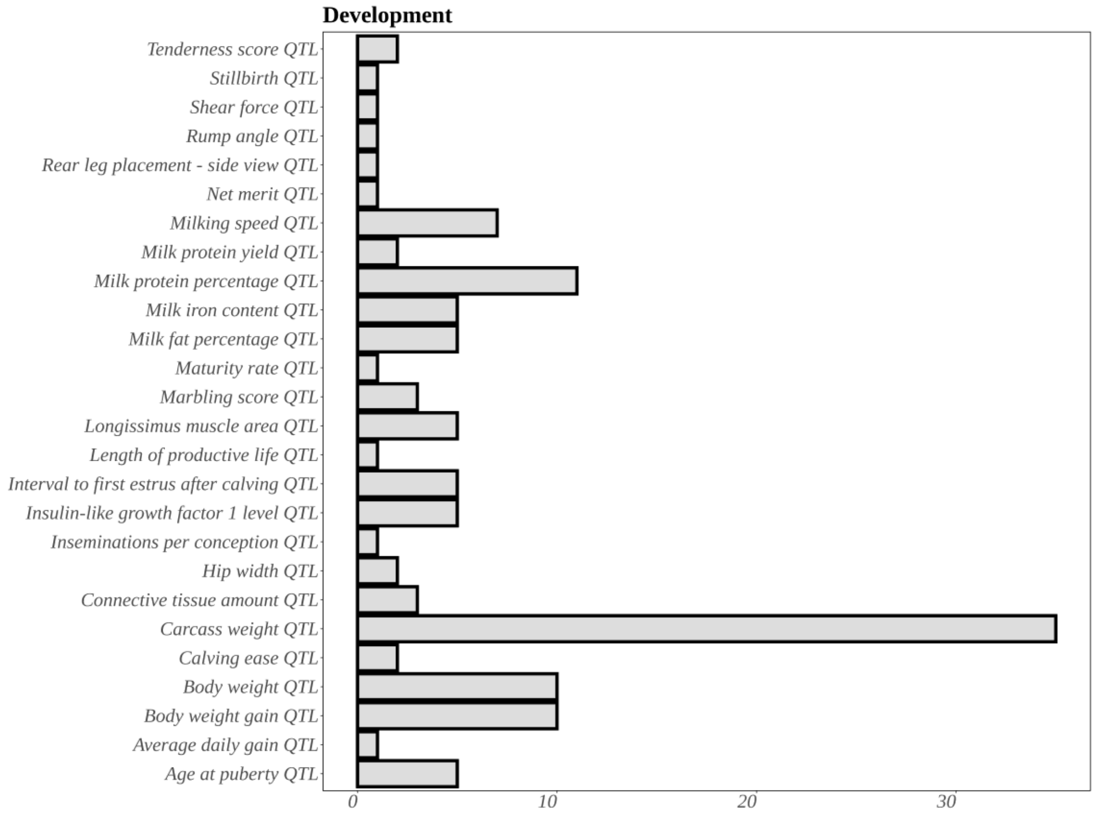



Dorsolumbar line length
Udder attachment QTL
Tenderness score QTL
Strength QTL
Stillbirth QTL
Shear force QTL
Rump angle QTL
Rear leg placement - side view QTL
Rear leg placement - rear view QTL
PTA type QTL
Offspring number QTL
Net merit QTL
Milking speed QTL
Milk zinc content QTL
Milk protein yield QTL
Milk protein percentage QTL
Milk lactose content QTL
Milk kappa-casein percentage QTL
Milk iron content QTL
Milk fat yield QTL
Milk fat percentage QTL
Milk conjugated linoleic acid content QTL
Milk calcium content QTL
Milk alpha-S2-casein percentage QTL
Milk alpha-S1-casein percentage QTL
Milk acidity/alkalinity QTL
Maturity rate QTL
Marbling score QTL
Longissimus muscle area QTL
Length of productive life QTL
Interval to first estrus after calving QTL
Insulin-like growth factor 1 level QTL
Inseminations per conception QTL
Foot angle QTL
Feet and leg conformation QTL
Curd firmness QTL
Connective tissue amount QTL
Conception rate QTL
Carcass weight QTL
Calving ease QTL
Body weight gain QTL
Body depth QTL
Average daily gain QTL
Age at puberty QTL
Age at first calving QTL
0
5
10
15
20
25


Rear width
Teat length QTL
Strength QTL
Stillbirth QTL
Somatic cell score QTL
Shear force QTL
Rump angle QTL
Rear leg placement - side view QTL
Pregnancy rate QTL
Net merit QTL
Milk protein yield QTL
Milk protein percentage QTL
Milk iron content QTL
Milk fat yield QTL
Milk fat percentage QTL
Methane production QTL
Length of productive life QTL
Inseminations per conception QTL
Foot angle QTL
Dairy form QTL
Calving ease QTL
0
20
40


Back width
Teat length QTL
Strength QTL
Stillbirth QTL
Somatic cell score QTL
Shear force QTL
Rump angle QTL
Rear leg placement - side view QTL
Pregnancy rate QTL
Net merit QTL
Milk protein yield QTL
Milk protein percentage QTL
Milk iron content QTL
Milk fat yield QTL
Milk fat percentage QTL
Methane production QTL
Length of productive life QTL
Inseminations per conception QTL
Foot angle QTL
Dairy form QTL
Calving ease QTL
0
10
20
30


Pelvic length
Teat length QTL
Strength QTL
Stillbirth QTL
Somatic cell score QTL
Shear force QTL
Rump angle QTL
Rear leg placement - side view QTL
Pregnancy rate QTL
Net merit QTL
Milk protein yield QTL
Milk protein percentage QTL
Milk linoleic acid content QTL
Milk iron content QTL
Milk fat yield QTL
Milk fat percentage QTL
Methane production QTL
Marbling score QTL
Length of productive life QTL
Inseminations per conception QTL
Foot angle QTL
Dairy form QTL
Calving ease QTL
Body weight QTL
0
10
20
30



4.15.2 Additional file 2 (.csv format)

Title: Top 20-SNP sliding windows explaining the largest proportion of genetic variance (>1%) for stayability (STAY2-8). Legend: The table includes trait name, chromosome (chr), percentage of variance explained, SNP range (start and stop), and physical position of each window (start and stop). Only windows explaining >1% variance and showing significant p-values after bootstrap validation are reported.

Additional file 2.csv

4.15.3 Additional file 3 (.csv format)

Title: Top 20-SNP sliding windows explaining the largest proportion of genetic variance (>1%) for fertility traits. Legend: The table includes trait name, chromosome (chr), percentage of variance explained, SNP range (start and stop), and physical position of each window (start and stop). Only windows explaining >1% variance and showing significant p-values after bootstrap validation are reported.

Additional file 3.csv

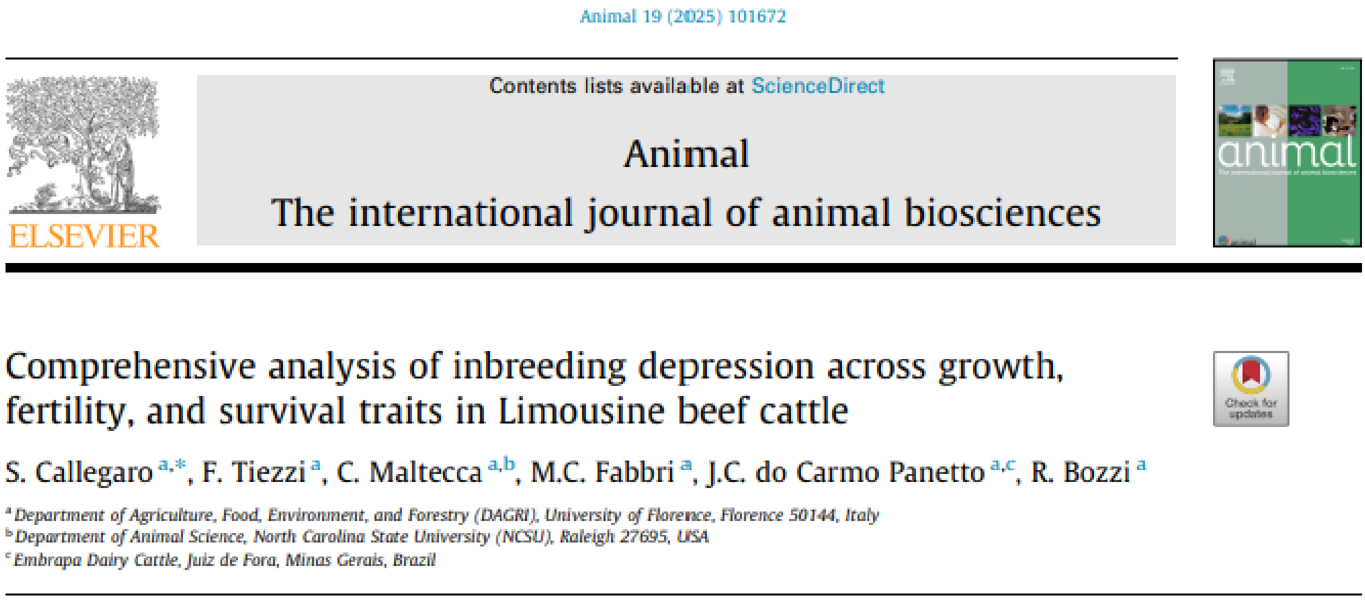
4.15.4 Additional file 4 (.csv format)

Title: Top 20-SNP sliding windows explaining the largest proportion of genetic variance (>1%) for conformation traits. Legend: The table includes trait name, chromosome (chr), percentage of variance explained, SNP range (start and stop), and physical position of each window (start and stop). Only windows explaining >1% variance and showing significant p-values after bootstrap validation are reported.

Additional file 4.csv

CHAPTER 5

5. Comprehensive analysis of inbreeding depression across growth, fertility, and survival traits in Limousine beef cattle



Animal 19 (2025) 101672

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Comprehensive analysis of inbreeding depression across growth, fertility, and survival traits in Limousine beef cattle

S. Callegaro [a,*], F. Tiezzi [a], C. Maltecca [a,b], M.C. Fabbri [a], J.C. do Carmo Panetto [a,c], R. Bozzi [a]

[a] Department of Agriculture, Food, Environment, and Forestry (DAGRI), University of Florence, Florence 50144, Italy
[b] Department of Animal Science, North Carolina State University (NCSU), Raleigh 27695, USA
[c] Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil

https://doi.org/10.1016/j.animal.2025.101672

Abstract

The Italian Limousine cattle population, with approximately 100 000 registered animals in the national herd book, plays a crucial role in the country's national beef industry, being one of the most widely spread breeds in Italy. Maintaining genetic diversity is essential to prevent the negative consequences of inbreeding, which can reduce animal performance for economically important traits such as growth, fertility, and longevity. Traditional pedigree-based inbreeding estimates have been previously used, but genomic tools offer the possibility of greater precision. This study evaluated the effects of inbreeding and inbreeding depression on key performance traits in Limousine cattle, considering both pedigree- and genomic-based inbreeding coefficients. Phenotypic comparisons between animals with low and high inbreeding levels revealed a negative effect of inbreeding on growth traits, including birth weight, weaning weight, yearling weight, and average daily gain. These effects were observed regardless of the inbreeding estimation method. Fertility traits were largely unaffected, except for age at first calving, which increased with higher inbreeding. Longevity, measured by the probability of survival across parities, was significantly reduced in inbred animals. Genomic inbreeding showed a greater impact on animals' fertility and longevity. Notably, when separating recent and ancient genomic inbreeding, the former had a more pronounced effect on growth traits. Similarly, recent inbreeding primarily impacted animal longevity. Genomic inbreeding coefficients provided more granular insights into inbreeding depression, allowing for a better identification of individuals at higher risk of performance reduction. The results highlight the detrimental effects of inbreeding on growth, fertility, and longevity in Limousine cattle, which could have implications for herd productivity and genetic diversity. The study underscores the importance of genomic tools to monitor and manage inbreeding levels. Implementing strategies to control inbreeding accumulation is vital for maintaining genetic variability and ensuring the long-term sustainability of beef cattle populations.

Keywords: genetic diversity, homozygosity, reproductive performance, fitness-related traits, genomic evaluation

Implications

Maintaining genetic diversity is essential for sustainable beef production. This study assessed how inbreeding affects growth, fertility, and longevity in Limousine beef cattle using pedigree and genomic data. Results showed that inbreeding, particularly recent genomic inbreeding, reduces growth performance, delays reproductive maturity, and shortens herd life. These findings are important for breeders and industry stakeholders, as they highlight the need for managing genetic diversity. Using genomic inbreeding measures, breeders can make informed mating decisions to avoid closely related pairings, reduce inbreeding depression, and preserve herd productivity. Strategic genetic management is essential to safeguard the long-term cattle sustainability.

5.1 Introduction

Limousine is a cosmopolitan beef cattle breed originating from France and has been extensively exported worldwide. In Italy, the Limousine population is widely distributed across the country, with approximately 100 000 animals raised on more than 2 500 farms. The breed is continuously expanding in population size and is now one of the most important beef breeds reared in Italy (Fabbri et al., 2024), playing a key role in the national beef industry being the fourth bovine population in the country (Sistema Informativo Veterinario, https://www.vetinfo.it/j6_statistiche/index.html#/). As selective breeding becomes more widespread to enhance herd productivity, there is a growing concern about the potential negative impact of inbreeding. Therefore, monitoring and analyzing the genetic diversity of the Italian Limousine population is crucial in understanding its genetic background, managing inbreeding-related risks, improving productivity and profitability, and ensuring sustainable breeding practices for the future.

The inbreeding coefficient is a key indicator to assess genetic diversity and evaluate the potential impact of inbreeding depression on economically important traits in livestock populations. It can quantify the probability that an individual carries allele that are identical by descent relative to a base population where all alleles are assumed to be unrelated (Wright, 1922). Alternatively, it can be defined as the correlation between homologous alleles resulting from the mating of related individuals (Wright, 1922). Both the inbreeding and relatedness coefficients play a crucial role in breeding programs, as they facilitate the characterization and efficient management of mating strategies within a population.

Inbreeding within cattle populations poses significant challenges to the breeding industry, as it can lead to a loss of genetic diversity, changes in effective population size and structure, and increased genetic drift. Specifically, increasing inbreeding is generally associated with a reduction in effective population size, as it reflects a decrease in the number of breeding individuals contributing genetically to the next generation (Lozada-Soto et al., 2022). These factors undermine the efficiency and long-term sustainability of breeding programs (Meuwissen et al., 2020; Curik et al., 2014; Charlesworth and Willis, 2009). As inbreeding accumulates, the mean phenotypic value of selected traits may shift in a negative direction due to inbreeding depression, which directly impacts herd profitability and productivity (Hill and Mackay, 2004; Hedrick and Garcia-Dorado, 2016). These phenotypic changes are primarily driven by the accumulation of deleterious homozygous variants at key loci. This accumulation, in turn, leads to a decline in the population mean when heterozygotes deviate from the average value of two homozygotes (Doekes et al., 2021). For breeders, these challenges require close monitoring and careful management to balance genetic diversity with the selection of desirable traits, ensuring the long-term success of breeding programs (Mota et al., 2024).

Pedigree-based inbreeding coefficients (**F_{PED}**) are widely used in breeding programs to monitor inbreeding levels, assess inbreeding depression, and guide mating strategies. These coefficients estimate the probability of alleles being identical by descent based on pedigree records. However, the accuracy depends on the completeness and depth of pedigree data, which can be diminished by unknown parentage or recording errors. Additionally, pedigree-based estimates do not account for Mendelian sampling variation and may underestimate inbreeding levels depending on the number of generations considered (Meuwissen et al., 2020). With the adoption of genomic selection in livestock breeding, genomic data enhances breeding value prediction and accelerates genetic progress by reducing generation intervals (Meuwissen et al., 2016). The availability of single-nucleotide polymorphism (**SNP**) data enables direct estimation of inbreeding, providing a more precise assessment than pedigree-based methods (Meuwissen et al., 2001; Maltecca et al., 2020). SNP-based measures capture homozygosity more effectively, account for Mendelian sampling variation, and distinguish individuals with similar recorded ancestry (Doekes et al., 2021; Keller et al., 2011). Moreover, they allow inbreeding estimation in populations with incomplete or missing pedigree records. Depending on population structure and study objectives, genomic approaches based on marker- or segment-based inbreeding can be used to assess inbreeding depression.

Inbreeding and inbreeding depression have been widely studied, though most genomic-based analyses have focused on dairy cattle. The slower adoption of genomic selection in beef cattle, in contrast to dairy, can be attributed to industry-specific challenges, such as difficulties in phenotype collection for selection candidates, the prevalence of sex-limited traits, and the common use of crossbreeding (Meuwissen et al., 2016). Nevertheless, genomic selection is becoming increasingly prevalent in beef cattle. For instance, routine genomic evaluations have been implemented in the American Angus breed since 2009 (Lozada-Soto et al., 2021). While genomic selection enhances breeding programs, it also raises concerns about the accumulation of inbreeding over generations. Genomic prediction can control generational inbreeding rates by accounting for Mendelian sampling through SNP data, but it also reduces generation intervals, leading to an increase in the annual rate of inbreeding (Daetwyler et al., 2007). In fact, yearly and generational rates of pedigree and genomic inbreeding have increased in almost all dairy cattle breeds (Doekes et al., 2019; Lozada-Soto et al., 2022; Doublet et al., 2019).

Furthermore, the impact of inbreeding varies, and not all forms necessarily result in inbreeding depression. The timing of inbreeding plays a crucial role, as ancient inbreeding potentially undergoes multiple generations of purging selection, which helps eliminate harmful alleles. In this context, inbreeding can be classified according to age, allowing for a comparative assessment of the effects of recent versus old inbreeding on inbreeding depression. For example, in Canadian Holsteins, authors found that recent inbreeding is more deleterious for milk production, reproduction, and health-related traits (Makanjuola et al., 2020). A study investigating inbreeding depression in U.S. dairy cattle reported that recent inbreeding measured through both pedigree and genomic was associated with a higher risk of reproductive diseases, especially metritis (Lozada-Soto et al., 2024). In contrast, ancient inbreeding either had no effect or reduced the probability of reproductive disease(s). Similarly, in beef cattle, Lozada-Soto al. (2021) reported that recent genomic inbreeding was more harmful to growth compared to ancient inbreeding.

Production, longevity, and fertility are key traits influencing the efficiency and profitability of beef cattle systems. These traits directly influence herd productivity, replacement, and costs. Longevity and survival contribute to the economic sustainability of beef herds by reducing the need for frequent replacements and improving overall farm efficiency. Fertility, in particular, plays a crucial role in herd sustainability, as it directly impacts reproductive performance and provides potential indirect indicators for early selection of longevity in breeding programs (Callegaro et al., 2024). However, inbreeding can negatively affect production, fertility, and longevity traits by prolonging calving intervals, delaying puberty, and ultimately reducing reproductive efficiency and lifetime productivity. Understanding these associations is essential for optimizing genetic diversity, refining selection strategies, and ensuring herd sustainability. Few studies in beef cattle have confirmed the detrimental effects of increased inbreeding on productive and reproductive performance (Mota et al., 2024; Lozada-Soto et al., 2021; Pereira et al., 2016; Sumreddee et al., 2019; Santana et al., 2010). The objectives of our study were to (1) characterize the Italian Limousine population in terms of pedigree and genomic inbreeding levels; (2) Evaluate the impact of inbreeding on growth, fertility, and longevity traits in Limousine beef cattle by comparing phenotypic performance across varying inbreeding levels; (3) differentiate the effects of recent and ancient inbreeding, quantifying their respective contributions in Limousine population.

5.2 Material and methods

5.2.1 Animals and data

All data for this study were provided by the National Italian Association of Limousine and Charolais Breeders. The raw pedigree file for the Limousine breed included 526 887 animals, comprising 15 025 sires and 123 701 dams. Pedigree statistics, such as the number of complete generations (**NCG**), complete generation equivalents (**CGE**), and pedigree completeness index (**PCI**), were calculated using the optiSel R package (Wellmann, 2019). The mean NCG was 2.03, with a maximum of 6. The CGE represents the sum of $\left(\frac{1}{2}\right)^n$ known ancestors for every individual, where n is the number of generations separating the individual from a given ancestor. This metric quantifies pedigree depth by summing the contributions of known ancestors. The PCI algorithm, as defined by MacCluer et al. (1983), is the harmonic mean of the pedigree completeness of an individual's parents and reflects the percentage of known ancestors over multiple generations. These pedigree metrics were used to filter individuals based on pedigree completeness to minimize potential biases in estimating inbreeding coefficients. Animals with NCG <= 3, CGE <= 5, and a PCI lower than 0.90 when considering four generations were excluded from further analysis. Although a higher threshold of filtering for CGE is commonly used in literature, our pedigree depth was lower with a maximum CGE of 8.02. Using a stricter threshold would have excluded a large portion of the animals. In addition, was considered a PCI lower than 0.90 that takes four generations into account, as this approach is consistent with previous studies (Lozada-Soto et al., 2021) and allows for a balance between completeness and sample size. After applying these criteria, 109 241 animals met the pedigree completeness thresholds and were retained for the study. The final pedigree dataset had an average NCG of 3.54 complete generations, an average CGE of 5.57 equivalent generations, and an average PCI of 0.97. The final average values for CGE and NCG reflect a common scenario in beef cattle populations, which often present less complete pedigree records compared to dairy breeds (Gutiérrez et al., 2003; Nyman et al., 2022).

Before the statistical analysis, genotyped individuals were imputed with a multi-step pipeline. A total of 421 sires, used as reference population, were genotyped using a panel of 119 854 SNPs (GeneSeek GGP Bovine 150K; Illumina Inc., San Diego, CA), while 8 482 individuals were genotyped with a panel of 28 299 SNPs (GeneSeek GGP Bovine LD v3; Illumina Inc.). The two panels had 13 984 SNPs in common. In the first step, individuals genotyped with the GeneSeek GGP Bovine LD v3 panel were imputed to the GeneSeek GGP Bovine 150K panel, leveraging the common set of 13 984 SNPs shared between the two panels. In the second step, an additional 13 670 nonoverlapping SNPs present on the GeneSeek GGP Bovine LD v3 panel were also imputed and integrated with the initial imputed set of 119 854 SNPs. This resulted in a total of 131 805 SNPs. Genotype imputation was carried out using FImpute v.3 with default parameters (Sargolzaei et al., 2014). The quality metrics of the imputation showed a high squared correlation (r^2) between allele frequencies across panels with a value of 0.99, indicating higher accuracy between imputed and true genotypes. After imputation, the Mendelian error rate increased slightly to 0.42%, and 98% of SNPs were retained after quality control filtering. The minor allele frequency remained stable, with 94–98% of SNPs exhibiting a minor allele frequency below 0.05. For subsequent animal analyses, SNPs with minor allele frequency of 0.05 and individuals with the largest genotype changes (> 1%) were removed to ensure data consistency. To further assess the accuracy of imputation, a validation step was performed. Specifically, a subset of genotyped markers was randomly masked and subsequently re-imputed using the same pipeline. The results of this masked-marker imputation, including quality metrics, are presented in the Supplementary Table S5.1.

For these analyses, quality control procedures were implemented to ensure data reliability. Individuals with a call rate below 90% and SNPs with a minor allele frequency below 0.05 and a call rate below 90% were removed. Additionally, SNPs located on non-autosomal chromosomes (e.g., sex chromosomes) were excluded. Following these filtering steps, the final dataset comprised 8 198 genotyped individuals and 116 124 SNP markers. Quality control was performed using preGSf90 software (Aguilar et al., 2014). The main regression analyses were conducted using imputed genotype data. To assess the consistency of the results, the same regression analyses were performed using SNP data from the low-density panel (GeneSeek GGP Bovine LD v3 array). This allowed for a direct comparison between the estimated low-density genotypes and the imputed data, indicating that imputation had minimal impact on the findings of the investigation. To maintain a fair comparison, the same 8 482 animals were included in both analyses.

5.2.2 Animals' traits

Growth traits. The traits analyzed in this study were birth weight (**BiW**, kg), weaning weight (**WW**, kg), yearling weight (**YW**, kg), and average daily gain (**ADG**, g/d). BiW was recorded within a range of 30–50 kg, while WW and YW were measured at ages between 170–250 and 290–440 days, respectively, in the Limousine population. These traits were selected due to their economic importance and their role in evaluating growth performance in beef cattle. Data were collected from animals born between 1991 and 2024, raised in herds distributed across the national territory. Contemporary groups were defined by combining herd information and birth year, and contemporary groups with fewer than five animals were removed. Additionally, phenotypic records outside the range of mean ±3.5 SD were excluded. After editing, the final dataset consisted of 47 598 records for ADG, 72 282 for BiW, 21 928 for WW, and 14 595 for YW. Only animals with known sire and dam were retained. Descriptive statistics, including the number of animals, mean values, median, SD, farm

ID, number of sires and dams for each growth trait, and the number of genotyped animals, are presented in Table 5.1.

Stayability and fertility traits. Stayability (**STAY**) is a key trait influencing farm profitability, reflecting herd efficiency and costs. It is defined as the probability of a cow remaining in the herd and producing until a specified age, provided that animals have the opportunity to reach that age. STAY is commonly expressed as a binary trait (1 = success, 0 = failure), based on the presence or absence of a calving record for each parity (Hudson and Van Vleck, 1981). In this study, STAY was assessed from the second to the fourth calving, with fourth cumulative binary traits assigned per cow (STAY2, STAY3, STAY4). Data editing retained only cows with age at first calving (**AFC**) between 700 and 1 400 days. Calving intervals outside the range of 290–550 days were excluded, and twin parities were considered as a single calving event. Records were censored if the last recorded calving occurred within 550 days of data extraction, as subsequent calvings could not be confirmed. Censored records were treated as missing data in the model.

Data on fertility traits were extracted from records of cows from the STAY dataset. Fertility traits considered were first calving interval (**FCI**, days) and AFC (days) and were treated as continuous responses. Values of FCI averaged 405.7 d (± 60.87 SD) and values of AFC averaged 1 026 d (± 143.69 SD), respectively. A summary of fertility traits is presented in Table 5.1. The number of cows and incidence of survival according to the number of records for each STAY are reported in Table 5.2. Data processing and editing for STAY and fertility traits followed the procedures described in Callegaro et al. (2024). In addition, descriptive statistics and incidence of survival rates for each STAY trait are comprehensively reported in Callegaro et al. (2024).

Table 5.1 Descriptive statistics and number of genotyped Limousine cattle for growth and fertility traits.

Descriptive statistics							
Traits	Number of animals with records	mean	SD	Herd	Sires	Dams	Genotyped
Growth traits[1]							
ADG, kg/d	47 598	1.00	0.27	1 975	3 552	21 503	5 321
WW, kg	21 928	254.20	46.23	1 617	2 823	13 589	2 490
YW, kg	14 595	335.40	62.28	1 553	2 571	10 504	1 891
BiW, kg	72 282	38.08	3.65	2 199	4 231	29 880	5 931
Fertility traits[2]							
AFC, days	38 188	1,026	143.79	2 471	6 194	28 482	2 298
FCI, days	25 526	405.70	60.87	2 042	4 958	19 887	1 880

Abbreviations: ADG = average daily gain; WW = weaning weight; YW = yearling weight; BiW = birth weight; AFC = age at first calving; FCI = first calving interval.

Table 5.2 Number of Limousine cows-and incidence of survival across parities for stayability (STAY).

Trait	Definition	N	N of cows survived	Incidence (survival %)
STAY1	Stayability as a first parity = 1; failed = 0	38 188	38 188	100.00
STAY2	Stayability as a second parity = 1; failed = 0	33 209	25 526	66.84
STAY3	Stayability as a third parity = 1; failed = 0	30 105	18 764	49.14
STAY4	Stayability as a fourth parity = 1; failed = 0	27 978	14 381	37.66

The total number of genotyped cows for STAY analyses was 2 062.

Abbreviations: STAY = Continuity in herd until the subsequent parity (Success = 1; Failure = 0); N = Total number of cows, including both those that survived and were culled.

5.2.3 Estimation of Inbreeding Measures

Different approaches were employed to assess the accumulation of inbreeding and its effect on production in the Italian Limousine population over time. These included inbreeding coefficients derived from pedigree data as well as genomic estimates, specifically those based on the genomic relationship matrix (**GRM**) using marker-by-marker (**F_{GRM}**) and runs of homozygosity (**ROH**) calculated with segment-based approaches (**F_{ROH}**). Genomic-based inbreeding measures were calculated using imputed SNPs.

Pedigree inbreeding measure. The F_{PED} was estimated using the INBUPGF90 program v1.47 from the BLUPF90 family (Aguilar and Misztal, 2008). This method uses a recursive algorithm and iterates to convergence based on minimal changes in inbreeding values across rounds. F_{PED} were estimated using the method proposed by Meuwissen and Luo (1992). The pedigree dataset used for these computations included 109 241 Limousine animals that met completeness criteria, as described in the previous animals and data section, to ensure accurate inbreeding estimates.

Genomic-based inbreeding measures. The first estimator of genomic-based inbreeding (F_{GRM}) was calculated using the diagonal elements of GRM computed following VanRaden's first method (VanRaden, 2008):

$$\mathrm{GRM} = \frac{\mathrm{ZZ'}}{\sum_{j=1}^{m} 2p_j(1-p_j)}, (1)$$

where $Z = X - 2p_j$, X is the n × m matrix of the genotypes coded as the number of the second allele, n is the number of genotyped animals, m is the number of markers, and p_j is the frequency of the second allele at locus j. The denominator represents the scaling factor. The F_{GRM} for individual i was taken as $\mathrm{diag}(G_{ii}) - 1$, where G is the SNP-derived GRM. BLUPF90 family programs (Aguilar et al., 2019) were used for the calculations of GRM.

The minor allele frequency of the markers used in GRM estimation does not accurately represent that of the base population due to the limited sample size in this study. Consequently, genomic inbreeding coefficients were computed under the assumption of a fixed allele frequency of 0.50. Several studies (VanRaden et al., 2011; Bjelland et al., 2013; Forni et al., 2011) have demonstrated that fixing allele frequencies at 0.50 enhances the concordance between F_{GRM} and pedigree- or ROH-based inbreeding estimates, and that was the reason beyond our choice. Additionally, fixing allele frequencies at 0.50 helps center SNP values in the GRM constructions, mitigating biases from rare alleles or unrepresentative sample frequencies. Rare alleles (close to 0 or 1) can disproportionately influence relationship estimates, potentially biasing the computation of genomic relationships and inbreeding coefficients.

Autozygosity across chromosomal regions was assessed through ROH, which were then used to obtain the proportion of the genome residing in ROH (F_{ROH}). To detect ROH segments from SNP data, the R package detectRUNS v. 0.9.5 was employed (Biscarini et al., 2018), implementing a sliding window method to prevent overestimation of shorter ROH segments (Purcell et al., 2007). The parameters used for ROH detection were as follows: (1) a minimum of 20 SNPs in a window (--homozyg-window-snp), (2) a minimum SNP density of 1 SNP per 1 000 kb (--homozyg-density), (3) a maximum gap of 1 megabases (Mb) between consecutive homozygous SNPs (--homozyg-gap), (4) a minimum base pair length of 1 000 kb (--homozyg-kb), and (5) a maximum of 2 opposite homozygous genotype allowed within the window (--homozyg-window-het).

The proportion of the autosomal genome covered by ROH is calculated as:

$$\mathrm{F_{ROH}} = \frac{\mathrm{L_{ROHi}}}{\mathrm{L_{Genome}}}, \quad (2)$$

where $\mathrm{L_{ROHi}}$ is the length of the i$^{\mathrm{th}}$ ROH segment identified for each animal (in base pairs) and $\mathrm{L_{Genome}}$ is the total length of the autosomes covered by the markers. This formula is used to calculate the inbreeding coefficient based on ROH (F_{ROH}). The length of an ROH segment provides insight into the age of inbreeding, with shorter segments generally indicating more ancient inbreeding. However, the exact length of an ROH segment originating from a specific generation can vary. To account for this, F_{ROH} was also estimated across four ROH length categories: 1–2 Mb ($F_{\mathrm{ROH1\text{–}2}}$), 2-4 Mb ($F_{\mathrm{ROH2\text{–}4}}$), 4-8 Mb ($F_{\mathrm{ROH4\text{–}8}}$), and > 8 Mb ($F_{\mathrm{ROH>8}}$), following criteria used in previous studies (Mota et al., 2024; Lozada-Soto et al., 2021).

5.2.4 Inbreeding depression for growth, fertility, and longevity traits

The impact of inbreeding on fertility, longevity, and growth traits was assessed separately for each trait using the different inbreeding coefficients described. Inbreeding depression was estimated by regressing phenotypic values on inbreeding coefficients and calculated as the effect of a 1% increase in pedigree and genomic inbreeding coefficients on the mean of the phenotype measured. Considering pedigree measure, inbreeding depression results were analyzed using F_{PED} estimates from two datasets: a full-dataset including all animals with phenotype information, and a reduced-dataset limited to genotyped animals. This approach allows for consistency checks, ensuring that restricting the analysis to genotyped animals does not significantly alter the results. Comparing pedigree-based estimates between the full and genotyped datasets can reveal the impact of pedigree completeness. Additionally, stronger inbreeding depression effects observed with genomic measures would suggest that this better capture recent or cryptic inbreeding.

In addition, to assess the expected phenotypic differences between animals with varying levels of inbreeding, we estimated predictions for growth and fertility traits using a Gaussian-linear model, while for STAY, predicted probabilities were obtained using a threshold-liability model. This approach allowed for a comparison between animals with high and low inbreeding levels, providing a more direct insight into the effects of inbreeding on growth, fertility, and functional longevity. Additionally, to quantify realized phenotypic depression in the Limousine population, we calculated the projected losses in growth traits (ADG, BiW, WW, YW), fertility (AFC), and STAY. These losses were estimated by comparing animals in the 10th percentile (low inbreeding) and 90th percentile (high inbreeding) for both pedigree- and genomic-based inbreeding coefficients. The difference between these groups was also computed to quantify the magnitude of inbreeding depression across these traits. Only traits showing statistically significant inbreeding depression were considered for the estimation of the expected phenotypic differences.

Since ROH detection is influenced by SNP density (Ferenčaković et al., 2013; Villanueva et al., 2021), analyses were conducted using genotypes from imputed data. In addition, analyses with a low-density panel were performed to evaluate whether the patterns observed in the imputed data panel were consistent with those obtained using a low-density dataset. To assess the effect of SNP density on ROH estimation and validate our findings, the same analyses were conducted in both imputed and non-imputed data. Results from low-density genotypes were compared to those from the imputed ones to determine whether inbreeding depression patterns remained consistent. The results from these two scenarios were similar, and therefore, low-density data will be presented in the supplementary material.

5.2.5 Genetic analyses

For growth traits the model was defined as follows:

$$\mathbf{y} = \beta_0 + \mathbf{Xb} + \beta_1 F + \mathbf{Za} + \mathbf{Wp} + \mathbf{e}, (3)$$

where y was the vector of phenotypic values of ADG, BiW, WW and YW; β_0 is the intercept; b is the vector of fixed effects of the sex, age of animals, and age of dam; F is a vector of inbreeding coefficients for F_{PED}, F_{GRM}, or F_{ROH}; β_1 is the linear regression coefficient for the regression coefficient, a is the vector of the random additive genetic effect, following a $\sim N(0, \mathbf{A}\sigma_a^2)$, where **A** is the numerator relationship matrix; p is the vector of the random effect of the contemporary group (herd-year), following p $\sim N(0, \mathbf{I}\sigma_{\text{p}}^2)$, where **I** is an identity matrix; e is the random residual, following $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$; and **X**, **Z**, and **W** are the incidence matrices for the fixed and random effect.

When BiW and WW were modeled in model 3 the maternal permanent environment effect was added, following mpe $\sim N(0, \mathbf{I}\sigma^2_{\mathrm{mpe}})$.

Fertility traits and STAY were analyzed fitting a univariate linear and threshold animal model. The fixed effects considered were the same:

$$\mathrm{y} = \beta_0 + \mathbf{X}\mathrm{b} + \beta_1 F + \mathbf{Z}\mathrm{a} + \mathbf{W}\mathrm{p} + \mathbf{e}, (4)$$

$$\lambda = \beta_0 + \mathbf{X}\mathrm{b} + \beta_1 F + \mathbf{Z}\mathrm{a} + \mathbf{W}\mathrm{p} + \mathbf{e}, (5)$$

where y is the linear vector of fertility traits (AFC, FCI) while λ represents the unobserved liability for threshold models for STAY traits; β_0 is the intercept; b is the vector associated with the fixed effects of year of calving (48 levels); β_1 is the linear regression coefficient for the inbreeding coefficient; a is the vector of animal additive genetic effects following a $\sim N(0, \mathbf{A}\sigma^2_a)$, where $\mathbf{A}$ is the numerator relationship matrix; p is the vector associated to the random effect of herd, following p $\sim N(0, \mathbf{I}\sigma^2_{\mathrm{p}})$, where $\mathbf{I}$ is an identity matrix; e is the vector associated with the random residual error following e $\sim N(0, \mathbf{I}\sigma^2_e)$; and $\mathbf{X}$, $\mathbf{Z}$, and $\mathbf{W}$ are incidence matrices for the fixed and random effects.

The variance components for STAY traits were fixed at values estimated from the single-step GBLUP assessed in the previous work in STAY on Limousine and Charolais beef cattle (Callegaro et al., 2024). For the growth and fertility traits, the variance components from models 3 and 4 are shown in Supplementary Table S5.2. The (co)variance components were estimated using the Gibbs sampling algorithm implemented in the BLUPF90 family software (Aguilar et al., 2019). Convergence was assessed by visual inspection of trace plots and throughout Geweke's test using the package coda (Plummer et al., 2006) in R. Models where STAY traits were the response variable were run using the THRGIBBS1F90 program, and those in which the response variable was a growth and fertility trait were run using the GIBBS3F90 program. All analyses were run for 100 000 cycles with a burn-in of 50 000 samples, and every 10th sample was stored for a total of 5 000 samples used for subsequent inference.

Best Linear Unbiased Estimates of the intercept and inbreeding slopes were used to estimate predictions for growth and fertility traits and predicted probabilities for STAY on the liability scale values across different inbreeding levels. Our predicted values were obtained from the regression equations using the solutions for the fixed effects obtained at each round of iterations to the phenotypic means. For STAY, predictions on the liability scale were converted to the probability scale by applying the inverse probit transformation (with the pnorm function in R), using the formula: $P_i = \Phi(p_i)$, (6) were Φ is the standard cumulative distribution function and p_i is the i_{th} predicted value of STAY on the liability scale. These predictions allowed us to assess the impact of inbreeding on trait values, providing insights into inbreeding depression across different levels of inbreeding.

5.3 Results

5.3.1 Inbreeding coefficients

The mean F_{PED} in the Limousine population was 1.99% (SD = 5.68%). Genomic inbreeding, estimated as F_{GRM} averaged 0.01 (SD = 0.03), while the mean of F_{ROH} was 0.19 (SD = 0.02), indicating a higher proportion of autozygosity captured by ROH. The mean partial inbreeding coefficients derived from ROH segments were 0.045 (SD = 0.029) for $F_{\mathrm{ROH1–2}}$, 0.029 (SD = 0.028) for $F_{\mathrm{ROH2–4}}$, 0.018 (SD = 0.028) for $F_{\mathrm{ROH4–8}}$, and 0.021 (SD = 0.034) for $F_{\mathrm{ROH>8}}$. These values reflect the contribution of ROH segments of different lengths to the overall inbreeding estimates. The mean CGE and NCG for the Limousine breed were 5.60 and 3.70, respectively. Furthermore, all animals included in the analyses had a PCI exceeding 90%, ensuring high data accuracy. The distributions of pedigree and genomic inbreeding coefficients are shown in Supplementary Fig. S5.1. Over time, F_{PED} exhibited an increasing trend from the first to the last birth year considered. In contrast, genomic inbreeding measures (F_{GRM} and F_{ROH}) remained relatively stable throughout the study period. Suggesting that genomic diversity may have remained relatively stable over time, possibly due to low selective pressure. Additionally, $\mathrm{F}_{\mathrm{GRM}}$ reflects the average genomic similarity across individuals and depends on allele frequencies and population structure, whereas $\mathrm{F}_{\mathrm{ROH}}$ captures the proportion of the genome in homozygous segments and is more sensitive to recent inbreeding. These differences may explain why their temporal trends sometimes are not fully aligned. Annual trends for each inbreeding measure are presented in Supplementary Fig. S5.2.

Pearson's correlation coefficients among all inbreeding measures are visualized in a heatmap (Figure 5.1). F_{PED} showed moderate to strong correlations with both F_{GRM} and F_{ROH}, with values ranging from 0.69 to 0.71. The highest correlation with F_{PED} was observed for F_{GRM} and $F_{\mathrm{ROH4–8}}$ (0.71), whereas the correlation between F_{PED} and F_{ROH} was slightly lower (0.69). Correlations between F_{PED} and short-segment F_{ROH} estimates ($F_{\mathrm{ROH1–2}}$ and $F_{\mathrm{ROH2–4}}$) were also high (0.70), with values remaining stable across increasing segment lengths. Genomic inbreeding measures ($\mathrm{F}_{\mathrm{GRM}}$ and $\mathrm{F}_{\mathrm{ROH}}$) showed consistently high correlations. Specifically, the correlation between $\mathrm{F}_{\mathrm{ROH}}$ and $\mathrm{F}_{\mathrm{GRM}}$ was 0.89, confirming the strong agreement between these two genomic inbreeding estimators. Correlations between F_{GRM} and different ROH segment classes ranged from 0.85 to 0.89, with a slight decline as segment length increased. This expected trend reflects the fact that shorter ROH segments capture more ancient inbreeding, whereas longer segments indicate more recent inbreeding events. While F_{GRM} correlates strongly with ROH-based measures, it captures homozygosity without explicitly distinguishing between recent and ancient inbreeding. Likewise, correlations among different $\mathrm{F}_{\mathrm{ROH}}$ segments remained consistently high (0.95–0.99), with the strongest correlations generally observed between segments of similar lengths, particularly between adjacent categories such as $F_{\mathrm{ROH}1\text{–}2}$ and $F_{\mathrm{ROH}2\text{–}4}$.

The distribution of ROH across 100 randomly selected animals revealed distinct patterns corresponding to different inbreeding timeframes (Figure 5.2a). While the accumulation of inbreeding persists across generations, ancient inbreeding segments have fragmented into shorter lengths over time. In our case, short ROH segments (1–2 Mb, 2–4 Mb) made up the largest proportion of the genome, suggesting that inbreeding accumulation has not accelerated dramatically in the last generations. In contrast, longer ROH segments (4–8 Mb, >8 Mb), associated with more recent inbreeding, accounted for a smaller proportion. Figure 5.2b shows the distribution of ROH across chromosomes, further confirming that short ROH segments (1–2 Mb, 2–4 Mb) are more frequent, indicating a higher prevalence of ancient inbreeding events. Regression analyses comparing genome-based inbreeding coefficients derived from imputed and non-imputed (low-density panel) data are presented in Supplementary Fig. S5.3. The estimated regression coefficients for F_{GRM} and F_{ROH} from imputed data against their counterparts from the low-density panel ranged from 0.93 to 0.96 for F_{GRM} and F_{ROH}, respectively, showing a high degree of concordance.

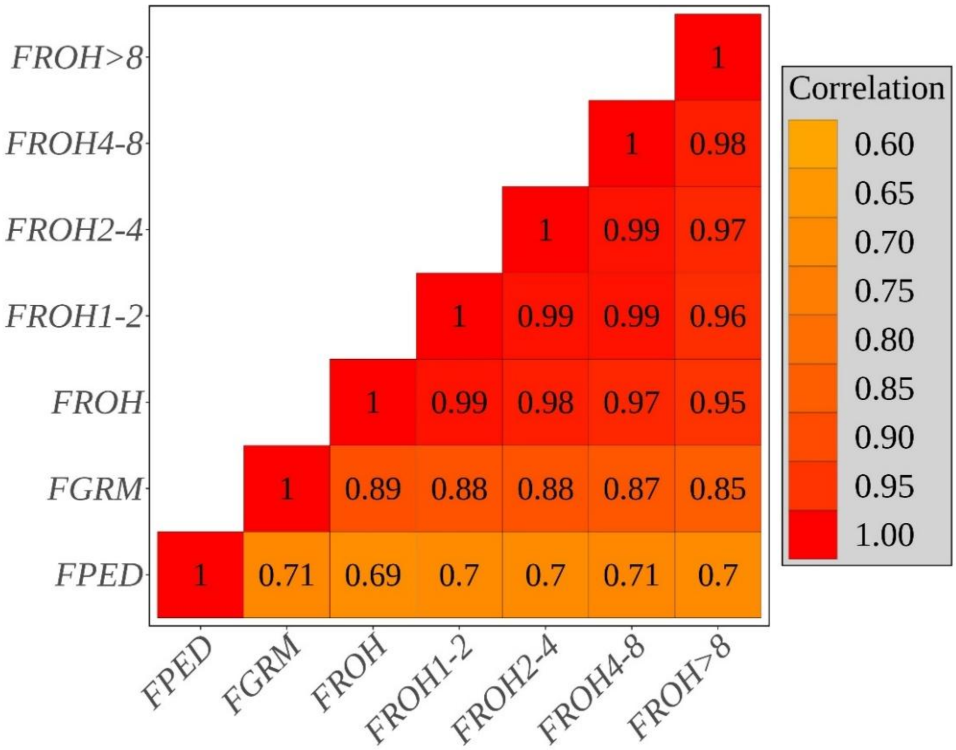


Figure 5.1 Pearson correlations between inbreeding measures in Limousine cattle. The plot shows the strength and direction of the correlation between inbreeding measures with darker colors indicate a stronger correlation (F_{PED} = total pedigree inbreeding; F_{GRM} = relationship matrix derived inbreeding; F_{ROH} = runs of homozygosity calculated with segment-based approaches; ROH of different length classes 1–2, 2–4, 4–8, and >8 Mb).

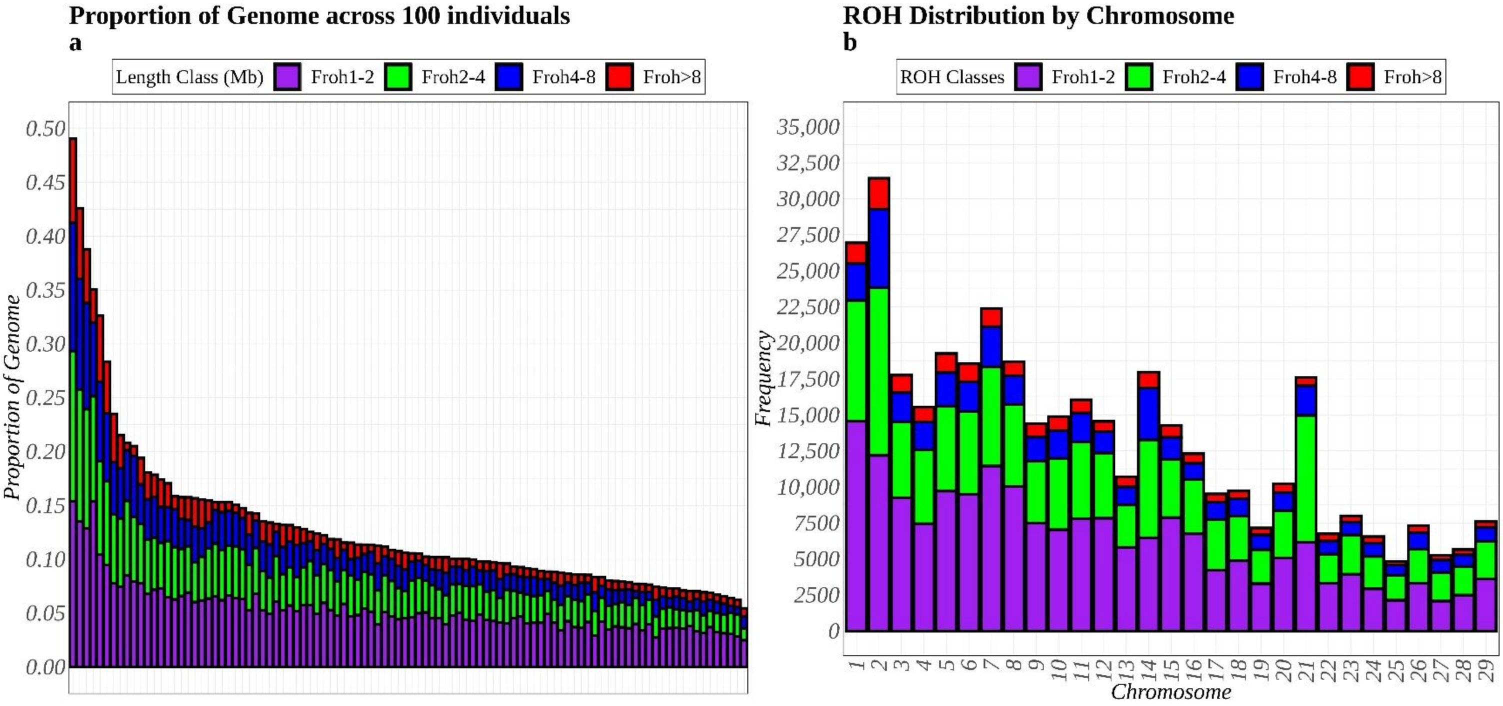


Figure 5.2 Runs of homozygosity (ROH) distribution and coverage in Limousine cattle. (a) Proportion of the genome covered by ROH of different length classes (1–2, 2–4, 4–8, >8 Mb) in 100 randomly sampled individuals. (b) Frequency distribution of the number of ROH per chromosome in different length classes (F_{ROH} = runs of homozygosity calculated with segment-based approaches).

5.3.2 Pedigree and genomic inbreeding depression

We quantified the effect of pedigree and genomic inbreeding on four growth traits (BiW, WW, YW, and ADG) in the Italian Limousine population. Furthermore, we estimated inbreeding depression for fertility and survival traits to provide a more comprehensive assessment of its impact. Fertility traits included AFC and FCI, while longevity was evaluated using STAY, specifically the probability of cows surviving from second to fourth parity (STAY2, STAY3, and STAY4). Inbreeding depression was estimated for all inbreeding measures, with effects expressed by a 1% increase in F_{PED}, F_{GRM}, and F_{ROH} in phenotypic units. The results are summarized in Table 5.3.

Across all measurements, inbreeding had a negative effect on growth performance, fertility, and longevity, though the magnitude of the effect varied depending on the inbreeding metric used. This variation was expected given the differences in mean inbreeding values among methods. Table 5.3 also reports F_{PED}-based inbreeding depression estimates for both the full-dataset and the reduced-dataset. Comparing these estimates ensures that restricting the analysis to genotyped animals does not significantly alter the results. Additionally, this comparison allows for evaluating whether genomic inbreeding measures capture greater variance in inbreeding depression effects than pedigree-based measures.

Inbreeding depression negatively affected all growth traits (BiW, WW, YW, and ADG) in the breed, although the magnitude varied depending on the inbreeding measure (Table 5.3). A 1% increase in F_{PED} reduced BiW by 0.016 kg in the full-dataset and by 0.06 kg when considering only genotyped animals. Similarly, BiW decreased by 0.07 kg for F_{GRM} and 0.05 kg for F_{ROH}. For WW, the reduction ranged from −0.25 kg (F_{PED}) to −0.93 kg (F_{GRM}), with F_{ROH} showing an intermediate effect (−0.41 kg). A similar trend was observed for YW, with reductions between −0.19 kg (F_{PED}, full-dataset) and −1.16 kg (F_{GRM}), although the only significant estimate was for F_{PED} in the full-dataset, other estimates were not significantly different from zero. Finally, ADG decreased by −1.20 g/d for F_{PED} (full-dataset), −4.03 g/d for F_{PED} (reduced-dataset), −3.19 g/d for F_{GRM}, and −1.56 g/d for F_{ROH}. Overall, genomic-based inbreeding measures predicted a greater reduction in growth performance with increasing inbreeding. Moreover, the non-random subset of genotyped animals and the imputation process, may have contributed to a slight inflation of genomic estimates.

The effects of pedigree-based and genomic inbreeding on female fertility and longevity traits are presented in Table 5.3. For fertility traits, no significant inbreeding depression was detected for FCI, with none of the four regression coefficients differing significantly from zero. In contrast, for AFC, an increase in inbreeding levels was associated with a delay in the onset of reproduction. This was evident from the positive regression coefficients for AFC, indicating that inbred animals took longer to conceive. Specifically, a 1% increase in inbreeding resulted in an increase in days to pregnancy of 0.57 days for F_{PED}, 2.51 days for F_{GRM}, and 1.30 days for F_{ROH}. However, when F_{PED} was estimated using only genotyped animals, the effect was not significant. Notably, genomic inbreeding measures showed a greater impact on inbreeding depression for AFC than pedigree-based estimates.

For longevity, analyzed using a threshold model, an increase in inbreeding level had a notable impact on STAY traits, specifically on the probability of a cow remaining in the herd for subsequent parities. Given that longevity is a key factor for farm profitability, estimating inbreeding depression for this trait is essential for developing effective breeding strategies. A 1% increase in F_{PED} was reported in Table 5.3 and resulted in a decrease in STAY liability for all the traits, indicating a reduction in the probability of survival of the cow at the next parity. When considering F_{PED} for the reduced-dataset, a significant effect was observed only for STAY2, with an estimate of −0.032. For both genomic inbreeding measures, the probability of cows failing to remain in the herd for the next parity increased more markedly compared to pedigree-based estimates. The effects of inbreeding depression per 1% increase in genomic inbreeding measures using the low-density panel are presented in Supplementary Table S5.3. The inbreeding depression estimates for F_{GRM} and F_{ROH} from non-imputed data were consistent with those obtained in Table 5.3 using the imputed panel.

Table 5.3 Inbreeding depression estimates for growth, fertility. and stayability traits in Limousine cattle, expressed as change in the phenotype per 1% increase in inbreeding level.

Trait	F_{PED} full-dataset		F_{PED} reduced-dataset		F_{GRM}		F_{ROH}	
	Slope (β)	95% HPDI	Slope (β)	95% HPDI	Slope (β)	95% HPDI	Slope (β)	95% HPDI
Growth traits								
ADG, g/d	-1.20	(-1.65; -0.75)	-4.03	(-7.31; -0.76)	-3.19	(-5.55; -0.83)	-1.56	(-2.98; -0.13)
WW, kg	-0.25	(-0.34; -0.17)	-0.92	(-1.57; -0.27)	-0.93	(-1.77; -0.09)	-0.41	(-0.69; -0.13)
YW, kg	-0.19	(-0.32; -0.06)	-0.65	(-1.77; 0.48)	-1.16	(-2.50; 0.17)	-0.27	(-1.10; 0.57)
BiW, kg	-0.016	(-0.021; -0.012)	-0.06	(-0.10; -0.02)	-0.07	(-0.13; -0.02)	-0.05	(-0.08; -0.02)
Fertility traits								
AFC, days	0.57	(0.22; 0.93)	-0.07	(-1.73; 1.60)	2.51	(1.19; 3.83)	1.30	(0.30; 2.20)
FCI, days	0.15	(-0.08; 0.39)	0.45	(-0.46; 1.36)	0.16	(-1.48; 1.80)	0.05	(-0.91; 1.01)
Stayability traits								
STAY2	-0.015	(-0.020; -0.010)	-0.032	(-0.053; -0.012)	-0.070	(-0.110; -0.024)	-0.043	(-0.068; -0.018)
STAY3	-0.014	(-0.019; -0.010)	-0.016	(-0.041; 0.008)	-0.056	(-0.088; -0.024)	-0.025	(-0.052; 0.003)
STAY4	-0.011	(-0.015; -0.007)	0.000	(-0.036; 0.038)	-0.063	(-0.104; -0.023)	-0.025	(-0.054; 0.004)

Abbreviations: F_{PED} = total pedigree inbreeding; F_{GRM} = relationship matrix derived inbreeding; F_{ROH} = runs of homozygosity calculated with segment-based approaches; HPDI = high posterior density interval; ADG = average daily gain; WW = weaning weight; YW = yearling weight; BiW = birth weight; AFC = age at first calving; STAY2 = stayability2; STAY3, stayability3; STAY4 staybility4.

5.3.3 Phenotypic depression differences between high and low inbreeding groups

To visualize the realized phenotypic depression in the studied population, we estimated the projected loss in growth traits for animals with low and high levels of pedigree and genomic inbreeding. To ensure direct and easy readability of results, inbreeding coefficients were scaled to pedigree inbreeding (%) to allow for direct comparison between pedigree and genomic inbreeding (F_{GRM} and F_{ROH}) in the same figures (Figure 5.3 and Figure 5.4). Using predictions, we modeled the expected values of growth traits across different levels of pedigree and genomic inbreeding (Figure 5.3). For the ADG, predicted values showed a sharp decline as inbreeding levels increased. A similar trend was evident for WW and YW, with higher inbreeding levels leading to substantial reductions in body weight. In contrast, BiW also declined with increasing inbreeding, though the reduction was less pronounced compared to other growth traits.

We also evaluated the projected impact of inbreeding on fertility and longevity traits, using the same approach. For AFC, we applied predictions, whereas for STAY, a threshold trait, probability marginal predictions were used (Figure 5.4a and Figure 5.4b, respectively). An increase in inbreeding was associated with a clear increase in the predicted AFC, indicating a negative effect on fertility. The strongest inbreeding depression effects on AFC were observed for genomic inbreeding measures, with calving age increasing from approximately 1 022 to 1 065 days for F_{GRM} and from 1 022 to 1 060 days for F_{ROH}. F_{PED} (reduced-dataset) displayed no effect, while F_{PED} (full-dataset) exhibited the mildest increase in AFC. These results suggest that genomic measures more accurately capture the adverse effects of inbreeding on fertility. For STAY, increasing inbreeding led to a consistent decline in the probability of survival to subsequent parities, confirming inbreeding depression in longevity. The reduction in STAY was most pronounced for F_{GRM} and F_{ROH}, followed by F_{PED} (reduced-dataset), while the least severe decline was observed for F_{PED} (full-dataset). Specifically, for F_{GRM}, the probability of survival decreased from 0.98 to 0.80 for STAY2, from 0.92 to 0.72 for STAY3, and from 0.84 to 0.52 for STAY4. Similarly, for F_{ROH}, survival probability declined from 0.98 to 0.77 for STAY2, from 0.92 to 0.76 for STAY3, and from 0.84 to 0.61 for STAY4.

To further investigate phenotypic depression, we compared growth traits between animals with low (10th percentile) and high (90th percentile) inbreeding levels (Table 5.4). Inbreeding had the greatest impact on WW, YW, and ADG, while BiW was the least affected. WW declined by up to 9.25 kg (F_{PED}, reduced-dataset), YW by 6.64 kg (F_{PED}, reduced-dataset), and ADG by 43.44 g/d (F_{PED}, reduced-dataset). The other most pronounced reductions were observed for genomic inbreeding measures, with F_{GRM} decreasing WW by 3.91 kg, YW by 5.18 kg, and ADG by 13.82 g/d. In contrast, BiW showed a modest decline, with reductions of less than 0.4 kg across all inbreeding metrics.

Similarly, the projected impact of inbreeding on fertility and longevity traits was assessed by comparing lowly (10th percentile) and highly (90th percentile) inbred animals (Table 5.4). For AFC, inbreeding increased the trait values, particularly for genomic measures, rising from 1 021 to 1 031 days (F_{GRM}) and from 1 021 to 1 030 days (F_{ROH}). In terms of STAY, survival probabilities showed a decline across all inbreeding metrics, with the strongest effects for genomic inbreeding. For STAY2, the probability dropped from 0.98 to 0.95 (F_{GRM}, F_{ROH}), while STAY3 declined from 0.93 to 0.88 (F_{GRM}) and from 0.93 to 0.89 (F_{ROH}). The most substantial reduction was observed for STAY4, where survival probability decreased from 0.85 to 0.77 (F_{GRM}) and from 0.84 to 0.79 (F_{ROH}). Overall, the strongest inbreeding depression effects were observed when inbreeding was measured using F_{GRM} and F_{ROH}, particularly for fertility and longevity traits.

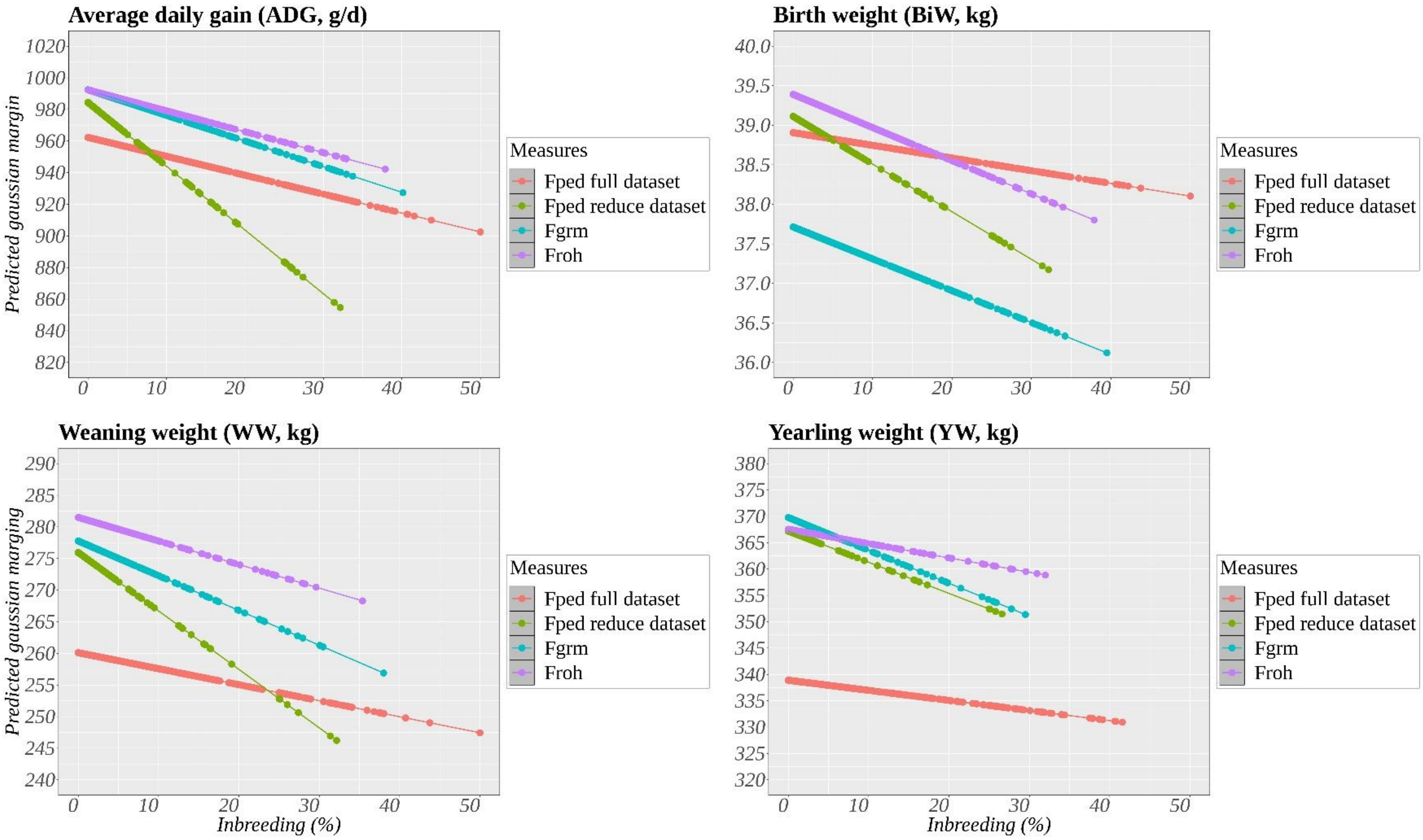


Figure 5.3 Expected phenotypic comparisons for growth traits between Limousine cattle with higher and lower inbreeding levels (gaussian prediction) (F_{PED} = total pedigree inbreeding; F_{GRM} = relationship matrix derived inbreeding; F_{ROH} = runs of homozygosity calculated with segment-based approaches).

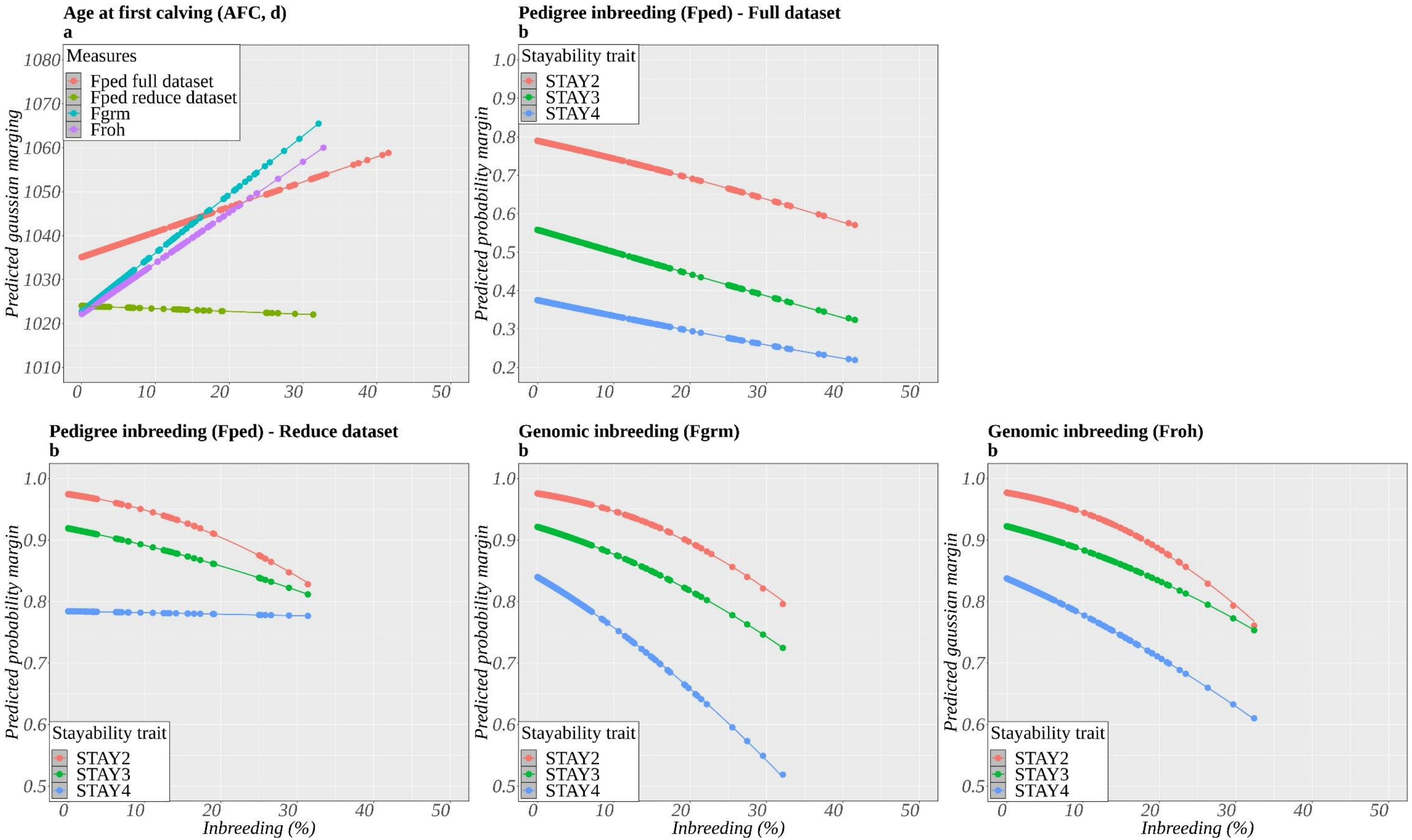


Figure 5.4 Predicted effects of inbreeding on age at first calving (AFC) and stayability (STAY) in Limousine cattle. (a) Predictions for AFC. (b) Probability marginal predictions for STAY (*F*$_{\text{PED}\,=}$ total pedigree inbreeding; *F*$_{\text{GRM}}$ = relationship matrix derived inbreeding; *F*$_{\text{ROH}}$ = runs of homozygosity calculated with segment-based approaches).

Table 5.4 Projected phenotypic depression in growth, fertility, and stayability traits for low (10th percentile) and high (90th percentile) inbreeding levels in Limousine cattle.

Trait	F_{PED} full-dataset			F_{PED} reduced-dataset			F_{GRM}			F_{ROH}		
	Low	High	Difference (95% HPDI)	Low	High	Difference (95% HPDI)	Low	High	Difference (95% HPDI)	Low	High	Difference (95% HPDI)
Growth traits												
ADG, g/d	962.33	943.13	19.02 to 19.37	984.05	940.61	41.00 to 45.88	994.53	980.71	12.94 to 14.69	993.79	982.75	10.34 to 11.74
WW, kg	260.10	256.00	4.04 to 4.15	275.95	266.70	8.48 to 10.03	273.25	269.34	3.55 to 4.27	281.87	279.03	2.57 to 3.10
YW, kg	330.85	327.47	3.33 to 3.43	364.98	358.34	6.00 to 7.30	368.52	363.34	4.60 to 5.75	371.78	370.77	0.90 to 1.11
BiW, kg	38.91	38.64	0.267 to 0.271	39.11	38.45	0.62 to 0.69	37.77	37.45	0.30 to 0.34	39.43	39.08	0.33 to 0.38
Fertility traits												
AFC, days	1 035	1 039	-3.97 to -3.86	1 024	1 023	0.35 to 0.40	1 021	1 031	-11.43 to -9.38	1 021	1 030	-9.81 to -8.08
Stayability traits												
STAY2	0.79	0.76	0.031 to 0.035	0.97	0.95	0.020 to 0.023	0.98	0.95	0.0234 to 0.0246	0.98	0.95	0.0286 to 0.0290
STAY3	0.56	0.52	0.038 to 0.042	0.92	0.90	0.021 to 0.025	0.93	0.88	0.0433 to 0.0436	0.93	0.89	0.0334 to 0.0338
STAY4	0.38	0.35	0.027 to 0.030	0.83	0.82	0.001 to 0.005	0.85	0.77	0.0765 to 0.0771	0.84	0.79	0.0545 to 0.0551

Abbreviations: F_{PED} = total pedigree inbreeding; F_{GRM} = relationship matrix derived inbreeding; F_{ROH} = runs of homozygosity calculated with segment-based approaches; HPDI = high posterior density interval; ADG = average daily gain; WW = weaning weight; YW = yearling weight; BiW = birth weight; AFC = age at first calving; STAY2 = stayability2; STAY3, stayability3; STAY4 staybility

5.3.4 Impact of recent and ancient inbreeding

We evaluated the effects of recent and ancient genomic inbreeding on growth and longevity traits. As expected, recent inbreeding had a stronger and more detrimental impact on studied traits compared to ancient inbreeding. The inbreeding depression estimated from homozygous segments was greater for longer segments ($F_{\mathrm{ROH}4\text{–}8}$ and $F_{\mathrm{ROH}>8}$), which indicates more recent inbreeding, compared to shorter segments ($F_{\mathrm{ROH}1\text{–}2}$ and $F_{\mathrm{ROH}2\text{–}4}$), which reflect more ancient inbreeding. The effects of a 1% increase in recent and ancient genomic inbreeding depression on growth and STAY traits are shown in Figure 5.5a and Figure 5.5b, respectively. The figures also display the highest posterior density intervals, which indicate the statistical significance of inbreeding depression estimates.

For growth traits, both recent and ancient inbreeding were significantly associated with phenotypic reductions in BiW, WW, and YW, while no significant effects were observed for ADG. Specifically, a 1% increase in recent inbreeding ($F_{\mathrm{ROH}4\text{–}8}$ and $F_{\mathrm{ROH}>8}$) reduced BiW by 0.055 kg and 0.070 kg, respectively, whereas an equivalent increase in ancient inbreeding ($F_{\mathrm{ROH}1\text{–}2}$ and $F_{\mathrm{ROH}2\text{–}4}$) led to reductions of 0.045 kg and 0.048 kg, respectively. For both WW and YW, inbreeding depression was more pronounced for recent inbreeding than for ancient inbreeding. A 1% increase in $F_{\mathrm{ROH}>8}$ reduced WW by 0.90 kg and YW by 0.93 kg, while the effect of ancient inbreeding ($F_{\mathrm{ROH}1\text{–}2}$) was smaller, with reductions of 0.53 kg for WW and 0.36 kg for YW. Notably, $F_{\mathrm{ROH}1\text{–}2}$ had no significant effect on YW.

For fertility traits, including AFC and FCI, no significant associations were detected for either recent or ancient inbreeding. These results are therefore not displayed in Figure 5.5. Regarding longevity, recent inbreeding significantly reduced the probability of survival to the next parity for all STAY traits. A 1% increase in inbreeding decreased the probability of survival by 0.054 for STAY2, 0.047 for STAY3, and 0.064 for STAY4 for recent inbreeding ($F_{\mathrm{ROH}>8}$). In contrast, an increase in ancient inbreeding ($F_{\mathrm{ROH}1\text{–}2}$ and $F_{\mathrm{ROH}2\text{–}4}$) had a smaller effect, reducing the probability of survival by 0.035 and 0.034 for STAY2 and by 0.025 for STAY4, while no significant effect was observed for STAY3. These results confirm the greater negative impact of recent inbreeding on longevity traits in Limousine cattle, with the strongest and cumulative effects observed in later parities.

Additional analyses using low-density SNP panel data are reported in Supplementary Fig. S5.4. To further illustrate the phenotypic consequences of recent and ancient inbreeding, we estimated the projected losses in growth and STAY traits for animals with high versus low genomic inbreeding levels. These predictions over the inbreeding levels are shown in Figure 5.6a for growth traits and Figure 5.6b for STAY traits. The results confirm that phenotypic losses were more pronounced in animals with high levels of recent inbreeding ($F_{\mathrm{ROH}4\text{–}8}$ and $F_{\mathrm{ROH}>8}$) than in those with high levels of ancient inbreeding ($F_{\mathrm{ROH}1\text{–}2}$ and $F_{\mathrm{ROH}2\text{–}4}$).

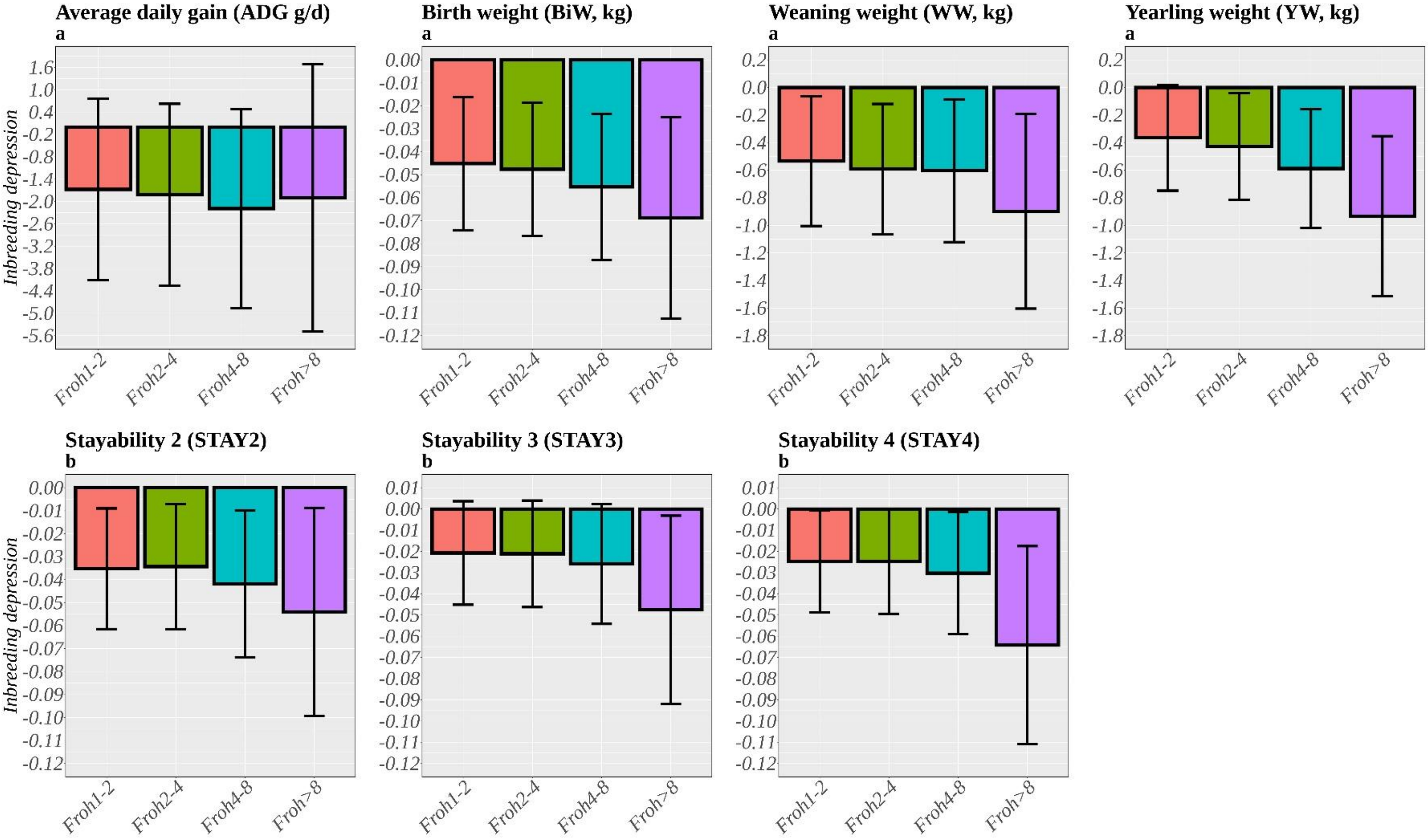


Figure 5.5 The effects of 1% increase in recent and ancient genomic inbreeding based on runs of homozygosity calculated with segment-based approaches (F_{ROH}) on (a) growth and (b) stayability traits in Limousine cattle. The range of highest posterior density interval (HPDI) is shown

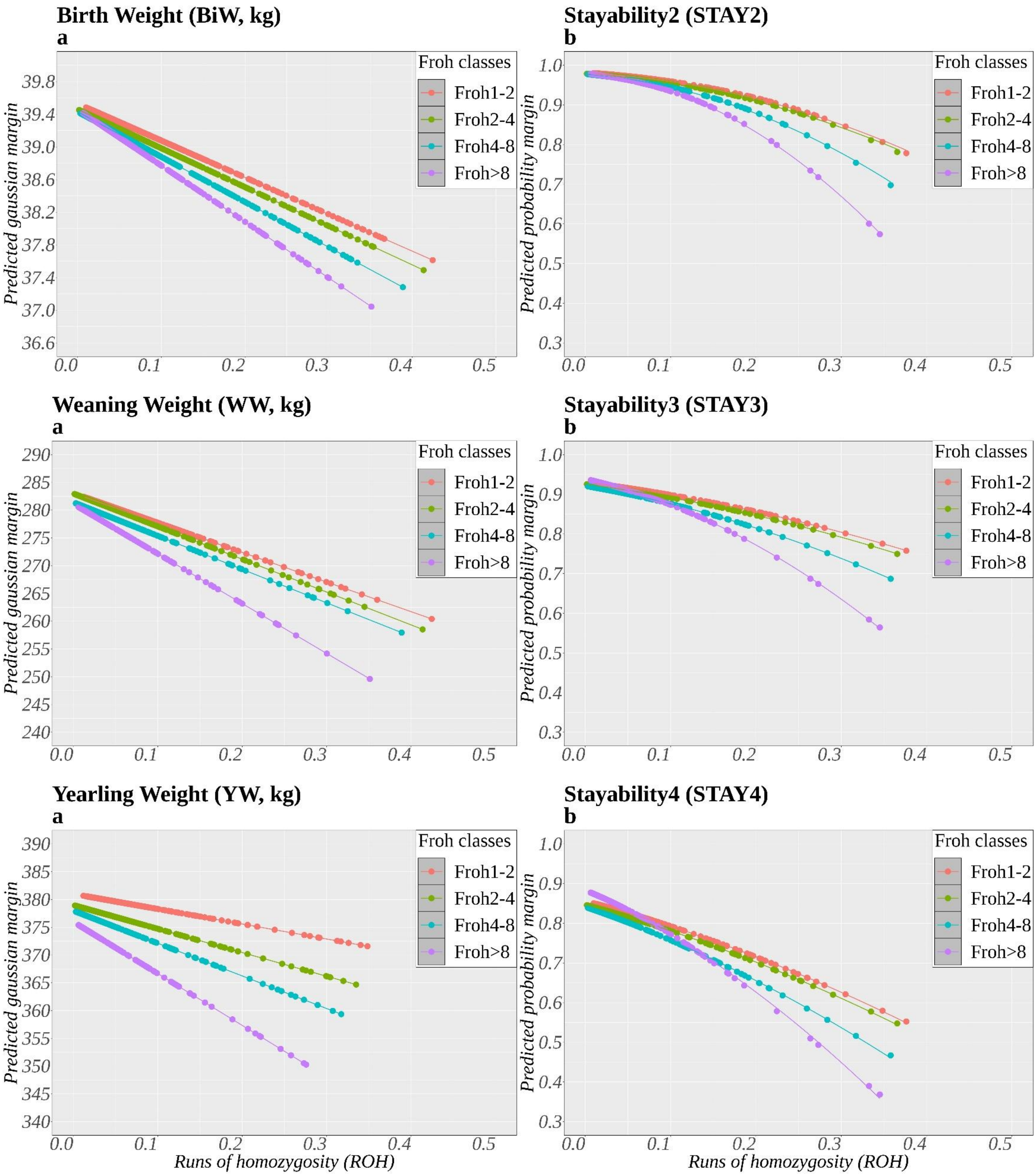


Figure 5.6 Projected phenotypic losses due to recent and ancient genomic inbreeding based on runs of homozygosity calculated with segment-based approaches (F_{ROH}) in Limousine cattle. (a) Growth traits (gaussian predictions). (b) Stayability traits (probability predictions).

5.4 Discussion

5.4.1 Inbreeding coefficients

In general, the mean F_{PED} observed in this study was slightly lower than values reported by Mota et al. (2024) in Nellore cattle and by Pereira et al. (2016) for Zebu breeds. The mean F_{PED} was also slightly lower than the value reported for American Angus cattle under genomic selection (Lozada-Soto et al., 2021). However, it aligned closely with findings from Santana et al. (2010), Peripolli et al. (2018) and Mulim et al. (2025), that reported values below 2%. Notably, the F_{PED} in our study was substantially lower than that reported for Line 1 Hereford cattle (29.2%) (Sumreddee et al., 2019) and Japanese black cattle (9.3%) (Nishio et al., 2023). It is not unexpected that pedigree inbreeding levels are higher in Line 1 Herefords compared to the Italian Limousine, given that the former have been maintained as a closed breeding line for over 85 years, originating from just two closely related bulls (paternal half-brothers) and 50 foundation cows (Sumreddee et al., 2019). The descriptive statistics for CGE and NCG further support the pedigree depth and completeness in our dataset, ensuring reliable estimates of inbreeding depression. However, partial incompleteness of pedigree information may have led to some underestimation of F_{PED}. When using genomic inbreeding measures, the mean F_{GRM} in our population was very similar to values reported in Nellore and Brazilian Angus cattle (Mota et al., 2024; Mulim et al., 2025). The mean F_{ROH} in our study was comparable to that estimated in American Angus cattle (Lozada-Soto et al., 2021) but higher than those observed in Nellore, Japanese black, and Brazilian Angus cattle (Mota et al., 2024; Nishio et al., 2023; Mulim et al., 2025). Although F_{ROH} estimated in the Hereford cattle was higher (23%) (Sumreddee et al., 2019).

Correlations between F_{PED} and genomic inbreeding measures (F_{GRM}, F_{ROH}) have been widely studied in different species. Specifically, some research found F_{PED}-F_{GRM} correlations ranging from 0.33 to 0.64, which were slightly lower than those reported in our study. Conversely, many other studies found F_{PED}-F_{ROH} correlations in the range 0.64–0.71, consistent with our findings. These studies were assessed across multiple beef and dairy cattle populations (Lozada-Soto et al., 2021; Doekes et al., 2019; Sumreddee et al., 2019; Nishio et al., 2023; Martikainen et al., 2017). Zhang et al. (2015) also documented correlations of 0.47 to 0.82 between F_{PED} and F_{ROH} in three dairy cattle breeds. In general, the observed correlations align with literature, with variation influenced by pedigree quality and ROH length. Pedigree depth and ROH segment length are key factors affecting F_{PED}-F_{ROH} correlations, which tend to increase with deeper pedigree records (Sumreddee et al., 2019; M. Ferenčaković et al., 2013). Additionally, VanRaden et al. (2011) found that fixing minor allele frequency at 0.50 improved F_{GRM}-F_{PED} correlations.

Our findings for genomic inbreeding measures align with previous studies, where strong correlations between F_{GRM} and F_{ROH} (0.81–0.97) have been reported in beef and dairy cattle (Lozada-Soto et al., 2021; Doekes et al., 2019; Bjelland et al., 2013; Forutan et al., 2018). However, lower correlations (0.62–0.66) were observed in Holstein and Jersey cattle (Pryce et al., 2014), likely due to differences in population structure and breeding history. Overall, our results are consistent with the literature, which reports moderate to high correlations between $\mathrm{F}_{\mathrm{GRM}}$ and $\mathrm{F}_{\mathrm{ROH}}$ in both beef and dairy cattle populations, as mentioned before. Contrary to our results, Mulim et al. (2025) reported negative correlations among $\mathrm{F}_{\mathrm{GRM}}$ and $\mathrm{F}_{\mathrm{ROH}}$ measures. This discrepancy may be explained by methodological aspects of $\mathrm{F}_{\mathrm{GRM}}$ estimation, which relies on allele frequencies. When these frequencies diverge from those of the founder population, the estimation of inbreeding may become biased (Nishio et al., 2023). Such discrepancies are more likely to emerge at genomic level, where allele frequency changes can be more pronounced, leading to reduced or even negative correlations between inbreeding coefficients (Villanueva et al., 2021; Mulim et al., 2022). Finally, as expected, F_{ROH} classes representing recent inbreeding showed stronger correlations with each other than with those reflecting ancient inbreeding, and vice versa, a trend also observed in American Angus and Nellore cattle (Mota et al., 2024; Lozada-Soto et al., 2021).

Considering F_{ROH} and the trend observed in the distribution of ROH segments (Figure 5.2), both within and across chromosomes, aligns with expectations that recombination progressively breaks down long homozygous segments over generations. The observed inter-individual variability suggests differences in inbreeding history within the population. The higher contribution of short ROH segments supports the hypothesis that inbreeding has accumulated over multiple ancestral generations rather than from recent mating of close relatives. The inter-chromosomal variability suggests that different chromosomes have experienced varying inbreeding histories. The higher prevalence of short ROH segments across chromosomes further supports the hypothesis that inbreeding has accumulated over multiple ancestral generations, rather than being a result of recent close relative matings.

5.4.2 Pedigree and genomic inbreeding depression

The use of genomic measures of autozygosity as an estimator of inbreeding has gained significant interest in livestock genetics. However, research on inbreeding depression based on genomic inbreeding coefficients remains limited, mainly due to the limited availability of genotyped animals across various species. This knowledge gap is particularly evident in beef cattle, since most studies on inbreeding effects have focused on dairy breeds (Bjelland et al., 2013).

In our study, the depressive effects of inbreeding were more pronounced for WW and YW compared to BiW. Similar trends were observed in American Angus cattle, where both pedigree-based and genomic-based inbreeding led to decreased growth from birth to post-weaning (Lozada-Soto et al., 2021). While some studies on Hereford and Angus reported no significant F_{PED} effects on BiW (Sumreddee et al., 2019; Davis and Simmen, 2010), they did detect depressive effects on WW and other post-weaning traits, consistent with our findings. In American Angus, inbreeding depression was greater than in our study, with reductions of 0.03 kg in BiW (for both males and females) and 0.47 kg in WW for females and 0.50 kg for males (Lozada-Soto et al., 2021). Other studies in beef cattle suggest that a 1% increase in F_{PED} reduces BiW by 0.02–0.06 kg and WW by 0.19–0.44 kg (Carolino and Gama, 2008; Carrillo and Siewerdt, 2010a). In Hereford, although no significant effects were found for BiW or WW, YW decreased by 1.06 kg per 1% F_{PED} increase, a larger effect than in our study (Sumreddee et al., 2019). This study also reported a significant reduction in ADG (0.006 kg/d), similar to what was reported in our results.

Although genomic inbreeding research in growth traits is limited, studies in beef populations such as Hereford, American Angus, and Nellore have reported inbreeding depression. In Herefords, a 1% increase in F_{GRM} was associated with a 0.53 kg decrease in WW, which is smaller than the effect observed in our study. For YW, the depression of −0.92 kg is similar to our findings. However, while a depressive effect was observed for YW using F_{GRM}, no significant depression was detected for F_{ROH} in the studied population. In contrast to our results, no depressive effects were found on BiW due to F_{GRM} or F_{ROH}, nor any effect on WW due to F_{ROH}. Regarding ADG, reductions similar to our findings were reported in Herefords, with values ranging from −0.003 to −0.006 kg/d for both F_{GRM} and F_{ROH} (Sumreddee et al., 2019). In American Angus, Lozada-Soto et al. (2021) found depressive effects of genomic inbreeding on growth, with BiW decreasing by 0.04–0.05 kg in both males and females. For WW, authors observed smaller decreases (0.61 kg in males and 0.59 kg in females) per 1% increase in F_{GRM}, but similar depression was observed with F_{ROH}. Finally, Garcia-Baccino et al. (2020), using homozygous SNPs (F_{HOM}) as a measure related to F_{GRM}, found a similar depressive effect of inbreeding on growth, reporting a decrease of 0.05 kg for BiW and 1.02 kg for WW.

Inbreeding depression for reproductive traits in both beef and dairy cattle has been well documented. However, contrary to our results, studies on Japanese Black cattle have reported no significant inbreeding depression, either using pedigree or genomic measures, for AFC (Nishio et al., 2023). Similarly, in Hereford cattle, no inbreeding depression for AFC was detected (Sumreddee et al., 2019). Conversely, studies in Alentejana cattle and Zebu, using pedigree and genomic inbreeding measures, have shown an increase in AFC, calving intervals, and days open (Pereira et al., 2016; Carolino and Gama, 2008). In comparison, in our results, genome-based inbreeding coefficients tended to yield larger estimates of inbreeding depression for fertility traits than pedigree-based measures. This discrepancy may be attributed to several factors, such as errors in pedigree records, pedigree depth, expected versus actual identical by descent, and number of records. Previous studies in dairy and beef cattle (Pryce et al., 2014; Nishio et al., 2023) suggested that F_{PED} might underestimate the inbreeding depression for female fertility traits

Few studies have evaluated the impact of inbreeding on longevity and survival in beef cattle. A study on Alentejana cattle found that increased inbreeding levels were associated with reduced longevity (Carolino and Gama, 2008). Similarly, a meta-analysis by Doekes et al. (2021), reviewing 154 studies across multiple livestock species, reported that a 1% increase in inbreeding led to an average reduction of 0.13% in trait mean values, including survival-related traits. This analysis also highlighted that genomic inbreeding measures were more effective in detecting inbreeding depression than pedigree-based estimates. Thompson et al. (2000) in dairy cattle found an increase in the level of inbreeding associated with a decrease in survival and concluded that survival and production represent a major challenge to the genetic programs of the US dairy industry. Additionally, a study on dairy cattle found that inbreeding had a statistically significant association with functional longevity (Sewalem et al., 2006). Our results, which highlight the detrimental effect of inbreeding depression on STAY traits, are consistent with those findings, as genomic inbreeding measures were more effective in detecting inbreeding depression in the Limousine population.

Inbreeding depression had a more harmful effect on fertility and longevity traits. Differences observed in pedigree-based estimates between the full and the reduced-dataset may reflect the influence of pedigree completeness. Meanwhile, stronger genomic inbreeding depression effects suggest that genomic measures offer a more accurate representation of inbreeding depression. Indeed, inbreeding depression is population-specific (Howard et al., 2017), and its extent varies depending on factors such as population structure, the method used to estimate inbreeding and inbreeding depression, allele frequencies, the strength of directional selection, and the marker panel density (Sumreddee et al., 2019; Reverter et al., 2017).

Our findings on inbreeding depression derived from imputed genotypes and the low-density panel align with Reverter et al. (2017), who consistently observed a negative impact of both marker- and segment-based genomic inbreeding on body weight in tropical cattle across marker panels of varying densities. This suggests that even with a lower-density marker panel, genomic inbreeding remains a reliable predictor of inbreeding depression in this context.

5.4.3 Phenotypic depression differences between high and low inbreeding groups

Our results indicate a clear phenotypic depression in growth traits associated with increased inbreeding. Across all inbreeding measures, animals in the high inbreeding group exhibited lower predicted performance compared to those in the low inbreeding group, confirming the detrimental effects of increased homozygosity on production and reproductive traits. Among growth traits, the most pronounced effects were observed for ADG, WW, and YW, while BiW showed a milder decline. The strong depressive effect was found on ADG and the associated reductions in WW and YW, likely stemming from a loss of heterozygosity at loci controlling postnatal growth. In contrast, BiW was less affected, possibly due to its lower environmental sensitivity compared to postnatal growth stages. BiW is strongly influenced by maternal effects (Carrillo and Siewerdt, 2010a), which may buffer the impact of inbreeding depression. This is consistent with findings in American Angus cattle, where post-weaning traits exhibited greater phenotypic depression than BiW (Lozada-Soto et al., 2021).

Similarly, our analysis of fertility and longevity traits reveals clear phenotypic depression associated with increased inbreeding levels. Inbreeding was linked to delayed sexual maturity, as indicated by the increase in AFC among the group of highly inbred animals. This delay in first calving, often leading to prolonged calving intervals, increases the risk of involuntary culling, negatively impacting reproductive efficiency (Parland et al., 2007). The strongest inbreeding depression effects were observed for genomic inbreeding measures (F_{GRM} and F_{ROH}), which showed the highest increase in AFC. This may reflect the ability of genomic metrics to better capture the realized homozygosity at deleterious loci compared to pedigree-based measures, particularly in populations with shallow pedigrees. However, it is important to consider that the estimation of inbreeding depression using genomic data in addition might be influenced by the non-random selection of genotyped animals and the imputation process. Although, several studies have highlighted the advantages of genomic-based measures in estimating inbreeding depression in respect to pedigree inbreeding (Keller et al., 2011; Howard et al., 2017). For longevity, increasing inbreeding levels were associated with reduced survival probability across parities, reinforcing the negative impact of inbreeding on long-term reproductive success. The decline in STAY was particularly evident for later parities (STAY3 and STAY4), suggesting the cumulative effect of inbreeding over time. The mildest reduction in survival probability was seen for F_{PED}, indicating that pedigree-based inbreeding measures may underestimate the long-term consequences of inbreeding on longevity. This aligns with studies showing that inbreeding depression in longevity becomes more pronounced as animals age, likely due to the accumulation of deleterious alleles affecting survival and reproduction (Parland et al., 2007; Leroy, 2014).

The difference between lowly and highly inbred animals was more evident when using genomic inbreeding measures (F_{GRM} and F_{ROH}) compared to F_{PED}. The stronger effects observed with genomic inbreeding measures likely reflect their ability to capture actual homozygosity rather than relying on expected values from pedigree calculations (Nishio et al., 2023). Our findings indicate that the accumulation of pedigree and genomic inbreeding in the Italian Limousine population is detrimental to growth performance, particularly ADG, as well as fertility and longevity, with AFC and STAY being the most affected traits. Therefore, careful monitoring of genetic diversity and inbreeding levels in the Limousine breed is essential to mitigate these negative effects and ensure sustainable productivity.

5.4.4 Impact of recent and ancient inbreeding

As expected, our results confirm the largely unfavorable effects of recent inbreeding on animal weight, highlighting its substantial impact on performance. Previous studies in beef cattle have reported that recent inbreeding (estimated from pedigree, ROH, and HBD segments) has a greater negative effect on BiW, WW, and post-weaning gain than ancient inbreeding (Lozada-Soto et al., 2021). Similarly, another study focusing on growth and carcass traits found that recent ROH-based inbreeding was more detrimental to beef cattle growth than ancient inbreeding (Mota et al., 2024). In dairy cattle, recent genomic inbreeding (measured through ROH and HBD) has also been shown to have stronger negative effects on production traits compared to ancient inbreeding (Doekes et al., 2019; Makanjuola et al., 2020). In another study in the German Holstein, the authors found that, for productive traits, the unfavourable effect of a 1% increase in inbreeding was larger when inbreeding was estimated using short ROH compared to longer ROH segments (Mugambe et al., 2024).

In our study, no significant effects of inbreeding were detected for fertility traits in the Italian Limousine population. This aligns with findings in American Angus cattle, where no significant effects of either recent or ancient inbreeding were observed for heifer pregnancy (Lozada-Soto et al., 2021). In dairy cattle, studies have yielded mixed results: some found no significant effects of recent or ancient inbreeding on fertility traits (Doekes et al., 2019) while others reported that recent pedigree and genomic inbreeding were more detrimental to fertility than ancient inbreeding (Maltecca et al., 2020; Makanjuola et al., 2020).

The observed negative effects of inbreeding on longevity traits (STAY) in the Limousine population align with the documented detrimental impact of inbreeding depression. However, our study provides novel insights by distinguishing the effects of recent and ancient inbreeding on functional longevity in beef cattle. Our findings demonstrate that recent inbreeding had a significantly stronger negative impact on STAY traits than ancient inbreeding, suggesting that recent inbreeding may lead to the fixation of recessive deleterious alleles with stronger detrimental effects on survival and reproductive longevity. The absence of significant associations between ancient inbreeding and

STAY3 further supports this hypothesis, as purging mechanisms may have eliminated the most harmful alleles affecting survival beyond early parities. The stronger inbreeding depression observed in later parities (STAY4) suggests a cumulative effect, where increased homozygosity progressively reduces an animal's ability to remain productive in the herd. This pattern is consistent with previous studies showing that inbreeding depression tends to intensify in traits related to fitness and survival over time (Parland et al., 2007). While no prior research has directly compared recent and ancient inbreeding for STAY traits, studies on inbreeding depression in fertility and survival traits in both beef and dairy cattle have consistently reported stronger effects of inbreeding on reproductive performance and longevity-related traits (Pryce et al., 2014). Given that STAY is influenced by both reproductive success and survival, it is reasonable to infer that recent inbreeding may reduce reproductive resilience, increasing the likelihood of early culling.

The stronger detrimental effects of recent inbreeding compared to ancient inbreeding in this study underscore its significant impact on both reproductive and production traits in the Limousine breed. Our results align with previous findings in beef and dairy cattle, where recent inbreeding was found to have more severe consequences than ancient inbreeding across various traits (Mota et al., 2024; Lozada-Soto et al., 2021; Doekes et al., 2019; Makanjuola et al., 2020). The stronger detrimental effects of recent inbreeding can be attributed to the progressive elimination of harmful alleles over multiple generations. Both natural and artificial selection play a role in decreasing the prevalence of deleterious alleles in older generations (Leroy, 2014). Conversely, longer ROH segments, indicative of recent inbreeding, are more likely to carry recessive or harmful alleles due to fewer recombination events, thus amplifying their negative impact on phenotypic traits (Charlesworth and Willis, 2009). Our findings highlight the importance of distinguishing between recent and ancient inbreeding when monitoring inbreeding trends in beef cattle populations.

5.5 Conclusions

Our findings confirm that inbreeding has a negative impact on the traits studied, leading to reduced reproductive efficiency and decreased lifetime productivity. Both pedigree and genomic inbreeding depression for growth were observed, with the depressive effects on growth becoming more pronounced from birth to weaning and continuing through post-weaning weight and ADG. Furthermore, recent genomic inbreeding had a more detrimental effect on growth compared to inbreeding accumulated over more ancient periods.

Inbreeding also reduced the probability of cows remaining in the herd for the next parity, as indicated by its impact on STAY. Fertility was similarly affected, with inbreeding depression particularly evident in the AFC. This reduction in longevity and survival negatively impacts herd sustainability by increasing replacement rates and production costs. Our results suggest that inbreeding negatively influences functional longevity, likely due to the expression of deleterious recessive alleles affecting health and fertility. Moreover, recent genomic inbreeding was found to have a more harmful effect on longevity than inbreeding accumulated over more ancient periods. Therefore, genetic management strategies that control inbreeding levels, especially recent genomic inbreeding, are essential for maintaining longevity and reproductive efficiency in beef cattle populations.

In addition to the potential loss of genetic diversity, inbreeding can reduce the mean fitness of individuals, exacerbating inbreeding depression. These findings underscore the need to manage genetic diversity and refine selection strategies to mitigate the negative effects of inbreeding, ensuring sustainable beef production. Specifically, genomic inbreeding coefficients may better reflect the depressing effects of realized inbreeding than pedigree-based methods. This allows breeders to implement more effective mating strategies and avoid pairing closely related animals, ultimately supporting sustainable breeding practices and the long-term productivity of livestock.

5.6 Ethics approval

The National Italian Association of Limousine and Charolais Breeders provided phenotypic, pedigree, and genomic information. The data used in this study were obtained from a pre-existing database; therefore, approval from the Animal Care and Use Committee was not required.

5.7 Data and model availability statement

The datasets generated and/or analysed during the current study are not publicly available due to being owned by a third party, ANACLI, Associazione Nazionale degli Allevatori delle razze bovine Charolaise e Limousine Italiane (http://www.anacli.it/) but are available from the corresponding author on reasonable request.

5.8 Declaration of generative AI and AI-assisted technologies in the writing process

None.

5.9 Author ORCIDs

Simone Callegaro: https://orcid.org/0000-0002-9270-1845

Francesco Tiezzi: https://orcid.org/0000-0002-4358-9236
Christian Maltecca: https://orcid.org/0000-0002-9996-4680
Maria Chiara Fabbri: https://orcid.org/0000-0002-3224-745X
João Cláudio do Carmo Panetto: https://orcid.org/0000-0002-9198-9728
Riccardo Bozzi: https://orcid.org/0000-0001-8854-0834

5.10 Authors' contributions

Simone Callegaro: Conceptualization, methodology, software, formal analysis, writing – original draft
Francesco Tiezzi: Conceptualization, methodology, supervision, writing - review & editing
Christian Maltecca: Conceptualization, methodology, supervision, writing - review & editing
Maria Chiara Fabbri: Conceptualization, Writing - review & editing
João Cláudio do Carmo Panetto: Conceptualization, methodology, writing - review & editing
Riccardo Bozzi: Resources, supervision, funding acquisition, writing - review & editing

5.11 Declaration of Interest

The authors declare that they have no competing interests.

5.12 Acknowledgements

We acknowledge Associazione Nazionale Allevatori delle razze bovine Charolais e Limousine for providing the data.

5.13 Financial support statement

This work was financially supported by Associazione Nazionale degli Allevatori delle razze bovine Charolaise e Limousine Italiane (Cup: J89H18000010005, URL: https://www.anacli.it/i-beef-2). The grant was received by RB. The founders had no role in study design and analysis, decision to publish, or preparation of the manuscript.

5.14 References

Aguilar, I., Misztal, I., 2008. Technical note: recursive algorithm for inbreeding coefficients assuming nonzero inbreeding of unknown parents. Journal of Dairy Science 91, 1669–1672. doi:10.3168/jds.2007-0575.

Aguilar, I., Misztal, I., Tsuruta, S., Legarra, A., Huiyu Wang, 2014. PREGSF90 – POSTGSF90: Computational Tools for the Implementation of Single-step Genomic Selection and Genome-wide Association with Ungenotyped Individuals in BLUPF90 Programs. Proceedings of the 10th World Congress of Genetics Applied to Livestock Production, 17-22 August 2014, Vancouver, Canada.

Aguilar, I., Tsuruta, S., Masuda, Y., Lourenco, D.A.L., Legarra, A., Misztal, I., 2019. BLUPF90 suite of programs for animal breeding with focus on genomics. Retrieved on 12 June 2025 from https://www.inia.uy/sites/default/files/publications/2024-10/blupf90-suite-programs-animal-breeding-focus-genomics.pdf.

Biscarini F, C.P., Gaspa G, Marras G., 2018. DetectRUNS: detect runs of homozygosity and runs of heterozygosity in diploid genomes. Retrieved on 17 September 2024 from https://cran.r-project.org/web/packages/detectRUNS/detectRUNS.pdf

Bjelland, D.W., Weigel, K.A., Vukasinovic, N., Nkrumah, J.D., 2013. Evaluation of inbreeding depression in Holstein cattle using whole-genome SNP markers and alternative measures of genomic inbreeding. Journal of Dairy Science 96, 4697–4706. doi:10.3168/jds.2012-6435

Callegaro, S., Tiezzi, F., Maltecca, C., Fabbri, M.C., Bozzi, R., 2024. Genetic parameters of functional longevity and associated traits in Italian Charolais and Limousine breeds. Journal of Animal Science 102, skae354. doi:10.1093/jas/skae354

Carolino, N., Gama, L.T., 2008. Inbreeding depression on beef cattle traits: Estimates, linearity of effects and heterogeneity among sire-families. Genetics Selection Evolution 40, 511–527. doi:10.1051/gse:2008018

Carrillo, J.A., Siewerdt, F., 2010a. Consequences of long-term inbreeding accumulation on preweaning traits in a closed nucleus Angus herd1. Journal of Animal Science 88, 87–95. doi:10.2527/jas.2009-1897

Charlesworth, D., Willis, J.H., 2009. The genetics of inbreeding depression. Nature Reviews Genetics 10, 783–796. doi:10.1038/nrg2664

Curik, I., Ferenčaković, M., Sölkner, J., 2014. Inbreeding and runs of homozygosity: A possible solution to an old problem. Livestock Science 166, 26–34. doi:10.1016/j.livsci.2014.05.034

Daetwyler, H.D., Villanueva, B., Bijma, P., Woolliams, J.A., 2007. Inbreeding in genome-wide selection. Journal of Animal Breeding and Genetics 124, 369–376. doi:10.1111/j.1439-0388.2007.00693.x

Davis, M.E., Simmen, R.C.M., 2010. Estimates of inbreeding depression for serum insulin-like growth factor I concentrations, body weights, and body weight gains in Angus beef cattle divergently selected for serum insulin-like growth factor I concentration1,2,3. Journal of Animal Science 88, 552–561. doi:10.2527/jas.2009-2232

Doekes, H.P., Bijma, P., Windig, J.J., 2021. How Depressing Is Inbreeding? A Meta-Analysis of 30 Years of Research on the Effects of Inbreeding in Livestock. Genes 12, 926. doi:10.3390/genes12060926

Doekes, H.P., Veerkamp, R.F., Bijma, P., De Jong, G., Hiemstra, S.J., Windig, J.J., 2019. Inbreeding depression due to recent and ancient inbreeding in Dutch Holstein–Friesian dairy cattle. Genetics Selection Evolution 51, 54. doi:10.1186/s12711-019-0497-z

Doublet, A.-C., Croiseau, P., Fritz, S., Michenet, A., Hozé, C., Danchin-Burge, C., Laloë, D., Restoux, G., 2019. The impact of genomic selection on genetic diversity and genetic gain in three French dairy cattle breeds. Genetics Selection Evolution 51, 52. doi:10.1186/s12711-019-0495-1

Fabbri, M.C., Tiezzi, F., Crovetti, A., Maltecca, C., Bozzi, R., 2024. Investigation of cosmopolitan and local Italian beef cattle breeds uncover common patterns of heterozygosity. animal 18, 101142. doi:10.1016/j.animal.2024.101142

Ferenčaković, M., Hamzić, E., Gredler, B., Solberg, T.R., Klemetsdal, G., Curik, I., Sölkner, J., 2013. Estimates of autozygosity derived from runs of homozygosity: empirical evidence from selected cattle populations. Journal of Animal Breeding and Genetics 130, 286–293. doi:10.1111/jbg.12012

Ferenčaković, Maja, Sölkner, J., Curik, I., 2013. Estimating autozygosity from high-throughput information: effects of SNP density and genotyping errors. Genetics Selection Evolution 45, 42. doi:10.1186/1297-9686-45-42

Forni, S., Aguilar, I., Misztal, I., 2011. Different genomic relationship matrices for single-step analysis using phenotypic, pedigree and genomic information. Genetics Selection Evolution 43, 1. doi:10.1186/1297-9686-43-1

Forutan, M., Ansari Mahyari, S., Baes, C., Melzer, N., Schenkel, F.S., Sargolzaei, M., 2018. Inbreeding and runs of homozygosity before and after genomic selection in North American Holstein cattle. BMC Genomics 19, 98. doi:10.1186/s12864-018-4453-z

Garcia-Baccino, C.A., Lourenco, D.A.L., Miller, S., Cantet, R.J.C., Vitezica, Z.G., 2020. Estimating dominance genetic variances for growth traits in American Angus males using genomic models. Journal of Animal Science 98, skz384. doi:10.1093/jas/skz384

Gutiérrez, J.P., Altarriba, J., Díaz, C., Quintanilla, R., Cañón, J., Piedrafita, J., 2003. Pedigree analysis of eight Spanish beef cattle breeds. Genetics Selection Evolution 35, 43. doi:10.1186/1297-9686-35-1-43

Hedrick, P.W., Garcia-Dorado, A., 2016. Understanding Inbreeding Depression, Purging, and Genetic Rescue. Trends in Ecology & Evolution 31, 940–952. doi:10.1016/j.tree.2016.09.005

Hill, W.G., Mackay, T.F.C., 2004. D. S. Falconer and Introduction to Quantitative Genetics. Genetics 167, 1529–1536. doi:10.1093/genetics/167.4.1529

Howard, J.T., Pryce, J.E., Baes, C., Maltecca, C., 2017. Invited review: Inbreeding in the genomics era: Inbreeding, inbreeding depression, and management of genomic variability. Journal of Dairy Science 100, 6009–6024. doi:10.3168/jds.2017-12787

Hudson, G.F.S., Van Vleck, L.D., 1981. Relationship Between Production and Stayability in Holstein Cattle. Journal of Dairy Science 64, 2246–2250. doi:10.3168/jds.S0022-0302(81)82836-6

Keller, M.C., Visscher, P.M., Goddard, M.E., 2011. Quantification of Inbreeding Due to Distant Ancestors and Its Detection Using Dense Single Nucleotide Polymorphism Data. Genetics 189, 237–249. doi:10.1534/genetics.111.130922

Leroy, G., 2014. Inbreeding depression in livestock species: review and meta-analysis. Animal Genetics 45, 618–628. doi:10.1111/age.12178

Lozada-Soto, E.A., Maltecca, C., Lu, D., Miller, S., Cole, J.B., Tiezzi, F., 2021. Trends in genetic diversity and the effect of inbreeding in American Angus cattle under genomic selection. Genetics Selection Evolution 53, 50. doi:10.1186/s12711-021-00644-z

Lozada-Soto, E.A., Parker Gaddis, K.L., Tiezzi, F., Jiang, J., Ma, L., Toghiani, S., VanRaden, P.M., Maltecca, C., 2024. Inbreeding depression for producer-recorded udder, metabolic, and reproductive diseases in US dairy cattle. Journal of Dairy Science 107, 3032–3046. doi:10.3168/jds.2023-23909

Lozada-Soto, E.A., Tiezzi, F., Jiang, J., Cole, J.B., VanRaden, P.M., Maltecca, C., 2022. Genomic characterization of autozygosity and recent inbreeding trends in all major breeds of US dairy cattle. Journal of Dairy Science 105, 8956–8971. doi:10.3168/jds.2022-22116

MacCluer, J.W., Boyce, A.J., Dyke, B., Weitkamp, L.R., Pfenning, D.W., Parsons, C.J., 1983. Inbreeding and pedigree structure in Standardbred horses. Journal of Heredity 74, 394–399. doi:10.1093/oxfordjournals.jhered.a109824

Makanjuola, B.O., Maltecca, C., Miglior, F., Schenkel, F.S., Baes, C.F., 2020. Effect of recent and ancient inbreeding on production and fertility traits in Canadian Holsteins. BMC Genomics 21, 605. doi:10.1186/s12864-020-07031-w

Maltecca, C., Tiezzi, F., Cole, J.B., Baes, C., 2020. Symposium review: Exploiting homozygosity in the era of genomics—Selection, inbreeding, and mating programs. Journal of Dairy Science 103, 5302–5313. doi:10.3168/jds.2019-17846

Martikainen, K., Tyrisevä, A.M., Matilainen, K., Pösö, J., Uimari, P., 2017. Estimation of inbreeding depression on female fertility in the Finnish Ayrshire population. Journal of Animal Breeding and Genetics 134, 383–392. doi:10.1111/jbg.12285

Meuwissen, T.H.E., Luo, Z., 1992. Computing inbreeding coefficients in large populations. Genetics Selection Evolution 24, 305–313. doi:10.1186/1297-9686-24-4-305

Meuwissen, T., Hayes, B., Goddard, M., 2016. Genomic selection: A paradigm shift in animal breeding. Animal Frontiers 6, 6–14. doi:10.2527/af.2016-0002

Meuwissen, T.H.E., Hayes, B.J., Goddard, M.E., 2001. Prediction of Total Genetic Value Using Genome-Wide Dense Marker Maps. Genetics 157, 1819–1829. doi:10.1093/genetics/157.4.1819

Meuwissen, T.H.E., Sonesson, A.K., Gebregiwergis, G., Woolliams, J.A., 2020. Management of Genetic Diversity in the Era of Genomics. Frontiers in Genetics 11, 880. doi:10.3389/fgene.2020.00880

Misztal, I., Lourenco, D., Aguilar, I., Legarra, A., Vitezica, Z., 2022. Manual for BLUPF90 family of programs. Retrieved on 12 June 2025 from https://nce.ads.uga.edu/html/projects/programs/docs/blupf90_all8.pdf

Mota, L.F.M., Carvajal, A.B., Silva Neto, J.B., Díaz, C., Carabaño, M.J., Baldi, F., Munari, D.P., 2024. Assessment of inbreeding coefficients and inbreeding depression on complex traits from genomic and pedigree data in Nelore cattle. BMC Genomics 25, 944. doi:10.1186/s12864-024-10842-w

Mugambe, J., Ahmed, R.H., Thaller, G., Schmidtmann, C., 2024. Impact of inbreeding on production, fertility, and health traits in German Holstein dairy cattle utilizing various inbreeding estimators. Journal of Dairy Science 107, 4714–4725. doi:10.3168/jds.2023-23728

Mulim, H.A., Brito, L.F., Batista Pinto, L.F., Moletta, J.L., Da Silva, L.R., Pedrosa, V.B., 2022. Genetic and Genomic Characterization of a New Beef Cattle Composite Breed (Purunã) Developed for Production in Pasture-Based Systems. Frontiers in Genetics 13, 858970. doi:10.3389/fgene.2022.858970

Mulim, H.A., Campos, G.S., Cardoso, F.F., Rojas De Oliveira, H., 2025. Exploring inbreeding depression in Brazilian Angus cattle population using pedigree and genomic data. Frontiers in Genetics 16, 1613820. doi:10.3389/fgene.2025.1613820

Nishio, M., Inoue, K., Ogawa, S., Ichinoseki, K., Arakawa, A., Fukuzawa, Y., Okamura, T., Kobayashi, E., Taniguchi, M., Oe, M., Ishii, K., 2023. Comparing pedigree and genomic inbreeding coefficients, and inbreeding depression of reproductive traits in Japanese Black cattle. BMC Genomics 24, 376. doi:10.1186/s12864-023-09480-5

Nyman, S., Johansson, A.M., Palucci, V., Schönherz, A.A., Guldbrandtsen, B., Hinrichs, D., De Koning, D.J., 2022. Inbreeding and pedigree analysis of the European red dairy cattle. Genetics Selection Evolution 54, 70. doi:10.1186/s12711-022-00761-3

Parland, S.M., Kearney, J.F., Rath, M., Berry, D.P., 2007. Inbreeding Effects on Milk Production, Calving Performance, Fertility, and Conformation in Irish Holstein-Friesians. Journal of Dairy Science 90, 4411–4419. doi:10.3168/jds.2007-0227

Pereira, R.J., Santana, M.L., Ayres, D.R., Bignardi, A.B., Menezes, G.R.O., Silva, L.O.C., Machado, C.H.C., Josahkian, L.A., Albuquerque, L.G., 2016. Inbreeding depression in Zebu cattle traits. Journal of Animal Breeding and Genetics 133, 523–533. doi:10.1111/jbg.12219

Peripolli, E., Metzger, J., De Lemos, M.V.A., Stafuzza, N.B., Kluska, S., Olivieri, B.F., Feitosa, F.L.B., Berton, M.P., Lopes, F.B., Munari, D.P., Lôbo, R.B., Magnabosco, C.D.U., Di Croce, F., Osterstock, J., Denise, S., Pereira, A.S.C., Baldi, F., 2018. Autozygosity islands and ROH patterns in Nellore lineages: evidence of selection for functionally important traits. BMC Genomics 19, 680. doi:10.1186/s12864-018-5060-8

Plummer, M., Best, N., Cowles, K., 2006. CODA: Convergence Diagnosis and Output Analysis for MCMC 6. https://CRAN.R-project.org/doc/Rnews/.

Pryce, J.E., Haile-Mariam, M., Goddard, M.E., Hayes, B.J., 2014. Identification of genomic regions associated with inbreeding depression in Holstein and Jersey dairy cattle. Genetics Selection Evolution 46, 71. doi:10.1186/s12711-014-0071-7

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., De Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. The American Journal of Human Genetics 81, 559–575. doi:10.1086/519795

Reverter, A., Porto-Neto, L.R., Fortes, M.R.S., Kasarapu, P., De Cara, M.A.R., Burrow, H.M., Lehnert, S.A., 2017. Genomic inbreeding depression for climatic adaptation of tropical beef cattle. Journal of Animal Science 95, 3809. doi:10.2527/jas2017.1643

Santana, M.L., Oliveira, P.S., Pedrosa, V.B., Eler, J.P., Groeneveld, E., Ferraz, J.B.S., 2010. Effect of inbreeding on growth and reproductive traits of Nellore cattle in Brazil. Livestock Science 131, 212–217. doi:10.1016/j.livsci.2010.04.003

Sargolzaei, M., Chesnais, J.P., Schenkel, F.S., 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics 15, 478. doi:10.1186/1471-2164-15-478

Sewalem, A., Kistemaker, G.J., Miglior, F., Van Doormaal, B.J., 2006. Analysis of Inbreeding and Its Relationship with Functional Longevity in Canadian Dairy Cattle. Journal of Dairy Science 89, 2210–2216. doi:10.3168/jds.S0022-0302(06)72291-3

Sumreddee, P., Toghiani, S., Hay, E.H., Roberts, A., Agrrey, S.E., Rekaya, R., 2019. Inbreeding depression in line 1 Hereford cattle population using pedigree and genomic information1. Journal of Animal Science 97, 1–18. doi:10.1093/jas/sky385

Thompson, J.R., Everett, R.W., Hammerschmidt, N.L., 2000. Effects of Inbreeding on Production and Survival in Holsteins. Journal of Dairy Science 83, 1856–1864. doi:10.3168/jds.S0022-0302(00)75057-0

VanRaden, P.M., 2008. Efficient Methods to Compute Genomic Predictions. Journal of Dairy Science 91, 4414–4423. doi:10.3168/jds.2007-0980

VanRaden, P.M., Olson, K.M., Wiggans, G.R., Cole, J.B., Tooker, M.E., 2011. Genomic inbreeding and relationships among Holsteins, Jerseys, and Brown Swiss. Journal of Dairy Science 94, 5673–5682. doi:10.3168/jds.2011-4500

Villanueva, B., Fernández, A., Saura, M., Caballero, A., Fernández, J., Morales-González, E., Toro, M.A., Pong-Wong, R., 2021. The value of genomic relationship matrices to estimate levels of inbreeding. Genetics Selection Evolution 53, 42. doi:10.1186/s12711-021-00635-0

Wellmann, R., 2019. Optimum contribution selection for animal breeding and conservation: the R package optiSel. BMC Bioinformatics 20, 25. doi:10.1186/s12859-018-2450-5

Wright, S., 1922. Coefficients of Inbreeding and Relationship. The American Naturalist 56, 330–338. doi:10.1086/279872

Zhang, Q., Calus, M.P., Guldbrandtsen, B., Lund, M.S., Sahana, G., 2015. Estimation of inbreeding using pedigree, 50k SNP chip genotypes and full sequence data in three cattle breeds. BMC Genetics 16, 88. doi:10.1186/s12863-015-0227-7

5.15 Supplementary Material

5.15.1 Supplementary Table

Supplementary Table S5.1 Comparison of imputation accuracy and quality metrics in Limousine cattle between full imputation and masked-marker validation approaches, including square correlation (r^2), mendelian errors, and single-nucleotide polymorphism (SNP) retention rates.

Quality metrics	Imputation (Full)	Imputation with Masked Markers
Square correlation (r^2)	0.99	0.98
Mendelian Errors (%)	0.42 %	0.75 %
SNP Retention (%)	98% successfully imputed	98% successfully imputed

Supplementary Table S5.2 Estimates of heritability and variance components for Limousine, considering growth and fertility traits, using a single trait model. Values within parentheses represent the highest posterior density interval (HPDI).

Traits	Heritability (h^2)	Variance components				
		σ^2_{a}	σ^2_{hy}	σ^2_{mpe}	σ^2_{h}	σ^2_{e}
Growth traits[1]						
ADG, kg/d	0.10 (0.09; 0.10)	70.83 (66.65; 75.36)	248.90 (240.80; 256.90)	-	-	410.85 (406.80; 414.50)
WW, kg	0.15 (0.14; 0.16)	210.13 (193.40; 224.20)	673.24 (648.10; 697.60)	14.18 (7.90; 20.21)	-	487.57 (476.90; 497.90)
YW, kg	0.17 (0.16; 0.18)	477.26 (439.70; 509.40)	1 513.44 (1 456.00; 1 566.00)	-	-	781.60 (756.40; 809.00)
BiW, kg	0.11 (0.10; 0.12)	1.43 (1.37; 1.50)	5.29 (5.15; 5.43)	0.22 (0.19; 0.25)	-	5.97 (5.92; 6.03)
Fertility traits[2]						
AFC, days	0.18 (0.15; 0.20)	388.10 (327.60; 435.00)	-	-	510.26 (466.00; 556.50)	1 274.94 (1 232.00; 1 320.00)
FCI, days	0.10 (0.07; 0.12)	368.62 (273.00; 453.20)	-	-	538.19 (472.00; 614.80)	2 865.88 (2 777.00; 2 956.00)

Abbreviations: σ^2_{a} = additive genetic variance; σ^2_{hy} = herd- year variance; σ^2_{mpe} = maternal permanent environmental variance; σ^2_{h} = herd variance; σ^2_{e} = residual variance; ADG = average daily gain; WW = weaning weight; YW = yearling weight; BiW = birth weight; AFC = age at first calving; FCI = first calving interval.

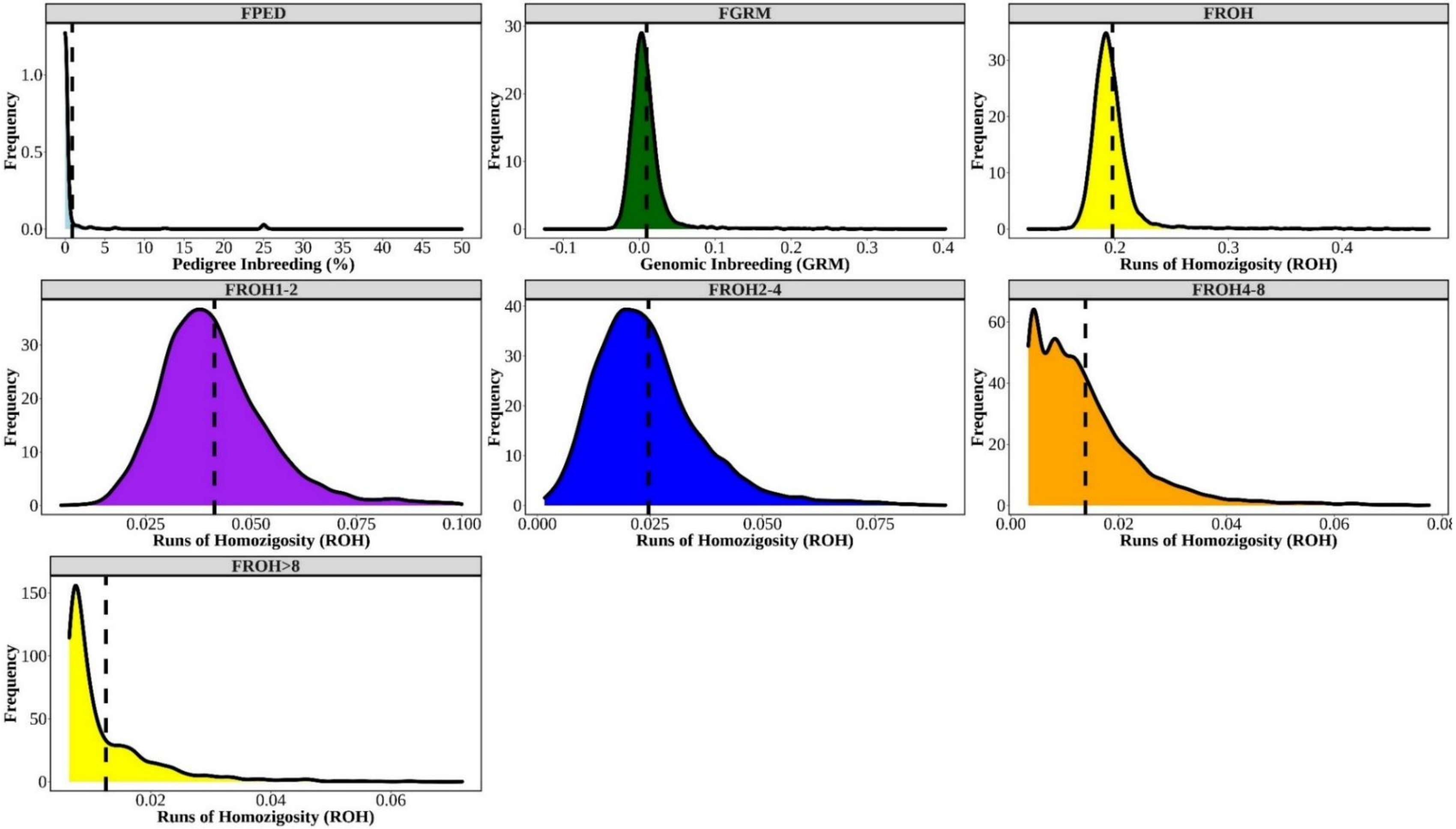
Supplementary Table S5.3 Inbreeding depression in Limousine cattle, expressed as the change in growth, fertility, and stayability traits per 1% increase in inbreeding. Estimates from the low-density SNP panel are included for comparison with results from imputed data to show consistent patterns.

Trait	F_{GRM}		F_{ROH}	
	Slope (β)	95% HPDI	Slope (β)	95% HPDI
Growth traits[1]				
ADG, g/d	-1.64	(-6.18; 2.88)	-0.47	(-2.69; 1.74)
WW, kg	-1.02	(-1.90; -0.13)	-0.26	(-0.69; 0.18)
YW, kg	-1.32	(-2.58; -0.07)	-0.25	(-0.76; 0.26)
BiW, kg	-0.07	(-0.13; -0.02)	-0.03	(-0.06; -0.01)
Fertility traits[2]				
AFC, days	1.90	(0.08; 3.71)	1.01	(0.23; 1.79)
FCI, days	0.09	(-1.95; 2.12)	-0.06	(-0.88; 0.76)
Stayability traits[3]				
STAY2	-0.11	(-0.16; -0.06)	-0.04	(-0.06; -0.02)
STAY3	-0.07	(-0.12; -0.03)	-0.03	(-0.06; -0.01)
STAY4	-0.09	(-0.14; -0.06)	-0.04	(-0.07; -0.01)

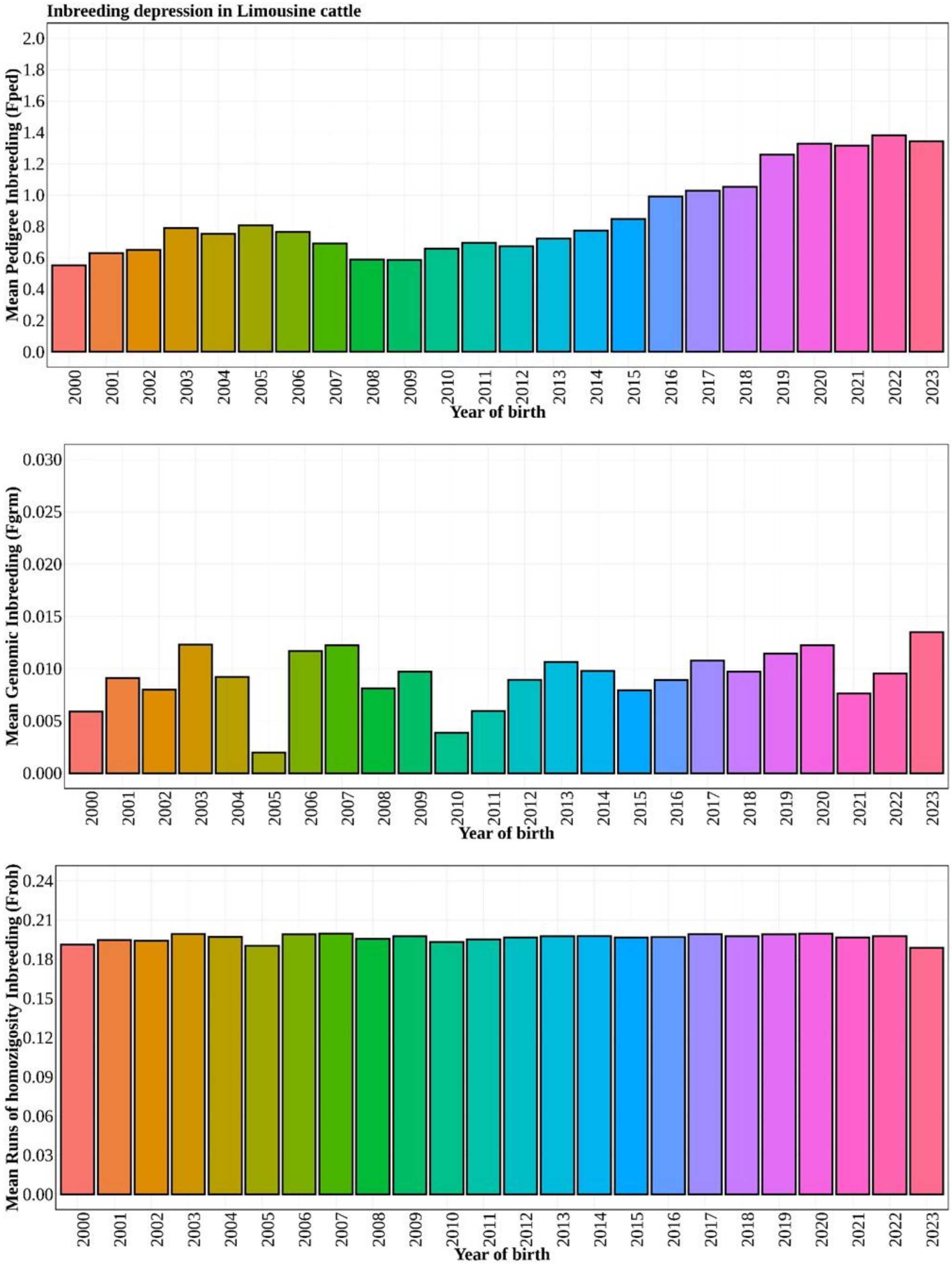
Abbreviations: F_{GRM} = relationship matrix derived inbreeding; F_{ROH} = runs of homozygosity calculated with segment-based approaches; HPDI = high posterior density intervals; ADG = average daily gain; WW = weaning weight; YW = yearling weight; BiW = birth weight; AFC = age at first calving; FCI = first calving interval; STAY2 = stayability2; STAY3 = stayability3; STAY4 = staybility4

5.15.2 Supplementary Figure

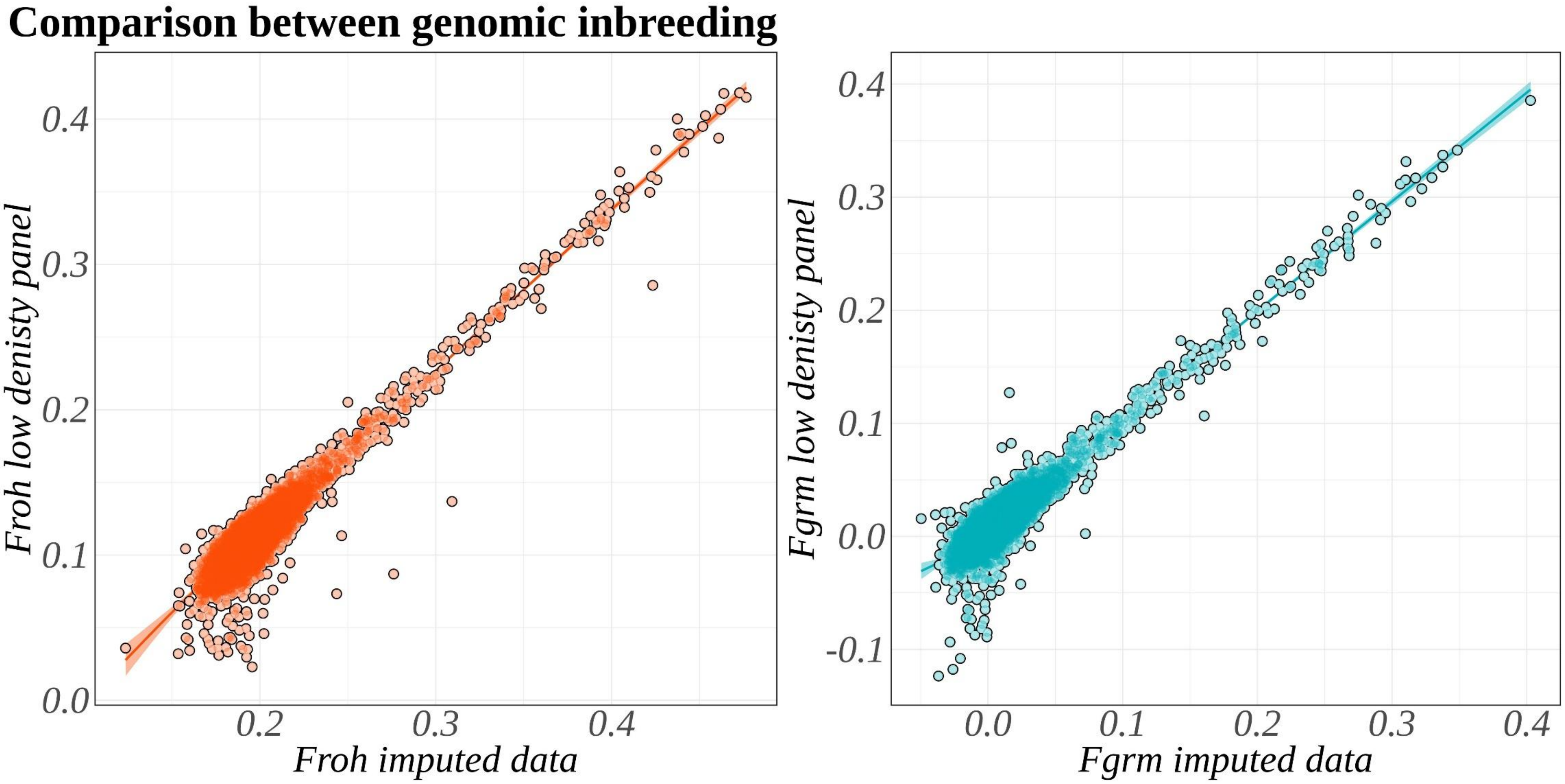
Supplementary Figure S5.1 Distribution of pedigree and genomic inbreeding in Limousine cattle. Genomic inbreeding is based on the diagonal of the genomic relationship matrix, based on Runs of Homozygosity (ROH) and partial ROH inbreeding (F_{PED} = total pedigree inbreeding; F_{GRM} = relationship matrix derived inbreeding; F_{ROH} = runs of homozygosity calculated with segment-based approaches)



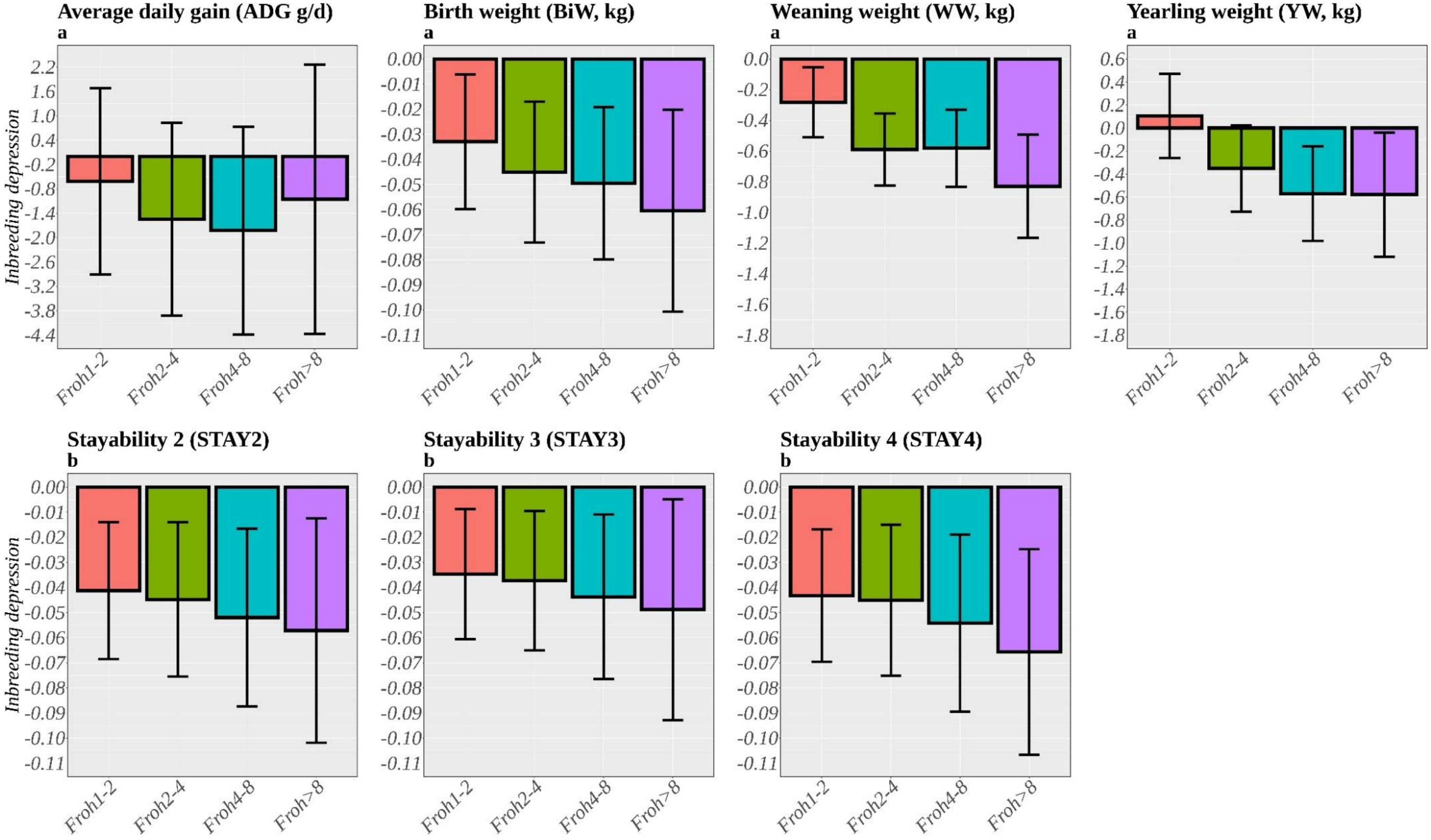
Supplementary Figure S5.2 Mean pedigree and genomic inbreeding in Limousine cattle from animals born 2000 to 2023. The inbreeding measures shown include pedigree inbreeding, genomic inbreeding, which is based on the diagonal of the genomic relationship matrix and Runs of Homozygosity (ROH).



Supplementary Figure S5.3 Estimated regression coefficients for genomic relationship matrix derived inbreeding (F_{GRM}) and Runs of Homozygosity calculated with segment-based approaches (F_{ROH}) from imputed data against their counterparts from the low-density panel in Limousine cattle (F_{ROH} = runs of homozygosity calculated with segment-based approaches*;* F_{GRM} = relationship matrix derived inbreeding).



Supplementary Figure S5.4 The effects of 1% increase in recent and ancient genomic inbreeding depression on growth and stayability traits in Limousine cattle, using low-density panel (F_{ROH} = runs of homozygosity calculated with segment-based approaches).



CHAPTER 6

6. Evaluating genotype by environment interaction for growth traits in Limousine cattle

Animal 18 (2024) 101344

ELSEVIER

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Evaluating genotype by environment interaction for growth traits in Limousine cattle

S. Callegaro[a], F. Tiezzi[a,*], M.C. Fabbri[a], S. Biffani[b], R. Bozzi[a]

[a] *Department of Agriculture, Food, Environment, and Forestry (DAGRI), University of Florence, Florence 50144, Italy*
[b] *Institute of Agricultural Biology and Biotechnology-CNR, Milano 20133, Italy*

https://doi.org/10.1016/j.animal.2024.101344

Abstract

Environmental conditions affect the growth and health of animals, making it crucial to quantify heat stress and the genetic basis of heat tolerance in animal breeding. The main objective of this study was to evaluate heat stress on beef cattle growth and investigate the genetic background of tolerance to harsh environmental conditions in the Italian Limousine beef cattle. Three growth traits were analysed: average daily gain (**ADG**), weaning weight (**WW**), and yearling weight (**YW**). Data were collected from animals raised between 1991 and 2022 and combined with 14 environmental covariates. Records for ADG, WW, and YW encompassed 108 205, 100 058, and 24 939 individuals, respectively, with 4 617, 4 670, and 2 048 genotyped individuals. Climatic variables were compared for inclusion in a linear mixed model using the Deviance Information Criterion. Multiple-trait models and genomic information incorporated environmental conditions with the largest impact on the studied traits Genotype by environment interaction (**G × E**) was detected in all the studied traits, showing substantial heterogeneity of the variance components across the different environments (**Env**). Heritability for WW remains constant among Env; instead, for ADG and YW decreased under uncomfortable environmental conditions. YW showed the lowest genetic correlation (0.28) between divergent conditions (Env 2 and Env 5,) for ADG and WW correlations dropped below 0.50 among Env. The values of genetic correlations indicate that growth traits are moderately to strongly affected by G × E. Eigenvalue decomposition of the additive genetic (co)variance matrix for ADG, WW, and YW indicated that three components accounted for over 0.80 of the proportion of the variance explained, suggesting different animal performances across Env. Spearman rank correlations showed potential re-ranking of genotyped sires, because ADG, WW, and YW showed correlations between Env below 0.80. The accuracy of single-step genomic EBV was higher compared to EBV for al traits. Overall, the result confirms the existence of G × E for growth traits in the Italian Limousine population. Including G × E in the model allows for more environment-aware predictions, helping breeders understand how different genetic bases respond to varying conditions. Genomic predictions incorporating G × E could accelerate genetic gains and improve response to selection for heat tolerance.

Keywords: growth, animal resilience, heat stress, multiple trait model, beef breed

Highlights

- Investigation of the genetic control of heat tolerance in Limousine cattle.
- Variance components for growth traits vary across environments.
- Spearman correlations confirmed possible sires re-ranking in different environment.

- Highlights the need for selecting animals that thrive under specific conditions.
- Results guide breeding resilient cattle, enhancing productivity in climate change.

Implications

This study investigated the genotype by environment interaction of three production traits (average daily gain, weaning weight, and yearling weight) in the Italian Limousine population. We found that growth is moderately to strongly determined by the genotype by environment interaction. Our findings show that growth performance varies significantly across environments, underscoring the importance of selecting animals that cope with specific conditions. These results can guide breeding programs to develop more resilient cattle, improving productivity and animal welfare in the context of climate change.

6.1 Introduction

Heat stress has been reported as a cause of decreased performance in livestock, resulting in significant economic losses and reduced animal health and welfare. Physiology, health, and metabolism could be severely damaged by increased temperatures (Nardone et al., 2010). For example, St.-Pierre et al. (2003) estimated $360 million in annual losses due to heat stress in the US beef sector. One effective strategy to overcome this problem is to select animals that are less sensitive to environmental stress and more adapted to harsh conditions (Renaudeau et al., 2012). The genetic control on tolerance to heat stress is attributable to the genotype by environment interaction (**G × E**) (Tiezzi and Maltecca, 2022). Therefore, genetic evaluation of G × E is constantly increasing in the livestock sector, especially for economically important traits.

Genotype by environment interaction imply that different genotypes may respond differently to environmental changes, affecting performance across different environments. Research on cattle and pigs has shown that the genetic component of heat stress is negatively correlated with production performance (Zumbach et al., 2008b; Biffani et al., 2016; Bradford et al., 2016). Additionally, studies have also demonstrated the existence of G × E for reproduction traits, showing a negative association between the genetic component of heat stress and the reproductive performances (Ravagnolo and Misztal, 2002a; Bernabucci et al., 2014; Tiezzi et al., 2020; Chen et al., 2021).

Quantifying the magnitude of G × E is a significant challenge. Therefore, developing strategies and tools to enhance the efficiency and sustainability of beef production is necessary to understand specific sector problems and the genetic background of sensitivity to environmental variation. One complication in modelling heat stress in beef cattle is that only the weight of animals at particular growth stages is often available. Another complication is compensatory growth, where animals can recover weight lost due to previous stress periods caused by heat stress (Beccari et al., 1983; Mader and Davis, 2004; Santana et al., 2016). One way to address these issues is to assume that heat load accumulates over the animals' lifetime or until a certain weight.

Generally, genetic analyses of heat stress in pigs (Zumbach et al., 2008a,b; Fragomeni et al., 2016a) and beef cattle (Bradford et al., 2015; Santana et al., 2016) are based on heat load function for live or carcass weight in growing/finishing animals. In selection, the presence of G × E is explored using multi-trait models (**MT**) or reaction norm models (Tiezzi and Maltecca, 2022). In the MT model, a trait measured in multiple environments is considered a different trait in each environment. Consequently, the animal's breeding values the environmental conditions, reflecting the heterogeneous impact of the genetic background across different environments. Environmental conditions must be treated as categorical variables so that the MT provides a breeding value estimate for each environment. This approach is called a multi-environment trial, where a set of genotypes or families are raised in several environments (Isik et al., 2017). Proper analyses of multi-environment trial can determine which animals perform better in a specific environment and understand the relationship among environments in terms of G × E patterns. Genetic correlations between environments lower than 0.80 have been proposed in animal breeding as evidence of G × E interactions (Hayes et al., 2016). Moreover, correlations below 0.70 suggest considerable reranking of selection candidates, confirming G × E (Mulder and Bijma, 2007). For this reason, it is recommended to assess separate breeding programs and consider the effect of the environment on the genetic or genomic model.

In cattle breeding programs in Italy, routine genetic evaluations do not account for G × E. This justifies a study to identify genetically superior individuals for tolerance to heat stress in the Limousine population, a French beef breed typically used in local or dairy crossbreeding systems or as a pure breed (Bouquet et al., 2011). It is widely raised in the Mediterranean, with a relevant presence in Italy. However, this population is distributed across the entire national territory, predominating in Central and Southern Italy. The diversity of Italian territory led to different farm management and environmental conditions, affecting the animals and exposing them to varying humidity and temperature levels. Consequently, animals' performance can be negatively affected. Understanding the extent of environmental variability in Italy is crucial. Animals can experience significant heat stress, especially during the summer months. In many regions, cattle may face over 50 days of high temperatures (CNR-ISAC, http://www.isac.cnr.it), leading to heat stress.

This context underscores the importance of considering G × E interactions in breeding programs, as it could significantly influence animal performance and welfare, guiding breeders towards G × Eaware breeding values. Therefore, the objectives of this study were: (a) to identify the environmental parameter with the most substantial impact on growth; (b) to estimate the impact of heat stress on genetic parameters; (c) to compare pedigree-BLUP and Single-Step genomic BLUP for the genetic evaluations for heat tolerance using a multiple-trait approach. The study was conducted on the Italian Limousine beef cattle population.

6.2 Material and methods

6.2.1 Animal data

Data were obtained from a pre-existing database; therefore, Animal Care and Use Committee approval was unnecessary. The National Italian Association of Limousine and Charolais Breeders provided phenotypic, pedigree, and genomic information. Records were collected on animals born between 1991 and 2022 and raised in herds in the national territory. The traits analysed were weaning weight (**WW**, kg), yearling weight (**YW**, kg), and average daily gain (**ADG**, g/d). Firstly, WW was measured considering a range of 170-250 days of age and YW at 290-440 days of age in the Limousine population. For each animal, ADG was calculated as the difference between two weights ranging from 30 days (minimum) to 150 days (maximum).

ADG, WW, and YW records of contemporary groups with less than five individuals were excluded from the dataset for data editing, with groups defined by concatenating the herd and the animal's birth year. Moreover, phenotypic records outside the interval of the mean +/-3.5 standard deviation units. After editing, the final database included 108 205, 100 058, and 24 939 animals for ADG, WW, and YW, respectively. For the subsequent analyses, the age of animals was grouped into two classes using the median (118, 205, and 323 days of age for ADG, WW, and YW, respectively). The age of the dam at calving was divided into five classes using the first four quintiles as discriminants to create a balanced number of observations in each class. Dividing the age of animals into two classes using the median reduces variability from individual age differences, which is useful where there is significant variation in age-related growth traits. It is also less sensitive to outliers, producing more robust and reliable results. Moreover, including fixed effects for age classes in our model can help control for age-related variation in weight.

We considered only animals with known sires and dams to analyse all the investigated traits. Finally, general information and descriptive statistics used in this study from the three final datasets were summarised in Table 6.1. In addition, Figure 6.1 illustrates the distribution of animals and herds for ADG, WW, and YW across the 20 Italian regions. Supplementary Table S6.1 provides the percentage distribution of herds and the number of animals for these growth traits in the seven most representative Italian regions.

Table 6.1 Descriptive statistics of traits, number of farms, contemporary groups (CG), sires, dams, and effects for cattle under study.

Traits	Descriptive statistics							
	N. of records	N. of animal with records	mean	SD	N. of Farm	N. of CG	N. sires	N. dams
ADG	108 205	108 205	1.04	0.28	829	5 712	3 783	39 014
WW	100 058	100 058	247.9	46.54	838	6 102	4 019	40 548
YW	24 939	24 939	354.5	65.61	550	2 030	2 162	16 090

Abbreviations: ADG = average daily gain; WW = weaning weight; YW = yearling weight.

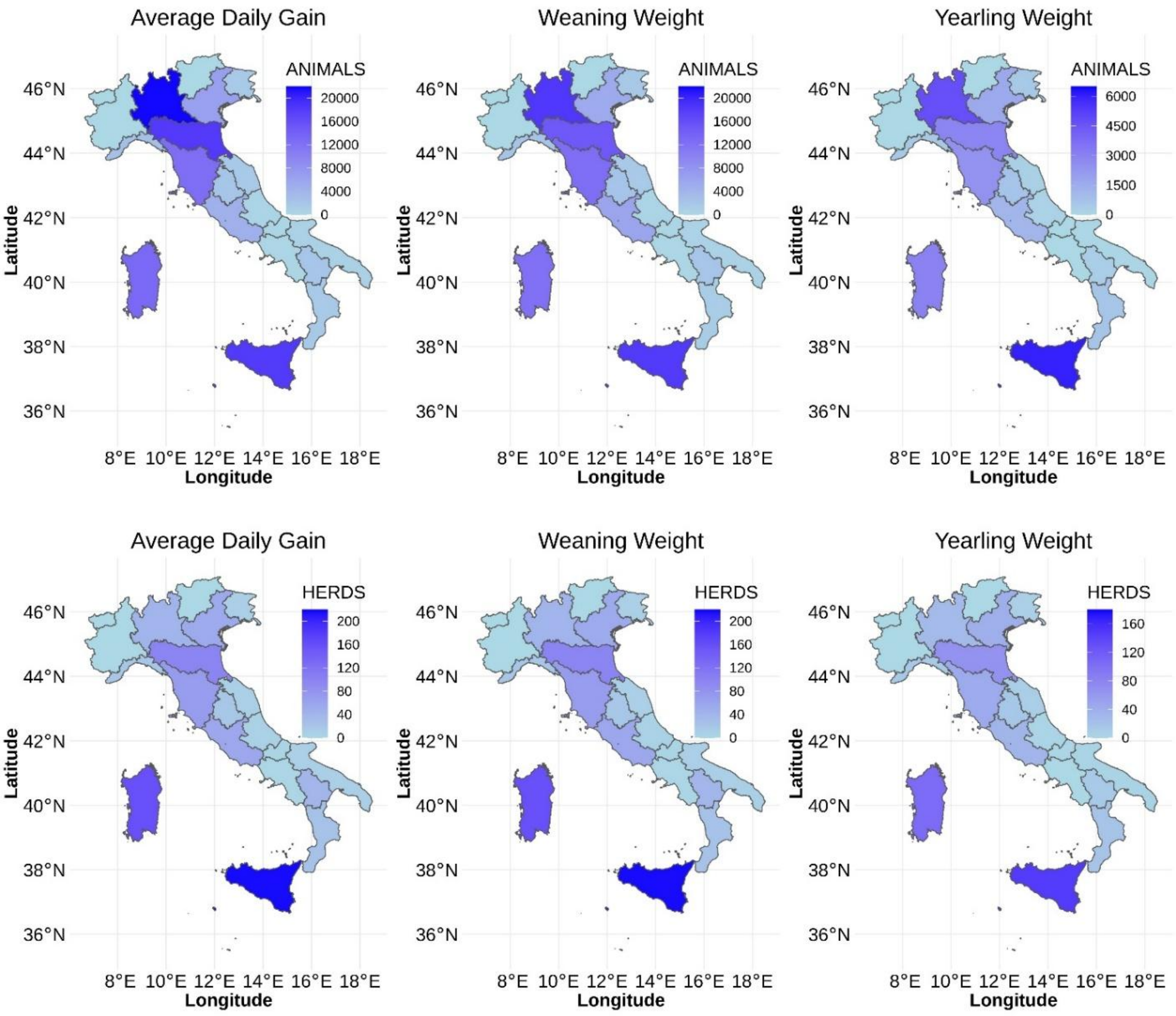


Figure 6.1 Frequency of cattle and herds across Italian regions for average daily gain (ADG, kg/d), weaning weight (WW, kg), and yearling weight (YW, kg).

6.2.2 Meteorological data and environmental descriptors

Several previous studies have used weather stations; however, data from meteorological stations could be limited for a specific time because the complete information is not always accessible. The National Aeronautics and Space Administration (NASA/POWER, https://power.larc.nasa.gov) provides a surrogate to the meteorological stations where weather data are obtained from satellite observations (Van Wart et al., 2015). Rockett et al. (2023) compared weather parameters and temperature humidity index (**THI**) values collected from weather stations against NASA/POWER estimates. The study showed that climatic data from the stations were highly correlated (with a Pearson correlation larger than 0.80) with data from NASA POWER; therefore, weather station data could be replaced.

Herds' positions were matched with their corresponding latitude and longitude coordinates to obtain data from NASA POWER. Meteorological data including average daily temperature (**Temp**, °C), relative humidity (**RH**, %), and the dew point (**DP**, °C Td) were downloaded. Subsequently, the average THI (National Oceanic and Atmospheric Administration, 1976) was calculated as:

$$\mathrm{THI} = (1.8 \times \mathrm{Temp} + 32) - (0.55 - 0.0055 \times \mathrm{RH}) \times (1.8 \times \mathrm{Temp} - 26)$$

Where Temp is the temperature in degrees Celsius, and RH is the relative humidity, expressed in percentage. Heat stress in animals was further characterised by calculating the heat load, which is the cumulative THI units above a certain threshold (70 or 75) over a specified period (Zumbach et al., 2008a; Santana et al., 2015; Bradford et al., 2016). This was applied to datasets ADG, WW, and YW. The heat load function was defined as follows:

$$\text{heat load } 70 = \max\left(0, \sum \mathrm{THI} - 70\right)$$

$$\text{heat load } 75 = \max\left(0, \sum \mathrm{THI} - 75\right)$$

THI was the accumulated THI for the animals on the days leading up to the weight measurement date or between the two dates for ADG. When THI was below the threshold, the model assumed that heat stress does not impact animal growth, and heat load was assigned to a value of 0. Conversely, when the average daily THI exceeds the threshold (either 70 or 75), heat load is assigned a positive value, reflecting the degree of heat stress experienced by the animals.

A range of 30 days before the weight data for WW was chosen based on Bradford et al. (2016). Instead, we reduce YW from 150 to 30 days because Bradford et al. (2016) found that cattle may have recovered to heat stress during the 150 days, resulting in no detectable G × E intersection. For ADG, a period of 30-150 days was chosen, assuming that in this gap, environmental parameters may negatively affect the growth of animals. Therefore, averaged weather data for the three analysed traits were calculated into these periods. This range was chosen based on the period during which significant weight gain occurs, reflecting the average environmental conditions affecting the cattle. This interval was also selected to assess the impact of heat stress on growth as reported in the literature these time frames are crucial for cattle accumulating stress due to environmental factors. Including this range for ADG was intentional to capture the impact of environmental stressors on cattle growth during these critical periods. Additionally, the mean values at the first and third quartile were considered because NASA POWER provides daily averages, not hourly values. Using only minimum and maximum would underestimate the effect of extreme conditions on the animals. Finally, 14 variables describing climatic conditions were obtained, as detailed in Supplementary Table S6.2.

6.2.3 Heat stress analysis model

The 14 environmental variables were categorised into 5 classes (Supplementary Table S6.2) to define ‘environmental conditions’ (**Env**). By grouping animals based on these classes, it is possible to evaluate the impact of different levels of environmental stress on growth traits (ADG, WW, and YW). The five classes of the Env were created using the first four quintiles as discriminants, allowing a balanced number of observations per each Env category. For each environmental variable, the 5 classes represent the different levels of environmental stress. Animals were grouped according to the environmental conditions at their respective locations. The 5 Env were defined as follows: Env 1 (extremely uncomfortable), Env 2 (moderately comfortable), Env 3 (comfortable), Env 4 (moderately uncomfortable), and Env 5 (extremely uncomfortable), respectively. An exception was for RH, where the 5 classes were defined differently: Env 1 (comfortable), Env 2 (moderately comfortable), Env 3 (moderately uncomfortable), Env 4 (uncomfortable), and Env (5 extremely uncomfortable), respectively. For heat load, the classes were treated differently: Env 1 (comfortable, with all heat load values equal to 0), and from env 2 until env 5, the classes were divided incrementally based on the heat accumulated by the animals.

Because most of the analysed environmental variables were derived from Temp and DP, Env 1 (extremely cold environment) and Env 5 (extremely hot environment) were both considered "extremely uncomfortable." Although cattle typically do not have problems with cold weather, extreme cold is still classified as uncomfortable due to its potential negative impact. Toghiani et al. (2020) found that cold stress as an environmental stressor could impact overall health and cattle productivity, in particular birth and weaning weight. The classification changes regarding to relative humidity, where the lowest values are the most comfortable.

6.2.4 Detection of best environmental covariates

The effect of the environmental conditions on ADG, WW, and YW was assessed using the following linear mixed model (Model 1 to evaluate the response of growth to a specific environment:

$$y_{ijklm} = \mu + env_i + sex_j + age_k + ageD_l + env_i * sex_j + env_i * age_k + env_i * ageD_l + age_k * ageD_l + hy_m + e_{ijklm} \quad [1]$$

Where y_{ijklm} was the measurements of ADG, WW, and YW, μ was the intercept of the model; env_i was the fixed effect of the environmental covariates divided into 5 classes i; sex_j was the fixed effect of the sex; age_k was the fixed effect of the age of the animals, divided into 2 levels using median (for YW we correct the model using age as a linear covariate); ageD, was the fixed effect of age dam class (5 levels); hy_m was the random effect of the herd year; e_{ijklm} was the residual error. The model also included all the two-way interactions among the fixed effects. These analyses were implemented using the function *lmer* of package 'lme4' (Bates, 2010) of the R software (R Core Team, 2020), using a maximum-likelihood estimator (option REML=FALSE in *lmer*). The models were compared based on the Deviance Information Criterion, with smaller values indicating better model fit.

6.2.5 Pedigree and genotypes

The raw pedigree file included 493 125 animals across more than 10 generations. Therefore, animals were traced back to 7 generations, including 44 304, 48 231, and 33 478 animals for ADG, WW, and YW, respectively. 269, 265, and 139 animals were genotyped with GeneSeek GGP Bovine 150K (HD, number of SNPs = 119 854) for ADG, WW, and YW, respectively. 4 348, 4 404, and 1 909 animals were genotyped with GeneSeek GGP Bovine LD v3 (number of SNPs = 28,299) for ADG, WW, and YW, respectively. The two panels shared 14 079 SNPs. Due to the low number of genotyped animals in HD compared to LD, animals genotyped with GeneSeek GGP Bovine 150K were imputed to GeneSeek GGP Bovine LD starting from the 14,079 SNPs shared. FImpute v.3 (Sargolzaei et al., 2014) was used with default parameters to impute genotypes.

Quality control and data filtering of genotype data were performed with PLINK v1.9 (Chang et al., 2015). Therefore, in the quality control autosomal SNPs and individuals with less than 10% of missing values, minor allele frequency higher than 0.01 and a call rate higher than 90% were retained. Thus, 22 910, 22 903, and 22 921 SNPs for 4 617, 4 670, and 2 048 animals for ADG, WW, and YW, respectively, were included in the successive analyses. Table 6.2 summarises the number of genotyped animals per category, considering males and females with phenotype, sires, and dams.

Table 6.2 Number of genotyped cattle across the three studied traits, considering animals with phenotype, sires, and dams.

Traits	Male with phenotype	Sires	Female with phenotype	Dams	Total
ADG	1 224	551	1 775	1 067	4 617
WW	1 127	675	1 565	1 303	4 670
YW	266	431	449	902	2 048

Abbreviations: ADG = average daily gain; WW = weaning weight; YW = yearling weight; Males with phenotype = are those individuals showing a phenotypic record in the dataset. They may appear as sires or other individuals; Sires = are those individuals not showing a phenotypic record in the dataset, but they appear as sires of individuals with phenotypes; Female with phenotype = are those individuals showing a phenotypic record in the dataset. They may appear as dams or other individuals; Dames = are those individuals not showing a phenotypic record in the dataset, but they appear as dams of individuals with phenotypes.

6.2.6 Model and analyses for variance components and breeding value estimates

Univariate model. Separate univariate models were used to mimic the national Limousine evaluation for growth traits without including the G × E effect. The models were defined as follows (Model 2):

$$y_{ijklm} = \mu + sex_i + age_j + ageD_k + a_l + cg_m + e_{ijklm} \; [2]$$

were y_{ijklm} is the value of ADG, WW, and YW; sex_i is the fixed effect of the sex; age_j is the fixed effect of the age of the animals (continuous covariate or in classes); $ageD_k$, is the fixed effect of age dam class (5 levels); a_l is the random additive direct genetic effect of the animal; cg_m is the random effect of the contemporary groups; e_{ijklm} is the random residual error. In matrix notation, the univariate model was represented as follows:

$$y = \mathbf{X}\beta + \mathbf{Z}u + \mathbf{W}m + e$$

where y is the vector of phenotypic values of ADG, WW, and YW; β is the vector of solutions for fixed effects**;** u is the vector of solutions for the random genetic effect of the animal; m is vector of solutions for the random effect of contemporary groups; e is the random residual error; and **X**, **Z**, **W** are the incidence matrices associated with fixed, direct genetic, and random genetic effects, respectively. In all the subsequent analyses for WW, the maternal permanent environmental effect was not incorporated in the models. This choice is related to the dam frequency and limited repeated records per dam in the Italian Limousine population. Also, Santana et al. (2016) omitted the permanent maternal environmental effect for one breed because of the limited repeated records per dam.

Multiple traits model including genotype by environment interaction. An MT animal model was used to estimate variance components for growth traits in 5 different Env, selected based on lower Deviance Information Criterion values from model 1. Utilising the covariance among traits, this model can generate breeding values for all traits of individuals. Consequently, variance-covariance structures can be applied to model G x E interactions. Records were assigned to the corresponding Env class based on the environmental covariate involved (Table 6.3). Fixed and random effects were the same as in Model 2.

Table 6.3 Distribution of cattle numbers for average daily gain, weaning weight, and yearling weight across Italy, divided into five different environments.

Traits	Covariate	Interval	Environment	N
ADG	maxRH_CLASS	[40.69 - 72.44]	Env 1	21 778
	maxRH_CLASS	(72.44 - 79.12]	Env 2	21 540
	maxRH_CLASS	(79.12 - 84.92]	Env 3	21 619
	maxRH_CLASS	(84.92 - 89.94]	Env 4	21 753
	maxRH_CLASS	(89.94 - 98.70]	Env 5	21 515
WW	T_CLASS	[-11.31 - 7.41]	Env 1	19 999
	T_CLASS	(7.41 - 12.35]	Env 2	20 024
	T_CLASS	(12.35 - 17.45]	Env 3	20 021
	T_CLASS	(17.45 - 22.72]	Env 4	20 002
	T_CLASS	(22.72 - 35.66]	Env 5	20 012
YW	DP_CLASS	[-8.65 - 3.88]	Env 1	4 990
	DP_CLASS	(3.88 - 7.17]	Env 2	4 985
	DP_CLASS	(7.17 - 10.25]	Env 3	4 992
	DP_CLASS	(10.25 - 13.89]	Env 4	4 984
	DP_CLASS	(13.59 - 23.15]	Env 5	4 988

Abbreviations: ADG = average daily gain; WW = weaning weight; YW = yearling weight; maxRH_CLASS = class of Maximum Relative Humidity; T_CLASS = class of Temperature; DP_CLASS = class of Dew Point.

In matrix notation, the following general model was used in 5 Env analyses:

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \end{bmatrix} = \begin{bmatrix} \mathbf{X_1} & 0 & 0 & 0 & 0 \\ 0 & \mathbf{X_2} & 0 & 0 & 0 \\ 0 & 0 & \mathbf{X_3} & 0 & 0 \\ 0 & 0 & 0 & \mathbf{X_4} & 0 \\ 0 & 0 & 0 & 0 & \mathbf{X_5} \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \\ b_4 \\ b_5 \end{bmatrix} + \begin{bmatrix} \mathbf{Z_1} & 0 & 0 & 0 & 0 \\ 0 & \mathbf{Z_2} & 0 & 0 & 0 \\ 0 & 0 & \mathbf{Z_3} & 0 & 0 \\ 0 & 0 & 0 & \mathbf{Z_4} & 0 \\ 0 & 0 & 0 & 0 & \mathbf{Z_5} \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \\ a_3 \\ a_4 \\ a_5 \end{bmatrix} +$$

$$\begin{bmatrix} \mathbf{W_1} & 0 & 0 & 0 & 0 \\ 0 & \mathbf{W_2} & 0 & 0 & 0 \\ 0 & 0 & \mathbf{W_3} & 0 & 0 \\ 0 & 0 & 0 & \mathbf{W_4} & 0 \\ 0 & 0 & 0 & 0 & \mathbf{W_5} \end{bmatrix} \begin{bmatrix} m_1 \\ m_2 \\ m_3 \\ m_4 \\ m_5 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ e_3 \\ e_4 \\ e_5 \end{bmatrix}$$

where y_1, y_2, y_3, y_4, and y_5 are trait records for genotypes in Env 1, 2, 3, 4, and 5, respectively, $\mathbf{X_1}$ to $\mathbf{X_5}$ are incidence matrices for fixed effect, $\mathbf{Z_1}$ to $\mathbf{Z_5}$ are the matrix of the additive genetic effect, $\mathbf{W_1}$ to $\mathbf{W_5}$ are the incidence matrices of the random effect of contemporary groups. The vectors of the systematic effects are represented by b_1 to b_5, a_1 to a_5 are vectors of additive genetic effect, m_1 to m_5 are vectors of the random effect of contemporary groups, and e_1 to e_5 are vectors of residual error. The assumptions regarding the (co)variance structure of random effects, and additive genetic effect in five Env are:

$$\begin{bmatrix} a_1 \\ a_2 \\ a_3 \\ a_4 \\ a_5 \end{bmatrix} \sim \mathrm{N}\left(0, \mathbf{A} \otimes \begin{bmatrix} \sigma_{a1}^2 & \sigma_{a12} & \sigma_{a13} & \sigma_{a14} & \sigma_{a15} \\ \sigma_{a21} & \sigma_{a2}^2 & \sigma_{a23} & \sigma_{a24} & \sigma_{a25} \\ \sigma_{a31} & \sigma_{a32} & \sigma_{a3}^2 & \sigma_{a34} & \sigma_{a35} \\ \sigma_{a41} & \sigma_{a42} & \sigma_{a43} & \sigma_{a4}^2 & \sigma_{a45} \\ \sigma_{a51} & \sigma_{a52} & \sigma_{a53} & \sigma_{a54} & \sigma_{a5}^2 \end{bmatrix}\right)$$

$$\begin{bmatrix} m_1 \\ m_2 \\ m_3 \\ m_4 \\ m_5 \end{bmatrix} \sim \mathrm{N}\left(0, \mathbf{I} \otimes \begin{bmatrix} \sigma_{m1}^2 & \sigma_{m12} & \sigma_{m13} & \sigma_{m14} & \sigma_{m15} \\ \sigma_{m21} & \sigma_{m2}^2 & \sigma_{m23} & \sigma_{m24} & \sigma_{m25} \\ \sigma_{m31} & \sigma_{m32} & \sigma_{m3}^2 & \sigma_{m34} & \sigma_{m35} \\ \sigma_{m41} & \sigma_{m42} & \sigma_{m43} & \sigma_{m4}^2 & \sigma_{m45} \\ \sigma_{m51} & \sigma_{m52} & \sigma_{m53} & \sigma_{m54} & \sigma_{m5}^2 \end{bmatrix}\right)$$

the random residuals (co)variance structure is:

$$\begin{bmatrix} e_1 \\ e_2 \\ e_3 \\ e_4 \\ e_5 \end{bmatrix} \sim \mathrm{N}\left(0, \mathbf{I} \otimes \begin{bmatrix} \sigma_{e1}^2 & 0 & 0 & 0 & 0 \\ 0 & \sigma_{e2}^2 & 0 & 0 & 0 \\ 0 & 0 & \sigma_{e3}^2 & 0 & 0 \\ 0 & 0 & 0 & \sigma_{e4}^2 & 0 \\ 0 & 0 & 0 & 0 & \sigma_{e5}^2 \end{bmatrix}\right)$$

where **A** is the matrix of additive genetic relationships among individuals in the pedigree, and **I** is the identity matrix. The residual covariance between the 5 Env is set to zero.

Multiple traits genomic-based single-step model including genotype by environment interaction. The SNP information can be included as a genomic relationships matrix among animals to obtain genomic EBV. Also, the MT can be extended with the information derived from SNP. The genomic approach combines matrix **A** (pedigree relationship matrix) with **G** (relationships matrix from SNP markers). Indeed, genomic EBV was estimated with single-step genomic BLUP (Aguilar et al., 2010), and the **H** matrix was composed by combining **A** with **G** (Legarra et al., 2014):

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

Where $\mathbf{A}^{-1}$ is the inverse of the numerator relationship matrix **A**, and $\mathbf{A}_{22}^{-1}$ is the inverse of the **A** matrix for the genotyped animals and $\mathbf{G}^{-1}$ represents the inverse of the SNP genomic relationship matrix, determined with the second method as in VanRaden (2008).

Multiple traits model using permutation. An additional model was assessed to evaluate the effective presence of G × E on the studied traits. MT models using permuted Env assignments (MT with permutations) were implemented, where y is the observation for the YW of the animal assigned randomly in the 5 Env (permutations). The fixed and random effects were the same in Model 2. Random assignment of the trait of animals in different Env minimises the environmental effect. The MT with permutations model was implemented only for YW, the trait with fewer available records. Ten permutations were carried out, estimating variance components for each.

6.2.6 Bayesian Inference, Model Comparison, and computation

Variance components and genetic parameters were estimated with an animal model through the GIBBS3F90 program, implemented in the BLUPF90 family of programs (Misztal et al., 2014). Therefore, a Gibbs chain of 1 000 000 iterations has been set, plus a burn-in period of 500 000 iterations and a sampling interval of 100 iterations. These constraints ensured model convergence for all traits analysed. Convergence of the parameters was confirmed by visual investigation of trace plots and throughout Geweke's test using the package 'coda' (Plummer et al., 2006) of the R software (R Core Team, 2020).

Heritability (h^2) and herd-year effect ($\mathrm{h_i}$) were defined as follows:

$$\mathrm{h}^2 = \frac{\sigma_{\mathrm{a}}^2}{\sigma_{\mathrm{a}}^2 + \sigma_{\mathrm{hy}}^2 + \sigma_{\mathrm{e}}^2}$$

$$\mathrm{h_i} = \frac{\sigma_{\mathrm{hy}}^2}{\sigma_{\mathrm{a}}^2 + \sigma_{\mathrm{hy}}^2 + \sigma_{\mathrm{e}}^2}$$

where σ_{a}^2 is the estimate of additive genetic variance, σ_{hy}^2 is the estimate of herd-year effect variance, and σ_{e}^2 is the estimate of residual variance. The posterior mean and 95% empirical confidence intervals of the posterior distribution were used as the estimate and its attached error. Confidence intervals were calculated using the R software package 'TeachingDemos' (Snow, 2020).

The accuracy of EBV and single-step genomic EBV for the trait *t* take into consideration of the animal *i* was estimated as follows:

$$\mathrm{Acc_{it}} = \sqrt{1 - \frac{\mathrm{SE_{it}^2}}{(1 + \mathrm{F_i})\sigma_{\mathrm{at}}^2}}$$

where $\mathrm{SE_{it}}$ is the standard error of EBV and single-step genomic EBV for animal i in the 5 Env, $\mathrm{F_i}$ is the inbreeding coefficient, and finally σ_{a}^2 is the estimated variance of animal genetic additive effect (Aguilar et al., 2020). A cross-validation using Linear Regression was performed to evaluate the single-step genomic BLUP and BLUP models (Legarra and Reverter, 2015). The evaluation was conducted by truncating the data to four years prior, using the complete pedigree information. The EBVs and single-step genomic EBVs and their accuracies were obtained by truncating the data to four years prior and using the complete pedigree. For the comparison between single-step genomic BLUP and BLUP, bulls with at least five progenies today and zero progeny four years ago were selected. The linear regression statistics (bias, slope, and accuracy) were calculated to assess the performance of the models between the whole and truncated datasets. The number of bulls used for the comparison were 619, 704, and 213, for ADG, WW, and YW, respectively.

To compute the genetic correlation between Env, the following equation was used for MT and single-step MT:

$$\mathrm{r_{gen}} = \frac{\sigma_{\mathrm{a,xz}}}{\sqrt{\sigma_{\mathrm{a,x}}^2 \times \sigma_{\mathrm{a,z}}^2}}$$

Where $\sigma_{\mathrm{a,xz}}$ is the genetic covariance between Env x and Env z, and $\sigma_{\mathrm{a,x}}^2$ and $\sigma_{\mathrm{a,z}}^2$ are the additive genetic variances. Genetic correlations were calculated among all the 5 Env.

Spearman's rank correlation was estimated between EBV and single-step genomic EBV across the five environmental conditions in the MT and single-step MT models to investigate a possible re-ranking among genotyped sires. Additionally, G × E and possible re-ranking between sires were evaluated by comparing EBV and single-step genomic EBV from univariate analyses without environmental effect and MT analyses. This study also investigated the impact of G × E interactions on animal growth in the Limousine population using eigenvalue decomposition of the genetic (co)variance matrix (Meyer, 2009). Eigenvalues indicate the amount of variation accounted for by each principal component, with larger eigenvalues indicating that the corresponding principal component captures more substantial variation. In G × E analysis, larger eigenvalues indicate significant patterns of variation across Env. The eigen decomposition of the additive genetic (co)variance matrix from the MT model was used to calculate the effective dimensionality of the genetic variation across the Env. The additive genetic (co)variance matrix summarises the genetic variation within several Env and the (co)variance among Env. To confirm the presence of G × E in growth traits, a comparison was made between the eigenvalue decomposition of additive genetic variance components from the MT and MT with permutations models for YW. The eigen-decomposition was performed using the *eigen* function of the R software.

6.3 Results

6.3.1 Characterisations of climatic conditions and detection of environmental parameters with great impact on growth

Average daily gain was 1.04 kg/d, WW was 247.9 kg, and YW was 354.5 kg. Standard deviation values were 0.28, 46.54, and 65.61 for ADG, WW, and YW, respectively. Environmental conditions were categorised into five environmental classes, with maximum RH class (%), average Temp class (°C), and average DP class (°C Td) provided the best model fit for ADG, WW, and YW, respectively. The Deviance Information Criterion values obtained for all the environmental covariates and traits assessed in Model 1 are provided in Supplementary Table S6.3. The distribution of the number of animals and environmental variables tested for ADG, WW, and YW in the five different Env are shown in Table 6.3.

6.3.2 Variance components

Estimates of variance components were obtained for all three traits with both MT and single-step MT. Heterogeneity in additive genetic variances ($\boldsymbol{\sigma_a^2}$) was observed across different environmental conditions (Table 6.4). For ADG, σ_a^2 estimates slightly decrease over maxRH levels, indicating lower magnitudes under extreme humidity conditions. The variance estimation for ADG was smaller when the conditions of RH were critical, and greater when the animals were exposed to the lowest RH levels (Table 6.4). Similar trends were observed for YW, with lower σ_a^2 estimates under stressful environmental conditions. (Table 6.4). Conversely, WW exhibited higher σ_a^2 estimates at extreme Temp. Detailed variance component estimates for ADG, WW, and YW across five environmental conditions are provided in Supplementary Table S3.4. Overall, the patterns and trends observed for variance components were consistent between MT and single-step MT analyses.

Table 6.4 Herd-year effect, heritability, and additive genetic variance of the three traits for Limousine cattle, obtained using a single trait, multiple trait, and single-step multiple trait models with environmental covariates having the lowest Deviance Information Criterion values, divided into five different environments. Values within parentheses represent Highest Posterior Density Interval (HPDI)

Trait	Single Trait BLUP			Single Trait single-step genomic BLUP			Environments	MT			single-step MT		
	h_i	h^2	σ_a^2	h_i	h^2	σ_a^2		h_i	h^2	σ_a^2	h_i	h^2	σ_a^2
ADG	$0.35^{(0.33; 0.36)}$	$0.08^{(0.07; 0.08)}$	$0.006^{(0.005; 0.006)}$	$0.34^{(0.33; 0.35)}$	$0.08^{(0.07; 0.08)}$	$0.006^{(0.005; 0.006)}$	Env 1	$0.47^{(0.46; 0.48)}$	$0.10^{(0.08; 0.12)}$	$0.008^{(0.007; 0.010)}$	$0.47^{(0.45; 0.49)}$	$0.10^{(0.08; 0.12)}$	$0.008^{(0.007; 0.009)}$
							Env 2	$0.47^{(0.46; 0.48)}$	$0.08^{(0.06; 0.10)}$	$0.006^{(0.005; 0.008)}$	$0.47^{(0.45; 0.49)}$	$0.08^{(0.06; 0.10)}$	$0.006^{(0.005; 0.008)}$
							Env 3	$0.47^{(0.46; 0.48)}$	$0.09^{(0.07; 0.11)}$	$0.007^{(0.006; 0.009)}$	$0.47^{(0.45; 0.49)}$	$0.09^{(0.07; 0.11)}$	$0.007^{(0.006; 0.009)}$
							Env 4	$0.46^{(0.45; 0.47)}$	$0.09^{(0.07; 0.11)}$	$0.007^{(0.006; 0.009)}$	$0.46^{(0.44; 0.48)}$	$0.10^{(0.08; 0.12)}$	$0.008^{(0.006; 0.009)}$
							Env 5	$0.44^{(0.43; 0.45)}$	$0.07^{(0.05; 0.09)}$	$0.005^{(0.004; 0.006)}$	$0.44^{(0.41; 0.46)}$	$0.08^{(0.06; 0.09)}$	$0.006^{(0.004; 0.007)}$
WW	$0.45^{(0.44; 0.46)}$	$0.12^{(0.11; 0.13)}$	$203.15^{(189.50; 219.10)}$	$0.43^{(0.41; 0.44)}$	$0.12^{(0.11; 0.13)}$	$197.86^{(183.90; 212.50)}$	Env 1	$0.48^{(0.47; 0.49)}$	$0.15^{(0.13; 0.18)}$	$249.94^{(213.40; 282.70)}$	$0.48^{(0.46; 0.50)}$	$0.15^{(0.13; 0.17)}$	$249.14^{(217.90; 282.50)}$
							Env 2	$0.52^{(0.52; 0.53)}$	$0.15^{(0.13; 0.17)}$	$260.47^{(226.40; 294.70)}$	$0.52^{(0.50; 0.54)}$	$0.15^{(0.13; 0.18)}$	$261.44^{(226.70; 300.40)}$
							Env 3	$0.53^{(0.52; 0.54)}$	$0.13^{(0.11; 0.15)}$	$222.49^{(189.70; 255.60)}$	$0.53^{(0.51; 0.55)}$	$0.13^{(0.10; 0.15)}$	$220.21^{(185.10; 259.10)}$
							Env 4	$0.54^{(0.53; 0.55)}$	$0.12^{(0.10\ 0.14)}$	$203.79^{(176.40; 244.20)}$	$0.54^{(0.52; 0.56)}$	$0.12^{(0.10; 0.14)}$	$201.80^{(172.10; 237.70)}$
							Env 5	$0.54^{(0.53; 0.55)}$	$0.14^{(0.12; 0.16)}$	$233.05^{(200.60; 265.10)}$	$0.54^{(0.52; 0.56)}$	$0.14^{(0.11; 0.16)}$	$228.10^{(190.20; 258.10)}$
YW	$0.58^{(0.56; 0.60)}$	$0.10^{(0.08; 0.12)}$	$335.61^{(276.50; 388.40)}$	$0.58^{(0.56; 0.59)}$	$0.10^{(0.08; 0.12)}$	$331.70^{(284.70; 389.90)}$	Env 1	$0.63^{(0.612; 0.64)}$	$0.09^{(0.06; 0.11)}$	$273.27^{(191.10; 353.00)}$	$0.62^{(0.60; 0.65)}$	$0.12^{(0.09; 0.15)}$	$362.15^{(269.30; 472.90)}$
							Env 2	$0.63^{(0.62; 0.64)}$	$0.15^{(0.11; 0.19)}$	$492.47^{(362.80; 634.00)}$	$0.63^{(0.60; 0.66)}$	$0.14^{(0.10; 0.18)}$	$453.30^{(336.80; 578.30)}$
							Env 3	$0.64^{(0.63; 0.65)}$	$0.14^{(0.10; 0.17)}$	$457.01^{(320.60; 571.20)}$	$0.64^{(0.61; 0.67)}$	$0.14^{(0.10; 0.17)}$	$469.17^{(353.30; 605.20)}$
							Env 4	$0.65^{(0.63; 0.66)}$	$0.14^{(0.10; 0.19)}$	$514.62^{(385.20; 657.40)}$	$0.64^{(0.61; 0.67)}$	$0.14^{(0.10; 0.19)}$	$509.13^{(369.90; 628.40)}$
							Env 5	$0.64^{(0.63; 0.65)}$	$0.13^{(0.10; 0.16)}$	$421.64^{(309.10; 517.70)}$	$0.65^{(0.62; 0.68)}$	$0.13^{(0.10; 0.16)}$	$423.15^{(324.80; 531.60)}$

Abbreviations: ADG = average daily gain; WW = weaning weight; YW = yearling weight; BLUP = best linear unbiased prediction; MT = multiple trait model; h_i= herd-year effect; h^2 = heritability; σ_a^2 = additive genetic variance.

6.3.3 Heritability and herd-year effect estimates

Table 6.4 presents heritability estimates for ADG, WW, and YW under different Env using MT and single-step MT models, along with univariate analysis results. These heritabilities were obtained in the five environments through MT models. Only the univariate model, used to mimic the national Limousine evaluation for growth traits, does not include the G × E effect. Heritability generally decreases for ADG and YW when the environmental conditions become less favourable, while remaining relatively stable for WW. Specifically, ADG showed low to moderate heritability across all RH Envs (0.07 to 0.10), while WW exhibited moderate heritability through Temp Envs (0.12 to 0.15), and YW showed low to moderate heritability across DP Envs (0.09 to 0.15). Lastly, univariate models showed heritability estimates of 0.08, 0.12,0.10 for ADG, WW, and YW, respectively.

The heritability estimates for ADG showed the highest value in the most comfortable climatic conditions (0.09 to 0.10) and slightly decreased with increasing RH levels (Table 6.4). Afterwards, YW heritability decreased in extreme DP Env but remained stable in intermediate conditions. Meanwhile WW heritability estimates remained constant across Temp Env, with a lower increase at extreme temperatures. The estimates and trends for heritability for ADG, WW, and YW remained consistent between MT and single-step MT analyses.

On the three traits, the herd-year effect (Table 6.4) was moderately low for ADG, modest for WW, and moderately high for YW in both univariate and MT analyses. Univariate models, on average, had a lower herd-year effect with estimates of 0.35, 0.45, and 0.58 for ADG, WW, and YW, respectively. Including environmental interaction for MT and single-step MT models, the herd-year effect increased, particularly for WW and YW, with the highest values observed in extreme conditions. Average daily gain exhibited a moderate herd-year effect (from 0.47 for Env 1 to 0.44 for Env 5), slightly decreasing over maxRH levels. A relatively higher herd-year effect was found for WW and YW. For WW, the herd-year effect increases over temperatures (0.48 Env1 to 0.54 Env 5). The highest herd-year effect was detected for YW, with values ranging between 0.62 and 0.65. For all three traits, MT and single-step MT models highlighted the same results.

6.3.4 Genetic correlations between environments and eigenvalue decompositions

The genetic correlations of additive genetic effects across different Env for the three traits, are summarised in Figure 6.2.

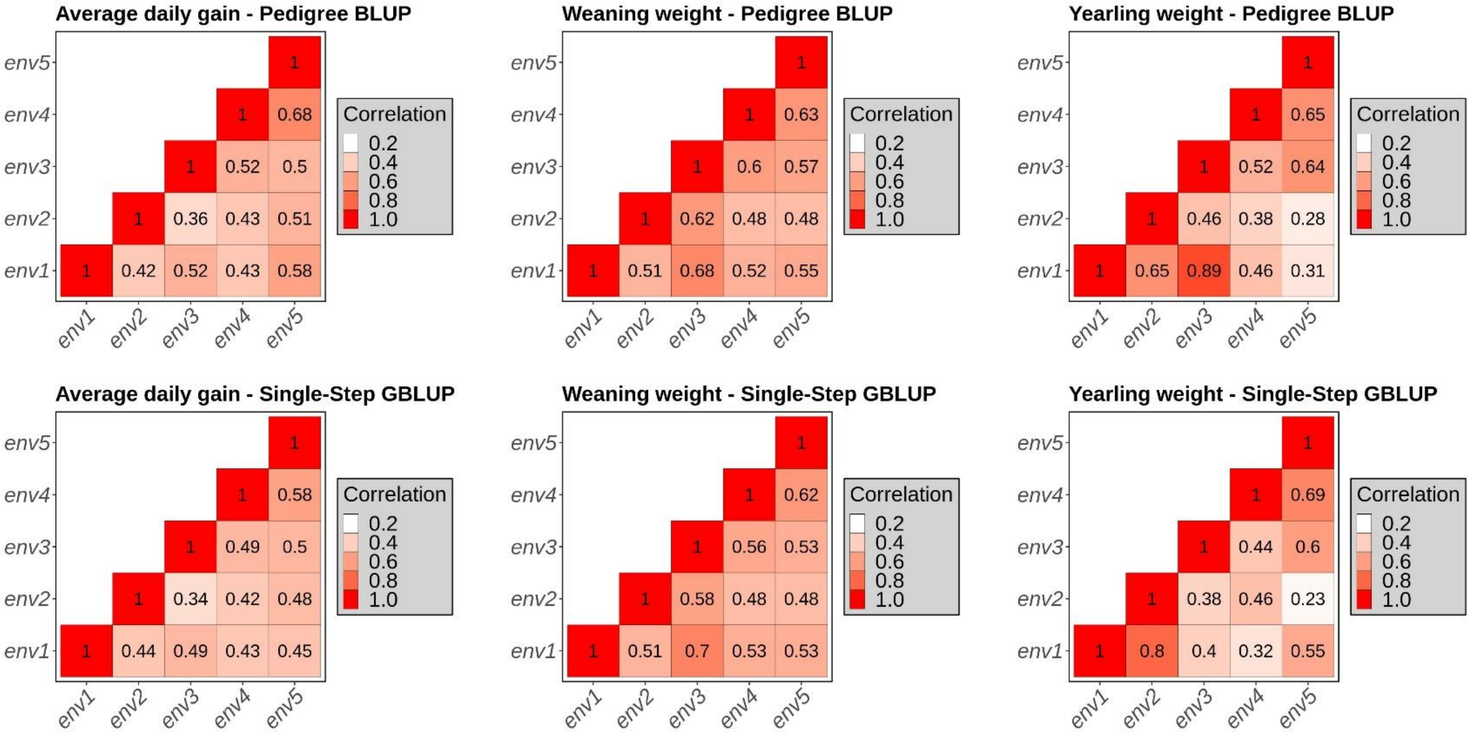


Figure 6.2 Genetic correlations between different traits for average daily gain (ADG, kg/d), weaning weight (WW, kg), and yearling weight (YW, kg) for Limousine cattle, using environmental covariates with lower Deviance Information Criterion values for multiple traits and single step multiple traits models, including genotype by environment interaction. Abbreviations: BLUP = best linear unbiased prediction; GBLUP = genomic best linear unbiased prediction.

As expected, the genetic correlation decreased gradually across Env (1 to 5) for WW and YW, with a weaker correlation between more distant Env. These correlations generally decreased as the environmental conditions became more divergent. For ADG, the genetic correlations showed the opposite trend, increasing from Env 1 to 5. Significant G × E interaction was detected for ADG, WW, and YW in the Limousine population, with a lower genetic correlation of 0.50 between the five Env. The lowest genetic correlation (0.28 ± 0.13) was detected for YW between Env 2 and 5, while the highest (0.89 ± 0.03) was between Env 1 and 3 of DP, suggesting limited G × E effects. Genetic correlations for ADG ranged from 0.36 ± 0.09 to 0.68 ± 0.06 across maxRH Env, indicating a moderate to remarkable G × E effect. Low to moderate interaction for WW was across Temp Env, with genetic correlations from 0.48 ± 0.07 to 0.68 ± 0.05, indicating a moderate G × E effect. For

YW genetic correlations between DP Envs ranged from 0.28 ± 0.13 to 0.89 ± 0.03, suggesting a negligible to remarkable G × E effect. Genomic correlations for single-step MT showed similar patterns to those of MT analyses. Figure 6.3 highlighted the presence of G × E on the three traits using eigenvalue decomposition of the additive genetic variance matrix. Each trait with 2 Env had an explained variance higher than 0.80, supporting G × E interactions. Supplementary Figure S6.1 confirms G × E for YW by comparing eigenvalues from MT models with 5 Env, and 10 random permutations. The permutations revealed substantial differences in the proportion of variance explained with one Env, showing that almost all variance was explained by the additive genetic (co)variance matrix.

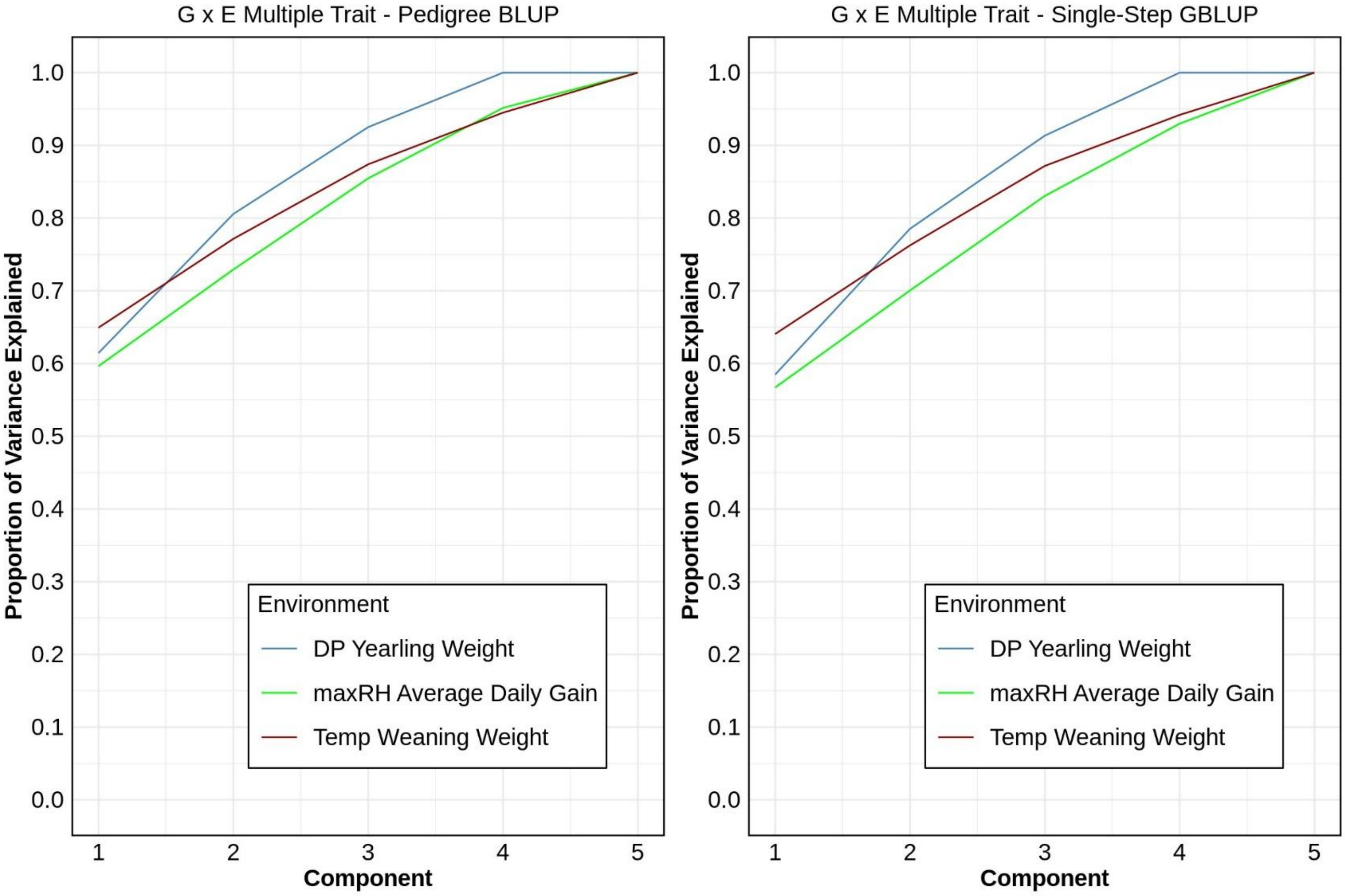


Figure 6.3 Eigenvalue decomposition of the (co)variance structure of the additive genetic variance matrix in beef cattle for average daily gain (ADG, kg/d), weaning weight (WW, kg), and yearling weight (YW, kg) using multiple traits and single-step multiple models traits including genotype by environment (G × E) interaction between five different environments. Abbreviations: maxRH = Maximum Relative Humidity; Temp = Temperature; DP = Dew Point; BLUP = best linear unbiased prediction; GBLUP = genomic best linear unbiased prediction.

6.3.5 Sire re-ranking across environments

The pattern of Spearman’s rank correlations between solutions and Env of the models are summarised as a heatmap in Figure 6.4. Rank correlations were calculated for genotyped sires using solutions from univariate, MT, and single-step MT models. Some combinations among Env for ADG, WW, and YW showed correlations lower than 0.80, indicating potential re-ranking of sires and G × E interactions among Env. For ADG, rank correlations across Env of maxRH on the MT model indicated moderate to strong re-ranking of sires, with some correlation below 0.70. The highest correlation was 0.92 between Env 4 and Env 5, while the lowest was 0.66 between Env 1 and Env 2. A similar pattern was observed for ADG in the single-step MT model, with a rank correlation lower than 0.70 between Env 2 and Env 3.

Similar rank correlations between MT and single-step MT models for WW were found across the 5 Temp Env. The highest Spearman’s rank correlation was equal to 0.92 between Env 1 and Env 3, and the lowest was 0.76 between Env 2 and Env 5. This suggests re-ranking of sires EBV and single-step genomic EBV could occur for WW under extreme Temp conditions. Strong rank correlations existed for YW across the closest DP Env for MT and single-step MT, with values ranging from 0.52 to 0.91. However, reranking could also occur among distant Env, especially under extreme conditions. Daughters of the same sire passing from cold to hot weather have different performances and ranked differently across Env for YW. Overall, the comparison of Spearman’s rank correlations was moderate between the univariate and MT models using pedigree and single-step analyses. Re-ranking of proven sires between models could occur for all growth traits, with the lowest correlations equal to 0.80, 0.82, and 0.80 for ADG, WW, and YW, respectively (Figure 6.4).

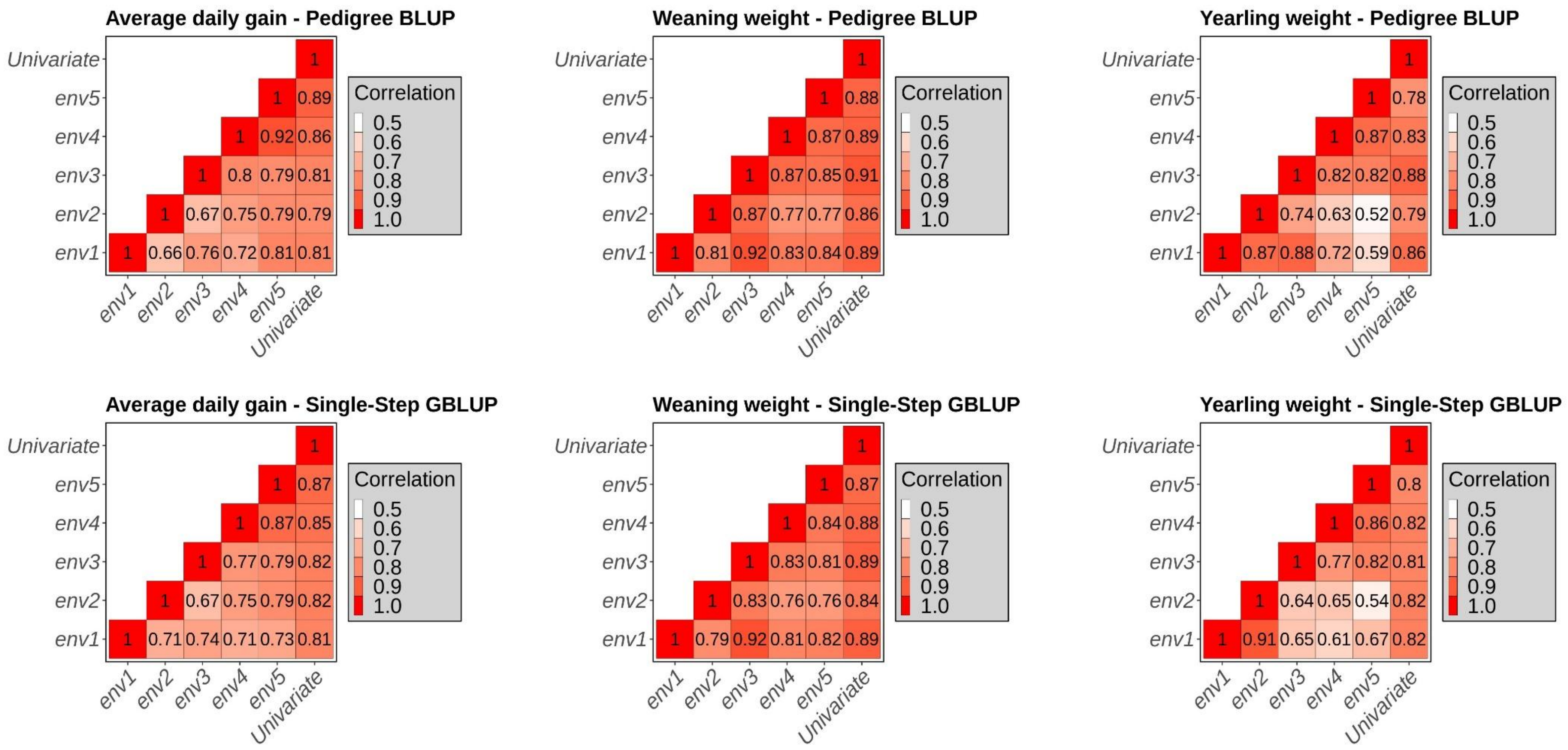


Figure 3.4 Heatmap of Spearman rank correlations between solutions for Limousine genotyped sires using univariate and multiple trait models including genotype by environment (G × E) interaction, for average daily gain (ADG, kg/d), weaning weight (WW, kg), and yearling weight (YW, kg). Abbreviations: BLUP = best linear unbiased prediction; GBLUP = genomic best linear unbiased prediction.

6.3.6 Comparison in accuracy of estimated breeding values and single-step genomic estimated breeding values for genotyped sires

EBV's Accuracy values were estimated for univariate, MT, single-step univariate, and single-step MT models. For ADG, the average accuracy of single-step genomic EBV (0.38) was higher than that of the EBV (0.34) (Figure 3.5). Considering WW, the average accuracy of (0.46) increased compared to EBV (0.41) (Figure 3.5). Similarly, for YW the average accuracy values increased with the inclusion of genomic information (0.35 for single-step genomic EBV compared to 0.32 for EBV) (Figure 3.5). In univariate analyses, single-step genomic EBV consistently showed higher average accuracies than EBV across all three traits. However, the standard deviation was greater than the difference in accuracy between the single-step and pedigree univariate analyses, making these differences not relevant.

For ADG, WW and YW, in the single-step MT analysis, single-step genomic EBV accuracies of the sires were slightly higher respect EBV accuracies across different Env (Figure 3.5). The accuracies increased on average by 0.02 to 0.04 points across Env. However, the standard deviation exceeded 0.1in all the Env, indicating that the differences in accuracy between single-step and pedigree models are not relevant. In all cases, including genomic information increased the sires' accuracies, potentially improving the response to selection for specific trait/environment combinations. For instance, in low environments, the accuracy for ADG increased from 0.21 to 0.25 (Env1) when using single-step genomic EBV compared to the univariate model. The accuracy of WW in intermediate environments increased from 0.29 to 0.33 (Env3). Similarly, for YW in extreme environments, the accuracy improved from 0.16 to 0.20 (Env5) with the inclusion of genomic information (Figure 6.5). The univariate model without the G × E effect showed larger accuracy compared to MT analyses. Results of linear regression cross-validation are summarized in the Supplementary Table S6.5.

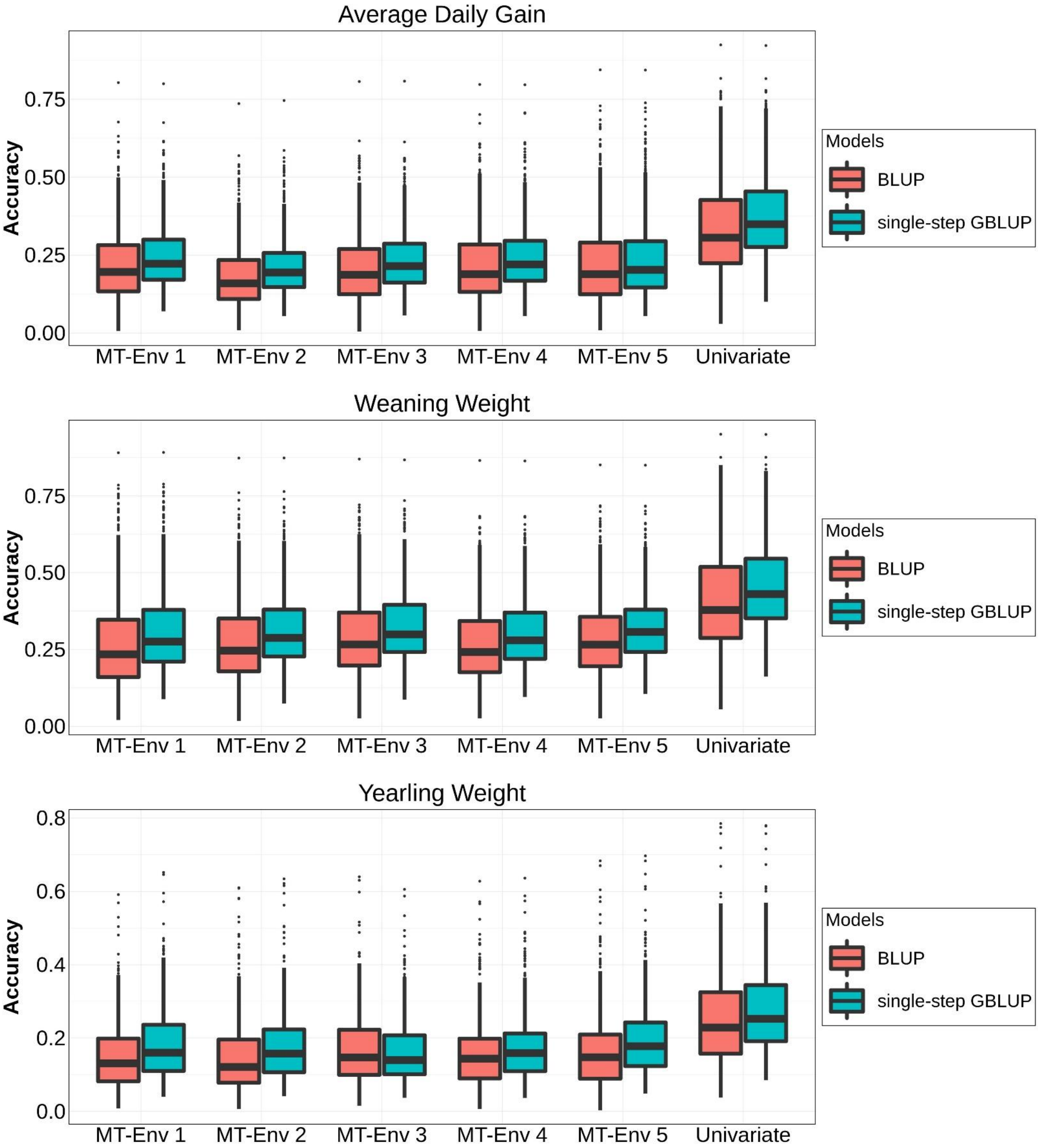


Figure 6.5 Comparison of the accuracy of Limousine genotyped sires between univariate models without genotype by environment (G × E) interaction, multiple traits (MT), and single-step multiple traits models for average daily gain (ADG, kg/d), weaning weight (WW, kg), and yearling weight (YW, kg). Abbreviations: BLUP = best linear unbiased prediction; GBLUP = genomic best linear unbiased prediction.

6.4 Discussion

Herds and animals included in this study were distributed around all the country, mostly in 7 regions (Lombardy, Sicily, Emilia-Romagna, Sardinia, Tuscany, Veneto, and Lazio), accounting for approximately 83-84% of the herds and 87-88% of the animals. The Limousine breed was introduced in Italy in 1985, and since then, the number of animals and herds has been consistently increasing. Because climate change is expected to modify the production environments in a shorter time (IPCC, 2007), understanding the genetic determination of heat tolerance in beef cattle is crucial. The main challenge in this study was identifying climatic parameters that significantly impact animal growth and quantifying G × E interactions on beef cattle. We explored G × E effects in the Limousine population using an MT model across 5 different environments, considering both pedigree (**A**), and genomic-pedigree (**H**) relationship matrices.

6.4.1 Selection of environmental covariates

For beef cattle, compensatory growth occurs after feed restriction or a physiologically stressed period and is generally expected to subsequently befall heat stress events (Mitlohner et al., 2001). Even though heat stress might temporarily reduce growth, cattle typically experience compensatory gain after heat stress subsides. In the beef sector heat stress could be confounded by seasonal changes since cattle graze outdoors (Paterson et al., 1995). Moreover, calculating proper heat stress and understanding which environmental covariates have a negative impact on growth is difficult. For these reasons, we included weaning and yearling weights because animals are exposed to a wide range of different climatic conditions during these periods.

Because of the cumulative nature of WW and YW and the type of farm management, heat load functions are mostly used in beef cattle. This approach has also been applied to dairy cattle (Ravagnolo and Misztal, 2000; Aguilar et al., 2009; Carrara et al., 2021) and swine (Zumbach et al., 2008a,b; Fragomeni et al., 2016a). Also, THI has been widely used in animal breeding, particularly for dairy cattle (Biffani et al., 2016; Nguyen et al., 2016; Ansari-Mahyari et al., 2019). Some studies have assessed different variables, such as temperature or humidity, to explore their impact on reproductive and productive traits in swine (Tiezzi et al., 2020; Usala et al., 2021; Freitas et al., 2021). For these reasons, one challenge was properly quantifying heat stress in beef cattle and identifying the meteorological parameters that most impact growth We evaluated 14 Env variables based on Temperature, RH, and DP to explore the impact of each of the evaluated traits. The most relevant environmental covariate for each trait was selected using Deviance Information Criterion applied to the baseline model (model 1). Lower values indicate a better fitting.

The selection of appropriate environmental parameters for assessing heat stress is crucial due to their significant physiological impacts on cattle. Elevated temperatures can cause thermal stress, leading to decreased feed intake, altered metabolism, and reduced growth rates. Chronic exposure to high temperatures can also impair immune function and increase disease susceptibility affecting overall health, reproduction, and productivity. Furthermore, cattle cannot lose heat efficiently through evaporation when RH is high because the air is almost saturated with water vapour. This inefficiency in heat loss occurs because high RH deters evaporative cooling through the skin (Baena et al., 2019). Combined with extreme temperatures, high RH can exacerbate heat stress in cattle, leading to further declines in performance and overall health (Baena et al., 2019). Understanding these physiological implications is essential for developing effective breeding and management strategies to improve cattle heat tolerance. By selecting more resilient animals for heat stress, the negative impacts of climate change on cattle productivity and welfare can be mitigated.

6.4.2 Variance components estimation

Generally, the values were similar between the BLUP and single-step genomic BLUP models, with a comparable pattern for variance components (Table 6.4). Moderate additive genetic estimates for ADG, WW, and YW suggest that heat stress will affect animal growth. A decrease in σ_a^2 was observed at extreme Env for ADG and YW, respectively, while σ_a^2 was low in most comfortable Env conditions for WW. In extreme environments, genetic variance is often observed to be lower due to stress effects, strong selective pressure, and G × E interaction. Also, fewer animals might perform well, leading to a reduced range of genetic variance. Under these conditions, the ability of individuals to express their genetic potential is diminished, leading to a reduction of genetic variance.

These results indicate the potential for different selection responses for these traits depending on maxRH, Temp, and DP under which the animals are raised. Variance components estimate can be used to evaluate the presence of G × E interactions; a variability suggests that the same genotype may perform differently based on environmental conditions, highlighting the importance of considering G × E interactions in breeding programs to improve animal performance across diverse Env. Moderate estimates from this study indicate significant genetic variability for traits like ADG, WW, and YW. This variability means selective breeding could be effective, depending on the specific environmental conditions the animals were subjected to.

6.4.3 Change in heritability and herd year effect across multi-environment analyses

Analyses using BLUP and single-step genomic BLUP showed similar heritability patterns. Across five different Env σ_a^2 varied, resulting in differences in heritability in the Limousine population (table 6.4). The heritability estimates suggest varying degrees of genetic control on growth traits under different environmental conditions, particularly in heat tolerance. Heritability estimates for univariate analyses were generally lower than those Bradford et al. (2016) reported for WW and YW in Angus cattle. Cardoso et al. (2011) found similar heritability (0.08 to 0.23) when including Env effects in a two-step model for post-weaning weight standardised at 345 days in Hereford cattle. Wiliams et al. (2012) observed changes in heritability of WW (0.26 and 0.28) and post-weaning weight (0.26 and 0.19) across different altitudes in Angus cattle using a multivariate analysis. Similarly, using a random regression model, Santana et al. (2016) and Bradford et al. (2016) reported higher heritability for WW across the Env gradient Oliveira et al. (2018) found higher heritability for YW compared to our study.

For ADG in different Env of maxRH, Cardoso and Tempelman (2012) found that the heritability increased in more favourable conditions for postweaning weight gain in Angus cattle, consistent with our finding. Likewise, for YW in Angus cattle, authors noted a reduction in direct heritability as environmental stress increased, using a random regression model (Bradford et al., 2016). Pegolo et al. (2011) observed an increase of the heritability in Nellore cattle in more favourable Env for weight at 450 days. On the contrary, using a reaction norm model, Santana et al., (2013, 2016) reported that WW had higher direct heritability in more favourable conditions in tropical composite cattle, Brangus, and Nellore. The differences in our results compared to other studies may be attributed to several factors. These include variations in the models used for heritability estimation and GxE analysis, the distinct genetic backgrounds and adaptations of the cattle breeds studied, fluctuations in environmental conditions such as climate and management practices, and differences in the structure and quality of the datasets. These disparities underscore the importance of considering breed-specific and environment-specific contexts in genetic studies.

Animals with moderate additive genetic estimates for growth traits may exhibit different physiological responses to environmental stressors. Understanding these responses can help in managing animal welfare more effectively. For instance, animals that maintain better growth rates under heat stress might have different metabolic or hormonal profiles compared to those that do not (Mujumdar et al., 2020; Smith et al., 2022). The herd-year effect (Table 6.4) indicates that management practices moderately impact growth traits. A higher herd-year effect in Env with stressful conditions slow down genetic progress. This moderate herd effect also contributes to G × E interactions, reflecting varying management practices and environmental conditions across different herds and years. This the expression of genetic potential and hinders the identification of superior genotypes.

6.4.4 Genetic correlation, eigenvalues, and genotype by environment interactions throughout the environment

Genetic correlations followed a similar pattern when using both MT and single-step MT models (Figure 6.2). A genetic correlation below 0.80 indicates evidence of G × E interactions. Genetic correlations did not decrease gradually across the 5 Env. In this study, the genetic correlation for the three traits reached values below 0.80, confirming the existence of G × E on Limousine growth traits under different environmental conditions. Using a MT approach, Carvalheiro et. al. (2019) observed G × E interactions for post-weaning weight gain, with a genetic correlation of 0.79 between harsh and favourable Env. Similar findings for WW were reported by Santana et al. (2016) in Brangus and Tropical Composite population, showing lower genetic correlations in extreme environments. Bradford et al. (2016) found genetic correlations below 0.50 for large heat load differences in Angus cattle, indicating that the Env gradient was not the same trait. Conversely, for YW no significant Env differences were found in the Angus population (Bradford et al., 2016). In swine, Zumbach et al. (2008) reported a genetic correlation of 0.42 for carcass weights between hot and cold months using two-trait analyses, and 0.02 when applying a random regression model for more distant heat load values. Usala et al. (2021) found a genetic correlation of 0.20 for average daily gain (ADG) between the 5th and 95th percentiles of relative humidity (RH).

Eigenvalue decomposition of the genetic additive matrix confirmed G × E interactions in the Italian Limousine population (Figure 6.3). For maxRH, Temp, and DP the proportion of variance explained by the first three eigenvalues was 0.86, 0.87, and 0.93 in MT and 0.83, 0.87, and 0.91 in single-step MT for ADG, WW, and YW, respectively. The remaining two eigenvalues accounted for less than 0.20 of the variances explained by the Env. The first eigenvalue absorbed less than 70% of the total variance, and the sum with the second seldom reached 80%. Eigenvalue decomposition on the permuted data (MT with permutations) did not show any G × E (Supplementary Fig. S4.1), supporting the findings from the actual data.

These estimates should interest breeders because the best-performing animals in one Env may not necessarily be the best in another, indicating different adaptability. These results could allow the development of targeted strategies to optimise animal performance and improve breeding and management strategies tailored to different environmental contexts. Thus, ADG, WW, and YW are moderately to largely affected by G × E, indicating that growth in the Limousine population in Italy varies across different Env of maxRH, Temp, and DP showing a potential G × E effect. Furthermore, we observed that the genetic correlations between the Env were often in different directions. This suggests varying relationships between average performance and environmental sensitivity of growth traits. Physiologically, this indicates that animals exhibiting high average performance might not necessarily show high environmental resilience. Different genetic mechanisms may control performance and sensitivity to environmental changes, affecting how traits manifest under varying conditions. Understanding these genetic correlations is crucial to developing breeding strategies that enhance performance and adaptability to diverse Env.

6.4.5 Reranking and identification of heat-tolerant sires

Considering the limited number of genotyped sires, re-ranking using Spearman rank correlation is expected under different Env, particularly when comparing animals in optimal versus extreme conditions. Numerous studies have documented changes in sire re-ranking in dairy cattle (Bernabucci et al., 2014; Carrara et al., 2021) and beef cattle for WW (Bradford et al., 2016). However, Bradford et al., 2016 reported rank correlation greater than 0.80 for YW, suggesting a low re-ranking of sire across environmental conditions. In swine, a weak rank correlation (0.18 to 0.54) for carcass traits under high and low Env indicates a higher physiological response to heat stress than beef cattle (Zumbach et al., 2008b).

In our study, many rank correlations for genotypes were below 0.80 between Env of maxRH, Temp, and DP. The findings proved that sire re-ranking across affects genetic gain. Environmental conditions influence an individual's breeding value, with sires excelling in favourable conditions potentially performing poorly and less resilient in extreme conditions. Selective breeding of sire that exhibit strong performance across diverse Env is expected to enhance overall productivity, health, and welfare, thereby increasing the profitability of cattle farms. Physiologically, sires that maintain high ranks in varying environmental conditions demonstrate resilience and adaptability, which are crucial for growth traits under environmental stressors.

6.4.6 Comparison in accuracy of estimated breeding values and single-step genomic estimated breeding values for genotyped sires

Genomic selection has better opportunities to exploit G × E interactions than traditional breeding by achieving high accuracy in unfavourable Env (Mulder, 2016). However, accuracies in extreme Env tend to be lower, and limited information is available for breeding value in these conditions (Mulder, 2016). Our study compared accuracies between single-step and pedigree-based analysis of genotyped sires across different Env. Results showed a slight improvement in accuracies for single-step MT compared to MT models for ADG, WW, and YW (Figure 6.5). Nevertheless, for all the three traits, the standard deviation exceeded the differences in accuracies between single-step and pedigree models, indicating non-significant differences. In addition, when univariate pedigree BLUP and single-step genomic BLUP were compared, accuracies increased with the inclusion of the H matrix. Conversely, single-step MT accuracies were lower compared to univariate pedigree BLUP without accounting for G × E. Accuracies using an H matrix were lower, probably because the number of genotyped animals was not large enough and using an MT approach, genotyped animals were distributed among the 5 Env.

These findings suggest that genomic selection might accelerate genetic gain for heat tolerance. Single-step genomic BLUP improved response to selection across different environments more than pedigree analyses for growth traits. The results imply that as more animals are genotyped, the accuracies of single-step analyses for heat tolerance could further improve. Mulder (2016) highlights that genomic selection enhances resilience compared to traditional breeding schemes. This is emphasised when the reference population is representative of diverse environmental conditions.

6.5 Conclusions

These findings provide the basis for the possible genetic or genomic selection development for heat tolerance in the Limousine cattle population. Heritability estimates demonstrate the tolerance to critical environmental conditions under genetic control. This suggests the feasibility of selecting individuals that are particularly tolerant to specific environmental and management conditions through direct genetic and genomic selection of growth traits. Furthermore, as evidence of G × E interactions, substantial heterogeneity was observed in the variance components for ADG, WW, and YW across different environmental conditions. A possible re-ranking in opposite Env of animals and sires was verified. Indeed, the best-performing genotypes in the less stressful Env may not excel under more challenging conditions These results underscore that selecting more tolerant animals can optimise the selection programs, showing that genomic selection could enhance the accuracy of breeding values across diverse Env. Despite these insights, further research is needed to better understand the genetic and genomic background of heat tolerance and the genetic mechanism of adaptability in beef cattle. This aspect could be explored by assessing future analyses by implementing a random regression model. When the number of environmental covariates exceeds one, multidimensional environmental data can be incorporated using Reproducing Kernel Hilbert Spaces regression. Exploring the relationship between genetics and environmental factors can provide relevant insights into heat tolerance and adaptability in beef cattle breeding programs.

6.6 Ethics approval

The data used in this study were part of a pre-existing dataset, so ethics approval was not needed.

6.7 Data and model availability statement

The datasets generated and/or analysed during the current study are not publicly available due to being owned by a third party, ANACLI, Associazione Nazionale degli Allevatori delle razze bovine Charolaise e Limousine Italiane (http://www.anacli.it/) but are available from the corresponding author on reasonable request.

6.8 Declaration of interest

None.

6.9 Acknowledgements

We acknowledge Associazione Nazionale Allevatori delle razze bovine Charolaise e Limousine (ANACLI) for providing the data.

6.10 Financial support statement

This work was financially supported by Associazione Nazionale degli Allevatori delle razze bovine Charolaise e Limousine Italiane (Cup: J89H18000010005, URL: http://www.anacli.it/ibeef). Grant was received by RB. The funders had no role in study design and analysis, decision to publish, or manuscript preparation.

6.11 Declaration of generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence assisted technologies in the writing process.

6.12 References

Aguilar, I., Misztal, I., Tsuruta, S., 2009. Genetic components of heat stress for dairy cattle with multiple lactations. Journal of Dairy Science 92, 5702-5711. https://doi.org/10.3168/jds.2008-1928.

Aguilar, I., Misztal, I., Johnson, D.L, Legarra, A., Tsuruta, S., 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. Journal of Dairy Science 93, 743–752. https://doi.org/10.3168/jds.2009-2730.

Aguilar, I., Fernandez, E.N., Blasco, A., Ravagnolo, O., and Legarra, A., 2020. Effects of Ignoring Inbreeding in Model-based Accuracy for BLUP and SSGBLUP. Journal Animal Breeding Genetics 137, 356–364. https://doi.org/10.1111/jbg.12470.

Ansari-Mahyari, S., Ojali, M.R., Forutan, M., Riasi, A., Brito, L.F., 2019. Investigating the genetic architecture of conception and non-return rates in Holstein cattle under heat stress conditions. Tropical Animal Health and Production 51, 1847-1853. https://doi.org/10.1007/s11250-019-01875-5.

Baccari Jr, F., Johnson, H.D., Hahn, G.L., 1983. Environmental heat effects on growth, plasma T3, and postheat compensatory effects on Holstein calves. Proceedings of the Society for Experimental Biology and Medicine 173, 312-318. https://doi.org/10.3181/00379727-173-41648.

Baena, M.M., Costa, A.C., Vieira, G.R., Rocha, R.D.F.B., Ribeiro, A.R.B., Ibelli, A.M.G., Meirelles, S.L.C., 2019. Heat tolerance responses in a Bos taurus cattle herd raised in a Brazilian climate. Journal of Thermal Biology 81, 162-169. https://doi.org/10.1016/j.jtherbio.2019.02.017.

Bates, D.M., 2010. lme4: Mixed-effects modeling with R. Retrieved on 17 February 2010 from https://cran.r-project.org/web/packages/lme4/vignettes/lmer.pdf.

Bernabucci, U., Biffani, S., Buggiotti, L., Vitali, A., Lacetera, N., Nardone, A., 2014. The effects of heat stress in Italian Holstein dairy cattle. Journal of Dairy Science 97, 471-486. https://doi.org/10.3168/jds.2013-6611.

Biffani, S., Bernabucci, U., Vitali, A., Lacetera, N., Nardone, A., 2016. Short communication: Effect of heat stress on nonreturn rate of Italian Holstein cows. Journal of Dairy Science 99, 5837-43. https://doi.org/10.3168/jds.2015-10491.

Bouquet, A., Venot, E., Laloë, D., Forabosco, F., Fogh, A., Pabiou, T., Moore, K., Eriksson, J.A., Renand, G., Phocas, F., 2011. Genetic structure of the European Charolais and Limousin cattle metapopulations using pedigree analyses. Journal of Animal Science 89, 1719–1730. https://doi.org/10.2527/jas.2010-3469.

Bradford, H.L., Fragomeni, B.O., Bertrand, J.K., Lourenco, D.A.L., Misztal, I., 2016. Genetic evaluations for growth heat tolerance in Angus cattle. Journal of Animal Science 94, 4143-4150. https://doi.org/10.2527/jas.2016-0707.

Cardoso, L.L., Braccini Neto, J., Cardoso, F.F., Cobuci, J.A., Biassus, I.D.O., Barcellos, J.O.J., 2011. Hierarchical Bayesian models for genotype × environment estimates in post-weaning gain of Hereford bovine via reaction norms. Revista Brasileira de Zootecnia 40, 294-300. https://doi.org/10.1590/S1516-35982011000200009.

Cardoso, F.F., Tempelman, R.J., 2012. Linear reaction norm models for genetic merit prediction of Angus cattle under genotype by environment interaction. Journal of Animal Science 90, 2130-2141. https://doi.org/10.2527/jas.2011-4333.

Carrara, E.R., Petrini, J., Salvian, M., de Oliveira, H.R., Rovadoscki, G.A., Iung, L. H.D.S., Miquilini, M., Machado, P.F., M., Mourão, G.B., 2021. Genetic parameters for milk yield and quality traits of Brazilian Holstein cows as a function of temperature and humidity index. Journal of Animal Breeding and Genetics 138, 643-654. https://doi.org/10.1111/jbg.12636.

Carvalheiro, R., Costilla, R., Neves, H.H., Albuquerque, L.G., Moore, S., Hayes, B.J., 2019. Unraveling genetic sensitivity of beef cattle to environmental variation under tropical conditions. Genetics Selection Evolution 51, 1-14. https://doi.org/10.1186/s12711-019-0470-x.

Chang C.C., Chow C.C., Tellier L.C., Vattikuti S., Purcell S.M., Lee J.J., 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience 4, 7. https://doi.org/10.1186/s13742-014-0038-1.

Fragomeni, B.O., Lourenco, D.A.L., Tsuruta, S., Andonov, S., Gray, K., Huang, Y., 2016a. Modelling response to heat stress in pigs from nucleus and commercial farms in different locations in the United States. Journal of Animal Science 94, 4789–4798. https://doi.org/10.2527/jas.2016-0536.

Freitas, P.H.F., Johnson, J.S., Chen, S., Oliveira, H.R., Tiezzi, F., Lázaro, S.F., Huang Y., Gu, Y., Schinckel A.P., Brito, L.F., 2021. Definition of environmental variables and critical periods to evaluate heat tolerance in Large White pigs based on single-step genomic reaction norms. Frontiers in Genetics 12, 717409. https://doi.org/10.3389/fgene.2021.717409.

Hayes, B.J., Daetwyler, H.D., Goddard, M.E., 2016. Models for genome × environment interaction: examples in livestock. Crop Science 56, 2251-2259. https://doi.org/10.2135/cropsci2015.07.0451.

IPCC, 2007. Climate Change 2007: The Physical Basis. Contribution of Working Group I to the Fourth Assessment of the Intergovernmental Panel on Climate Change. IPCC Secretariat, Geneva, Switzerland.

Isik, F., Holland, J., Maltecca, C., 2017. Genetic data analysis for plant and animal breeding (Vol. 400). Cham, Switzerland: Springer International Publishing. https://doi.org/10.1007/978-3-319-55177-7.

Legarra, A., Christensen, O.F., Aguilar, I., Misztal, I., 2014. Single step, a general approach for genomic selection. Livestock Science 166, 54–65. https://doi.org/10.1016/j.livsci.2014.04.029.

Legarra, A., Reverter, A., 2018. Semi-parametric estimates of population accuracy and bias of predictions of breeding values and future phenotypes using the LR method. Genetics Selection Evolution, 50, 53. https://doi.org/10.1186/s12711-018-0426-6

Mader, T.L., Davis, M.S., 2004. Effect of management strategies on reducing heat stress of feedlot cattle: feed and water intake. Journal of Animal Science 82, 3077–3087. https://doi.org/10.2527/2004.82103077x.

Majumdar, A., Burch, K.S., Haldar, T., Sankararaman, S., Pasaniuc, B., Gauderman, W.J., Witte, J.S., 2020. A two-step approach to testing overall effect of gene–environment interaction for multiple phenotypes. Bioinformatics 36, 5640-5648. https://doi.org/10.1093/bioinformatics/btaa1083.

Meyer, K., 2009. Factor-analytic models for genotype × environment type problems and structured covariance matrices. Genetic Selection Evolution 41, 21. https://doi.org/10.1186/1297-9686-41-21.

Misztal, I., Tsuruta, S., Lourenco, D.A.L., Aguilar, I., Legarra, A., Vitezica, Z., 2014. Manual for BLUPF90 family of programs. Retrieved on 12 May 2014 from http://nce.ads.uga.edu/wiki/lib/exe/fetch.php?media=blupf90_.

Mitlöhner, F.M., Morrow, J.L., Dailey, J.W., Wilson, S.C., Galyean, M.L., Miller, M.F., McGlone, J.J., 2001. Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle. Journal of Animal Science 79, 2327-2335. https://doi.org/10.2527/2001.7992327x.

Mulder, H.A., Bijma, P., 2007. Effects of genotype x environment interaction on genetic gain in breeding programs. Journal of Animal Science 83, 49–61. https://doi: 10.2527/2005.83149x.

Nardone, A., Ronchi, B., Lacetera, N., Bernabucci, U., 2006. Climatic effects on productive traits in livestock. Veterinary Research Communications 30, 75. DOI: 10.1007/s11259-006-0016-x.

Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M.S., Bernabucci, U., 2010. Effects of climate changes on animal production and sustainability of livestock systems. Livestock Science 130, 157-69. https://doi.org/10.1016/j.livsci.2010.02.011.

Nguyen, T.T., Bowman, P.J., Haile-Mariam, M., Pryce, J.E., Hayes, B.J., 2016. Genomic selection for tolerance to heat stress in Australian dairy cattle. Journal of Dairy Science 99, 2849-2862. https://doi.org/10.3168/jds.2015-9685.

Paterson, J., Forcherio, C., Larson, B., Samford, M., Kerley, M., 1995. The effects of fescue toxicosis on beef cattle productivity. Journal of Animal Science 73, 889-898. https://doi.org/10.2527/1995.733889x.

Pegolo, N.T., Albuquerque, L.G.D., Lôbo, R.B., de Oliveira, H.N., 2011. Effects of sex and age on genotype × environment interaction for beef cattle body weight studied using reaction norm models. Journal of Animal Science 89, 3410-3425. https://doi.org/10.2527/jas.2010-3520.

Plummer, M., Best, N., Cowles, K., Vines, K., 2006. CODA: Convergence Diagnosis and Output Analysis for MCMC. R News 6, 7-11. https://CRAN.R-project.org/doc/ Rnews/.

Ravagnolo, O., Misztal, I., 2000. Genetic component of heat stress in dairy cattle, parameter estimation. Journal of Dairy Science 83, 2126–2130. https://doi.org/10.3168/jds.S0022-0302(00)75095-8.

Ravagnolo, O., Misztal, I., 2002. Effect of heat stress on nonreturn rate in Holstein cows: Genetic analyses. Journal of Dairy Science 85, 3092-3100. https://doi.org/10.3168/jds.S0022-0302(02)74396-8.

R Core Team. 2020. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Renaudeau, D., Collin, A., Yahav, S., de Basilio, V., Gourdine, J.L., Collier, R.J., 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal 6, 707:728. https://doi.org/10.1017/S1751731111002448.

Rockett, P.L., Campos, I.L., Baes, C.F., Tulpan, D., Miglior, F., Schenkel, F.S., 2023. Phenotypic analysis of heat stress in Holsteins using test-day production records and NASA POWER meteorological data. Journal of Dairy Science 106, 1142-1158. https://doi.org/10.3168/jds.2022-22370.

Sargolzaei, M., Chesnais, J.P., Schenkel, F.S., 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics 15, 478. https://doi.org/10.1186/1471-2164-15-478.

Santana, M.L., Eler, J.P., Cardoso, F.F., Albuquerque, L.G.D., Ferraz, J.B.S., 2013. Phenotypic plasticity of composite beef cattle performance using reaction norms model with unknown covariate. Animal 7, 202-210. https://doi.org/10.1017/S1751731112001711.

Santana Jr, M.L., Bignardi, A.B., Eler, J.P., Ferraz, J.B.S., 2016. Genetic variation of the weaning weight of beef cattle as a function of accumulated heat stress. Journal of Animal Breeding and Genetics 133, 92-104. https://doi.org/10.1111/jbg.12169.

Smith, J.L., Wilson, M.L., Nilson, S.M., Rowan, T.N., Schnabel, R.D., Decker, J.E., Seabury, C.M., 2022. Genome-wide association and genotype by environment interactions for growth traits in US Red Angus cattle. BMC genomics, 23, 517. https://doi.org/10.1186/s12864-022-08667-6.

Snow, G., 2020. _TeachingDemos: Demonstrations for Teaching and Learning_. R package version 2.12. Retrieved on 7 April 2020 from https://CRAN.R-project.org/package=TeachingDemos.

St.-Pierre, N.R., Cobanov, B., Schnitkey, G., 2003. Economic losses from heat stress by US livestock industries. Journal of Dairy Science 86, E52–E77. https://doi.org/10.3168/jds.S0022-0302(03)74040-5.

Oliveira, D.P., Lourenco, D.A.L., Tsuruta, S., Misztal, I., Santos, D.J.A., de Araújo Neto, F.R., Aspilcueta-Borquis R.R., Baldi F., Carvalheiro R., de Camargo G.M.F., Albuquerque L.G., Tonhati, H. 2018., Reaction norm for yearling weight in beef cattle using single-step genomic evaluation. Journal of Animal Science 96, 27-34. https://doi.org/10.1093/jas/skx006.

Tiezzi, F., Brito, L.F., Howard, J., Huang, Y.J., Gray, K., Schwab, C., Fix, J., Maltecca, C., 2020. Genomics of heat tolerance in reproductive performance investigated in four independent maternal lines of pigs. Frontiers in Genetics 11, 629. https://doi.org/10.3389/fgene.2020.00629.

Tiezzi, F., Maltecca, C., 2022. Genotype by Environment Interactions in Livestock Farming. In Animal Breeding and Genetics. Encyclopedia of Sustainability Science and Technology Series (ed. Spangler, M.L.). Springer, New York, NY, USA, pp. 77-97. https://doi.org/10.1007/978-1-0716-2460-9_1115.

Toghiani, S., Roberts, A., Rekaya, R., 2020. Impact of cold stress on birth and weaning weight in a composite beef cattle breed. Livestock Science 236, 104053. https://doi.org/10.1016/j.livsci.2020.104053.

Usala, M., Macciotta, N.P.P., Bergamaschi, M., Maltecca, C., Fix, J., Schwab, C., Shull, C., Tiezzi, F., 2021. Genetic parameters for tolerance to heat stress in crossbred swine carcass traits. Frontiers in Genetics 11, 612815. https://doi.org/10.3389/fgene.2020.612815.

VanRaden, P.M., 2008. Efficient Methods to Compute Genomic Predictions. Journal Dairy Science. 91, 4414–4423. https://doi.org/10.3168/jds.2007-0980.

Williams, J.L., Bertrand, J.K., Misztal, I., Łukaszewicz, M., 2012. Genotype by environment interaction for growth due to altitude in United States Angus cattle. Journal of Animal Science 90, 2152-2158. https://doi.org/10.2527/jas.2011-4365.

Zumbach, B., Misztal, I., Tsuruta, S., Sanchez, J.P., Azain, M., Herring, W., 2008a. Genetic components of heat stress in finishing pigs: development of a heat load function. Journal of Animal Science 86, 2082–2088. https://doi.org/10.2527/jas.2007-0523.

Zumbach, B., Misztal, I., Tsuruta, S., Sanchez, J.P., Azain, M., Herring, W., Holl, J., Long, T., Culbertson, M., 2008b. Genetic components of heat stress in finishing pigs: Parameter estimation. Journal of Animal Science 86, 2076–2081. https://doi.org/10.2527/jas.2007-0282.

6.13 Supplementary Material

6.13.1 Supplementary Table

Supplementary Table S6.1 Percentage (%) distribution of herd and numbers of Limousine cattle population for the three growth traits across the seven most representative Italian regions.

Regions	Location	ADG		WW		YW	
		% of total herds	% of total animals	% of total herds	% of total animals	% of total herds	% of total animals
Lombardy	North centre	5.10	20.10	4.90	18.40	5.10	18.90
Sicily	South	24.40	16.80	25.80	18.20	26.90	24.20
Emilia- Romagna	North centre	12.40	16.80	11.80	14.90	12.20	11.70
Sardinia	Southwest	19.90	12.15	19.00	12.20	19.10	12.10
Tuscany	Centre	8.20	11.50	8.30	12.50	8.00	9.70
Veneto	Northeast	6.60	6.00	6.80	5.40	7.10	6.50
Lazio	Centre	6.30	4.25	6.40	6.00	6.00	4.60
	TOTAL %	82.90	87.60	83.00	87.60	84.40	87.70

Abbreviations: ADG = average daily gain; WW = weaning weight; YW = yearling weight.

Supplementary Table S6.2 Description of the environmental parameters analysed in this study for the evaluation of their impact on beef cattle.

Environmental variables	Description
Temperature (T, °C)	average daily mean
Relative Humidity (RH, %)	average daily mean
Dew Point (DP, °C Td)	average daily mean
Temperature Humidity Index (THI)	average daily mean
Heat load (HL)	sum of the THI over the days prior weight date and between the two weights for ADG
Minimum Temperature (minT, °C)	average daily mean first quantile of the normal distribution
Maximum Temperature (maxT, °C)	average daily mean first quantile of the normal distribution
Minimum Relative Humidity (minRH, %)	average daily mean first quantile of the normal distribution
Maximum Relative Humidity (maxRH, %)	average daily mean first quantile of the normal distribution
Minimum Dew Point (minDP, °C Td).	average daily mean first quantile of the normal distribution
Maximum Dew Point (maxDP, °C Td)	average daily mean first quantile of the normal distribution
Minimum Temperature Humidity Index (minTHI)	average daily mean first quantile of the normal distribution
Maximum Temperature Humidity Index (maxTHI)	average daily mean first quantile of the normal distribution
Class Temperature (T_CLASS, °C)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Relative Humidity (RH_CLASS, %)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Dew Point (DP_CLASS, °C Td)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Temperature Humidity Index (THI_CLASS)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Heat Load (HL_CLASS)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Minimum Temperature (minT_CLASS, °C)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Maximum Temperature (maxT_CLASS, °C)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Minimum Relative Humidity (minRH_CLASS, %)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Maximum Relative Humidity (maxRH, %)	five classes using the first four quartiles as breaking values of the benchmark variable

Class Minimum Dew Point (minDP_CLASS, °C Td)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Maximum Dew Point (maxDP_CLASS, °C Td)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Minimum Temperature Humidity Index (minTHI_CLASS)	five classes using the first four quartiles as breaking values of the benchmark variable
Class Maximum Temperature Humidity Index (maxTHI_CLASS)	five classes using the first four quartiles as breaking values of the benchmark variable

Supplementary Table S6.3 Deviance Information Criterion values for model 1, assessing the impact of different environmental covariates on beef cattle growth traits.

Environmental Descriptors	Time Period	Values
ADG		
Class Temperature (T_CLASS, °C)	0-150, d	1 487 358.0
Class Relative Humidity (RH_CLASS, %)	0-150, d	1 487 305.0
Class Dew Point (DP_CLASS, °C Td).	0-150, d	1 487 340.0
Class Temperature Humidity Index (THI_CLASS)	0-150, d	1 487 361.0
Class Heat Load > 70 (HL_CLASS_70)	0-150, d	1 487 405.0
Class Heat Load > 75 (HL_CLASS_75)	0-150, d	1 487 361.0
Class Minimum Temperature (minT_CLASS,°C)	0-150, d	1 487 340.0
Class Maximum Temperature (maxT_CLASS,°C)	0-150, d	1 487 359.0
Class Minimum Relative Humidity (minRH_CLASS, %)	0-150, d	1 487 304.0
Class Maximum Relative Humidity (maxRH, CLASS %)	0-150, d	1 487 256.0
Class Minimum Dew Point (minDP_CLASS, °C Td).	0-150, d	1 487 343.0
Class Maximum Dew Point (maxDP_CLASS, °C Td).	0-150, d	1 487 349.0
Class Minimum Temperature Humidity Index (minTHI_CLASS)	0-150, d	1 487 347.0
Class Maximum Temperature Humidity Index (maxTHI_CLASS)	0-150, d	1 487 361.0
WW		
Class Temperature (T_CLASS, °C)	30 d prior weight date	978 670.4
Class Relative Humidity (RH_CLASS, %)	30 d prior weight date	978 724.6
Class Dew Point (DP_CLASS, °C Td).	30 d prior weight date	978 737.1
Class Temperature Humidity Index (THI_CLASS)	30 d prior weight date	978 671.2
Class Heat Load > 70 (HL_CLASS_70)	30 d prior weight date	978 764.4
Class Heat Load > 75 (HL_CLASS_75)	30 d prior weight date	978 798.2
Class Minimum Temperature (minT_CLASS,°C)	30 d prior weight date	978 689.4
Class Maximum Temperature (maxT_CLASS,°C)	30 d prior weight date	978 692.1
Class Minimum Relative Humidity (minRH_CLASS, %)	30 d prior weight date	978 791.8
Class Maximum Relative Humidity (maxRH, CLASS %)	30 d prior weight date	978 723.8
Class Minimum Dew Point (minDP_CLASS, °C Td).	30 d prior weight date	978 736.0
Class Maximum Dew Point (maxDP_CLASS, °C Td).	30 d prior weight date	978 756.9
Class Minimum Temperature Humidity Index (minTHI_CLASS)	30 d prior weight date	978 696.3
Class Maximum Temperature Humidity Index (maxTHI_CLASS)	30 d prior weight date	978 681.7
YW		
Class Temperature (T_CLASS, °C)	30 d prior weight date	255 278.7
Class Relative Humidity (RH_CLASS, %)	30 d prior weight date	255 282.7
Class Dew Point (DP_CLASS, °C Td).	30 d prior weight date	**255 242.9**

Class Temperature Humidity Index (THI_CLASS)	30 d prior weight date	255 277.5
Class Heat Load > 70 (HL_CLASS_70)	30 d prior weight date	255 296.2
Class Heat Load > 75 (HL_CLASS_75)	30 d prior weight date	255 247.1
Class Minimum Temperature (minT_CLASS, °C)	30 d prior weight date	255 281.5
Class Maximum Temperature (maxT_CLASS, °C)	30 d prior weight date	255 246.6
Class Minimum Relative Humidity (minRH_CLASS, %)	30 d prior weight date	255 255.2
Class Maximum Relative Humidity (maxRH, CLASS %)	30 d prior weight date	255 274.7
Class Minimum Dew Point (minDP_CLASS, °C Td).	30 d prior weight date	255 259.8
Class Maximum Dew Point (maxDP_CLASS, °C Td).	30 d prior weight date	255 265.2
Class Minimum Temperature Humidity Index (minTHI_CLASS)	30 d prior weight date	255 266.9
Class Maximum Temperature Humidity Index (maxTHI_CLASS)	30 d prior weight date	255 258.9

Abbreviations: ADG = average daily gain; WW = weaning weight; YW = yearling weight.

Supplementary Table S6.4 Variance components of the three traits for cattle under study. considering single trait analyses (both BLUP and Single-step genomic BLUP), multiple trait, and single step multiple trait models. Environmental covariates with the lowest Deviance Information Criterion values are used, divided into five different environments. Values within parentheses represent the Highest Posterior Density Interval (HPDI).

Trait	Variance components				Environment	Variance components			
	Single Trait BLUP		Single Trait single-step genomic BLUP			MT		single-step MT	
	σ^2_{hy}	σ^2_{e}	σ^2_{hy}	σ^2_{e}		σ^2_{hy}	σ^2_{e}	σ^2_{hy}	σ^2_{e}
ADG (kg/d)	0.026 $^{(0.025;\ 0.028)}$	0.044 $^{(0.044;\ 0.045)}$	0.026 $^{(0.025;\ 0.027)}$	0.044 $^{(0.044;\ 0.045)}$	Env 1	0.037 $^{(0.036;\ 0.038)}$	0.034 $^{(0.032;\ 0.035)}$	0.037 $^{(0.036;\ 0.040)}$	0.034 $^{(0.032;\ 0.035)}$
					Env 2	0.036 $^{(0.035;\ 0.037)}$	0.035 $^{(0.034;\ 0.036)}$	0.036 $^{(0.033;\ 0.038)}$	0.035 $^{(0.034;\ 0.036)}$
					Env 3	0.038 $^{(0.037;\ 0.040)}$	0.035 $^{(0.034;\ 0.036)}$	0.038 $^{(0.036;\ 0.041)}$	0.035 $^{(0.034;\ 0.036)}$
					Env 4	0.037 $^{(0.036;\ 0.038)}$	0.036 $^{(0.034;\ 0.037)}$	0.037 $^{(0.034;\ 0.039)}$	0.035 $^{(0.034;\ 0.037)}$
					Env 5	0.031 $^{(0.030;\ 0.032)}$	0.035 $^{(0.034;\ 0.036)}$	0.031 $^{(0.028;\ 0.033)}$	0.035 $^{(0.033;\ 0.036)}$
WW (kg/d)	699.99 $^{(672.30;\ 735.20)}$	738.04 $^{(726.80;\ 749.90)}$	700.31 $^{(669.80;\ 727.30)}$	742.45 $^{(731.00;\ 754.30)}$	Env 1	777.09 $^{(757.30;\ 798.80)}$	587.92 $^{(559.80;\ 616.40)}$	778.13 $^{(719.70;\ 836.10)}$	590.52 $^{(566.40;\ 615.60)}$
					Env 2	888.65 $^{(865.90;\ 910.20)}$	542.13 $^{(514.20;\ 569.80)}$	886.53 $^{(832.10;\ 943.40)}$	542.97 $^{(512.90;\ 570.40)}$
					Env 3	904.96 $^{(883.70;\ 927.80)}$	581.70 $^{(549.30;\ 605.10)}$	907.97 $^{(851.00;\ 972.20)}$	583.23 $^{(553.60;\ 613.90)}$
					Env 4	888.10 $^{(868.00;\ 911.40)}$	543.23 $^{(515.70;\ 567.20)}$	889.05 $^{(832.30;\ 945.60)}$	546.37 $^{(518.90;\ 573.90)}$
					Env 5	905.92 $^{(885.40;\ 929.90)}$	529.07 $^{(501.30;\ 556.50)}$	909.09 $^{(852.40;\ 964.80)}$	534.91 $^{(503.80;\ 558.80)}$
YW (kg/d)	1 901.14 $^{(1\ 756.00;\ 2\ 029.00)}$	1 036.63 $^{(993.30;\ 1\ 079.00)}$	1 893.36 $^{(1\ 751.00;\ 2\ 019.00)}$	1 050.45 $^{(1\ 007.00;\ 1\ 094.00)}$	Env 1	1 931.35 $^{(1\ 884.00;\ 1984.00)}$	869.31 $^{(791.70;\ 942.10)}$	1 927.40 $^{(1\ 731.00;\ 2148.00)}$	807.25 $^{(719.80;\ 889.60)}$
					Env 2	2 043.49 $^{(1\ 989.00;\ 2099.00)}$	695.90 $^{(603.20;\ 816.30)}$	2 034.68 $^{(1\ 835.00;\ 2289.00)}$	732.43 $^{(633.00;\ 816.60)}$
					Env 3	2 168.04 $^{(2\ 105.00;\ 2221.00)}$	757.12 $^{(651.00;\ 865.20)}$	2 168.30 $^{(1\ 925.00;\ 2402.00)}$	744.64 $^{(636.70;\ 837.20)}$
					Env 4	2 371.29 $^{(2\ 314.00;\ 2439.00)}$	763.75 $^{(658.40;\ 886.80)}$	2 290.93 $^{(2\ 088.00;\ 2506.00)}$	779.38 $^{(670.00;\ 880.30)}$
					Env 5	2 099.68 $^{(2\ 046.00;\ 2159.00)}$	740.54 $^{(657.10;\ 825.20)}$	2 168.94 $^{(1\ 972.00;\ 2412.00)}$	741.79 $^{(651.00;\ 832.20)}$

Abbreviations: ADG = average daily gain; WW = weaning weight; YW = yearling weight; BLUP = best linear unbiased prediction; σ^2_{hy} = permanent environmental variance (herd-year); σ^2_{e} = residual variance; MT = multiple trait model.

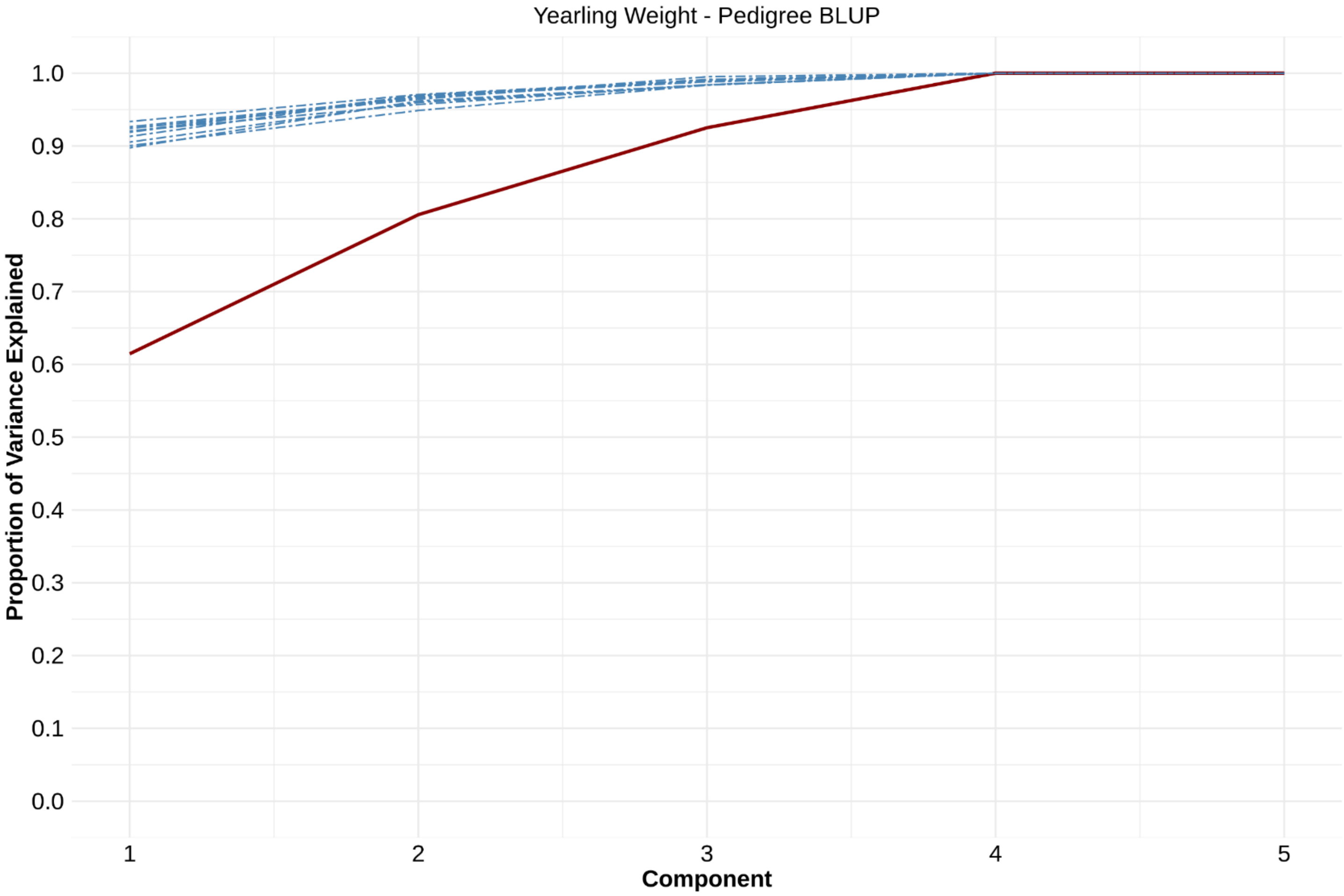
Supplementary Table S6.5 Linear regression statistics (bias, slope, and accuracy) for BLUP and Single-step genomic BLUP models for beef cattle, comparing their performance on the whole dataset with complete pedigree information and on a dataset truncated to four years prior.

Trait	Environment	EBV			Single-step genomic EBV		
		Accuracy	Bias	Slope	Accuracy	Bias	Slope
ADG (kg/d)	Env1	0.80	-0.003	0.70	0.84	-0.0007	0.70
	Env2	0.83	-0.003	0.79	0.85	-0.0008	0.78
	Env3	0.82	-0.004	0.78	0.84	-0.0008	0.77
	Env4	0.83	-0.005	0.76	0.85	-0.003	0.76
	Env5	0.80	-0.004	0.75	0.83	-0.001	0.75
	Univariate	0.81	0.007	0.79	0.83	0.003	0.85
WW (kg)	Env1	0.83	-0.75	0.83	0.84	-0.05	0.82
	Env2	0.79	0.45	0.77	0.81	0.05	0.76
	Env3	0.85	-0.38	0.84	0.85	-0.04	0.82
	Env4	0.82	-0.57	0.79	0.81	-0.38	0.77
	Env5	0.83	-0.25	0.82	0.83	-0.09	0.82
	Univariate	0.80	-2.70	1.15	0.82	0.43	0.81
YW (kg)	Env1	0.94	-0.93	0.76	0.88	-0.60	0.76
	Env2	0.89	-0.69	0.73	0.87	-0.59	0.65
	Env3	0.92	-1.97	0.78	0.93	-1.74	0.71
	Env4	0.90	-2.36	0.81	0.89	-1.90	0.79
	Env5	0.91	-1.73	0.92	0.90	-1.53	0.87
	Univariate	0.90	2.36	1.55	0.90	-0.98	0.96

Abbreviations: ADG = average daily gain; WW = weaning weight; YW = yearling weight; BLUP = best linear unbiased prediction; EBV = estimated breeding value

6.13.2 Supplementary Figure

Supplementary Figure S6.1 Comparison of eigenvalue decomposition of the G matrix of multiple trait model for yearling weight (YW, kg) across five environments of dew point (red) and ten permutations (dashed blue) in the Limousine population. Abbreviations: BLUP = best linear unbiased prediction.



CHAPTER 7

7. General Conclusions and Implications

7.1 Conclusions

This thesis provided an integrated evaluation of the genetic and genomic basis of sustainability and resilience in Italian beef cattle. Through complementary studies focused on functional longevity, fertility, growth, inbreeding, and genotype-by-environment interaction, the thesis demonstrated how quantitative and genomic approaches can be combined to enhance the efficiency, adaptability, and long-term viability of beef production systems. The overall findings contribute to a deeper understanding of how genetic variability highlights productivity, resilience, and sustainability in populations subjected to environmental and management constraints.

The central aim of this research was to improve both the understanding and application of genomic methodologies in the evaluation of traits contributing to sustainable beef cattle breeding. Collectively, the studies highlight that achieving sustainability in beef production requires a multifaceted approach, one that integrates productivity with resilience, genetic diversity, and adaptability to changing environments. This thesis underscores that sustainability is not solely a matter of output efficiency but of balancing short-term performance gains with long-term population robustness.

7.1.1 Integration of findings across studies. Across all analyses, a scenario emerged: genetic variation for resilience and efficiency-related traits exists and can be exploited through genomic evaluation. The combined use of quantitative and genomic models revealed that functional traits such as longevity and fertility, although complex and moderately heritable, are genetically structured and responsive to selection. Integrating these traits with morphological and performance information provides a broader perspective on animal robustness and lifetime productivity, enabling the identification of individuals capable of maintaining efficiency across diverse physiological and environmental conditions.

The incorporation of genomic information also provided biological insight into the architecture of these traits. Genomic regions and candidate genes associated with immune response, metabolic balance, and reproductive physiology supported the polygenic nature of these traits. These results promote a more informed use of genomic data for selection strategies targeting functional and adaptive mechanisms.

The analysis of genomic inbreeding and inbreeding depression emphasized the importance of maintaining genetics to achieve long-term sustainability. The observed negative effects of inbreeding on growth and fertility confirmed that selection must be accompanied by effective genetic management. Genomic inbreeding coefficients proved to be more sensitive than pedigree-based measures, reinforcing the need for continuous genomic monitoring in selection programs. Moreover, distinguishing between recent and ancient inbreeding provided valuable insights into the temporal dynamics of genetic erosion, highlighting the necessity of controlling recent inbreeding to prevent immediate performance losses while ensuring long-term adaptability.

In the context of early growth traits, such as weaning weight, the exclusion of maternal additive effects in some models, consistent with current national genetic evaluation schemes and driven by data structure limitations, should be acknowledged as a potential source of variance redistribution. Maternal genetic contributions may partly influence estimates of direct genetic effects, inbreeding depression, and genotype-by-environment interaction, suggesting that future evaluations could benefit from enhanced data recording, wider genotyping, and alternative model formulations that explicitly account for maternal effects.

The evaluation of genotype-by-environment interaction further demonstrated that resilience to climatic stress could be estimated. Detecting genotypes that lost or maintain stable productivity under climatic stress conditions confirmed that environmental sensitivity varies across animals. Incorporating genotype-by-environment interaction into evaluations allows for the identification of both robust genotypes, suited to a range of environments, and specialized ones adapted to specific climatic conditions. This evaluation is an essential step toward climate-resilient breeding.

*7.1.2 Implications for sustainable breeding and livestock systems***.** The findings of this thesis emphasize that resilience and sustainability can be integrated into breeding programs by jointly considering functional, fertility, and adaptive traits. Genomic prediction enables more accurate estimation of breeding values even for traits of low heritability, supporting earlier and more reliable selection decisions. Including stayability and fertility traits in national selection indices can reduce replacement rates, enhance herd efficiency, and improve both economic and environmental sustainability. Furthermore, identifying genomic regions linked to productivity and resilience opens opportunities for genomic and marker-assisted selection to accelerate progress in these key traits.

Sustainable breeding also requires active management of genetic diversity. Continuous monitoring of genomic inbreeding, together with the use of optimal contribution or genomic selection, can balance genetic gain with the preservation of variability. The inclusion of genotype-by-environment effects in national evaluations would further strengthen the robustness of selection, allowing the identification of animals that perform consistently across systems or exhibit specific adaptation to heat or extensive conditions. These strategies align with the broader objectives of sustainable livestock production, in which animal welfare, responsible environmental management, and economic viability are mutually dependent goals supported by genetic improvement.

7.1.3 Future perspectives. Future research should aim to refine the definition and measurement of resilience by integrating precision livestock data, such as sensor-derived information on behavior, activity, or thermal response, into genomic evaluations. Linking longitudinal phenotypic data with genomic information will improve the characterization of individual adaptive capacity and facilitate selection for robustness and recovery traits. Expanding genome-wide association analyses through functional genomics approaches, including transcriptomics, epigenetics, and metabolomics, will provide deeper insight into the biological pathways connecting genetic variation to phenotypic resilience. Furthermore, multi-breed and international collaborations could increase the accuracy of genomic predictions, promote the exchange of genetic material, and support the development of globally resilient beef populations.

In summary, this thesis establishes a multidimensional framework for breeding resilient and sustainable beef cattle. By integrating quantitative genetics, genomics, and environmental information, it demonstrates that modern selection can simultaneously enhance productivity, animal welfare, and environmental efficiency. The approaches developed and validated in this work offer a solid scientific basis for advancing breeding programs that are not only economically viable but also ethically and ecologically sustainable in the context of future global challenges.

List of scientific contributions

Peer-reviewed publications

- **Callegaro, S**., F. Tiezzi, M.C. Fabbri, S. Biffani, R. Bozzi. 2024. Evaluating genotype by environment interaction for growth traits in Limousine cattle. Animal, 18:101344. https://doi.org/10.1016/j.animal.2024.101344.
- **Callegaro, S**., F. Tiezzi, C. Maltecca, M.C. Fabbri, R. Bozzi. 2024. Genetic parameters of functional longevity and associated traits in Italian Charolais and Limousine breeds. Journal of Animal Science. 102:354. https://doi.org/10.1093/jas/skae354.
- **Callegaro, S**., F. Tiezzi, C. Maltecca, M.C. Fabbri, J.C. do Carmo Panetto, R. Bozzi. 2025. Comprehensive analysis of inbreeding depression across growth, fertility, and survival traits in Limousine beef cattle. Animal. 101672. https://doi.org/10.1016/j.animal.2025.101672.

Under review

- **Callegaro, S**., C. Maltecca, F. Tiezzi, M.C. Fabbri, R. Bozzi. 2025. Genomic insights into female productivity in Limousine cattle: a single-step genome-wide association on longevity, fertility, and conformation traits. Submitted to BMC Genomics.

Conferences

- **Callegaro, S.**, F. Tiezzi, M.C. Fabbri, R. Bozzi. 2023. Different methods to handle preferential treatment in slaughter age in Italian Limousine. In: Book of Abstracts of the XXV National Congress of the Animal Science and Production Association (ASPA), June, Bari, Italy. Oral presentation.
- **Callegaro, S.,** S. Biffani, F. Tiezzi, M.C. Fabbri, R. Bozzi. 2023. Impact of heat stress on growth of Italian Limousine and Charolais. In: Book of Abstracts of the XXV National Congress of the Animal Science and Production Association (ASPA), June, Bari, Italy. Oral presentation.
- **Callegaro, S.,** F. Tiezzi, M.C. Fabbri, C. Maltecca, R. Bozzi. 2024. Genotype by environment interaction for growth traits in the Italian Limousine population. Book of Abstracts of the 75th Annual Meeting of the European Federation of Animal Science (EAAP), 1 September – 5 September, Firenze, Italy. Oral presentation.
- **Callegaro, S.,** F. Tiezzi, C. Maltecca, M.C. Fabbri, R. Bozzi. 2025. Comprehensive analysis of inbreeding depression across growth, fertility, and survival traits in Charolais and Limousine beef cattle. In: Book of Abstracts of the XXVI National Congress of the Animal Science and Production Association (ASPA), June, Torino, Italy. Oral presentation.

Acknowledgements

Prima di tutto vorrei ringraziare l'Associazione Nazionale Allevatori delle razze bovine Charolaise e Limousine Italiane e il direttore Stefano Saleppichi, perché senza di loro questo dottorato non sarebbe stato possibile. Estendo la mia più sincera gratitudine al Piano di Sviluppo Rurale Nazionale (PSRN), che ha permesso lo sviluppo e l'implementazione di nuove tematiche per le due razze oggetto di studio nella tesi. Di conseguenza, vorrei anche ringraziare gli allevatori e il loro lavoro, perché senza di loro la ricerca in campo zootecnico non sarebbe possibile.

Sono sinceramente grato al mio supervisore, prof. Riccardo Bozzi, per la sua guida e il supporto durante il mio percorso. Soprattutto lo ringrazio per aver accolto un nordico padano alla corte dei Medici in terra fiorentina, nel bellissimo "Gran Ducato di Toscana". Un ringraziamento speciale va al prof. Francesco Tiezzi, co-supervisore della mia tesi, per il suo supporto, le sue idee e la sua immensa pazienza nel lavorare con me e nel rispondere alle migliaia di domande (talvolta anche poco sensate) che gli ho posto in questi tre anni. Vi ringrazio profondamente per avermi fatto conoscere, apprezzare e, perché no, a volte anche odiare questo affascinante mondo della genetica quantitativa.

Un altro ringraziamento speciale va al prof. Christian Maltecca, che mi ha ospitato negli Stati Uniti durante il mio periodo all'estero, a Raleigh, in North Carolina, presso la NC State University. Grazie per la tua disponibilità e ospitalità e, soprattutto, per avermi dato l'opportunità di trascorrere un periodo negli Stati Uniti e di condividere le tue conoscenze con me.

Un ringraziamento va anche al mio gruppo di ricerca e ai colleghi con cui ho avuto la possibilità di lavorare, collaborare e divertirmi. Infatti, non posso non ringraziare Ale, le sue birre e il Chiosco dell'Oliveta degli Etruschi a Sesto Fiorentino. Non ci posso fare nulla, la birra, oltre a essere buonissima, è un ottimo compagno di viaggio, sempre presente.

Ai miei amici di sempre. Vi conosco dall'asilo e dalle scuole e, nonostante tutto, tutt'ora siamo ancora uniti e legati come una volta. Quante esperienze abbiamo condiviso e quanti momenti indimenticabili abbiamo vissuto insieme? Forse anche troppi… so che potrò sempre contare su di voi, se mai ne avrò bisogno. Infine, un pensiero speciale per te, Anna, la mia migliore amica, che hai deciso di lasciarci poco tempo fa. Una scelta che tutt'ora faccio fatica a comprendere. Sappi che ti porterò sempre nel cuore; posso solo dirti grazie per tutto quello che abbiamo passato insieme. Anche se non ci sei più, voglio condividere con te questo traguardo che ho raggiunto, per me importantissimo. Ciao Anna, ti voglio bene. Riposa in pace, Simo.

Sono profondamente grato alla mia famiglia. Il vostro amore, i vostri principi, la vostra pazienza e il vostro supporto sono stati fonte di ispirazione. Grazie per credere in me e per sostenere ogni mia

scelta; senza di voi non avrei mai potuto raggiungere questi traguardi. Vi sono infinitamente grato per tutto, perciò voglio dirvi, dal profondo del mio cuore, grazie mamma, papà, fratellone e sorellona. In particolare, mamma e papà, grazie per tutti i sacrifici che avete fatto per me; spero che questo traguardo e questa tesi vi rendano fieri di me.

Un grazie speciale va anche a mio zio Sergio, che anni fa, in mezzo ai suoi animali, ha deciso di adottarmi e insegnarmi tutto ciò che riguarda il mondo dei cavalli. Grazie a te ho conosciuto persone straordinarie. In particolare, sarò sempre grato ad Adriano, suo papà Vittorio e sua mamma Giuseppa, per avermi ospitato come uno di famiglia e fatto lavorare nel loro bellissimo allevamento di pecore. Zio, come dicesti al momento della mia laurea magistrale: “Questa laurea è un po’ anche mia, metà del tuo voto lo prendo io” … anche col dottorato sarà così?

Ed eccoci qua, all’ultima persona. Una persona speciale: la mia morosa, il mio criceto (a volte un po’ mannaro, ma questo è un altro discorso). Da dove cominciare? Forse dirti semplicemente grazie sarebbe banale e superficiale… Ma grazie per tutto il supporto che mi hai sempre dato, grazie per essermi stata sempre vicina nonostante io sia una persona che si chiude in sé stessa e difficilmente si apre. Grazie perché con te posso essere sempre me stesso, anche se ogni tanto sono un po’ “rotto” (porta pazienza :)). Il più grande grazie va al fatto che hai deciso di starmi sempre vicina in questo mio viaggio, di supportare questa mia scelta nonostante la lontananza e le diverse città che ci hanno separati. Senza di te e il tuo supporto non avrei mai potuto raggiungere questo traguardo. Infine, un grazie speciale a tua mamma Marina e a tuo papà Ettore, che mi hanno accolto e accettato per quello che sono nella vostra famiglia.

Sono una persona che esprime molto di rado i propri sentimenti e difficilmente li condivido con qualcuno. Ma ora è arrivato il momento di farlo, e dunque di ringraziare tutti voi. Perciò, a ognuno di voi, un ultimo GRAZIE per aver contribuito a questo mio viaggio.

To conclude, I would like to sincerely thank the external reviewers who will read this thesis. I am very grateful for your time, expertise, and constructive feedback, which will be invaluable in improving this work. Your careful evaluation and insights are truly appreciated.

"It is not the strongest of the species that survives, nor the most intelligent,
but the one most responsive to change."
Charles Darwin

"Life is suffering. It's hard. The world is cursed.
But still, you find reasons to keep living."
Hayao Miyazaki, *Princess Mononoke*

As the bamboo bends with the wind yet never breaks,
so must science adapt, evolve, and find strength through change.