CHRISTHIAN BORGES DE SOUZA

GENETIC EFFECTS OF DOUBLE MUSCLE AND SLICK HAIR MUTATIONS ON SELECTION CRITERIA AND GENOMIC STUDY FOR WATER EFFICIENCY IN SENEPOL CATTLE

Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal of Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Simone Eliza Facioni Guimarães

Co-advisers: Gilberto Romeiro de Oliveira Menezes
Delvan Alves da Silva

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Adviser

```
dedicatoria = function(leitor)
{
  if (leitor == "meus pais")
  {print ("Para meus pais: João e ionilda")
    }
if (leitor == "minha namorada")
{
  print ("Para: Luana")
}
  if (leitor=="Professores | pesquisadores")
  {
  print ("Para: Simone, Gilberto, Paulo, Delvan, Renata, Daniele")
  }
if(leitor=="amigos")
{
  print ("Para todos apaixonados por genética e melhorameto")
 }
}
```

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“$\leftrightarrow A =$ *sucesso,* $\therefore A = (X + Y + Z)$*. O trabalho é* X*;* Y *é o lazer; e* Z *é manter a boca fechada.” (Albert Einstein)*

ABSTRACT

SOUZA, Christhian Borges, M.Sc., Universidade Federal de Viçosa, February 2024. **Genetic effects of double muscle and slick hair mutations on selection criteria and genomic study for water efficiency in senepol cattle**. Advisor: Simone Eliza Facioni Guimarães. Co-advisors: Gilberto Romeiro de Oliveira Menezes and Delvan Alves da Silva.

Concerns about climate change, water resources, and scarcity encourage new research and solutions in all industry sectors, and the livestock is no exception. The possibility of practicing genetic selection to reduce the water footprint of beef may be a viable alternative. However, the inclusion of new traits in breeding programs requires a prior study of their impact on already established traits such as weight gain. Thus, the aim of this study was to describe the genetic parameters of water efficiency measures and conduct a Genome-Wide Association Study (GWAS), as well as Post-GWAS, to assess the effect of *Double Muscle* (DM) and *Slick Hair* mutations on selection criteria. Data were provided by the Brazilian Association of Senepol Cattle Breeders - ABCB Senepol and the Embrapa Geneplus Program. It was observed that individuals carrying *Slick Hair* and not carrying DM are the most recommended. Heritability averages were found for water efficiency measures, and the phenotypic correlation values between weight gain and residual water intake were null. In addition, showed a genetic association of medium to high magnitude with residual feed intake, making them more attractive for selection. This is different from gross measurements that demonstrate a medium to high genetic association with weight gain. Water intake and dry matter intake demonstrated high genetic association (0.85). Only for water conversion ratio were significant SNPs found, reinforcing the polygenic nature of these traits. Enrichment analyses revealed that various biological processes are involved in the expression of this phenotype. In general, there is genetic variability for water efficiency selection; these traits demonstrate a polygenic nature, and several biological processes linked to saliva production, heat stress, water transport, imune sistem, lipid metabolism and oxidoreductase activity are involved with phenotype.

Keywords: Adapted Taurine. Genetic Parameters. Genome Wide Association Study. Post-GWAS.

SOUZA, Christhian Borges, M.Sc., Universidade Federal de Viçosa, Fevereiro 2024. **Efeitos genéticos das mutações de dupla musculatura e pelo liso em critérios de seleção e estudo genômico para eficiência hídrica em bovinos da raça Senepol**. Orientador (a): Simone Eliza Facioni Guimarães. Co-orientadores: Gilberto Romeiro de Oliveira Menezes and Delvan Alves da Silva.

A preocupação quanto as mudanças climáticas, os recursos hídricos e a escassez incentivam novas pesquisas e soluções em todos os setores, com a pecuria não é diferente. A possibilidade de praticar a seleção genética para diminuir a pegada hídrica da carne bovina pode ser uma alternativa viável. No entanto, a inclusão de novas características nos programas de melhoramento requer estudo prévio do impacto destas em características já consolidadas como ganho de peso. Assim, o objetivo deste estudo foi descrever os parâmetros genéticos das medidas de eficiência hídrica e realizar um estudo de associação genômica ampla (GWAS), bem como Pós-Gwas e verificar efeito das mutações *Double Muscle* (DM) e *Slick Hair* em critérios de seleção. Os dados foram cedidos pela Associação Brasileira de Criadores de Bovinos da raça Senepol – ABCB Senepol e Programa Embrapa Geneplus. Foi observado que individuos portadores do *Slick Hair* e não portadores da DM são os mais indicados. Foram encontradas médias herdabilidade para medidas de eficiência hídrica, os valores de correlação fenotipa entre ganho de peso e as medidas residuais de ingestão de agua foram nulas, além de apresetarem associação genética de média a alta magnitude com o consumo alimentar residual, tornando-as mais atrativas a seleção. Diferente das medidas brutas que demonstram de média a alta associação genética com ganho de peso. A ingestão de agua e o consumo de matéria seca demonstram alta associação genética (0.85). Apenas para conversão hídrica foi encotrado SNPs significaticos reforçando a natureza poligênica destas características. As análises de enriquecimento revelaram que diversos processos biológicos estão envolvidos na expressão deste fenótipo. Em linhas gerais, há variabilidade genética para seleção de eficiência hídrica, estas características demonstram natureza poligênica e diversos processos biológicos ligados a produção de saliva, stress térmico, transporte de água, sistema imunológico, metabolismo de lipídios e atividades de oxidoreductase estão envolvidos com a expressão do fenótipo.

Palavras-chave: Estudo de Associação Genômica Ampla. Parâmetros genéticos. Pós-Gwas.

SUMMARY

INTRODUCTION

Among the guidelines of sustainable development, there is a rooted concern regarding available water resources. In this way, the beef production chain must seek actions that promote productivity in an environmentally resilient manner. It is important to highlight some goals of the United Nations to be aimed in 2030, in order to improve the efficiency of freshwater use and minimize the number of people suffering from water scarcity (UN, 2024). According to the 2021 World Economic Forum, the natural resources crisis is among the ten most serious global risks for the next 10 years (McLennan, 2021).

From a genetic perspective, Ahlberg et al. (2019) demonstrated genetic variability in beef cattle for different measures related to water intake, with heritability values found between 0.39 and 0.37. This indicates the possibility of selection based on cattle that are more efficient in water consumption for meat production. The study of new traits requires the estimation of genetic parameters, as it is essential to understand the genetic variability and associations of a particular economically important trait with others used in the selection process, which inform decision-making in breeding programs. Additionally, Genome Wide Association Study (GWAS) allow the identification of loci for quantitative traits (QTL) associated with economically important traits and related genes present in these regions. These can be used in post-GWAS studies to better understand the mechanisms involved in phenotype expression.

Some genes single can have a significant effect on the phenotype of an individual, as is the case Double Muscling (DM); a hereditary disorder caused by a gene mutation in the GDF-8 (Growth Differentiation Factor 8) gene, encoding myostatin (URQUIZA et al., 2017). Several studies, such as Wiener et al. (2009), show significant differences between carriers of the DM mutant allele and non-carriers for performance and carcass traits. It also impairs the reproductive development and fertility of individuals carrying the mutation (SIQUEIRA et al., 2015). Another mutation with notable phenotypic impact is *slick hair*, located in the PRLR gene region, resulting in the phenotype of short, sleek hair with larger sweat glands (HUSON, 2014). This phenotype reflects animals with greater resistance to thermal stress and potentially better productive and reproductive indices in tropical conditions (OLSON et al., 2003).

Therefore, it is important to quantify the difference between carriers and non-carriers of DM and slick hair mutations on several traits to support practical decisions in selection processes based on scientific information. Thus, its very important determine the most suitable genotype for selection in a resilient manner, considering the selection criteria already consolidated in the breed. In addition, verify the feasibility of selection for water efficiency and assessed the genetic relationships with feed efficiency and weight gain, with aim to identify genes and biological processes associated with these traits.

CHAPTER 1 – GENERAL CONSIDERATION

1. LITERATURE REVIEW

1.1 Double muscling

The double muscling (DM) is a hereditary condition responsible for increased hypertrophy and hyperplasia of muscle fibers, resulting from a genetic mutation in the GDF-8 gene (Growth Differentiation Factor 8), encoding myostatin (URQUIZA et al., 2017). Specifically in the Senepol cattle, Xavier (2014) demonstrated in SNP prospecting work the mutation known as nt821 located in exon III, which renders the action of myostatin inactive. In active form, myostatin binds to the Activin IIB receptor to exert its biological function in the regulation of myogenesis (AOKI, 2008). According to Backingham et al. (2003), myogenesis basically involves the transformation of somites into myoblasts. The action of myostatin regulates this process by blocking the expression of the PAX 3 transcriptional paired box 3 factor (MCFALARME et al., 2006), a factor responsible for the transcriptional control of cells directing them in tissue formation (BACKINGHAM & RELAIX, 2007).

The first description of the syndrome in cattle was reported in 1807 in England, in Shorthorn breed (CHARLIER et al., 1995). This disorder was later also found in other breeds such as Belgian Blue, Piemontese, Asturiana de los Valles, Maine Anjou, Charolais, Limousin, Parthenaise, Rubea Gallega, and Marchigiana (TEIXEIRA et al., 2006). Regarding the phenotype, affected animals show all muscles in the body pronouncedly, delayed development of reproductive organs, high incidence of dystocic births, reduced fertility, lower carcass fat concentration, increased susceptibility to respiratory diseases, and macroglossia in newborn calves (SIQUEIRA, 2015).

Studies like Wiener et al. (2009) analyzed 146 South Devon cattle, divided into homozygous wild tipe (dmdm), heterozygotes with one copy of DM (DMdm), and homozygous carriers (DMDM). The authors observed that DM carriers had lower growth rates and reduced subcutaneous fat in the carcass. Regarding meat sensory traits, they reported that DMDM animals produced less juicy and less flavorful meat than dmdm. However, DMDM individuals had a higher hot carcass weight (367.5 kg) compared to DMdm animals (356 kg), while dmdm were lower than the others (339 kg).

Aldai et al. (2006) evaluated carcass traits of 12 Asturiana de los Valles bulls and 4 Asturiana de la Montaña, observing that dmdm individuals had higher values for subcutaneous and intramuscular fat compared to DMdm, which in turn were higher than DMDM. The authors reported that there was no significant difference between final slaughter weight and percentage of bone in the carcass among the evaluated genotypes. Allais et al., (2010), conducted a study on carcass traits in the Charolais breed and observed that heterozygous individuals show higher carcass yield and REA, and lower carcass fat content than homozygotes non-carrier. Regarding birth weight, Casas et al. (1999) concluded that raising cattle homozygous for DM in extensive systems is unfeasible due to the high incidence of dystocic births. However, individuals carrying the DM gene show higher weaning weight and demonstrate higher live weight at slaughter compared to those without the mutation (CASAS et al., 2004). Therefore, DM can directly impact the expression of some traits of interest, so the choice of animals carrying or not carrying the allele should be considered according to the selection goal.

1.2 Slick hair

The existence of the slick hair gene in Senepol cattle was highlighted by Olson et al. (2003), who observed the ability of animals to maintain a lower rectal temperature than non-carriers of the gene. This gene causes the phenotype of short, sleek hair with larger sweat glands, resulting in individuals with greater resistance to thermal stress and potentially possessing better productive and reproductive indices in tropical conditions (OLSON et al., 2003).

The gene in question is classified as dominantly inherited, a factor that allows the progeny to exhibit the characteristic phenotype. The recurrent mutation is located in the region between 37.5 and 39.5 Mb on BTA20, where the PRLR and SPF2 genes are located, which in turn are associated with reproduction and milk production (HUSON, 2014). Bertipaglia (2007) evaluated the effect of coat type on productive traits in Braford breed cows and observed that selecting individuals with shorter hair is related to a reduction in calving interval. In addition, *slick hair* can helps to improve reproductive performance (POOLE et al., 2014). In beef cattle, hair length shows negative correlation with growth traits in tropical conditions (WILLIAMS et al., 2006).

In dairy cattle Dickmen et al. (2014), for instance, assessed different lactation periods in Holstein cows carrying the Slick Hair and observed that carrier animals showed lower loss on daily milk production in summer compared to winter. Heterozygous cows exhibited a reduction of 1.3 kg of milk, while non-carrier cows expressed a 3.7 91 kg decline in production. The improvements observed in cattle with *slick hair* are linked to their ability to regulate body temperature during heat stress conditions, showcasing a reduced respiratory rate and internal temperature, along with an increased sweating rate (DICKMEN et al., 2008). However, studies evaluating the effect of *slick hair* in beef cattle are still limited in the scientific literature.

The productivity of animals subjected to heat stress is restricted through physiological changes that modulate the organism to maintain vital functions in challenging environments (ADBUCH, 2021). The expression of reproductive potential is highly influenced by the environment; the conception rate in females exposed to uncomfortable temperature conditions is significantly reduced (DE RENSIS et al., 2002), as well as in milk production and quality (MELO et al., 2016), and has a notable detrimental impact on weight performance in beef cattle (MADER, 2003). Therefore, similar to DM, slick hair can impact the expression of certain phenotypes and should be considered as a selection criterion for breeding in tropical climates.

1.3 Water efficiency

In relation the beef production environmental concerns, the use of natural resources has been consistently highlighted. These issues contribute to the growth of social movements related to dietary habits change, such as veganism and vegetarianism, which constantly put pressure on the livestock (NUNES, 2020). Thus, changes must occur in production practices. The optimization of water use by productive sectors with the aim of preserving water resources and reducing water scarcity is one of the main goal to achieve sustainable development according to the UN (UN, 2024).

In this context, it is crucial to understand and improve the "water footprint" of a specific product. The term refers to the total volume of water used to produce a particular product, with a global average ranging from 15,415 to 15,497 liters per kilogram of beef (RAN et al., 2016). These values are high when compared to other activities in the livestock sector (MEKONNEN & HOEKSTRA, 2012). The water footprint is divided into water used for the production of forages and grains for animal nutrition (green water), water directly designated for animal ingestion (blue water), and finally, the portion attributed to pollutant dilution (grey water) (RAN et al., 2016). Nardone et al. (2010) describe the efficient use of water resources through the selection and management of individuals adapted to adverse conditions of thermal and water stress as a crucial strategy related to the sustainability of livestock systems.

Thus, genetic improvement assumes a fundamental role. Several studies conducted over the years related to feed efficiency elucidate the ability to obtain more efficient animals in the use of food resources, which have a positive socioenvironmental impact (MEDEIROS et al., 2013). The optimization of the efficiency of natural resources used by the beef cattle industry, supported by selection processes, should follow the footsteps of traits related to productive and reproductive efficiency consolidated by undeniable genetic advances in recent decades (MARQUES, 2018; TEIXEIRA et al., 2018).

However, regarding the selection of beef cattle based on water intake, information is still scarce. In laboratory animals, Bachmanov et al. (2002) evaluated 28 strains of mice to identify differences in water and food intake among individuals. The authors reported heritability values of 0.69 for water intake. Subsequently, Ahlberg et al. (2019) demonstrated in beef cattle the existence of genetic variability for different measures related to water intake, with heritability values of 0.39 and 0.37. These values indicate the possibility of selection based on more water-efficient cattle.

Recently, with the aim of estimating genetic parameters for traits related to water intake in Senepol cattle, Pereira et al. (2021) observed heritability values ranging from 0.09±0.05 to 0.37±0.10. For the genetic correlations observed in the study, the authors reported a value of 0.79 between water intake and feed consumption. This association between traits demonstrates that selection based on lower water consumption indirectly reflects the selection for lower dry feed intake.

The measure of efficiency called residual water intake (RWI) is calculated as the difference between observed water intake and water intake predicted by the regression equation based on dry matter intake and average metabolic weight RWI_{DMI} (AHLBERG et al., 2019). Or through the regression equation based on average daily weight gain and average metabolic weight RWI_{ADG}. According to Pereira et al. (2021), both measures have a high correlation of 0.98 ± 0.02, and they also show a positive genetic association with residual feed intake of 0.51±0.29 and 0.64±0.24 for RWI_{DMI} and RWI_{ADG}, respectively.

The above results support the concept that selection based on water efficiency measures can reduce the water footprint of beef production. It is important to emphasize the need for a better understanding of the behavior of these traits and their correlations with other criteria used for selection. To ensure that genetic gains in water efficiency do not compromise the advancement of other traits and to support strategies such as the use of selection indices and correlated response. Thus, choose the most resilient measures to be used as a new selection criterion.

1.4 Genetic parameters estimation

The understanding of the behavior and nature of the traits is obtained through the estimation of genetic parameters; quantifying heritability and correlations is crucial for promoting efficient selection (MENDONÇA et al., 2012). The determination of phenotypic variation in a population due to genetic or environmental factors is verified through the estimation of heritability (FALCONER AND MACKAY, 2002). Thus, checking the feasibility of selection for a particular criterion. Quantitative traits are generally polygenic and show genetic, phenotypic, and environmental correlations with others; concerning genetic correlations, there are two causal agents: pleiotropy and linkage disequilibrium (LD) (TABORDA, 2021).

Pleiotropy is the effect that a specific gene can have on more than one trait, while linkage disequilibrium (LD) constitutes the non-random association of alleles at distinct loci that do not segregate independently (FALCONER AND MACKAY, 2002). According to Qanbari (2020), the causes of LD are the physical proximity between loci and evolutionary processes linked to mutation, selection, genetic drift, migration, and effective population size.

In the presence of these effects, traits of interest are positively correlated when selection for a particular trait induces another in the same direction. When both traits follow opposite directions, the correlation is negative (FALCONER AND MACKAY, 2002). In addition, the influence of one trait on another varies according to magnitude expressed by correlation value. Therefore, it is essential to understand the genetic relationship between traits adopted in the selection process to ensure better strategies and decisions to achieve selection goals.

Among the several methodologies proposed over the years to obtain estimators of variance components, Restricted Maximum Likelihood (REML) proposed by Patterson & Thompson (1971) is widely used by breeding programs and research. REML involves maximizing the logarithm of the probability density function of observations, considering the loss of degrees of freedom of fixed effects by deriving the random part of the function with respect to the parameters (SEARLE et al., 1992). The iterative process of the method starts from an arbitrary value until the estimated parameters converge to the point of maximum probability occurrence (SEARLE et al., 1992).

1.5 Genome wide association study and post-GWAS

Additionally, the estimation of genetic parameters for understanding new traits, Genome-Wide Association Study (GWAS) can be used to expand knowledge about the nature of traits by observing genomic regions responsible for phenotypic expression. This methodology is one of the benefits of genomics, a branch of science that involves the comprehensive study of the genome of a particular organism, based on DNA molecule information, including marker information, gene mapping, bioinformatics, and gene expression analysis (JORGE, 2013). This science has provided several benefits to research, and its application in animal production reflects gains in the accuracy of genetic information, a reduction in generation intervals, and consequently an increase in genetic gain, especially in traits with low heritability and costly phenotyping (MENEZES et al., 2013).

Genetic markers are capable of identifying heritable polymorphisms located in a specific DNA region, and any genetic polymorphism between two or more individuals is termed a marker (ZOLET et al., 2017). Among the markers used for studies involving cattle traits and the genome, Single Nucleotide Polymorphisms (SNPs) stand out (CAETANO, 2009). SNPs are single nucleotide polymorphisms resulting from a mutation inherited as an allelic variant (ZOLET et al., 2017). Characterization is done through DNA fragment sequencing, by comparing it with the reference base strand for the species under study. The interest in using SNPs lies in these markers being dispersed throughout the genome, capturing a large volume of information (CAETANO, 2009). Therefore, GWAS allows the identification of SNPs associated with genes that influence traits of interest; this information supports a better biological understanding of the studied trait (MAGALHÃES et al., 2016). In order to determine Quantitative Trait Loci (QTL) in linkage disequilibrium with a particular SNP (PRYCE et al., 2010).

Subsequently, with the identification of QTL regions by GWAS, the use of databases and gene networks enables the identification of candidate genes in QTL regions that explain part of the genetic variance of a particular trait, allowing for a biological understanding of the studied trait (OTTO et al., 2020). This understanding is essential because GWAS does not consider that genes act in gene networks for various biological processes (DADOUSIS et al., 2017). Thus, the enrichment of gene networks acting in different biological processes allows us to understand the mechanisms that act on the phenotype.

1.6 Senepol history

In 1918, on the island of Saint Croix, located in the Caribbean Sea region, Red Poll cattle were imported into an N'Dama herd with the aim of producing precocity, polled, docile individuals and heat tolerant with red coat. The crossbreeding between these two breeds laid the foundation for the emergence of the Senepol cattle. Around the 2000s, they arrived in Brazil and formed the largest herd of the breed today (ABCB SENEPOL, 2024).

The Senepol breed is categorized as a tropically adapted taurine breed, and in general, this breed has distinctive and beneficial traits such as high performance, docility, precocity, meat tenderness, and resistance to parasites (MENEZES et al., 2016). In addition, the Senepol breed demonstrates the ability to realized natural mating in the grazing tropical system (SILVA et al., 2018). These positive aspects make the breed an attractive option for use in crossbreeding systems or as a purebred, particularly for beef production purposes. This is especially relevant when considering that the majority of beef cattle production in Brazil is conducted in grazing systems (ABIEC, 2023).

Adaptability to heat and docility are indispensable factors for modern livestock. The rise in temperatures compels the livestock industry to seek new alternatives, such as animals more resilient to heat, to prevent declines in productivity. While, the temperament of the animals directly impacts animal welfare. Both themes are linked to sustainability, and it is essential to explore new alternatives to ensure productivity in a resilient manner towards the environment

REFERENCES

ABCB Senepol – **Brazilian Association of Senepol Breeders**. Available: <História da Raça – ABCB Senepol>. Accessed 29 Feb 2024.

ABIEC – **Brazilian beef Exporters Association**. 2023. Available: Beef Report 2023 | Capítulo 04 | A pecuária do Brasil - ABIEC. Accessed 29 Feb 2024.

ADBUCH, N. G. **Efeito do estresse térmico em caraterísticas fisiológicas, heatológicas e hormonais em bovinos da raça Caracu.** 57 f. Dissertação (Mestrado em Zootecnia) – Instituto de Zootecnia, APTA/SAA, São Paulo, Nova Odessa, 2021.

AHLBERG, C. M.; ALLWARDT, K.; BROOKS, A.; BRUNO, K.; TAYLOR, A.; MCPHILIPS, L.; KREBIEL, C. R.; LORENZO, M. K.; RICHARDS, C. J.; PLACE, S. E.; DE SILVA, U.; OVERBEKE, D. L. V.; MATEESCU, R. G.; KUEHN, L. A.; WEABER, R.; BORMANN, J.; ROLF, M. M. Characterization of water intake and water efficiency in beef cattle. **Journal of Animal Science**, p.4770-4782, 2019.

ALLAIS, S.; LEVÉZIEL, H.; PAYET-DUPRAT, N.; HOCQUETTE, J. F.; LEPETIT, J.; ROUSSET, S.; DENOYELLE, C.; BERNARD-CAPEL, C.; JOURNAUX, L.; BONNOT, A.; RENAND, G. The two mutations, Q204X and nt821, of the myostatin gene affect carcass and meat quality in young heterozygous bulls of French beef breeds. **Journal of Animal Science**, v. 88, n. 2, p.446-454, 2010.

ALDAI, N.; MURRAY, B. E.; OLIVÁN, M.; MARTINÉZ, A.; TRÓIA, D. J.; OSORO, K.; NÁJERA, A.I. The influence of breed and mh-genotype on carcass conformation, meat physico-chemical characteristics, and the fatty acid profile of muscle from yearling bulls. **Meat Science**. v. 72, p. 486-495, 2006.

AOKI, M. S.; SANTOS, A. R.; LEAL, M. L. Adaptações moleculares ao treinamento de força: recentes descobertas sobre o papel da miostatina. **Revista Mackenzie Educação Física e Esporte**. v. 7, n. 1, p. 161-167, 2008.

BACKINGHAM, M. The formation of skeletal muscle: from somite to limb. **Journal of anatomy.** v. 202, n. 1, p. 59-68, 2003.

BACKINGHAM, M & RELAIX, F. The Role of Pax Genes in the Development of Tissues and Organs: Pax3 and Pax7 Regulate Muscle Progenitor Cell Functions. **Annual Review of cell and Developmental Biology.** v. 23, p. 645-673, 2007.

BACHMANOV, A. A.; REED, D. R.; BEAUCHAMP, G. K.; TORDOFF, M. G. Food Intake, Water Intake, and Drinking Spout Side Preference of 28 Mouse Strains. **Behavior Genetics.** v. 32, n. 6, p. 435-443, 2002.

BERTIPAGLIA, E. C. A. **Efeitos das características de pelame e da taxa de sudação sobre parâmetros reprodutivos em vacas da raça Braford.** 163f. Tese (Doutorado em Medicina Veterinária) – Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, São Paulo, Jaboticabal, 2007.

BINDEA, G.; MLECNIK, H.; CHAROENTONG, P.; TOSOLINI, M.; KIRILOVSKY, A.; FRIDMAN, W. H.; PAGÉS, F.; TRAJANOSK, Z.; GALON, J. ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. **Bioinformatics**, v. 25, n. 8, p. 1091–1093, 2009.

CAETANO, A. R. Marcadores SNP: Conceitos básicos, aplicações no manejo e no melhoramento animal e perspectivas para o futuro. **Revista Brasileira de Zootecnia**, v. 38, p. 64–71, 2009.

CASAS, E.; BENNETT, G. L.; SMITH, T. P.; CUNDIFF, L. V. Association of myostatin on early calf mortality, growth, and carcass composition traits in crossbred cattle. **Journal of Animal Science**, v. 82, n. 10, p. 2913-2918, 2004.

CASAS, E.; KEELE, J. W.; FAHRENKRUG, S. C.; SMITH, T. P.; CUNDIFF, L. V.; STONE, R. T. Quantitative analysis of birth, weaning, and yearling weights and calving difficulty in Piedmontese crossbreds segregating an inactive myostatin allele. **Journal of Animal Science**, v. 77, n. 7, p. 1686-1692, 1999.

CHARLIER, C.; COPPIETERS, W.; FARNIR, F.; GROBET, L.; LEROY, P. L.; MICHAUX, C.; MNI, M.; SCHWERS, VANMASHOVEN, P.; HANSET, R.; GEORGES, M. The mh gene causing double-muscling in the cattle maps to bovine chromosome 2. **Mammalian Genome.** v. 6, n. 11, p. 788-792, 1995.

DADOUSIS, C.; PEGOLO, S.; ROSA, G. J. M.; BITTANTE, G.; CECCHINATO, A. Genome-wide association and pathway-based analysis using latent variables related to milk protein composition and cheesemaking traits in dairy cattle. **Journal of Dairy Science**. v. 100, n. 11, p. 9085–9102, 2017. Disponível em: http://dx.doi.org/10.3168/jds.2017-13219.

DE RENSIS, F.; MARCONI, P.; CAPELLI, T.; GATTI, R.; FACCIOLONGO, F.; FRANZINI, F.; SCARAMUZZI, R. J. Fertility in postpaetum dairy cows in winter or summer followinng estrus sychromization and fixed tima AI fter the induction of na LH surge whith GnRH or hCG.**Theriogenology.** v. 58, n. 9, p. 1675-1687, 2002.

DIKMEN, S. E. R. D. A. L., ALAVA, E., PONTES, E., MEDO, J. M., DIKMEN, B. Y., OLSON, T. A., & HANSEN, P. J. Differences in Thermoregulatory Ability Between Slick-Haired and Wild-Type Lactating Holstein Cows in Response to Acute Heat Stress. **Journal of Dairy Science**, v. 91; n.9, p. 3395 – 3402, 2008.

DIKMEN, S., KHAN, F. A., HUSON, H. J., SONSTEGARD, T. S., MOSS, J. I., DAHL, G. E., HANSEN, P. J. The SLICK hair locus derived from Senepol cattle confers thermotolerance to intensively managed lactating Holstein cows. **Journal of Dairy Science**. v.97, p. 5508-5520, 2014.

FALCONER D. S. & MACKAY T. F. C. 2002. **Introducción a la genética cuatitativa**. Editorial Acribia, S. A: Zaragoza.

HUSON, H. J.; KIM E. S.; GODFREY, E. S et al. Genome-wide association study and ancestral origins of the slick-hair coat in tropically adapted cattle. **Frontiers in Genetics**. v. 5, p. 1-12, 2014.

JORGE, W. A genômica bovina – origem e evolução dos taurinos e zebuinos. **Vet e Zootec.** v. 20, n. 2, p. 217-237, 2013.

MADER, T. L. Enviromental strees in confined beef cattle. **Journal of Animal Science**. v. 81, n. 14, p. 110-119, 2003.

MAGALHÃES, A. F. B.; CAMARGO, G. M. F.; FERNADES JÚNIOR, E. A.; GORDO, D. G. M.; TONUSSI, L. R. et al. Genome-Wide Association Study of Meat Quality Traits in Nellore Cattle. **PloS one**. V. 11, n. 6, p. e0157845, 2016

MARQUES, E. G. **Evolução Fenotípica da Raça Nelore na Associação Brasileira de Criadores de Zebu.** 54f. Dissertação (Mestrado em Zootecnia) – Universidade Federal de Viçosa, Minas Gerais, Viçosa, 2018.

MCLENNAN, M. O Global Risks Report 2021 16th Edition. Cology, Suíça, **World Economic Forum**. 2021.

MCFARLANE, C.; PLUMMER, E.; THOMAS, M.; HENNEBRY, A.; ASHBY, M.; LING, N.; SMITH, H.; SHARMA, M.; KAMBADUR, R. Myostatin induces cachexia by activating the ubiquitin proteolytic system through an NF-kappaB-independent, FoxO1-dependent mechanism. **Journal of Cellular Physiology.** v. 209, n. 2, p. 501-514, 2006.

MEDEIROS, R. S. GOMES, R. C.; NASCIMENTO, M. L.; ALBERTINI, T. Z.; SOUZA, A. R. D. L.; REIS, S. F.; PAULINO, P. V. R.; LANNA, D. P. Eficiência nutricional: Chave sustentável para a produção de carne bovina. In: ROSA, A. N. F. et al. (Ed.) **Melhoramento aplicado em gado de corte: Programa Embrapa Geneplus**. Brasilia: Embrapa., Campo-Grande: Embrapa Gado de Corte, p. 61-74, 2013.

MEKONNEN, M. M. & HOESTRA, A. Y. A Global Assessment of the Water Footprint of Farm Animal Products. **Ecosystems**. v. 15, n. 13, p. 401-415, 2012.

MELO, A. F.; MOREIRA, J. M.; ATAÍDES, D. S.; GUIMARÃES, R. A. M.; LOIOLA, J. L.; SARDINHA, H. C. Efeitos do estresse térmico na produção de vacas leiteiras: Revisão. **Pubvet**. v. 10, n.10, p. 721-730, 2016.

MENDOÇA, P. T.; LOPES, P. S.; BRACCINI NETO, J.; CARNEIRO, P. L. S.; TORRES, R. D. A.; GUIMARÃES, S. E. F.; VERONEZE, R. Estimação de parâmetros genéticos de uma população F2 de suínos. **Revista Brasileira de Saúde e Produção Animal**. v. 13, p. 330-343, 2012.

MENEZES, G. R. O.; REGITANO, L. C. A.; SILVA, M. V. G. B.; CARDOSO, F. F.; SILVA, L. O. C.; SIQUEIRA, F.; EGITO, A. A. Genômica aplicada ao melhoramento genético de gado de corte.

(Ed.), **Melhoramento genético aplicado em gado de corte: Programa Embrapa – Geneplus.** Brasília: Embrapa., Campo Grande: Embrapa Gado de Corte, p. 213-225, 2013.

MENEZES, G. R. O.; NOBRE, P. R. C.; TORRES JUNIOR, R. A. A.; GONDO, A.; SILVA, L. O. C.; SILVA, L. N. **Sumário de touros Senepol Embrapa Gado de Corte livro - técnico.** Brasília - DF: (INFOTECA-E). Disponível em: <http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1066876>, 2016.

NARDORNE, A.; RONCHI, B.; RANIERI, M. S.; BERNABUCCI, U. Effects of climate changes o animal production and sustainability of livestock system. Livestok Science. v. 130, n. 1-3, p. 57-69, 2010.

NUNES, E. L. M. Transição ecológica: Uma proposta baseada no comum, no mucipalismo libertário e no veganismo ablicionista. 168f. Tese (Doutorado em ciências) – Instituto de Energia e Ambiente da Universidade de São Paulo, São Paulo, 2020.

OLSON, T. A.; LUCENA, C.; CHASE, C. C.; HAMMOND, A. C. Evidence of a major gene influencing hair lenght and heat tolerance in bos taurus cattle. **Journal of Animal Science**. v. 81, n. 1, p. 80-90, 2003.

ON – **United nations**. Available: <Home - United Nations Sustainable Development>. Acesso em: 29 de Janeiro de 2024.

OTTO, P. I.; GUIMARÃES, S. E. F.; CALUS, M. P. L.; VANDENPLAS, J.; MACHADO, M. A.; PANETTO, J. C. C.; DA SILVA, M. V. G. B. Single-step genome-wide association studies (GWAS) and post-GWAS analyses to identify genomic regions and candidate genes for milk yield in Brazilian Girolando cattle. **Journal of Dairy Science**, v. 103, n. 11, p. 10347–10360, 2020.

PATTERSON, H. D. & THOMPSON, R. Recovery of Inter-Block Information when Block Sizes are Unequal. **Biometrika**. v.38, pp. 545-554, 1971.

PEREIRA, G. M.; EGITO, A. A.; GOMES, R. C.; RIBAS, N. M.; TORRES JUNIOR, R. A. A.; FERNANDES JUNIOR, J. A.; MENEZES, G. R. O. Water requirements of beef production can be reduce by genetic selection. **Animal The international journal of animal biosciences.** v. 15, n. 3, p. 100142, 2021.

POOLE, R. K., DEVINE, T. L., MAYBERRY, K. J., EISEMANN, J. H., POORE, M. H., LONG, N. M., & POOLE, D. H. Impact of slick hair trait on physiological and reproductive performance in beef heifers consuming ergot alkaloids from endophyte-infected tall fescue. **Journal of animal science**, v. 97. n.4, 1456-1467, 2019

PRYCE, J. E.; BOLORMAA, S.; CHAMBERLAIN, A. J.; BOWMAN, P. J.; SAVIN, K.; GODDARD, M. E.; HAYES, B. J. A. validated genome-wide association study in 2 dairy cattle breeds for milk production and fertility traits using variable length haplotypes. **Journal of Dairy Science**, v. 93, n. 7, p. 3331–3345, 2010.

QANBARI, S. On the Extent of Linkage Disequilibrium in the Genome of Farm Animals. **Frontiers in Genetics**, v. 10, p. 1–11, 2020.

RAN, Y.; LANNERSTAD, M.; VAN MIDDELAAR, C. E.; DE BOER, I. J. Assessing water resource use in livestock production: A review of methods. **Livestock Science**. v. 187, p. 68-79, 2016.

SEARLE, S. R., CASELLA, G., MCCULLOCH, C. E. **Variance components**. Jhon Willey & Sons, INC., Publication, 1992.

SILVA, A. L.; SATO, G. Y. P.; BORDIN, R. A.; REIS, H. M. G. A raça Senepol como opção para melhoramento genético em adaptabilidade ao meio tropical. **Tekhne e logos**. v. 9, n. 1, p. 16-30, 2018.

SIQUEIRA, F. Musculatura dupla em bovinos. In: MENEZES, G. R. O. et al. **Sumário de touros Senepol Geneplus Embrapa.** Brasília – DF: Embrapa. (ALICE), p. 28-29. Disponível em: <Sumario-Senepol-2015-2.pdf (embrapa.br) >. 2015. Acesso em: 23 de julho de 2022.

TABORDA, P. A. B. **Temperamento de vacas cruzadas Holandês-Gir: Resposta ao treinamento para a primeira ordenha e estiativa de parametros genéticos.** 91f. Tese (Doutorado em Genética e Melhoramento Animal) – Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Vetrerinárias, Jaboticabal, 2017.

TEIXEIRA, C. S.; OLIVEIRA, D. A. A.; QUIRINO, C. R. Musculatura dupla: II –Determinação genética. **Archivos Latinoamericanos de Produção Animal**. v. 14, n. 1 p. 17-23, 2006.

TEIXEIRA, B. B. M.; MACNEIL, M. D.; DA COSTA, R. F.; DIONELLO, N. J. L.; YOKKO, M. J.; CARDOSO, F. F. Genetic parameters and trends for traits of the Hereford and Braford breeds in Brazil. **Livestock Science**. v. 208, p. 60-66, 2018.

URQUIZA, A. S. C. **Avaliação e aplicação de métodos de genotipagem para estudo da síndrome da musculatura dupla em bovinos da raça Senepol.** 69 f. Dissertação (Mestrado em Zootecnia) – Universidade Estadual de Mato Grosso do Sul, Aquidauana, 2017.

WIENER, P. et al. The effects of a mutation in the myostatin gene on meat and carcass quality. **Meat Science**. v. 83, n.1, p.127-134, 2009.

WILLIAMS, J. L., GARRICK, D. J., ENNS, R. M., SHIRLEY, K. L. Inheritance of hair slickness score and its correlation with growth. **Proceedings, Western Section, American Society of Animal Science.** v. 57, p. 29 – 31, 2006.

XAVIER, S. R. **Prospecção de polimorfismos no gene da miostatina em bovinos da raça Senepol.** 60f. Dissertação (Mestrado em Ciência Animal) – Universidade Federal de Mato Grosso do Sul, Campo Grande, 2014.

ZOLET, A. C. T.; TURCHETTO, C.; ZANELLA, C. M.; PASSAIA, G. Marcadores Moleculares na Era da Genômica: Metodologias e Aplicações. **Sociedade Brasileira de Genética** - São Paulo, Ribeirão Preto, 2017.

CHAPTER 2 – SHORT COMMUNICATION: QUANTITATIVE ANALISYS OF SEVEN YEARLING TRAITS IN SENEPOL CATTLE *SLICK HAIR* CARRIER

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ABSTRACT

Senepol cattle carrying the Slick hair gene exhibit the phenotype of short and sleek hair, which consequently provides these animals with better resistance to heat stress in tropical conditions. In light of this, to assess the impact of slick hair on growth, carcass, precocity, and feed efficiency traits in Senepol cattle, records from 5,176 purebred Senepol cattle from commercial performance tests in Brazil were used. The animals were classified as carriers of *Slick hair* (SS), heterozygous (Ss), and non-carriers (ss). Regarding phenotypic results, heterozygous individuals showed superiority ($P < 0.05$) for yearling weight compared to homozygotes, which did not differ from each other ($P > 0.05$). In terms of results expressed in Expected Progeny Difference (EPD), SS individuals were superior to ss ($P < 0.05$) for ribeye area, and for yearling weight, Ss individuals were superior to SS ($P < 0.05$). Similarly, for carcass conformation score, Ss individuals exhibited superiority ($P < 0.05$) compared to others. Thus, genetic improvement programs for the Senepol breed should seek actions that promote Slick hair, aiming for adaptability to tropical conditions without compromising progress in other traits.

Keywords:

Animal breeding; Heat stress; Major gene; Rusticity; Tropical livestock

Guidelines (Livestock Science)

1. INTRODUCTION

The utilization of crossbreeding strategies between *Bos taurus taurus* and *Bos taurus indicus* cattle directly contributes to productive intensification of livestock in tropical regions (Mendonça et al., 2019). However, the raising of *Bos taurus taurus* in such conditions demonstrates limitations due to heat stress; on the other hand, genetically adapted taurine breeds can be explored as a viable alternative (Hernández et al., 2021). Additionally, in Senepol cattle, a tropically adapted breed, Olson et al. (2003) identified a major gene know as *Slick Hair* that provides better resistance to thermal stress in *Bos taurus taurus*. In Brazil, Senepol cattle homozygous without the *slick hair* gene being an eliminatory criterion for the granting of definitive genealogical registration by ABCB Senepol (MAPA, 2021).

The promoter mutation of *Slick Hair* is located on BTA 20 and consists of a single-base deletion (20:39136558 GC > G) in exon 10. The deletion introduces a premature stop codon (p.Leu462*), specifically in the prolactin receptor (PRLR), resulting in a phenotype characterized by short and sleek hair with larger sweat glands (Littlejohn et al., 2014). Such phenotypic changes promote better resilience to heat stress in cattle.

The low performance exhibited by animals under thermal stress is associated with physiological changes that modulate the organism to maintain vital functions in challenging environments (Pires et al., 2019). The expression of reproductive potential is strongly influenced by environmental conditions; conception rates in females exposed to uncomfortable temperature conditions are significantly reduced (De Rensis et al., 2002), as well as feed intake (Brown-Brandl et al., 2006), and growth in beef cattle (Mader, 2003). Thus, the thermotolerance of cattle carrying the slick mutation allows them to express their productive potential in adverse heat conditions (Olson et al., 2003). However, selection for adaptability without checking the impact of the mutation on selection criteria can be a risky practice, as studies in beef cattle have not yet quantified these effects. Therefore, this study aimed to evaluate the effect of the *slick hair* gene on growth, carcass, precocity, and feed efficiency traits in Senepol cattle.

2. MATERIAL E METHODS

2.1. Data and genetic evaluation

The dataset was provided by the Embrapa Geneplus Program and Brazilian Association of Senepol Cattle Breeders – ABCB Senepol. Records from 5,176 Senepol cattle were used, the animals genotyped using the SNP chip GGP Bovine 50K and 100K from Neogen®. Wich reports each genotype for *Slick Hair*, the animals were classified into 3,337 homozygous carriers of *Slick Hair* (SS), 1,689 heterozygotes (Ss), and 150 homozygotes free of mutation (ss). The data were obtained from a compilation of commercial performance tests coordinated by Embrapa Geneplus Program in Brazil, between 2011 and 2021. The animals had *ad libitum* access to food and water, and finished the tests with an average age of 546±87 days. The dataset contain information expressed as Expected Progeny Difference (EPD) as phenotype. These EPD values were obtained from the Brazilian Senepol Genomic Evaluation 2022 describe by ABCB - Senepol (2022).

2.2. Slick hair analysis

For the assessmet of *Slick Hair* mutation, carcass traits were collected by ultrasonography measurements in the *Longissimus dorsi* muscle between the 12th and 13th ribs: ribeye area in squared centimeters (REA), backfat thickness in millimeters (BF) and intramuscular fat expressed in percentage (IMF). In addition, in relation to carcass the score carcass conformation (SCC) described by Wenceslau et al. (2012), as a visual assessment in scores of (1 – 6) for precocity, muscularity, and structure was used. As growth trait was assessed weight (YW) expressed in kilograms (kg), and as precocity trait the scrotal circumference (SC) in centimeters (cm). Finally, as a yearling measure of feed efficiency, the residual feed intake (RFI) in kilograms was assessed (Koch et al., 1993).

Table 1.

Description of yearling phenotypes collected by traits according genotype.

Traits	Genotype				
	ss	Ss	SS	Total	CG
YW (kg)	69	758	1569	2396	255
SC (cm^2)	20	246	440	706	79
SCC (1 – 6)	67	752	1512	2331	240
REA (cm^2)	59	715	1432	2206	209
BF (mm)	55	657	1378	2090	203
IMF (%)	56	693	1414	2163	204
RFI (kg)	42	480	1001	1523	136

YW= weight, SC = scrotal circumference, SCC = score of carcass conformation, REA = ribeye area, BF = backfat thickness, IMF = intramuscular fat, RFI = residual feed intake, ss = *slick hair* homozygous non-carrier, Ss = Heterozygous, SS = homozygous carriers of *slick hair*, GC = number of contemporary group for traits.

Firstly, the data were assessed for normality and variance homogeneity using the Kolmogorov-Smirnov and Bartlett's tests, respectively. The effect of genotype on traits expressed in phenotype was evaluated through a fixed model with genotype, contemporary group containing phenotypes within ±3 standard deviations and least 3 animals for group, and the linear age of the animal as a covariate, as described below:

$$y_{ij} = \mu + \alpha_i + \beta_j + b_1\left(X_{ij} - \bar{X}\right) + e_{ij} \qquad (1)$$

Where $\boldsymbol{y_{ij}}$ is the phenotypic observation of traits, $\boldsymbol{\mu}$ is the overall mean, $\boldsymbol{\alpha_i}$ is the *ith* genotype (1: SS, 2:Ss, 3:ss), $\boldsymbol{\beta_j}$ is the j^{th} contemporary group, $\boldsymbol{b_1}$ is the linear regression coefficient for phenotype in function age on the day of measurement, $\boldsymbol{e_{ij}}$ is the random residual error

The genetic values expressed in EPD, were assessed using a fixed model, with genotype effect for double muscling, described with:

$$y_{ij} = \mu + \alpha_i + e_{ij} \quad (2)$$

Where $\boldsymbol{y_{ij}}$ is the observed value for the traits expressed in EPD, $\boldsymbol{\mu}$ is the overall mean for the EPD, $\boldsymbol{\alpha_i}$ is the i^{th} genotype (DMdm or dmdm), $\boldsymbol{e_{ij}}$ is the random residual error.

The statistical models were implemented through the analysis of variance methodology (ANOVA II) by R software (R Development Core Team, 2023), with the support of the Car package (Fox et al., 2022). In addition, the Tukey-Kramer multiple comparisons test with a significance level of 5% was used to assess significant differences between the evaluated genotypes.

3. Results and discussion

Our work was conducted to investigate the impact of the *Slick Hair* gene on several traits adopted as selection criteria in the Senepol cattle. In dairy cattle, advantages of selection based on individuals carrying the allele in question have been demonstrated. Dickmen et al. (2014), for instance, assessed different lactation periods in Holstein cows carrying the *Slick Hair* and observed that carrier animals showed lower loss on daily milk production in summer compared to winter. However, studies evaluating the effect of *Slick Hair* in beef cattle are still limited in the scientific literature.

The phenotypic results are described in Table 2, and the evaluated genotypes showed differences only for YW, with Ss demonstrating superiority ($P < 0.05$) compared to the other genotypes, which did not differ from each other.

The tropical conditions that animals were exposed allowed *Slick Hair* carriers to demonstrate better results, likely due to their enhanced ability to dissipate heat, making them less suffering under such conditions (Hernández et al., 2021). Regarding other traits expressed in phenotype, no differences were detected across genotypes ($P > 0.05$). In addition, it is important to highlight the physiological characteristics exhibited by *Slick Hair* carriers, such as lower respiratory frequency and rectal and skin temperatures compared to non-carrier animals in heat stress situations (Dickmen et al., 2014). Therefore, *Slick Hair* carriers may express their productive potential in adverse high-temperature conditions due to their increased resilience to such environmental challenges.

The results expressed in EPD, as described in Table 3, show that Ss individuals were superior ($P < 0.05$) to the other evaluated genotypes, which did not differ from each other for SCCg. A similar pattern was observed for YWg, where Ss animals exhibited higher values ($P < 0.05$) compared to SS animals, while ss animals showed intermediate values, not differing from the other genotypes.

Table 2.

Effect of *Slick Hair* mutation on yearling traits expressed in phenotype.

Traits	Genotype		
	ss	Ss	SS
YW (kg)	419.64±9.89b	431.53±2.69a	422.56±1.87b
SC (cm^2)	33.47±0.94	34.27±0.22	34.28±0.18
SCC (1 – 6)	3.88±0.16	4.14±0.05	4.00±0.03
REA (cm^2)	69.49±2.00	69.97±0.47	68.78±0.32
BF (mm)	5.34±0.39	5.91±0.11	5.83±0.08
IMF (%)	2.82±0.16	2.72±0.05	2.80±0.03
RFI (kg)	0.01±0.10	0.01±0.03	-0.03±0.02

YW= phenotype for weight, SC = phenotype for scrotal circumference, SCC = phenotype for score of carcass conformation, REA = phenotype for ribeye area, BF = phenotype for backfat thickness, IMF = phenotype for intramuscular fat, RFI = phenotype for residual feed intake, ss = *slick hair* homozygous non-carrier, Ss = Heterozygous, SS = homozygous carriers of *slick hair*. Means within the same row with different superscripts differ by Tukey-Kramer test ($\alpha = 0.05$).

Finally, for REAg, both Ss and SS animals presented higher values ($P < 0.05$) compared to ss individuals. Thus, Slick Hair animals demonstrate a better genetic potential for REAg, an important indicator of muscle deposition in the carcass. While for the other evaluated traits, there was no significant difference across genotypes ($P > 0.05$). The results obtained in this study demonstrate that the selection for Slick Hair with aim of improving thermotolerance in Senepol cattle can be conducted without adversely affecting the evaluated selection.

It is important to emphasize the climate changes that have occurred over the years and the increasing concern regarding heat stress and challenges for the beef chain. Therefore, it is essential to seek new production strategies and more resilient animals, especially in relation to taurine breeds and their respective crosses with zebu cattle (Ortiz-Colón et al., 2018). Global warming directly impacts production, reproduction, and animal welfare in beef cattle husbandry (Berman, 2011).

Table 3.

Effect of *Slick Hair* mutation on yearling traits expressed in Expected Progeny Difference (EPD).

Traits	Genotype		
	ss	Ss	SS
YWg (kg)	2.194±054ab	3.046±0.16a	1.647±0.12b
SCg (cm^2)	-0.016±0.03	-0.018±0.01	-0.077±0.01
SCCg (1 – 6)	0.066±0.01b	0.090±0.01a	0.068±0.01b
REAg (cm^2)	-0.602±0.12b	-0.124±0.03ab	-0.06±0.02a
BFg (mm)	0.065±0.03	0.050±0.01	0.030±0.01
IMFg (%)	0.009±0.001	0.007±0.01	-0.002±0.012
RFIg (kg)	0.008±0.001	0.002±0.00	-0.002±0.00

YWg= EPD for weight, SCg = EPD for scrotal circumference, SCCg = EPD for score of carcass conformation, REAg = EPD for ribeye area, BFg = EPD for backfat thickness, IMFg = EPD for intramuscular fat, RFIg = EPD for residual feed intake, ss = *slick hair* homozygous non-carrier, Ss = Heterozygous, SS = homozygous carriers of *slick hair*. Means within the same row with different superscripts differ by Tukey-Kramer test (α = 0.05).

Thus, selection based on individuals more resistant to such conditions contributes directly to improvements in meat production in tropical regions.

4. Conclusion

Heterozygous Senepol cattle carrying the *Slick Hair* gene demonstrated improvements in yearling weight, as well as a better genetic potential for ribeye area and carcass conformation. Therefore, selection processes in the Senepol breed should aim for initiatives that promote the slick hair coat, seeking adaptability to tropical conditions without adversely affecting the genetic progress of other traits.

CRediT authorship contribution statement

Christhian B. Souza: Writing – original draft, Formal analysis. **Simone E.F. Guimarães**: Resources, Conceptualization, Supervision, Writing – review and editing. **Andrea Gondo:** data preparation and editing. **Gilberto R.O. Menezes**: Formal analysis, Software, Writing – review and editing.

Declaration of Competing Interest

All authors declare that there are no conflicts of interest that could inappropriately bias this work, as action detrimental to impartiality of the research.

REFERENCES

ABCB – Senepol. The Brazilian Senepol Genomic Evaluation year 2022. URL: https://senepol.org.br/sumarios/. [Accessed 04 July 2023].

Berman, A., 2011. Invited review: Are adaptations present to support dairy cattle productivity in warm climates?. Journal of Dairy Science. 94, 2147 – 2158. https://doi.org/10.3168/jds.2010-3962.

Brown - Brandl, T. M., Nienaber, J. A., Eigenberg, R. A., Mader, T. L., Morrow, J. L., Dailey, J. W., 2006. Comparison of heat tolerance of feedlot heifers of different breeds. Livestock Science. 105, 19-26. https://doi.org/10.1016/j.livsci.2006.04.012.

De Rensis, F., Marconi, P., Capelli, T., Gatti, R.; Facciolongo, F., Franzini, F., Scaramuzzi, R. J., 2002. Fertility in postpaetum dairy cows in winter or summer followinng estrus sychromization and fixed tima AI fter the induction of na LH surge whith GnRH or hCG. Theriogenology. 58, 1675-1687. https://doi.org/10.1016/S0093-691X(02)01075-0.

Dikmen, S., Khan, F. A., Huson, H. J., Sonstegard, T. S., Moss, J. I., Dahl, G. E., Hansen, P. J., 2014.

The SLICK hair locus derived from Senepol cattle confers thermotolerance to intensively managed lactating Holstein cows. Journal of Dairy Science. 97, 5508-5520. https://doi.org/10.3168/jds.2014-8087.

Fox, J., 2022. Companion to Applied Regression. R Package Version 3.1-1. URL: https://cran.r-project.org/package=car.

Hernández, L. A. J., Nava, S. Z., Verde, O., Santini, P. L., Urdaneta, M. M., Fonseca, J. P. H., Morales, C. F., Sonstegard, T. S., Huson, H. J., Olson, T. a., 2021. Heat stress response in slick vs normal-haired Criollo Limonero heifers in a tropical environment. Tropical Animal health and production. 53, 445. https://doi.org/10.1007/s11250-021-02856-3.

Koch, R. M., Swinger, L. A., Chambers, D., Gregory, K. E., 1963. Efficiency of Feed Use in Beef Cattle. Journal of Animal Science. 22, 486-494. https://doi.org/10.2527/jas1963.222486x.

Littlejhon, M. D., Henty, K. M., Tiplady, K., Johnson, T., Lopdell, T., Sherlock, R. G., Li, W., Lukefahr, S. D., Shanks, B. C., Garrick, D. J., Russel, S. R., Spelman, R. J., Davis, S. R., 2014. Functionally reciprocal mutations of the prolactin signalling pathway define hairy and slick cattle. Nature communications. 5, 5861. https://doi.org/10.1038/ncomms6861.

Mader, T. L., 2003. Enviromental strees in confined beef cattle. Journal of Animal Science. 81,10-119, https://doi.org/10.2527/2003.8114_suppl_2E110x.

MAPA – Brazilian ministry of agriculture and livestock. Avaliação Zoogenética Senepol - Ministério da Agricultura e Pecuária (www.gov.br). [Acessed 05 January 2024].

Ortiz – Colón, G., Fain, S. J., Perés, I. K., Curbelo – Rodruíguez, J., Jiménez – Cabán, E., Pagán – Morales, M., Gould, G. A., 2018. Assessing climate vulnerabilities and adaptive strategies for resilient beef and dairy operations in the tropics. Climate Change, 146, 47-58. https://doi.org/10.1007/s10584-017-2110-1.

Olson, T. A., Lucena, C., Chase, C. C., Hammond, A. C. 2003. Evidence of a major gene influencing hair lenght and heat tolerance in bos taurus cattle. Journal of Animal Science. 81, 80-90.

https://doi.org/10.2527/2003.81180x.

Pires, B. V., Stafuzza, N. B., Lima, S. B. G. P. N. P., Negrão, J. A., Paz, C. C. P., 2019. Differential espression of heat shock protein genes associated with heat stress in Nelore and Caracu beef cattle. Livestock Science, 230, 103839. https://doi.org/10.1016/j.livsci.2019.103839.

R Development core team, 2023. R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3- 900051-07-0. https://www.R-project.org. [Accessed 21 February 2023].

Wenceslau, R. R., Felipe, V. P. S., Valente, B. D., Rosa, A. N., Nobre, P. R. C., Martin Nieto, L., Silva, M. A., 2012. Variance component estimates for weight and visual scores of slaughter conformation in Nellore cattle. Arq. Bras. Med. Vet. Zootec, 64, 443 – 449. https://doi.org/10.1590/S0102-09352012000200026.

CHAPTER 3 - EFFECT OF ONE COPY OF NT821 MUTATION IN MYOSTATIN GENE ON SEVEN YEARLING TRAITS IN SENEPOL CATTLE

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ABSTRACT

Several mutations in the myostatin gene cause double musculature in beef cattle, and several studies have indicated a significant effect of these mutations on traits used as selection criteria. In Senepol cattle this mutation is known as nt821, thus is important understand better effects of double muscle on interested traits. Records from 5.235 purebred Senepol cattle from commercial performance tests in Brazil was used. Phenotypes and Expected Difference Progeny (EPD) were assessed for Weight (YW), Scrotal Circumference (SC), Score Carcass Conformation (SCC), Ribeye Area (REA), Backfat Thickness (BF), Intramuscular Fat (IMF), Residual Feed Intake (RFI). A copy of the nt821 mutation causes losses ($P < 0.05$) for SC, BF, and IMF. On the other hand, the nt821 mutation provides better results for REA ($P < 0.05$). However, no differences were detected ($P > 0.05$) for YW, SCC, and RFI across genotyped assessed. Heterozygous animals presented a larger musculature on carcass with less subcutaneous and intramuscular fat, in addition to losses for indicators of precocity compared to the ones with no copy of the mutation.

Keywords:

Animal breeding; Meat quality; Myostatin; *GDF-8*

Guidelines (Livestock Science)

1. INTRODUCTION

The double musculature (DM) is responsible for increased hypertrophy and hyperplasia of muscle fibers, resulting from a genetic mutation in the *GDF-8* gene (Growth Differentiation Factor 8), that coding myostatin responsible for muscle hypertrophy (Mcpherron and Lee, 1997). Several distinct mutations have been identified that explain the increased muscling in cattle, Xavier (2014) showed that DM in Senepol cattle in Brazil is due to a mutation that leads to an 11 – base pair deletion in the gene, known as nt821 mutation in exon III, which truncated inactive protein.

As an alternative to tropical meat production systems, the Senepol breed, known as a tropically adapted taurine *(Bos taurus taurus)*, demonstrates several interesting traits for meat production, such as high performance, precocity and meat tenderness (Schatz et al., 2020). In addittion, Senepol cattle bulls can naturally mate in tropical pasture conditions (Sosa et al., 2021), faciliting their use in crossbreeding strategies with *Bos taurus indicus*.

However, the occurrence of DM has been reported in the Senepol breed since its arrival in Brazil in the year 2000, being an eliminatory criterion for the granting of definitive genealogical registration by ABCB Senepol (MAPA, 2021).

According to Casas and Kehrli (2016) concerning the phenotype, DM animals demonstrate higher muscular hypertrophy, dystocic deliveries and lower concentration of carcass fat. In addition, there is intolerance to stress, susceptibility to respiratory disease and macroglossia in newborn calf (Webb and Casey, 2010), as well as delayed development of the reproductive organs and reduced fertility (Hoflack et al., 2006). However, shown improvements in growth traits (Casas et al., 2004). Thus, its essential assess the effects of one copy nt821 mutation on selection criteria for Senepol cattle to support decisions in selection process, according to each goals.

Since 2018, Senepol breeders in Brazil have had a genomic test available that determines if the individual carries the nt821 mutation, discriminating if is free homozygous, heterozygous or carrier homozygous (ABCB – Senepol, 2018). Thus, this study aimed to evaluate the effect of one copy of nt821 mutation in myostatin gene on traits linked to growth, precocity, feed eficiency, carcass and meat quality in Senepol cattle.

2. MATERIAL AND METHODS

2.1. Data

Dataset was provided by Embrapa Geneplus Program and Brazilian Association of Senepol Cattle Breeders – ABCB Senepol. Records from 5,235 purebred Senepol individuals (*Bos taurus taurus*) were used, the animals were genotyped using the GGP Bovine 50K and 100K SNP chip from Neogen®, which reports each genotype for DM, the distribution of the animals in this study are shows in table 1. The data were obtained from a compilation of commercial performance tests coordinated by Embrapa Geneplus Program in Brazil, between 2011 and 2021. The animals had *ad libitum* access to food and water, and finished the tests with an average age of 546±87 days.

Table 1.

Description of genotyped population according to sex and genotype for nt821 mutation.

Genotype	Sex		
	Female	Male	Total
dmdm	2926	1448	4374
DMdm	547	314	861
Total	3473	1762	5235

dmdm = No copy of nt821 mutation, DMdm = One copy of nt821 mutation.

2.2. Traits

The yearling traits evaluated in this study were recorded at the end of the tests. The carcass traits were collected by ultrasonography measurements in the *Longissimus dorsi* muscle between the 12th and 13th ribs: ribeye area in squared centimeters (REA), backfat thickness in millimeters (BF) and intramuscular fat expressed in percentage (IMF). In addition, in relation to carcass the score carcass conformation (SCC) described by Wenceslau et al. (2012) as a visual assessment in scores of (1 – 6) for precocity, muscularity, and structure was used. As growth trait was assessed weight (YW) expressed in kilograms (kg), and as precocity trait the scrotal circumference (SC) in centimeters (cm). Finally, as a yearling measure of feed efficiency, the residual feed intake (RFI) in kilograms per day was assessed. In addition to phenotypic data, expected progeny difference (EPD) for all individuals and traits were used. The EPDs, were obtained from the 2022´s Brazilian Senepol Genomic Evaluation conducted by Embrapa Geneplus Program and Brazilian Senepol Breeders Association (Embrapa Geneplus, 2022). Using the BLUPF90 *software* (Misztal et al., 2002), with Single-Step GBLUP methodology (Aguilar et al., 2010).

2.3. Double muscling analysis

For the assessment of the nt821 mutation on traits of interest, the quality control for phenotypic dataset were realized by contemporary group with ±3 standard deviation and least 3 animals for group. Table 2 shows the collected phenotypes in this study according to genotype.

To analyze the effect of genotype on phenotypes, a fixed model was assessed, with effects of genotype for DM, contemporary group, and as a linear covariate age of the animal on the day of measurement:

$$y_{ij} = \mu + \alpha_i + \beta_j + b_1 \left(X_{ij} - \bar{X}\right) + e_{ij} \qquad (1)$$

Where $\boldsymbol{y_{ij}}$ is the phenotypic observation of traits, $\boldsymbol{\mu}$ is the overall mean, $\boldsymbol{\alpha_i}$ is the i^{th} genotype (DMdm or dmdm), $\boldsymbol{\beta_j}$ is the j^{th} contemporary group, $\boldsymbol{b_1}$ is the linear regression coefficient for phenotype in function age on the day of measurement, $\boldsymbol{e_{ij}}$ is the random residual error.

The genetic values expressed in EPD, were assessed using a fixed model, with genotype effect for double muscling, described with:

$$y_{ij} = \mu + \alpha_i + e_{ij} \qquad (2)$$

Where $\boldsymbol{y_{ij}}$ is the observed value for the traits expressed in EPD, $\boldsymbol{\mu}$ is the overall mean for the EPD, $\boldsymbol{\alpha_i}$ is the i^{th} genotype (DMdm or dmdm), $\boldsymbol{e_{ij}}$ is the random residual error.

Table 2.

Description of yearling phenotypes collected by traits according genotype.

Traits	Genotype			
	dmdm	DMdm	Total	CG
YW (kg)	2332	428	2760	364
SC (cm)	601	132	733	83
SCC (1-6)	2025	359	2384	246
REA (cm^2)	1917	343	2260	216
BF (mm)	1819	325	2144	210
IMF (%)	1872	341	2213	210
RFI (kg)	1328	218	1546	138

YW= yearling weight, SC = scrotal circumference, SCC = score carcass conformation, REA = ribeye area, BF = backfat thickness, IMF = intramuscular fat, RFI = residual feed intake, dmdm = No copy of nt821 mutation, DMdm = One copy of nt821 mutation, GC = number of contemporary group for traits.

Analyses were performed using the R software (R Development Core Team, 2023) with the support of Car packages (Fox et al., 2022) and Agricolae packages (Mendiburu, 2021). Data were tested for normality and homogeneity of variance using the Kolmogorov-Smirnov test and Bartlett's test, respectively. Thus, models 1 and 2 were implemented through the analysis of variance methodology (ANOVA II). In addition, the Tukey-Kramer multiple comparison test at 5% significance level was used to compare significant differences between genotypes.

3. RESULTS

Significant differences were observed across the genotypes evaluated for all carcass traits (Table 3). The dmdm animals demonstrated superiority ($P < 0.05$) for IMF, BF and SC to DMdm. While for REA, DMdm expressed higher values ($P < 0.05$) compared to dmdm individuals. As for the results for RFI, SCC, and YW, no significant differences were observed across the evaluated genotypes (P > 0.05).

Table 3.

Effect of mutation nt821 on yearling traits expressed in phenotype.

Traits	Genotype	
	dmdm	DMdm
YW (kg)	423.885±1.53	428.978±3.62
SC (cm)	34.449±0.15a	33.169±0.32b
SCC (1-6)	4.023±0.03	4.091±0.07
REA (cm^2)	68.525±0.27b	72.561±0.70a
BF (mm)	5.923±0.07a	5.214±0.15b
IMF (%)	2.837±0.02a	2.376±0.05b
RFI (kg)	-0.014±0.02	-0.067±0.04

YW= phenotype for weight, SC = phenotype for scrotal circumference, SCC = phenotype for score carcass conformation, REA = phenotype for ribeye area, BF = phenotype for backfat thickness, IMF = phenotype for intramuscular fat, RFI = phenotype for residual feed intake, dmdm = No copy of nt821 mutation, DMdm = One copy of nt821 mutation. Means within the same row with different superscripts differ by Tukey-Kramer test (α = 0.05).

Results for EPDs obtained in the current study showed the same trend as the results expressed in phenotype described in Table 3. Therefore, on average, the progenies of dmdm individuals are expected to express better results ($P < 0.05$) for SC, BF, and IMF in relation to DMdm progenies. However, the genotypes no differ ($P > 0.05$) in genetic values for YWg, RFIg, and SCCg, similar to phenotypic results. On the other hand, DMdm individuals showed higher values ($P < 0.05$) for REA$_g$.

Table 4.

Effect of mutation nt821 on yearling traits expressed in Expected Progeny Difference (EPD).

Traits	Genotype	
	dmdm	DMdm
YWg (kg)	1.985±0.11	2.801±0.22
SCg (cm)	-0.004±0.01a	-0.090±0.02b
SCCg (1-6)	0.073±0.01	0.085±0.01
REAg (cm^2)	-0.247±0.02b	0.640±0.05a
BFg (mm)	0.066±0.01a	-0.102±0.01b
IMFg (%)	0.007±0.01a	-0.030±0.01b
RFIg (kg)	-0.001±0.00	0.003±0.00

YWg= EPD for weight, SCg = EPD for scrotal circumference, SCCg = EPD for score carcass conformation, REAg = EPD for ribeye area, BFg = EPD for backfat thickness, IMFg = EPD for intramuscular fat, RFIg = EPD for residual feed intake, dmdm = No copy of nt821 mutation, DMdm = One copy of nt821 mutation. Means within the same row with different superscripts differ by Tukey-Kramer test (α = 0.05).

4. DISCUSSION

In the current study, we evaluated the effect of the nt821 myostatin mutation in Senepol purebred animals raised in tropical conditions. Thus, this mutation influenced all carcass traits evaluated by ultrasonography, in which dmdm individuals demonstrate better BF and IMF expressed in both phenotypic and EPD evaluations. Therefore, DM reflects qualitative losses in the final product, since that DM animals have lower carcass fat deposition. These results corroborate Wiener et al. (2009), which analyzed 146 South Devon cattle and observed that individuals with DM showed a reduction in BF. As well as the results obtained by Aldai et al. (2006), with Asturiana de los Valles and Asturiana de la Montaña breeds, observed dmdm individuals had higher values in BF and IMF compared to DMdm. In this work, the authors also demonstrated that DMdm animals express superiority in BF and IMF when compared to individuals homozygous for double muscle (DMDM). Regarding REA, Sellick et al. (2007) demonstrated that DM cattle are superior to mutation-free individuals, corroborating the results obtained in our study.

According to Silva et al. (2017), carcasses with lower levels of BF exposed to low temperatures in the cold chambers suffer from *Cold Shortening* inducing hardness meat, while IMF avoids losses in softness and darkening of meat. Given this, studies such as Wiener et al. (2009) evaluated the effect of DM on the quality of the carcass and beef and observed that the DM carriers produce meat with lower juiciness and flavor compared to mutation-free animals. In adition, it is worth mentioning that BF is an indicative of sexual precocity, and individuals with greater fat deposition reach desired levels of carcass fat coverage at younger ages, reducing age at slaughter and reproduction (Suguisawa et al., 2013). Thus, DM animals have more difficulty in depositing fat and causes higher protein and energy requirements compared to free-mutations animals for beef fattening (Campeneere et al., 2001). So, DM animals can implies additional costs and time in process preparation for slaughter industry.

On the other hand, the results for REA demonstrate that DMdm animals show higher values in both phenotype and EPD. The use of REA as a selection criterion that aims to evaluate the muscle of the carcass for select superior animals for muscling to improve the proportion of noble cuts (Bertrand et al., 2001). Since DM directly affects myogenesis regulation, carrier individuals have amplified the growth potential of all body muscles. However, no difference was observed for visual conformation analysis (SCC) evaluated in this study. According to Wenceslau et al. (2012), SCC is the average of the score attributed from 1 to 6 for muscling, precocity, and structure, being the criterion used to select individuals with harmonic carcasses between these three analyzed variables. Concerning growth traits, there was no significant difference across the genotypes evaluated for YW, so the selection for dmdm Senepol cattle did not confer losses in performance compared to selection based on the DMdm animals. Our results are also similar with Casas et al. (2004), that not observed significant differences across the genotypes for post-weaning weight gain.

Used as a selection criterion in beef cattle SC serves as indication of sexual precocity. Bulls with higher SC values tend to produce daughters that conception at a younger age (Eller et al., 2010). In this study, dmdm Senepol cattle expressed higher values for SC as EPD and phenotype. Therefore, selection for non-carrier DM animals improves sexual precocity, as individuals DMdm demonstrate lower values for SC and BF, that are the main criteria used as indicative of precocity. Hoflack et al. (2006), in their evaluation of reproductive traits and semen quality in DM Belgian Blue, demonstrated that 93.7% of bulls failed the reproductive soundness at 13.4 months compared to 59.3% for non-carrier DM Holstein Frisian animals. These DM animals showed qualitative losses related to sperm mortality and morphology. Ancient studies already demonstrated the negative effect of DM on reproductive traits, as Michaux and Hanset (1981) reported problems of genital infantilism as long males as females.

Regarding the feed efficiency RFI, described by Koch et al. (1963) to identify animals with lower nutritional requirements to achieve certain gains, the RFI results obtained, expressed both as phenotypes and as EPDs showed no significant differences across the evaluated genotypes. Therefore, the DM mutation does not influence selection to improve feed efficiency in the Senepol breed. This differs from the results obtained in Piemontese cattle for feed conversion by Short et al. (2002), in which DM carriers presented better results for feed efficiency compared to non-carriers.

Important to emphasize that the Senepol breed in the tropics is used in crossbreeding systems with zebu cattle to produce individuals with superior meat quality. Thus, double muscling may be identified in crossed animals, affecting the traits as described here; so selection of the bulls to be used in such crossing schemes should be based on individuals free from the nt821 mutation.

5. CONCLUSION

A copy of the nt821 mutation in Senepol cattle impairs precocity losses and fat deposition in the carcass. However, it provides carcasses with better muscle deposition and does not affect performance and feed efficiency.

CRediT authorship contribuition statement

Christhian B. Souza: Writing – original draft, Formal analysis. **Simone E.F. Guimarães**: Resources, Conceptualization, Supervision, Writing – review and editing. **Gilberto R.O. Menezes**: Formal analysis, Software, Writing – review and editing.

Declaration of Competing Interest

All authors declare that there are no conflicts of interest that could inappropriately bias this work, as action detrimental to impartiality of the research.

REFERENCES

ABCB Senepol - The Brazilian Senepol Genomic Evaluation year 2019. Sumário de Touros Senepol 2018. [Acessed 05 Jenuary 2024].

ABCB Senepol - The Brazilian Senepol Genomic Evaluation year 2019. sumario-de-femeas-senepol-pmgs-2019_2020.pdf. [Acessed 07 February 2023].

ABCB Senepol – Brazilian Senepol Breeders Association. História da Raça - ABCB Senepol [Acessed 05 march 2023].

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, 743–52. https://doi.org/10.3168/jds.2009-2730.

Aldai, N., Murray, B. E., Oliván, M., Martínez, A., Troy, J. D., Osoro, K., Nájera, I. A., 2006. The influence of breed and mh-genotype on carcass conformation, meat physico-chemical characteristics, and the fatty acid profile of muscle from yearling bulls. Meat Science. 72, p. 486-495.

https://doi.org/10.1016/ j.meatsci.2005.08.016.

Bertrand, J. K., Verde, R. D., Hering, W. O., Moser, D. W., 2001. Genetic evaluation for beef carcass traits. Journal of Animal Science. 79, 190-200. https://doi.org/10.2527/jas2001.79E-SupplE190x.

Campeneere, S., Fiems, L. O., Boucqué, V., 2001. Energy and protein requirements of Belgian Blue double-muscled bulls. Animal feed science and technology. 90, 153-167. https://doi.org/10.1016/S0377-8401(01)00217-6.

Casas, E., Bennett, G. L., Smith, T. P. L., Cundiff, L. V., 2004. Association of myostatin on early calf mortality, growth, and carcass composition traits in crossbred cattle. Journal of Animal Science. 82, 2913-2918. https://doi.org/10.2527/2004.82102913x.

Casas, E., Kehrli Jr, M. E., 2016. A Review of Selected Genes with Known Effects on Performance and Health of Cattle. Frontiers in Veterinary Science. 3, 113. https://doi.org/10.3389/fvets.2016.00113.

Chalier, C., Coppieters, W., Famir, F., Grobet, L., Leroy, P. L., Michaux, C., Mni, M., Schwers, A., Vanmanshoven, P., Hanset, R., Georges, M., 1995. The mh gene causing double-muscling in the cattle maps tobovine chromosome 2. Mammalian Genome. 6, 788-792. https://doi.org/10.1007/BF00539005.

Eler, J. P., Ferraz, J. B. S., Teixeira, L. A., 2010. Selação para precocidade sexual em novilhas. In: Pires, A. V. (Eds.), Bovinocutulra de corte. FEALQ., Piracicaba, 2, pp. 801-811.

Embrapa Geneplus Program, 2022. The Brazilian Senepol Genomic Evaluation year 2022. GP Plus ON - Estatísticas Senepol (geneplus.com.br). [Acessed 27 February 2023].

Fox, J., 2022. Companion to Applied Regression. R Package Version 3.1-1. URL: https://cran.r-project.org/package=car.

Grobet, L., Royo, L. J., Poncelet D., Pirottin, D., Brouwers, B., Riquet, J., Schoeberlin, A., Dunner, S., Ménissier, F., Massanbanda, J., Fries, R., Hanset, R., Georges, M.,1997. A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nature genetics. 17, 71-74.

https://doi.org/10.1038/ng0997-71.

Hoflack, G., Somm, V. A., Maes, D., Kruif, A., Opsomer, G., Duchateau, L., 2006. Breeding soundness and libido examination of Belgian Blue and Holstein Friesian artificial insemination bulls in Belgium and The Netherlands. Theriogenology. 66, 207-216. https://doi.org/10.1016/j.theriogenology.2005.11.003.

Koch, R. M., Swinger, L. A., Chambers, D., Gregory, K. E., 1963. Efficiency of Feed Use in Beef Cattle. Journal of Animal Science. 22, 486-494. https://doi.org/10.2527/jas1963.222486x.

Mcpherron, C. A., Lee, S., 1997. Double muscling in cattle due to mutations in the myostatin gene. Proc. Natl. Sci, USA 94,12457-12461. https://doi.org/10.1073/pnas.94.23.12457.

Mendburu, F., 2021. Statistical Procedures for Agricultural Research. R Package Version 1.3-5. URL: https://cran.r-project.org/package=agricolae.

MAPA – Brazilian ministry of agriculture and livestock. Avaliação Zoogenética Senepol — Ministério da Agricultura e Pecuária (www.gov.br). [Acessed 05 January 2024].

Misztal, I., Tsuruta, S., Strabel, T., Auvray, B., Druet, T., Lee, D. H., 2002. BLUPF90 and related programs (BGF90). In: World congress on genetics applied to livestock production. Proceedings. Montpellier: INRA CIRAD. pp.19-23.

Michaux, C., Hanset, R., 1981. Sexual Development of Double-Muscled and Conventional Bulls. Journal of Animal Breeding and Genetics. 98, 29-37. https://doi.org/10.1111/j.1439-0388.1981.tb00326.x.

R Development core team, 2023. R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3- 900051-07-0. https://www.R-project.org. [Accessed 21 February 2023].

Schatz, T., Tomás, S., Reed, S., Hearnden, M., 2020. Crossbreeding with a tropically adapted Bos taurus breed (Senepol) to improve meat quality and production from Brahman herds in Northern

Australia. 1. Steer performance. Animal Production Science. 60, 487 - 491. https://doi.org/10.1071/AN18609.

Short, R. E., ManNeil, M. D., Groz, M. D., Gerrard, D. E., Grings, E. E., 2002. Pleiotropic effects in Hereford, Limousin, and Piedmontese F2 crossbred calves of genes controlling muscularity including the Piedmontese myostatin allele. Journal of Animal Science. 80, 1 – 11. https://doi.org/10.2527/2002.8011.

Sellick, G. S., Pitchford, W. S., Moris, C. A., Cullen N. G., Crawford, H. W., Raadsma, H. W., Bottema, C. D. K., 2007. Effect of myostatin yield carcass in cattle. Animal Genetics. 38, 440-446. https://doi.org/10.1111/j.1365-2052.2007.01623.x.

Silva, L. H P., Paulino, P. V. R., Assis, G. J. F., Assia, D. E. F., Estrada, M. M., Silva, J. C., Martins, T. S., Valadares Filho, S. C., Paulino, M. F., Chizzotti, M. L., 2017, Effect of post-weaning growth rate on carcass traits and meat quality of Nellore cattle. Meat Science. 123, 192-197. https://doi.org/10.1016/j. meatsci.2016.10.005.

Sosa, F., Carmickle, A. T., Jiménez-Cabán, E., Ortega, M. S., Dikmen, S., Negrón-Pérez, V., Jannaman, E. A., Baktula, A., Rincón, G., Larson, C. C., Pagán-Morales, M., Denicol, A. C., Sonstegard, T. S., Hansen, P. J., 2021. Inheritance of the SLICK1 allele of PRLR in cattle. Animal Genetics. 52, 887 – 890. https://doi.org/10.1111/age.13145.

Suguisawa, L., Matos, B. C., Suguisawa, J. M., 2013. Uso da ultrassonografia na avaliação de características de carcaça e de qualidade de carne, In: Rosa, A. N. F., Martins, E. N., Silva, L. O. C., (Ed.), Melhoramento genético aplicado em gado de corte: Programa Embrapa – Geneplus. Campo Grande, Brazil, pp. 97-107.

Webb, E. C., Casey, N. H., 2010. Physiological limits to growth and the related effects on meat quality. Livestock Science. 130, 33-40. https://doi.org/ 10.1016/j.livsci.2010.02.008.

Wiener, P., Lãs, J. A., Frank –Lawane, A., Ryan, M., Richardson, R. I., Nute, G. R., Madeira, J. D., Homero, D., Williams, J. L., 2009. The effects of a mutation in the myostatin gene on meat and carcass quality. Meat Science. 83, 127-134. https://doi.org/10.1016/j.meatsci.2009.04.010.

Wenceslau, R. R., Felipe, V. P. S., Valente, B. D., Rosa, A. N., Nobre, P. R. C., Martin Nieto, L., Silva, M. A., 2012. Variance component estimates for weight and visual scores of slaughter conformation in Nellore cattle. Arq. Bras. Med. Vet. Zootec, 64, 443 – 449. https://doi.org/10.1590/S0102-09352012000200026.

CHAPTER 4 – GWAS AND POST-GWAS FOR WATER EFFICIENCY IN BEEF CATTLE

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Guidelines (Genetic Selection Evolution)

Abstract

Background

The need to produce in an environmentally resilient manner drives new research aiming to achieve sustainability in beef production systems. The water footprint of beef is a concern that needs improvement, and the inefficiency of water use within the production chain is alarming. One way to contribute is through the possibility of conducting genetic selection for water efficiency in beef cattle populations. However, it is essential to understand the genetic architecture and mechanisms involved in the expression of this phenotype to support the choice of selection criteria. Thus, the aim of our study was to estimate genetic parameters for water efficiency measures, conduct a genome-wide association study (GWAS), and identify genetic networks and biological processes involved.

Results

A population of 1762 purebred Senepol cattle was phenotyped for water intake (WI). The evaluated water efficiency measures included gross water efficiency (GWE), water conversion ratio (WCR), residual water intake based on average daily gain (RWI_{ADG}), and residual water intake based on dry matter intake (RWI_{DMI}). A subset of 1342 animals was genotyped, and the heritability values found for WI, GWE, WCR, RWI_{ADG}, and RWI_{DMI} were 0.36±0.06, 0.26±0.05, 0.22±0.05, 0.24±0.05, and 0.20±0.05, respectively. Phenotypic correlations between average daily gain (ADG) and the residuals RWI_{DMI} and RWI_{ADG} are zero, unlike the raw measures; all water efficiency measures are moderately to highly correlated with each other. We conducted genome-wide association studies (GWAS) to estimate the effect of 79860 single nucleotide polymorphisms (SNPs), and only WCR showed significant SNPs. The assessment of gene enrichment in the significant regions reveals various biological processes involved, such as saliva production, water transport, renal system, and immune system

Conclusions

Genetic selection for water efficiency traits in Senepol cattle is feasible and can reduce the water requirements of meat production. Water efficiency measures are of polygenic nature, and various biological processes act simultaneously for the expression of the phenotype.

1. BACKGROUND

Political and social pressures lead to new challenges for the beef production chain, such as sustainable development goals of the United Nations (1), strategies for enteric methane mitigation in ruminants (2), deforestation for new cultivable areas (3), use of natural resources and water footprint (4). Therefore, the need to produce environmentally resilient way drives new research in order to promote sustainable production (5). Changes in production systems have been studied and implemented over the years, as integrated crop-livestock-forestry system (6), conscious use of livestock waste to produce biogas, biofertilizer and bioenergy (7, 8), grazing management (9) and others.

The identification of environmental impact selection criteria can directly contribute to sustainability process in the beef chain. Therefore, it is extremely important to study the genetic architecture of possible environmental impact traits by estimation of (co)variance components to assess the feasibility of genetic selection. As the use of feed efficiency measures has been begun by breeding programs, obtaining significant genetic gains over the years in cattle (10, 11, 12). As well as, studies with enteric methane emission traits (13,14).

Another point of notable environmental concern is the conscious use of water by agricultural and livestock sectors, therefore, it is essential improve the efficiency of freshwater use in animal husbandry (15). Given this, it is extremely important to significantly reduce the water footprint of animal origin products, the term refers to total volume of water used to produce a given product, on a global mean 15.415 to 15.497 l/kg for beef (4). According (16), the value of the water footprint beef is considered high in relation to others agricultural sector. Further, studies have shown that different breed types and genders shows differences in WI (17), as well weather change, feed management and body weight demonstrated effect in beef cattle for WI (18, 19). On the other hand, can be differences between similar animals by genetics effects (20), thus the identification of more efficient individuals in using water to express certain levels of production, encouraged new studies for WI traits in beef cattle (21, 22). Both studies demonstrated genetic variability for water intake (WI) measurements.

The use of genomic data in estimating genetic values enabled genome-wide association studies (GWAS), capable of identifying SNPs associated with quantitative trait loci (QTL) (23). Thus, the identification candidate genes in QTL regions and subsequent application of post-GWAS study to evaluate genes found through genetic networks in cellular components, molecular functions and biological processes with goal clarify the genetic mechanisms of traits (24). Thus, the aim of this work was to estimate genetic parameters, perform GWAS and post-GWAS for WI traits in Senepol cattle, a tropically adapted taurine breed.

2. METHODS

2.1 Phenotyping and Description of the traits

For this study, no ethical approval was required because we did not conduct any experiments on animals. Pedigree data related to 5.314 purebred Senepol cattle (*Bos taurus taurus*) were used, provided by the Brazilian Association of Senepol Breeders and the Embrapa Geneplus Beef Cattle Breeding Program. Phenotype data includes information from 2.751 individuals referring to the study population, divided into males (n = 1.199) and females (n = 1.552) who started the tests at an average age and weight of 561±64 days and 474.11±124 kg for males, while the females 487±68 days and 378.95±77 kg, respectively. The phenotypes were obtained from a compilation of twelve commercial performance tests conducted on the Santo Antônio da Grama farm, Pirajuí, São Paulo, Brazil (21° 59′ S; 49° 27′ W), between 2016 and 2022. To form groups, the animals were divided into males or females and a maximum range of 90 days. Thus, the animals were housed in collective pens over approximately 70 days, plus a minimum of 14 days of adaptation period, with access *ad libitum* to water and total mixed ration.

The individual records for water intake and feed intake, were obtained by electronic feeders and electronic water bins within the Intergado system (Intergado Ltd., Contagem, Minas Gerais, Brasil) (25). Data from all valid test days provided information on water intake (WI l/day) and dry matter intake (DMI, kg/day), with a minimum of 35 days valid for each animal. The difference between fasting weighing at the beginning of the test and end of the tests divided by the number of days, yielded average daily gain (ADG). The average metabolic weight ($BW^{0.75}$), was calculated as the mean between the initial and final weight to the power of 0.75. Using these variables, water efficiency and feed efficiency measures were computed. The feed conversion ratio (FCR, DMI/ADG), gross feed efficiency (GFE, ADG/DMI), water conversion ratio (WCR, WI/ADG), gross water efficiency (GWE, ADG/WI x 100) and residual feed intake (RFI) by Koch et al. (26).

Table 1 Descriptive statistical of all traits in Senepol cattle

Traits	Mean ± SD	Minimum	Maximum	Phenotype number
GWE	4.24±1.33	0.21	9.81	1603
GFE	0.142±0.04	0.05	0.40	2367
WCR	26.143± 12.43	8.59	149.07	1589
FCR	7.912±4.25	2.26	56.40	2346
RFI	0.141±0.72	-3.10	3.82	2368
RWI_{ADG}	0.138±3.33	-10.74	11.61	1602
RWI_{DMI}	-0.463±4.30	-24.31	12.23	1502
WI	29.44±7.74	11.64	55.84	1762
DMI	8.322±1.60	2.74	15.73	2580
ADG	1.604±0.42	0.04	2.87	2522
$BW^{0.75}$	343.0±0.43	162.90	590.70	2522

GWE gross water efficiency, *GFE* gross feed efficiency, *WCR* water conversion ratio, *FCR* feed conversion ratio, *RFI* residual feed intake, *RWI_{ADG}* residual water intake based on ADG, *RWI_{DMI}* residual feed intake based on DMI, *WI* average daily water intake, *DMI* average daily feed intake, *ADG* average daily weight gain, $BW^{0.75}$ mid-test metabolic body weight.

Additionally, residual water intake based on ADG (RWI_{ADG}), estimated as the residual of linear regression equation of WI on ADG and $BW^{0.75}$ (22). The residual water intake based on DMI (RWI_{DMI}), estimated as the residual of the linear regression equation of WI on DMI and $BW^{0.75}$ (21). The regression models used for measure RFI, RWI_{ADG} and RWI_{DMI} were adjusted separately for each group. The GWE was multiplied by 100 to adjust the scale the results that exhibited very small numbers. The quality control for phenotypes, included contemporaneous groups (CG) with a minimum 3 animals and records outside the range of ±3.5 standard deviation (SD) from CG, resulting 109 CGs at the end. The CGs were defined as test group, farm of origin. Data manipulation was performed by R 4.3.1 *software* (27), and descripted statistical are provided in Table1.

2.2 Genotype data

A subset 1342 Senepol cattle was genotyped with the GGP Bovine 50K and 100K SNP Chip (Neogen GeneSeek Operations, Lincoln, NE). Using Beagle *software* (28), the 50K SNP genotypes were then impute to 100K with on based to positions *Bos taurus* ARS-UCD 1.2 genome reference. In order to remove variants with low imputation accuracy, only SNPs with a $R^2 > 0.3$ and a MAF > 2% were retained. The quality control for genomic data was performed by BLUPF90+ *software* (29). Included autosomal SNPs only, and samples with *call rate* < 90% were removed, as well as SNPs with *call rate* < 90% or minor allelic frequency (MAF) < 5%, and SNPs with deviation of Hardy-Weinberg equilibrium > 0.15 were removed. 79860 SNPs remained for further analysis.

2.3 Estimating genetics parameters

The (co)variance components was obtained by restricted maximum likelihood (REML), using the average information algorithm (AI-REML) and combining genomic information and pedigree via *Single-step*GBLUB (30), by BLUPF90+ s*oftware*. Bivariate analyses were chosen due to difficulty of estimating a positive definite matrix. An animal model was used that included direct additive genetics and residual effect as random and the contemporary group as fixed effects, in addition to the linear covariate of initial weight animal nested CG, was used. The model described below was used for each pair of traits:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

Where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ are vectors of phenotypic measurements of traits 1 and 2; $\boldsymbol{b_1}$ e $\boldsymbol{b_2}$ are vectors of fixed effects; $\boldsymbol{a_1}$e $\boldsymbol{a_2}$ are vectors of random direct additive genetic effect, $\boldsymbol{a} \sim NMV\ (\boldsymbol{0}, \boldsymbol{H} \otimes\ \boldsymbol{G_0})$, and $\boldsymbol{H}$ is the relationship matrix that combine pedigree and genomic information and $\boldsymbol{G_0}$ is the matrix of genetic variance and (co)variance between traits; $\boldsymbol{e_1}$e $\boldsymbol{e_2}$ are vectors of random residual effects, $\boldsymbol{e} \sim NMV\ (\boldsymbol{0},\ \boldsymbol{I} \otimes\ \boldsymbol{R_0})$ and $\boldsymbol{R_0}$ is the matrix of residual variance and (co)variance between traits; $\boldsymbol{X_1}$ and $\boldsymbol{X_2}$ are design matrices of fixed effects; $\boldsymbol{Z_1}$ and $\boldsymbol{Z_2}$ are design matrix of random additive genetics effects.

The genetic correlations were obtained based on estimating (co)variance, while heritability values for each trait were calculated as a mean using SDs from each analyses as weights. Finally, the SD of heritabilities were obtained thought a simple mean across the SDs estimated in each bivariate analyze for a given trait.

2.4 Genomic wide association

To execute the GWAS, single-step GWAS methodology was used (31), by BLUPF90+ and postGSf90 *software* (32). A single-trait animal model was implemented using the same effects adopted in the estimation of the (co)variance components, as described below:

$$y = X_\beta + Z_\alpha + e$$

Where $\boldsymbol{y}$ is the vector of phenotypic measurements of trait; $\boldsymbol{\beta}$ is the vector of fixed effects; $\boldsymbol{\alpha}$ is the vector of random direct additive genetic effect, $\boldsymbol{\alpha} \sim N\ (\mathbf{0}, \boldsymbol{H} \otimes\ \boldsymbol{G_0})$; $\boldsymbol{e}$ is the vector of random residual effects, $\boldsymbol{e} \sim N\ (\mathbf{0},\ \boldsymbol{I} \otimes\ \boldsymbol{R_0})$; $\boldsymbol{X}$ is the design matrices of fixed effects; $\boldsymbol{Z}$ is the design matrix of random additive genetics effects.

To account for multiple tests, Bonferroni threshold was used which verify significant SNPs. The p-value was determined as 0.05/79860, equivalent to 6.2 on –log10 scale. The results obtained in the GWAS were plotted in the form of a Manhattan plot using the R 4.3.1 *software* (27).

2.5 QTL regions and candidate genes

QTL regions were defined base on the localization of significant SNPs discovered in GWAS and linkage disequilibrium (LD) expressed in r^2 (33). For compute LD between SNPs, the PLINK 1.9, was used. With r^2 values obtained, the LD decay was plotted by R *software* with support ggplot2 and dplyr packages (34, 35). The value 0.16 for LD discovered with distance of 50kb between SNPs, thus QTL region was defined in 50kb upstream and downstream from each significant SNP. Potential candidate genes were listed based on *Bos taurus* ARS-UCD 1.2 genome reference using genome browser (https://www.ensembl.org/Bos_taurus/Info/Index). Additionally, were selected QTLs previously associated with traits of interest in cattle described in Animal QTL database (36).

2.6 Biologic process network

The list of genes associated with the traits was investigated through by VarElct NGS Phenotyper (37). The toll VarElect (https://varelect.genecards.org/), prioritizes genes based on the phenotype of interest through shared pathways, interaction networks, and paralogous genes (38). Thus, VarElect can increase or decrease the list of genes associated with the traits of interest. Subsequently, all listed genes were enrichment in gene ontology (GO) terms, using the plug-in ClueGO 2.5.10 (39) for Cytoscape 3.10.0 (40).Were used a selection of levels 1 to 5 of the GO hierarchy, a gene set was considered to be enriched if the $P < 0.05$ associated with the hypergeometric test using *Bonferroni* correction. GO terms were clustered in functional groups if the Kappa statistic > 0,4 and the percentage of associated genes > 10% in each biologic process.

3. RESULTS

3.1 Genetics parameters

The heritability estimates ($\boldsymbol{h^2}$) for water and feed efficiency traits are presented in Table 3. The $\boldsymbol{h^2}$ values range from 0.16 to 0.19 for feed efficiency traits, while values obtained for water efficiency traits range from 0.20 to 0.26, highlighting the feasibility of genetic selection for water efficiency traits. The average daily gain (ADG) showed a moderate $\boldsymbol{h^2}$ of 0.30±0.04, and the genetic and phenotypic correlations between ADG and water and feed efficiency traits are described in the table 2. However, efficiency measures based on equation residuals showed a null phenotypic correlation. The observed values for genetic correlations between these measures and ADG ranged from 0.29 to 0.37.

The water intake (WI) demonstrated a high genetic (0.85) and phenotypic association (0.60) with dry matter intake (DMI). While the genetic correlations between WI and water efficiency measures range from -0.13 to 0.91, and phenotypic correlations range from -0.25 to 0.47, the genetic correlations between DMI and water efficiency measures vary from -0.38 to 0.47, and phenotypic correlations range from -0.19 to 0.47. Regarding the gross water efficiency (GWE), strong genetic associations were detected with gross feed efficiency (GFE) and feed conversion ratio (FCR) of 0.83 and -0.63, respectively. Similarly, for water conversion ratio (WCR), the genetic correlations with GFE and FCR were -0.66 and 0.91, respectively. The genetic (0.70) and phenotypic correlation (0.17) estimates between residual feed intake (RFI) and residual water intake adjusted for ADG (RWI_{ADG}) were higher in relation to the genetic (0.43) and phenotypic correlations (0.04) obtained between RWI_{DMI} and

RFI. Although these water efficiency measures demonstrate a high genetic association of 0.95 and a phenotypic association of 0.60.

Table 2 Phenotypic and genetic correlations between average daily gain (ADG) with feed and water efficiency traits.

Traits	ADG	
	Phenotypic (±SE)	Genetic (±SD)
GWE	0.64±0.02	0.73±0.08
GFE	0.80±0.01	0.93±0.12
WCR	-0.60±0.02	-0.57±0.11
FCR	-0.67±0.01	-0.74±0.08
RFI	0.01±0.02	0.33±0.15
RWI_{ADG}	0.02±0.02	0.29±0.15
RWI_{DMI}	0.01±0.02	0.37±0.17
WI	0.53±0.02	0.64±0.09
DMI	0.40±0.02	0.81±0.06

GWE gross water efficiency, *GFE* gross feed efficiency, *WCR* water conversion ratio, *FCR* feed conversion ratio, *RFI* residual feed intake, RWI_{ADG} residual water intake based on ADG, RWI_{DMI} residual feed intake based on DMI, *WI* average daily water intake, *DMI* average daily feed intake, *SD* standart deviation, *SE* standart error.

Table 3 Heritabilities (±SD) in the diagonal, genetic correlations above (±SD) and phenotypic correlations below (±SE) between feed and water efficiency traits.

	GWE	GFE	WCR	FCR	RFI	$\mathrm{RWI}_{\mathrm{ADG}}$	$\mathrm{RWI}_{\mathrm{DMI}}$	WI	DMI
GWE	0.26±0.05	0.83±0.07	-0.80±0.08	-0.63±0.14	-0.16±0.18	-0.34±0.17	-0.28±0.18	-0.05±0.18	0.33±0.15
GFE	0.73±0.02	0.19±0.04	-0.66±0.12	0.79±0.08	0.03±0.19	0.13±0.19	0.23±0.20	0.45±0.14	0.57±0.12
WCR	-0.78±0.01	-0.66±0.02	0.22±0.05	0.91±0.05	0.26±0.19	0.28±0.18	0.19±0.20	-0.13±0.17	-0.38±0.15
FCR	-0.61±0.02	-0.76±0.01	0.92±0.01	0.13±0.03	0.18±0.20	-0.09±0.21	-0.25±0.22	-0.40±0.16	-0.38±0.16
RFI	-0.25±0.02	-0.25±0.02	0.15±0.02	0.17±0.02	0.16±0.04	0.70±0.12	0.43±0.20	0.64±0.12	0.82±0.05
$\mathrm{RWI}_{\mathrm{ADG}}$	-0.38±0.02	-0.09±0.02	0.24±0.02	0.04±0.02	0.17±0.02	0.24±0.05	0.95±0.03	0.89±0.03	0.47±0.13
$\mathrm{RWI}_{\mathrm{DMI}}$	-0.12±0.02	-0.08±0.03	0.10±0.03	-0.02±0.02	0.04±0.02	0.60±0.02	0.20±0.05	0.91±0.05	0.30±0.17
WI	-0.25±0.02	0.06±0.02	0.26±0.02	-0.24±0.02	-0.02±0.02	0.47±0.02	0.15±0.02	0.36±0.06	0.85±0.05
DMI	-0.06±0.02	0.01±0.01	-0.19±0.01	0.08±0.02	0.47±0.02	0.21±0.02	-0.14±0.02	0.60±0.01	0.27±0.04

GWE gross water efficiency, *GFE* gross feed efficiency, *WCR* water conversion ratio, *FCR* feed conversion ratio, *RFI* residual feed intake, RWI_{ADG} residual water intake based on ADG, RWI_{DMI} residual feed intake based on DMI, *WI* average daily water intake, *DMI* average daily feed intake.

3.2 QTLs regions and associated genes

For feed efficiency traits, the Manhattan plot are in Additional file 1; however, significant SNPs were only found for (FCR), as illustrated in the Manhattan plot [see Additional file 1]. The QTL regions were defined based on the position of significant SNPs (–log10 *P-value* > 6.20) with 50 Kb upstream and 50 Kb downstream according to the linkage disequilibrium decay, as detailed in the supplementary file [see Additional file 2]. This approach led to the identification of four genes associated with FCR, as described in Table 4.

Table 4 Associated genes with significantly SNPs for feed conversion ratio (FCR).

-log10(*P*-value)	Snp	BTA	Position (pb)	Gene symbol	Gene name
7,482	39943	11	68137353	ANXA4	annexin A4
7,482	39943	11	68137353	GMCL1	germ cell-less 1, spermatosis associated
6,776	75656	27	15758723	PDLIM3	PDZ and LIM domain
6,776	75657	27	15810792	SORBS2	sorbin and SH3 domain containing 2

Based on the results reported in CattleQTLdb, 20 QTLs were previously documented in the same regions as the ANXA4 and GMCL1 genes identified in this study [see Additional file 3: Table S1] for FCR. The majority of these QTLs are associated with milk production and type traits in dairy cattle, but none are directly related to FCR. Regarding the growth trait ADG, no significant SNPs were found, as well as for the raw measures of DMI and WI. Concerning water efficiency traits, significant SNPs were found only for WCR (–log10 *P-value* > 6.20), as shown in Figure 1. In the QTL regions where the SNPs were identified, 20 genes were found in these regions, as described in Table 5. Additionally, in these identified regions, 67 QTLs were previously reported in CattleQTLdb [see Additional file 3: Table S2]. In general, these QTLs are related to milk production, reproduction, growth, meat and carcass quality, and type traits. However, none SNP was simultaneously significant for both FCR and WCR.

Table 5 Associated genes with significantly SNPs for water conversion ratio (WCR).

-log10(*P*-value)	Snp	BTA	Position (pb)	Gene symbol	Gene name
8,278	51939	15	76150043	PHF21A	PHD finger protein 21A
7,690	72810	25	22622802	ARHGAP17	Rho GTPase activating protein 17
6,870	10008	3	27397345	SLC22A15	solute carrier family 22 member 15
6,870	10009	3	27454599	NHLH2	nescient helix-loop-helix 2
6,472	22496	6	58912805	PDS5A	PDS5 cohesin associated factor A
6,286	22590	6	61733082	GRXCR1	glutaredoxin and cysteine rich domain containing 1
6,457	27671	7	107496188	FBXL17	F-box and leucine rich repeat protein 17
6,225	29245	8	48217731	C8H9orf85	chromosome 8 C9orf85 homolog
6,490	29938	8	71655186	ADAMDEC1	ADAM-like, decysin 1
6,490	29938	8	71655186	ADAM7	ADAM metallopeptidase domain 7
6,298	47472	14	21479119	RB1CC1	RB1 inducible coiled-coil 1
6,647	50864	15	43015289	SWAP70	switching B cell complex subunit SWAP70
6,543	50936	15	45066548	OVCH2	ovochymase 2
6,543	50936	15	45066548	CYB5R2	cytochrome b5 reductase 2

6,543	50936	15	45066548	PPFIBP2	PPFIA binding protein 2
6,211	51516	15	63530260	PRRG4	proline rich and Gla domain 4
6,211	51516	15	63530260	QSER1	glutamine and serine rich 1
6,307	79825	29	49641551	TNNT3	troponin T3, fast skeletal type
6,307	79825	29	49641551	PRR33	proline rich 33
6,307	79825	29	49641551	LSP1	lymphocyte specific protein 1

Due to small number of genes identified for FCR, only the genes related to WCR were used in subsequent ontology analyses. The genes associated with WCR were investigated using Varlect with the keywords "water" and "conversion." Thus, based on the 20 annotated genes, the Varlect tool indirectly associated additionally 61 protein-coding genes. This resulted in a total of 81 genes for further analyses. It is important to note that considering only the 20 genes found in the GWAS is not sufficient for enriching GO terms, for that ClueGO statistical parameters had been respected.

3.3 Biologic process and molecular function

The ClueGo enrichment results for cellular components demonstrated that the related genes generally function in the membrane [see Additional 4]. The genes were grouped into three main clusters, with the cellular component containing the highest number of genes being the apical part of the cell, including 12 genes, followed by apical plasma membrane (n = 8), nuclear transcription factor complex (n = 6), RNA polymerase II transcription factor complex (n = 6), basolateral plasma membrane (n = 5), plasma membrane raft (n = 4), and caveola (n = 3).

The results of molecular functions are illustrated in Figure 1. The genes were enriched in 20 GO terms with an average of 4 genes, grouped into 4 functional groups: Water channel activity, activating transcription factor binding, disordered domain-specific binding, and protease binding. The percentage of genes associated with the molecular functions glycerol transmembrane transporter activity and polyol transmembrane transporter activity was 100%, while water channel activity showed that 44.44% of annotated genes were associated. For water transmembrane transporter activity, a value of 40% was observed. The molecular function with the highest number of associated genes was RNA polymerase II transcription factor binding, which includes 7 genes.

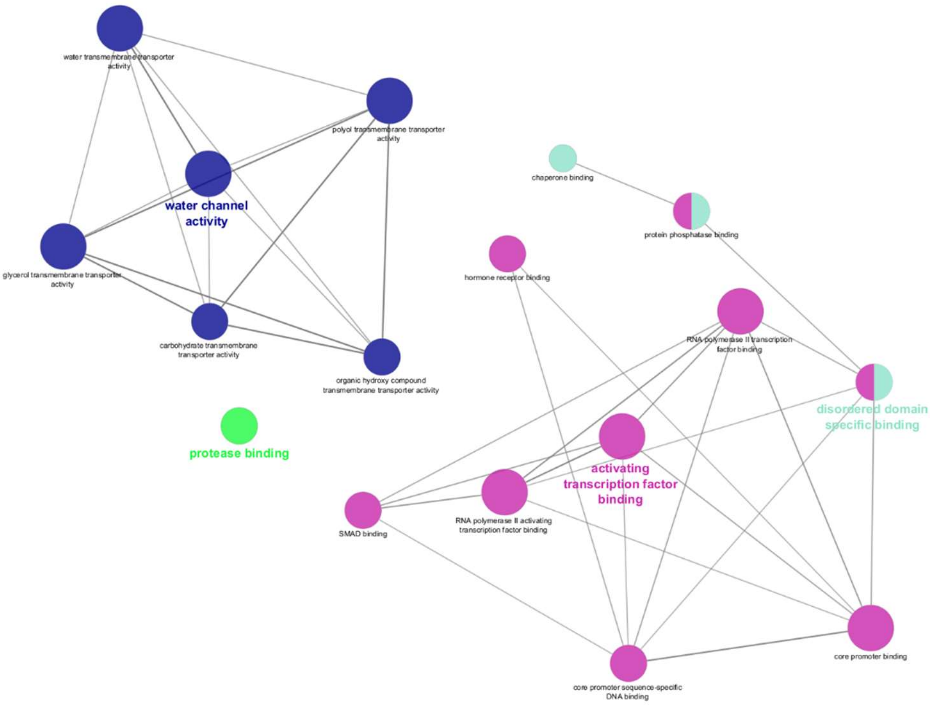
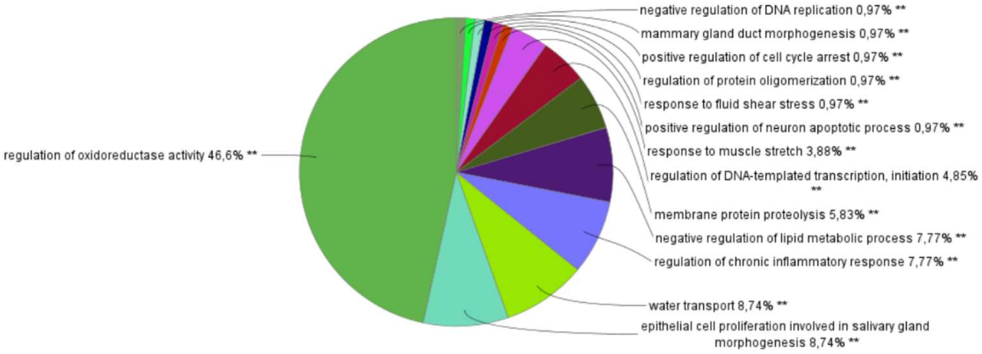


Fig. 1 Network interaction between GO terms from molecular function for WCR.

The results obtained for biological processes are illustrated in Figure 3, and the interaction networks between the processes are shown in Figure 3. On average, the 103 GO terms found contained 17 genes and were grouped into 13 functional groups, summarized in Figure 2. The biological processes containing the highest number of genes were the negative regulation of lipid metabolic process, including 7 genes. The water transport process was the significantly most enriched process ($P - value$ = 2.3E-09), followed by the significantly enriched fluid transport process ($P - value$ = 6.6E-09), and the largest functional group was represented by 48 GO terms.

Fig. 2 Percentage of GO terms by functional groups for WCR.



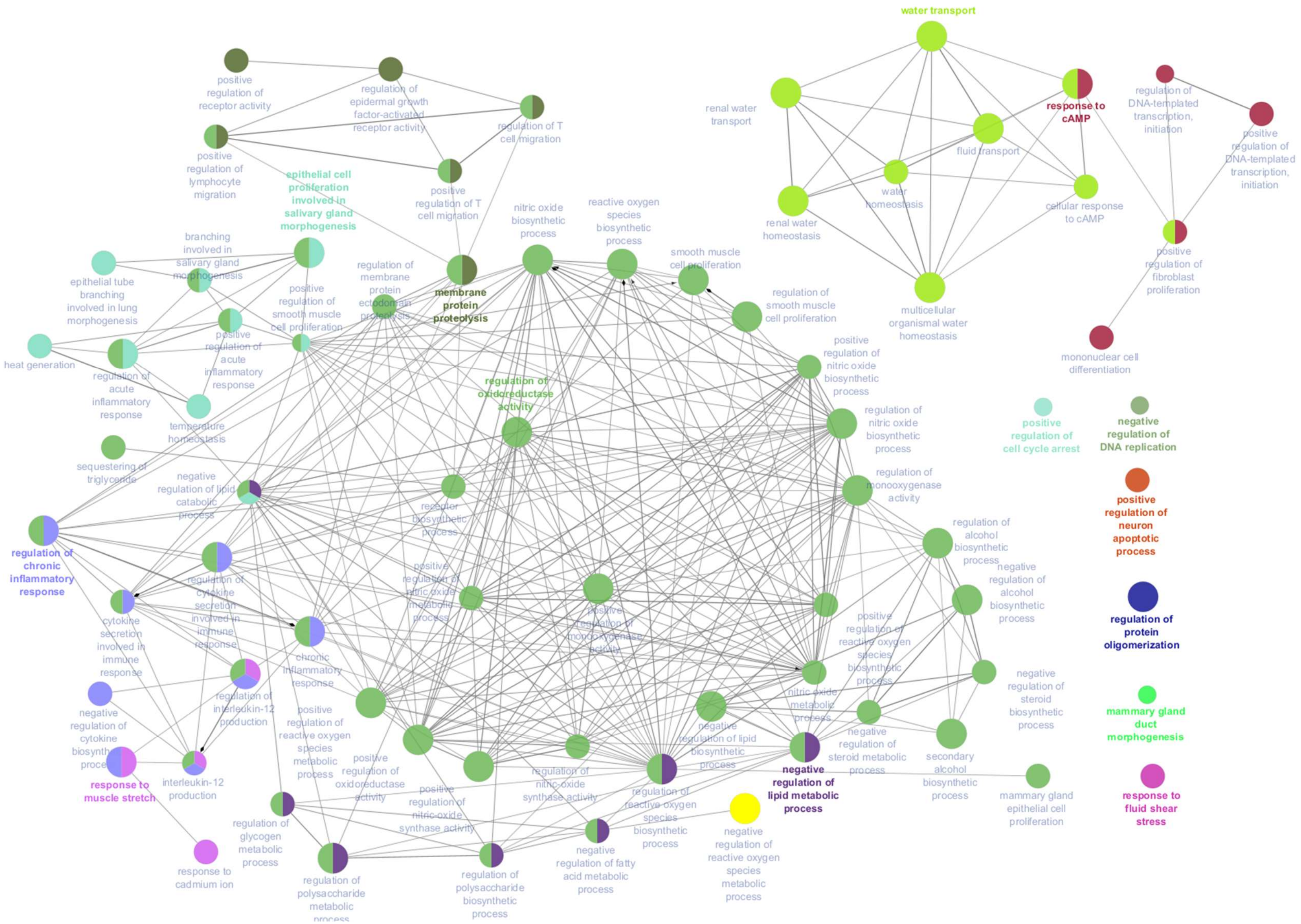


Fig 3. Network interaction between GO term for biologic process from WCR.

4. DISCUSSION

4.1 Genetics parameters

In our study, we report parameter estimates for water efficiency traits in beef cattle. The moderate heritability values for WI and water efficiency measures demonstrate the feasibility of selection based on these traits and confirm previous findings (21, 22). Thus, it is possible to include water efficiency traits in beef cattle selection processes, with aim to increase the efficiency of freshwater use by beef cattle and consequently reduce the water footprint of beef over the years. The raw water efficiency measures, GWE, and WCR showed a high genetic association with the evaluated growth trait ADG. In other words, selection based on these two traits may result in individuals with reduced growth, following the same principle as feed efficiency measures. However, concerning RWI_{ADG} and RWI_{DMI} the estimated genetic correlations were moderate, making them more easily exploitable in breeding programs without compromising animal performance. Moreover, these measures are phenotypically independent of growth, similar to RFI. When handled correctly and combined with other selection criteria and used in selection indices, these traits can be effective.

The high genetic (0.91) and phenotypic (0.60) association between WI and DMI indicates that selection for one can directly impact the expression of the other. As suggested by Pereira et al. (21), it is possible to select for water efficiency to achieve correlated genetic gains for feed efficiency, because one Intergado System's automatic water drinker, for a same period of time, can evaluate 35 animals while an electronic feed bin only eight. Thus, collecting phenotypes for WI incurs lower costs and allows testing more individuals in the same space and time compared to measuring feed intake. Various factors affect WI in cattle, but the genetic literature is still scarce, with only two studies evaluating different water efficiency measures (21, 22). However, these studies were limited to genetic parameters only, while our study progressed to GWAS and post-GWAS, providing a better understanding of the genetic mechanisms involved in the traits through the underlying biological processes.

4.2 Genome wide association

Only significant SNPs (–log10 *P-value* > 6.20) were found for WCR and FCR. Thus, the other traits exhibit a highly polygenic nature, aligning with the theory of the infinitesimal model for quantitative traits. While other studies identified significant SNPs for RFI, ADG, and DMI (41, 42), these markers explain only a small portion of the additive genetic variance and are scattered throughout the genome, influencing various biological processes. Despite advancements in animal genetic breeding, the databases used in feed efficiency research are still relatively small and lack robustness, leading to genomic predictions of low accuracy (43). Therefore, caution is recommended in interpreting the results.

In general, feed efficiency is controlled by multiple genes of small effect involved in various biological processes (41). The nature of these traits makes it challenging to identify QTL regions, and the measures of water efficiency may follow the same principle. Both measures are highly correlated (Table 3), indicating that common genes are simultaneously influencing both traits. Consequently, no significant SNPs were found in our study for GWE, WI, RWI_{ADG}, and RWI_{DMI}. Recently, (44) investigated water intake behavioral traits using GWAS methodology and found significant SNPs of small effect for several traits, demonstrating the polygenic nature of these traits related to water intake, consistent with our results. However, as described in Table 4, our study identified four genes in flanking regions of significant SNPs for FCR. The ANXA4 gene encodes a major calcium-dependent phospholipid-binding protein that plays a role in type II alveolar cells. Additionally, calcium ions participate in the light exchange regulation of photoreceptor cells, and the effects of calcium in these processes are mediated by proteins such as annexin 4 (45, 46). However, its relationship with feed efficiency is not clear and straightforward.

On the other hand, the GMCL1 gene encodes a protein involved in the process of spermatogenesis. However, ANXA4 and GMCL1 are in a QTL region that influences various type traits, milk production, and reproduction in dairy cattle (47). The PDLIM3 gene is involved in cytoskeleton assembly and is present in bovine skeletal muscle (48), and it has been found in a QTL region for intramuscular fat by Hay and Roberts (49), in a composite breed of beef cattle. As well as, SORBS2 that can be explained by the genetic association between FCR and marbling (50). Additionally, both were found by Silva (51) and associated with RFI. Another interesting fact is that SORBS2 acts in skeletal muscle cells (52), and skeletal muscle metabolism is positively correlated with basal energy expenditure (53). This is one explanation for the animals have been ability to be more efficient, as they require fewer nutrients for maintenance (54).

While for WCR, genes were found on various chromosomes and enriched in diverse biological processes. Therefore, the new trait in question demonstrates a highly polygenic nature, with several low-effect genes influencing WCR. The SLC22A15 gene is responsible for transporting organic ions and functions in transmembrane transporter activity, as described in GeneCards (55), playing a role in solute transport such as carnitine (56). This is interesting because carnitine is involved in ATP generation (57), and ATP generation is directly linked to animal performance (58). It is important to note that ADG is the denominator of WCR and show high genetic association with WCR.

In Varlect, SLC22A15 and CYB5R2 were associated with aquaporin precursor genes that facilitate water transport through the water channel. In this way, the presence of these genes may facilitate water transport and its utilization by cells. In plants, aquaporins confer resistance to water stress (59), and it is possible that, in a similar manner, the small effects of these genes enable animals to perform with lower water intake and demonstrate greater resistance through osmotic adjustment. The genes SWAP70, RB1CC1, ADAM7, and LSP1 have functions related to the immune system, as described in GeneCards (55), similar to results describes for Dressler et al. (44) where the authors also reported different genes associated with the immune system. Therefore, animal health can directly affect water consumption and its respective efficiency measures. As well as, water intake is used as an indicator of animal health (60).

4.3 Biologic process

For a better understanding of the biological functions of all identified genes, we conducted analyses of enrichment for biological processes and molecular functions. This work allowed us to confirm the effects of genes found in GWAS and gain a better understanding of the mechanisms involved in WCR. Firstly, the focus will be on molecular functions. The network of molecular functions for WCR was enriched for water channel activity, responsible for fluid transport across the plasma membrane carried out by aquaporins, maintaining cellular balance (61). In the digestive system, water transport is fundamental to ensuring the proper functioning of digestive and absorptive functions of nutrients, and transmembrane water channel proteins are crucial for this process (62). In addition, reports by Xu et al. (59) indicating that the same water channel proteins confer greater resistance to water stress, maintain osmotic balance, and improve ion distribution. Another molecular function was activating transcription factor binding, playing a role in DNA binding for transcriptional regulation (63). Transcription factors can repress or induce gene expression (64), and their presence may be related to the organism ability to adapt to environmental stimuli such as the diet provided to the animals (65). Some transcription factors are directly linked to the modulation of the organism in situations of water stress (66).

The enriched networks of biological processes for WCR are linked to the molecular functions in which the identified genes are involved. Concomitantly, the networks of biological processes were enriched for water transport, fluid transport, water homeostasis, and renal water transport. In general, water balance results from water intake and excretion via the urinary system, mainly dependent on the processes of reabsorption and filtration related to water transport (67). The network was also enriched for biological processes linked to DNA transcription, which is connected to the transcription mechanism, and response to cAMP, resulting in a change in the metabolism state or activity, such as gene expression (68). These processes act as initial physiological responses to external stimuli (69).

The network highlighted the biological process of membrane protein proteolysis, where proteolysis in general, promotes the maintenance of cellular functions, ensuring that the membrane performs its functions (70). It is essential to note that many biological processes and molecular functions highlighted for WCR occur on or depend on the membrane. Processes related to the salivary gland were enriched, such as epithelial cell proliferation involved in salivary gland morphogenesis and branching involved in salivary gland morphogenesis. Saliva is one of the means of water loss for animals, its production is linked to water consumption and plays a fundamental role in the digestive process of food (71). Losses associated with saliva and sweat production can represent about 18% (72).

In this context, our study also enriched processes related to heat stress and temperature homeostasis, which are related to sweat production, evapotranspiration, and the consumption of water to cope with physiological stress (60). Water consumption is the quickest way to reduce body temperature in thermal stress situations, as observed in studies like Arias and Mader (73), where animals consumed almost twice as much water in summer (32.4±0.13 L/day) compared to winter (17.3±0.08 L/day). Similarly, Meyer et al. (74) observed in dairy cows that for each increase in ambient temperature expressed in degrees Celsius, water intake increased by 1.52 kg/day.

Some processes related to the immune system were enriched for WCR network, such as the regulation of chronic inflammatory response. The inflammation process is the primary mechanism for eliminating infectious agents and tissue repair, ultimately aiming for the restoration of health (75). As mentioned previously, animal health is closely related to water intake (60). As well as, behavioral traits of water intake are affected by genes related to the immune system (44). The network also enriched the process response to muscle stretch, which is related to postnatal growth (76). Growth, in turn, is one of the factors that influence the water needs of the animal; growing individuals consume more water, and water restriction can compromise the body weight of animals in the growth phase (60).

The network enriched another process: negative regulation of lipid metabolic process. An interesting fact is that animals with better feed efficient tend to demonstrate a lower percentage of fat in the carcass, lower EGS and MAR (54). As described in our results, feed efficiency measures show a moderate to high genetic and phenotypic association with WCR (Table 3). Huang et al. (77) found differentially expressed genes in adipose tissue between Wagyu and Holstein in their study, and after enrichment, the network also presented the biological process lipid metabolism process. As the Wagyu breed is known for its high capacity for fat deposition, unlike Holstein cattle specialized in milk production, this finding clearly highlights the role of this process among others in phenotype expression. Therefore, it is interesting for new studies to explore water efficiency measures with carcass traits and meat quality. Our results show that WCR is associated with the process regulation of oxidoreductase activity, among various other processes involving oxidoreductases. Oxidoreductases are a group of enzymes responsible for catalyzing the transfer of electrons from one molecule to another (78). Among these enzymes, oxidase is responsible for catalyzing oxygen and reducing it to water (79). These processes may contribute to the production of metabolic water, which contributes to body hydration, in addition to directly ingested water and water present in the feed (60).

5. Conclusions

The water efficiency traits demonstrate that can be explored by genetic selection with the goal of producing more sustainably. Measures based on the residuals of equations show better due to their relationship with ADG, as well as RFI. Although all traits can be explored with the help of selection indices, and all water efficiency traits show genetic associations with feed efficiency traits, and can be implemented with a view to using correlated response.

All water efficiency measures exhibit a polygenic nature. The water conversion ratio, the only enriched trait evaluated at the level of biological processes, demonstrates that, just like multiple genes influence it, several biological processes linked to saliva production, heat stress, water transport, imune sistem, lipid metabolism and oxidoreductase activity are involved with WCR.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AG performed imputation and genotype data preprocessing. PVBR performed phenotype data preprocessing. CBS performed analyses of genetic parameters, GWAS, post-Gwas and network analyses, and original draft. GROM and SEFG performed conceptualization, supervision, writing – review and editing.

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REFERENCES

1. UN: The United Nations. sustenable development goals. Home - United Nations Sustainable Development.

2. Ribeiro Pereira LG, Machado FS, Campos MM, Guimaraes Júnior R, Tomich TR, Reis LG, et al. Enteric methane mitigation strategies in ruminants: a review. Rev Colomb Cienc Pecu. 2015;28(2):124–43.

3. Doggart N, Morgan-Brown T, Lyimo E, Mbilinyi B, Meshack CK, Sallu SM, et al. Agriculture is the main driver of deforestation in Tanzania. Environ Res Lett. 2020;15(3):034028.

4. Ran Y, Lannerstad M, Herrero M, Van Middelaar CE, De Boer IJM. Assessing water resource use in livestock production: A review of methods. Livest Sci. 2016;187:68–79.

5. Rosa AN, Menezes GRO, Egito A. Recursos genéticos e estratégias de melhoramneto. In :Rosa AN, Martins EN, Menezes GRO, Silva LOC. Melhoramento Genético Aplicado em Gado de Corte: Programa Geneplus-Embrapa. Embrapa; 2013.

6. Alves BJR, Madari BE, Boddey RM. Integrated crop–livestock–forestry systems: prospects for a sustainable agricultural intensification. Nutr Cycl Agroecosystems. 2017;108(1):1–4.

7. Guares SA, Lima JD de, Oliveira GA. Techno-economic model to appraise the use of cattle manure in biodigesters in the generation of electrical energy and biofertilizer. Biomass Bioenergy. 2021;150:106107.

8. Lu J, Gao X. Biogas: Potential, challenges, and perspectives in a changing China. Biomass Bioenergy. 2021;150:106127.

9. Cardoso A da S, Barbero RP, Romanzini EP, Teobaldo RW, Ongaratto F, Fernandes MHM da R, et al. Intensification: A Key Strategy to Achieve Great Animal and Environmental Beef Cattle Production Sustainability in Brachiaria Grasslands. Sustainability. 2020;12(16):6656.

10. American Angus Association. 2024. Genetic Trend EPD/$Value by Birth Year (angus.org). Acesseed 15 Jan 2024.

11. Bolormaa S, MacLeod IM, Khansefid M, Marett LC, Wales WJ, Nieuwhof GJ, et al. Evaluation of updated Feed Saved breeding values developed in Australian Holstein dairy cattle. JDS Commun. 2022;3(2):114–9.

12. Bouquet A, Fouilloux MN, Renand G, Phocas F. Genetic parameters for growth, muscularity, feed efficiency and carcass traits of young beef bulls. Livest Sci. 2010;129(1):38–48.

13. Lassen J, Løvendahl P. Heritability estimates for enteric methane emissions from Holstein cattle measured using noninvasive methods. J Dairy Sci. 2016;99(3):1959–67.

14. Pickering NK, Chagunda MGG, Banos G, Mrode R, McEwan JC, Wall E. Genetic parameters for predicted methane production and laser methane detector measurements1. J Anim Sci. 2015;93(1):11–20.

15. Nardone A, Ronchi B, Lacetera N, Ranieri MS, Bernabucci U. Effects of climate changes on animal production and sustainability of livestock systems. Livest Sci. 2010;130(1):57–69.

16. Mekonnen MM, Hoekstra AY. A Global Assessment of the Water Footprint of Farm Animal Products. Ecosystems. 2012;15(3):401–15.

17. Brew MN, Myer RO, Hersom MJ, Carter JN, Elzo MA, Hansen GR, et al. Water intake and factors affecting water intake of growing beef cattle. Livest Sci. 2011;140(1):297–300.

18. Ahlberg CM, Allwardt K, Broocks A, Bruno K, McPhillips L, Taylor A, et al. Environmental effects on water intake and water intake prediction in growing beef cattle1,2. J Anim Sci. 2018;96(10):4368–84.

19. Water In: Nutrient Requirements of Beef Cattle, 8th Revised Edition. Washington, D.C.: National Academies Press; 2016. p. 153-159.

20. Menezes GRO, Gomes RC, Ribas MN, Junior JAF, Pereira GM, Fávero R, et al. Genetic and phenotypic parameters for feed and water efficiency in Senepol cattle. 2. Cooperation, networking and global interactions in the animal production sector; Bryant J et al, 2018.

21. Pereira GM, Egito AA, Gomes RC, Ribas MN, Torres Junior RAA, Fernandes Junior JA, et al. Water requirements of beef production can be reduced by genetic selection. Animal. 2021;15(3):100142.

22. Ahlberg CM, Allwardt K, Broocks A, Bruno K, Taylor A, Mcphillips L, et al. Characterization of water intake and water efficiency in beef cattle1,2. J Anim Sci. 2019;97(12):4770–82.

23. Wang H, Misztal I, Aguilar I, Legarra A, Muir WM. Genome-wide association mapping including phenotypes from relatives without genotypes. Genet Res. 2012;94(2):73–83.

24. Verardo LL, Silva FF, Varona L, Resende MDV, Bastiaansen JWM, Lopes PS, et al. Bayesian GWAS and network analysis revealed new candidate genes for number of teats in pigs. J Appl Genet. 2015;56(1):123–32.

25. Oliveira BR, Ribas MN, Machado FS, Lima JAM, Cavalcanti LFL, Chizzotti ML, et al. Validation of a system for monitoring individual feeding and drinking behaviour and intake in young cattle. Animal. 2018;12(3):634–9.

26. Koch RM, Swiger LA, Chambers D, Gregory KE. Efficiency of Feed Use in Beef Cattle. J Anim Sci. 1963;22(2):486–94.

27. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2024.

28. Browning BL, Tian X, Zhou Y, Browning SR. Fast two-stage phasing of large-scale sequence data. Am J Hum Genet. 2021;108(10):1880–90.

29. Misztal I, Tsuruta S, Strabel T, Auvray B, Druet T, Lee D. BLUPF90 and related programs. Vol. Vol. 28, Proceedings of the 7th World Congress on Genetics Applied to Livestock Production. 2002. 743 p.

30. Aguilar I, Misztal I, Johnson DL, Legarra A, Tsuruta S, Lawlor TJ. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score1. J Dairy Sci. 2010;93(2):743–52.

31. Aguilar I, Legarra A, Cardoso F, Masuda Y, Lourenco D, Misztal I. Frequentist p-values for large-scale-single step genome-wide association, with an application to birth weight in American Angus cattle. Genet Sel Evol. 2019;51(1):28.

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

33. Sved JA. Linkage disequilibrium and homozygosity of chromosome segments in finite populations. Theor Popul Biol. 1971;2(2):125–41.

34. Wickhan H, Chang W, Henry L, Pedersen LT. ggplot2 package: Create Elegant Data Visualisations Using the Grammar of Graphics. 2023. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics (r-project.org). Acesseed 15 Jan 2024.

35. Wickhan H, François R, Henry L, Muller K, Vaughan, D. dplyr package: A Grammar of Data Manipulation. 2023. dplyr: A Grammar of Data Manipulation (r-project.org). Acesseed 15 Jan 2024.

36. Hu ZL, Park CA, Reecy JM. Bringing the Animal QTLdb and CorrDB into the future: meeting new challenges and providing updated services. Nucleic Acids Res. 2022;50(D1):D956–61.

37. Stelzer G, Plaschkes I, Oz-Levi D, Alkelai A, Olender T, Zimmerman S, et al. VarElect: the phenotype-based variation prioritizer of the GeneCards Suite. BMC Genomics. 2016;17(2):444.

38. Rocha R de FB, Garcia AO, Otto PI, dos Santos MG, da Silva MVB, Martins MF, et al. Single-step genome-wide association studies and post-GWAS analyses for the number of oocytes and embryos in Gir cattle. Mamm Genome. 2023;34(3):497–508.

39. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics. 2009;25(8):1091–3.

40. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504.

41. Zhang F, Wang Y, Mukiibi R, Chen L, Vinsky M, Plastow G, et al. Genetic architecture of quantitative traits in beef cattle revealed by genome wide association studies of imputed whole genome sequence variants: I: feed efficiency and component traits. BMC Genomics. 2020;21(1):36.

42. Seabury CM, Oldeschulte DL, Saatchi M, Beever JE, Decker JE, Halley YA, et al. Genome-wide association study for feed efficiency and growth traits in U.S. beef cattle. BMC Genomics. 2017;18(1):386.

43. Lu D, Akanno EC, Crowley JJ, Schenkel F, Li H, De Pauw M, et al. Accuracy of genomic predictions for feed efficiency traits of beef cattle using 50K and imputed HD genotypes1. J Anim Sci. 2016;94(4):1342–53.

44. Dressler EA, Shaffer W, Bruno K, Krehbiel CR, Calvo-Lorenzo M, Richards CJ, et al. Heritability and variance component estimation for feed and water intake behaviors of feedlot cattle. J Anim Sci. 2023;101:skad386.

45. Zernii EYu, Tikhomirova NK, Philippov PP, Senin II. Detection of Annexin IV in Bovine Retinal Rods. Biochem Mosc. 2003;68(1):129–34.

46. Sohma H, Matsushima N, Watanabe T, Hattori A, Kuroki Y, Akino T. Ca2+-dependent binding of annexin IV to surfactant protein A and lamellar bodies in alveolar type II cells. Biochem J. 1995;312(1):175–81.

47. Cole JB, Wiggans GR, Ma L, Sonstegard TS, Lawlor TJ, Crooker BA, et al. Genome-wide association analysis of thirty one production, health, reproduction and body conformation traits in contemporary U.S. Holstein cows. BMC Genomics. 2011;12(1):408.

48. Reverter A, Hudson NJ, Wang Y, Tan SH, Barris W, Byrne KA, et al. A gene coexpression network for bovine skeletal muscle inferred from microarray data. Physiol Genomics. 2006;28(1):76–83.

49. Hay EH, Roberts A. Genome-wide association study for carcass traits in a composite beef cattle breed. Livest Sci. 2018;213:35–43.

50. Hoque MA, Arthur PF, Hiramoto K, Oikawa T. Genetic parameters for carcass traits of field progeny and their relationships with feed efficiency traits of their sire population for Japanese Black cattle. Livest Sci. 2006;100(2):251–60.

51. Silva BPM. Arquitetura genética do consumo alimentar residual em bovinos Nelore. Msc Dissertation, Paulista state university - UNESP. 2018.

52. Anekal PV, Yong J, Manser E. Arg Kinase-binding Protein 2 (ArgBP2) Interaction with α-Actinin and Actin Stress Fibers Inhibits Cell Migration. J Biol Chem. 2015;290(4):2112–25.

53. Zurlo F, Larson K, Bogardus C, Ravussin E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. J Clin Invest. 1990;86(5):1423–7.

54. Santana MH de A, Gomes R da C, Ferraz JBS, Junior PR. Medidas de eficiência alimentar para avaliação de bovinos de corte. Sci Agrar Parana. 2014;13(2):95–107.

55. GeneCards - The Human Gene Database. GeneCards - Human Genes | Gene Database | Gene Search. Acesseed 15 Jan 2024.

56. Yee SW, Buitrago D, Stecula A, Ngo HX, Chien HC, Zou L, et al. Deorphaning a Solute Carrier 22 family member, SLC22A15, through functional genomic studies. FASEB J Off Publ Fed Am Soc Exp Biol. 2020;34(12):15734–52.

57. Stoppoloni D, Politi L, Dalla Vedova P, Messano M, Koverech A, Scandurra R, et al. l-Carnitine enhances extracellular matrix synthesis in human primary chondrocytes. Rheumatol Int. 2013;33(9):2399–403.

58. Salin K, Auer SK, Rey B, Selman C, Metcalfe NB. Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. Proc R Soc B Biol Sci. 2015;282(1812):20151028.

59. Xu Y, Hu W, Liu J, Zhang J, Jia C, Miao H, et al. A banana aquaporin gene, MaPIP1;1, is involved in tolerance to drought and salt stresses. BMC Plant Biol. 2014;14(1):59.

60. Golher DM, Patel BHM, Bhoite SH, Syed MI, Panchbhai GJ, Thirumurugan P. Factors influencing water intake in dairy cows: a review. Int J Biometeorol. 2021;65(4):617–25.

61. Verkman AS, Mitra AK. Structure and function of aquaporin water channels. Am J Physiol-Ren Physiol. 2000;278(1):F13–28.

62. Laforenza U. Water channel proteins in the gastrointestinal tract. Mol Aspects Med. 2012;33(5):642–50.

63. Boeva V. Analysis of Genomic Sequence Motifs for Deciphering Transcription Factor Binding and Transcriptional Regulation in Eukaryotic Cells. Front Genet. 2016;7:24.

64. Morgunova E, Taipale J. Structural perspective of cooperative transcription factor binding. Curr Opin Struct Biol. 2017;47:1–8.

65. Yang X, Wang J, Ma X, Du J, Mei C, Zan L. Transcriptome-wide N6-Methyladenosine Methylome Profiling Reveals m6A Regulation of Skeletal Myoblast Differentiation in Cattle (Bos taurus). Front Cell Dev Biol. 2021;9:785380.

66. Frenkel L, Freudenthal R, Romano A, Nahmod VE, Maldonado H, Delorenzi A. Angiotensin II and the transcription factor Rel/NF-κB link environmental water shortage with memory improvement. Neuroscience. 2002;115(4):1079–87.

67. Feraille E, Sassi A, Olivier V, Arnoux G, Martin PY. Renal water transport in health and disease. Pflüg Arch - Eur J Physiol. 2022;474(8):841–52.

68. Sands WA, Palmer TM. Regulating gene transcription in response to cyclic AMP elevation. Cell Signal. 2008;20(3):460–6.

69. Lefkimmiatis K, Zaccolo M. cAMP signaling in subcellular compartments. Pharmacol Ther. 2014;143(3):295–304.

70. Arnold I, Langer T. Membrane protein degradation by AAA proteases in mitochondria. Biochim Biophys Acta BBA - Mol Cell Res. 2002;1592(1):89–96.

71. Van Thang T, Sunagawa K, Nagamine I, Kishi T, Ogura G. A Physiological Stimulating Factor of Water Intake during and after Dry Forage Feeding in Large-type Goats. Asian-Australas J Anim Sci. 2012;25(4):502–14.

72. Holter JB, Urban WE. Water Partitioning and Intake Prediction in Dry and Lactating Holstein Cows1. J Dairy Sci. 1992;75(6):1472–9.

73. Arias RA, Mader TL. Environmental factors affecting daily water intake on cattle finished in feedlots. J Anim Sci. 2011;89(1):245–51.

74. Meyer U, Everinghoff M, Gädeken D, Flachowsky G. Investigations on the water intake of lactating dairy cows. Livest Prod Sci. 2004;90(2):117–21.

75. Fioranelli M, Roccia MG, Flavin D, Cota L. Regulation of Inflammatory Reaction in Health and Disease. Int J Mol Sci. 2021;22(10):5277.

76. Goldspink G, Williams P, Simpson H. Gene Expression in Response to Muscle Stretch. Clin Orthop Relat Res. 2002;403:S146.

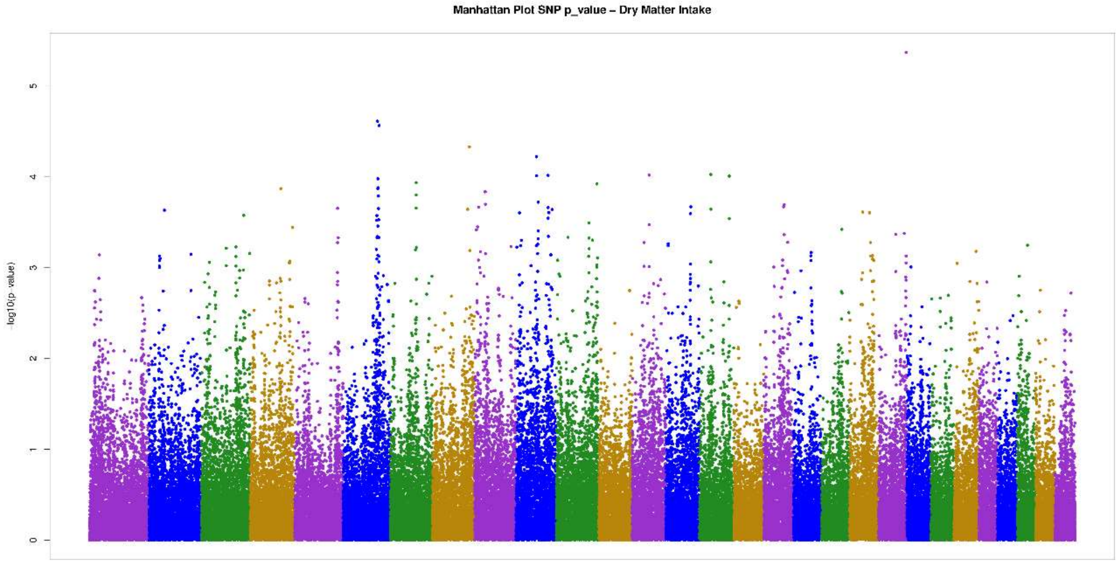
77. Huang W, Guo Y, Du W, Zhang X, Li A, Miao X. Global transcriptome analysis identifies differentially expressed genes related to lipid metabolism in Wagyu and Holstein cattle. Sci Rep. 2017 Jul 13;7(1):5278.

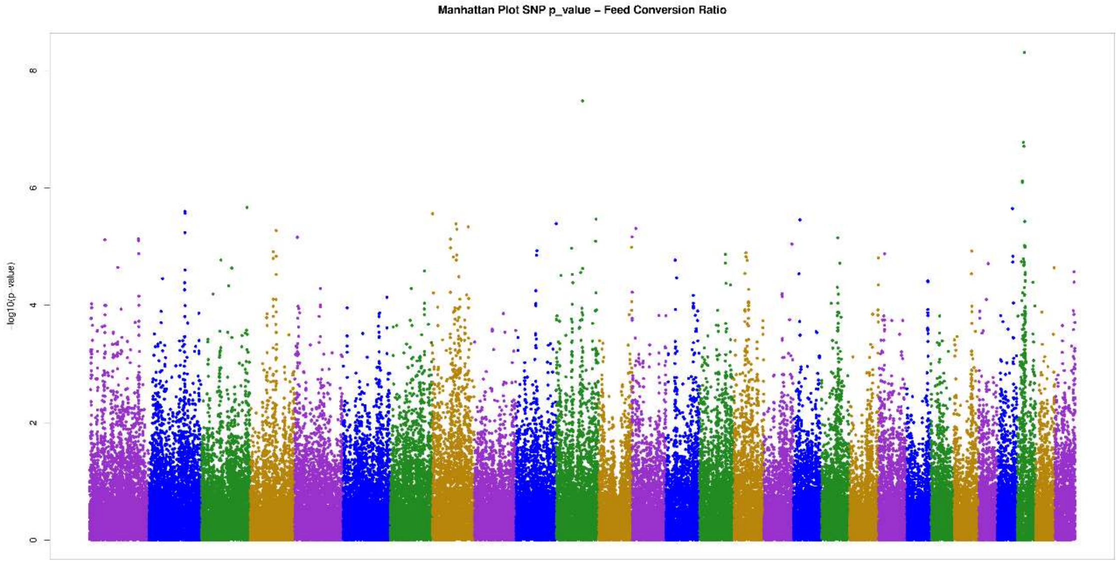
78. Ramli ANM, Hong PK, Abdul Manas NH, Wan Azelee NI. Chapter 25 - An overview of enzyme technology used in food industry. In: Kuddus M, Aguilar CN, editors. Value-Addition in Food Products and Processing Through Enzyme Technology. Academic Press; 2022. p. 333–45.

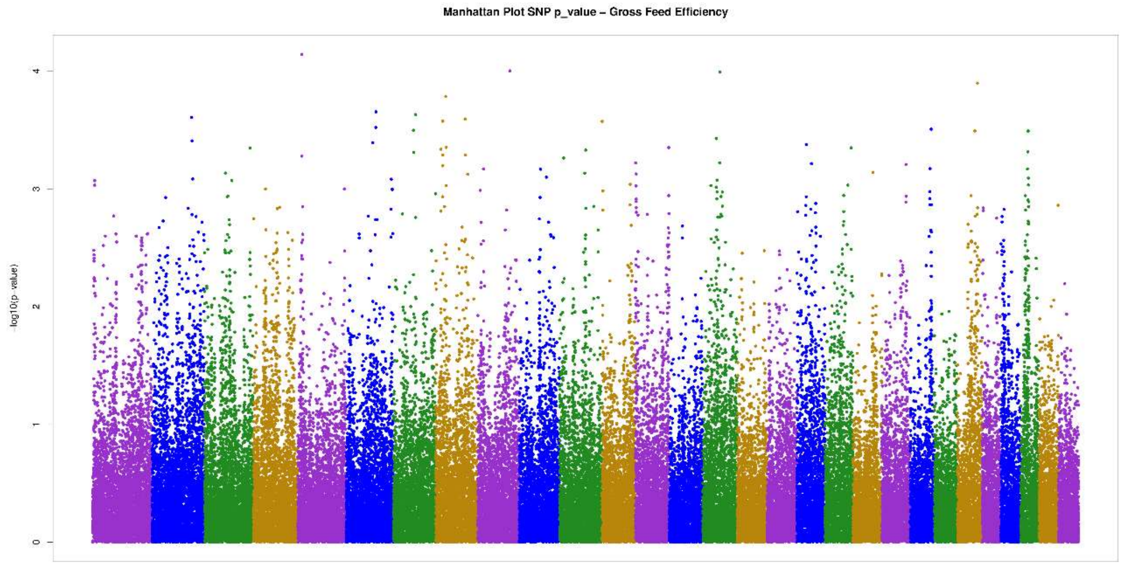
79. McDonald AE, Gospodaryov DV. Alternative NAD(P)H dehydrogenase and alternative oxidase: Proposed physiological roles in animals. Mitochondrion. 2019 Mar 1;45:7–17.

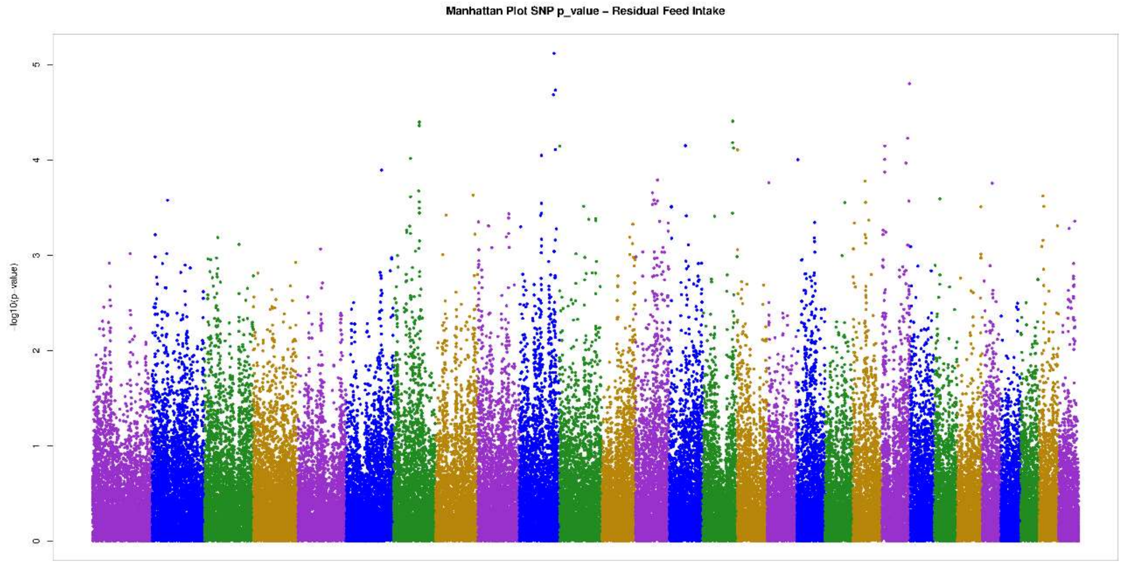
ADDITIONAL FILES

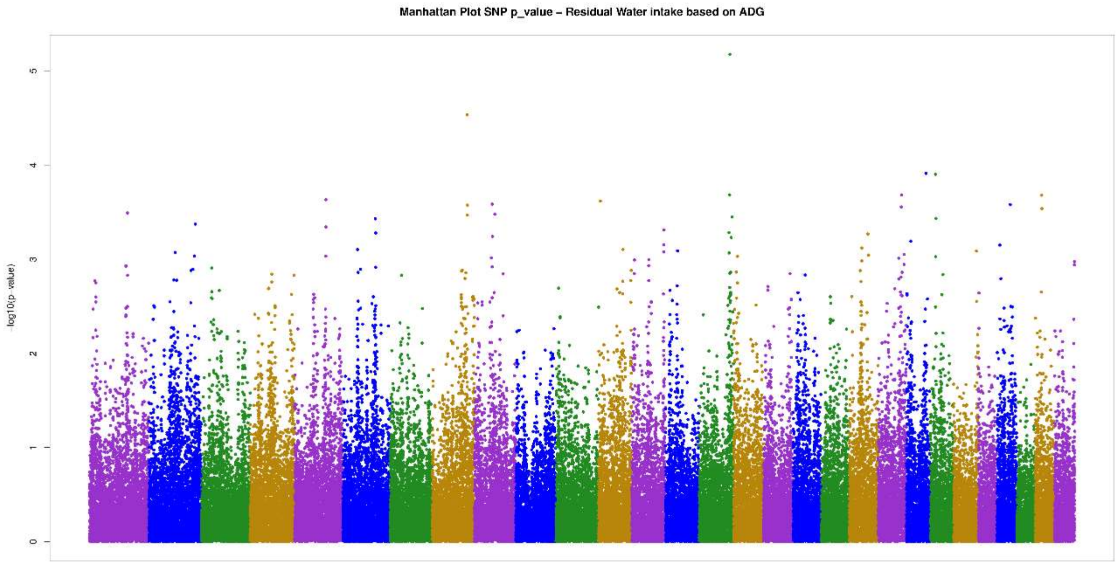
Additional file 1 - Manhattan plot. An additional file shows Manhattan plot for all feed and water efficiency traits evaluated.

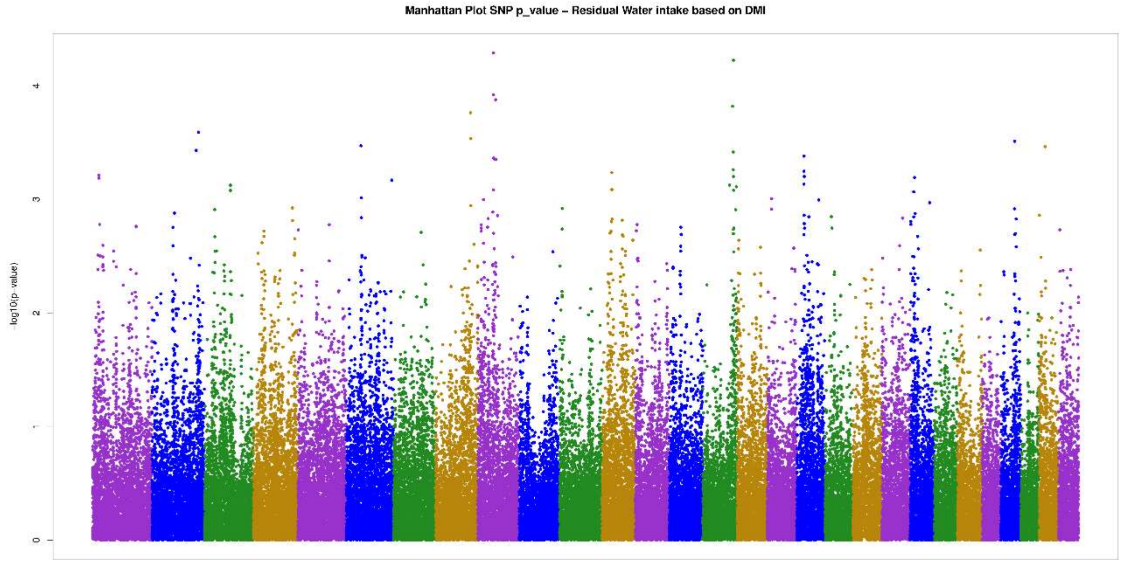


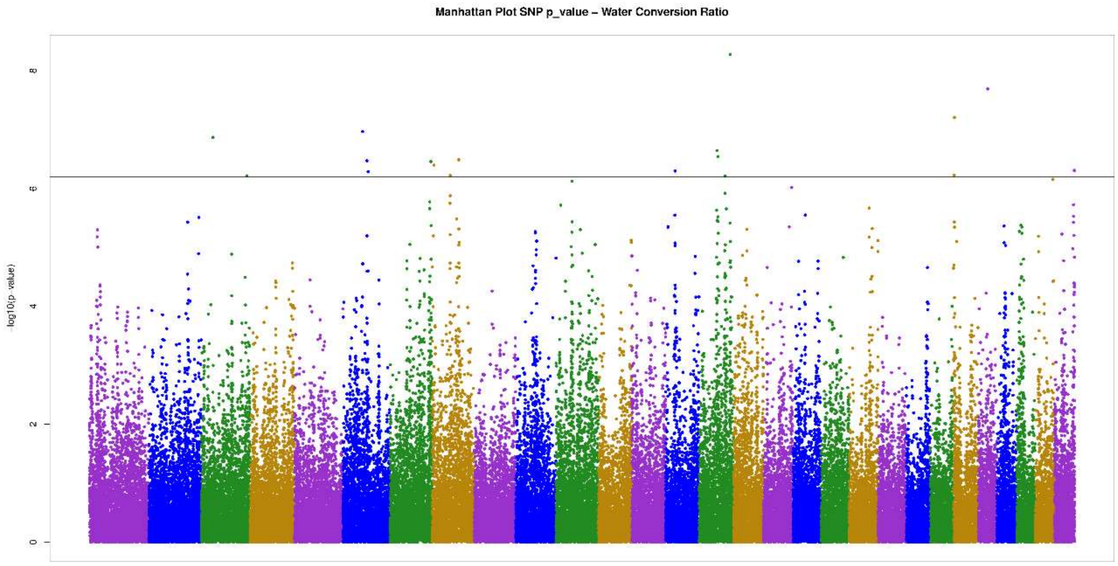


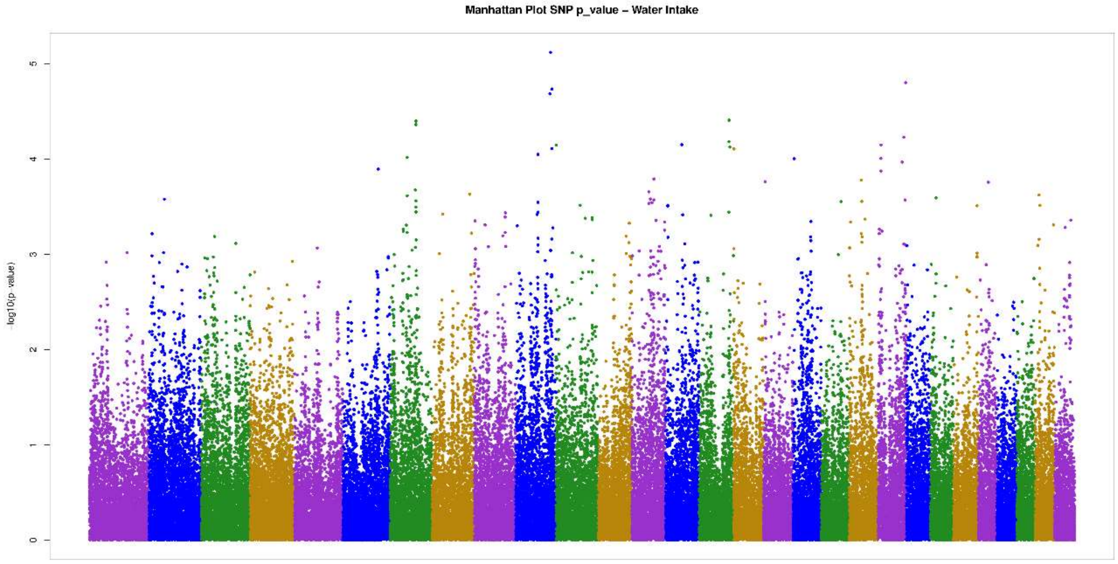

Manhattan Plot SNP p_value – Gross Feed Efficiency
-log10(p_value)
0
1
2
3
4


Manhattan Plot SNP p_value – Residual Feed Intake
-log10(p_value)
0
1
2
3
4
5


Manhattan Plot SNP p_value – Residual Water intake based on ADG
-log10(p_value)
0
1
2
3
4
5


Manhattan Plot SNP p_value – Residual Water intake based on DMI
-log10(p_value)
0
1
2
3
4


Manhattan Plot SNP p_value – Water Conversion Ratio
-log10(p_value)
0
2
4
6
8


Manhattan Plot SNP p_value – Water Intake
-log10(p_value)
0
1
2
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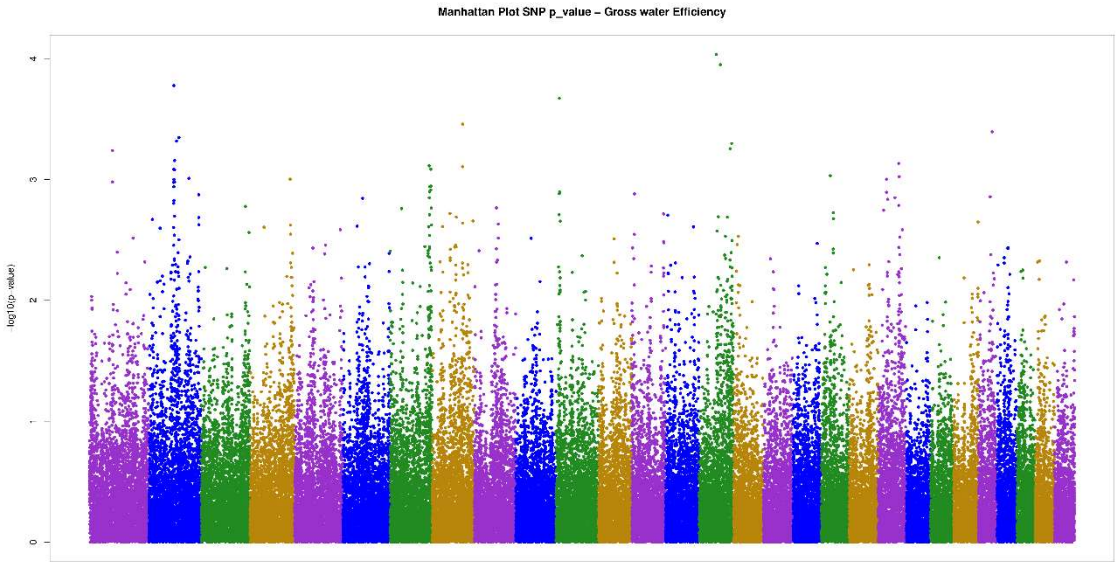
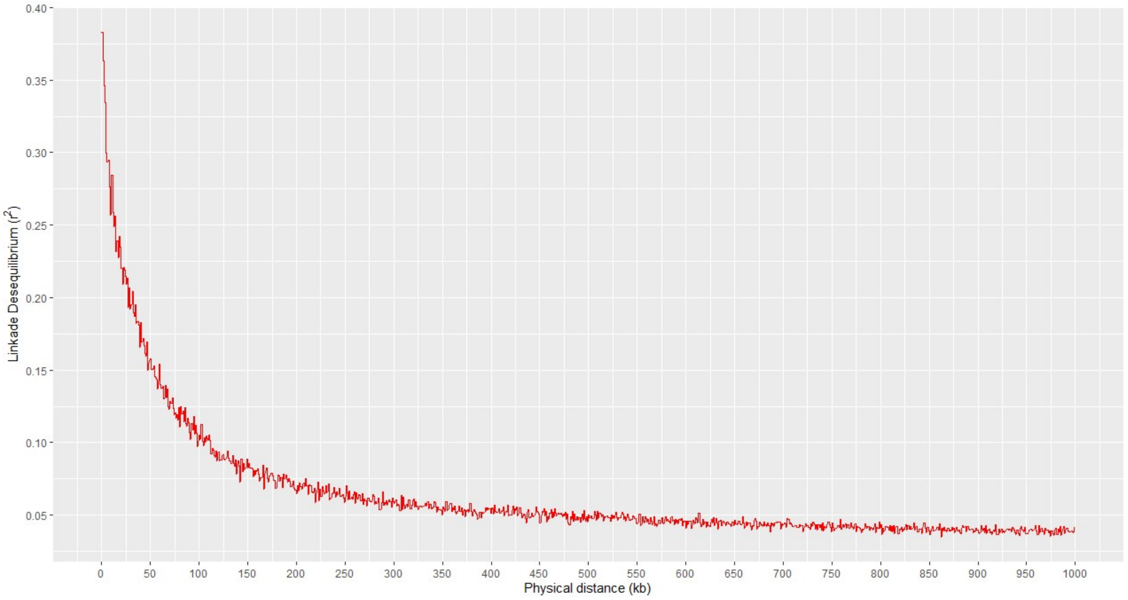


Additional file 2 - Linkage Disequilibrium. An additional file shows linkage disequilibrium decay by physical distance between SNPs.



Additional file 3: Table S1 - Quantitative Trait Loci. An additional file shows list of QTLs found for feed conversion ratio (FCR).

QTL	Gene
Milk kappa-casein percentage QTL (113111)	ANXA4/GMCL1
Milk kappa-casein percentage QTL (113112)	
Milk kappa-casein percentage QTL (113113)	
Milk kappa-casein percentage QTL (113114)	
Milk kappa-casein percentage QTL (113115)	
Body depth QTL (45037)	
Dairy form QTL (45038)	

Pregnancy rate QTL (45039)	
PTA type QTL (45040)	
Rear leg placement - rear view QTL (45041)	
Udder height QTL (45042)	
Rump width QTL (45043)	
Stature QTL (45044)	
Strength QTL (45045)	
Udder cleft QTL (45046)	
Milking speed QTL (157876)	
Stature QTL (125966)	
Milk kappa-casein percentage QTL (112666)	
Milk fat yield QTL (244377)	

QTL quantitative trait loci, *Gene* the gene name present in this QTL region.

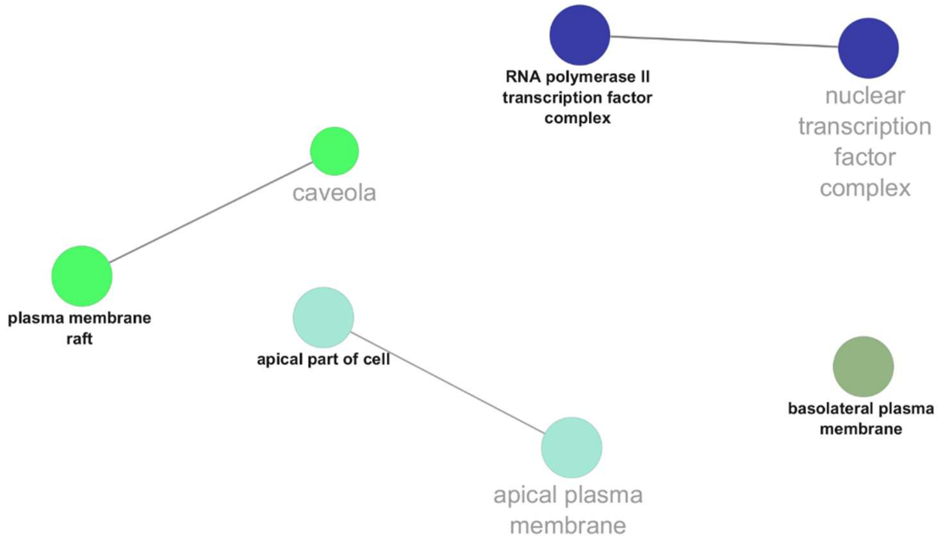
Additional file 3: Table S2 - Quantitative Trait Loci. An additional file shows list of QTLs found for water conversion ratio (WCR).

QTL	GENE
Milk tridecylic acid content QTL (155890)	SLC22A15
Foot angle QTL (39769)	
Net merit QTL (39770)	
Milk protein percentage QTL (39771)	
Rear leg placement - side view QTL (39772)	
Calving ease QTL (39773)	
Foot angle QTL (39769)	NHLH2
Net merit QTL (39770)	
Milk protein percentage QTL (39771)	
Rear leg placement - side view QTL (39772)	
Calving ease QTL (39773)	
Connective tissue amount QTL (233388)	ND
Milking speed QTL (157397)	
Milk protein percentage QTL (137868)	
Milk fat percentage QTL (138248)	
Carcass weight QTL (151373)	
Milk protein percentage QTL (138152)	
Tenderness score QTL (228263)	
Milk kappa-casein percentage QTL (113445)	GRXCR1
Milk kappa-casein percentage QTL (113446)	
Milk kappa-casein percentage QTL (113447)	
Milk kappa-casein percentage QTL (113448)	
Milk yield QTL (220169)	
Milk yield QTL (220170)	
Milk kappa-casein percentage QTL (111115)	
Milk glycosylated kappa-casein percentage QTL (116669)	
Milk kappa-casein percentage QTL (111083)	
Milk glycosylated kappa-casein percentage QTL (116807)	
Average daily gain QTL (131068)	ND

Average daily gain QTL (131054)	ADAMDEC1/ADAM7
Marbling score QTL (231874)	
Marbling score QTL (232450)	
Milk fat percentage QTL (247563)	RB1CC1
Milk protein percentage QTL (252043)	
Milk yield QTL (241600)	
Milk fat percentage QTL (247473)	
Milk yield QTL (241466)	
Milk fat percentage QTL (248244)	
Milk protein percentage QTL (252864)	
Milk yield QTL (241625)	
Subcutaneous fat thickness QTL (154001)	
Heifer pregnancy QTL (211597)	
Milk fat percentage QTL (247201)	
Milk yield QTL (241449)	SWAP70
Milk protein percentage QTL (253274)	
Body weight gain QTL (68726)	
Milk protein percentage QTL (253012)	OVCH2/CYB5R2/PPFIBP2
Milk yield QTL (242403)	
Milk protein percentage QTL (253254)	
Milk protein percentage QTL (252971)	
Interval from first to last insemination QTL (143091)	PRRG4/QSER1
Conception rate QTL (106860)	
Milk fat percentage QTL (26673)	
Milk capric acid content QTL (126212)	
Milk lauric acid content QTL (126223)	
Facial pigmentation QTL (37416)	PHF21A
Conception rate QTL (177168)	ARHGAP17
Average daily gain QTL (102144)	
Milk unglycosylated kappa-casein percentage QTL (118492)	
Pregnancy rate QTL (107053)	TNNT3/PRR33/LSP1
Conception rate QTL (107230)	
Tenderness score QTL (230194)	
Body weight gain QTL (69458)	
Udder balance QTL (214890)	
Stature QTL (154237)	
Stature QTL (154359)	
Inseminations per conception QTL (181563)	

QTL quantitative trait loci, *Gene* the gene name present in this QTL region.

Additional file 4 - Cellular Components Network. An additional file shows the network interaction between GO terms from cellular component for WCR



FINAL CONSIDERATIONS

Water efficiency traits show feasibility of selection, these traits demonstrate a polygenic nature with several biological processes acting simultaneously. Measurements based on the residuals of the equations, such as residual water intake, show a phenotypic null relationship with weight gain and a high genetic association with residual feed intake. These behaviors are positive points for the use of these traits in genetic improvement programs.

The genetic relationship between water intake and feed intake can be explored in selection strategies such as indices and correlated response. In addition, the fact that collecting phenotypes for water intake is easier than feed intake may contribute to expanding the phenotypic database, aiming to improve prediction accuracy and open up new pathways.

It is essential to investigate the relationship of these water efficiency traits with other selection criteria, such as those related to carcass and reproduction. Finally, in Senepol cattle, breeders and breeding programs should pursue actions that promote selection for *Slick Hair*, as well as for individuals non-carrier *double muscling*.