GMR
The Original by FUNPEC-RP

# Polymorphisms in genes coding milk proteins and protein hormones involved in milk production traits in Brazilian Guzerá cattle

**R.S. Steinberg[1,2], M.R.S. Carvalho[1], I.C. Rosse[1,3], M.A. Machado[4] and M.G.C.D. Peixoto[4]**

[1] Departamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
[2] Instituto Federal de Minas Gerais – *Campus* Bambuí, Bambuí, MG, Brasil
[3] Departamento de Farmácia, Escola de Farmácia, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brasil
[4] Embrapa Gado de Leite, Juiz de Fora, MG, Brasil

Corresponding author: R.S. Steinberg
E-mail: raphael.silva@ifmg.edu.br

Genet. Mol. Res. 21 (3): gmr19046
Received March 15, 2022
Accepted July 13, 2022
Published July 31, 2022
DOI http://dx.doi.org/10.4238/gmr19046

**ABSTRACT.** Research on genes affecting phenotypic variation in milk production and composition from indicine (*Bos indicus*) cattle is imperative, since these breeds are important tropical genetic resources, and there have been few studies investigating the genetic basis of these traits. We identified polymorphisms in κ-casein (*CSN3*), β-lactoglobulin (*LGB*), thyroglobulin (*TG*) and prolactin (*PRL*) and examined their effect on milk and composition traits in the Guzerá breed. DNA samples of 260 Guzerá cattle selected for dual purpose use were genotyped. Allele frequencies observed for the A allele were 0.83, 0.18 and 0.25 respectively for *CSN3*, *LGB* and *PRL* genes, while for the *TG* gene T allele had an allele frequency of 0.09. For all polymorphisms evaluated, observed genotypic frequencies were in agreement with those expected according to the Hardy-Weinberg Equilibrium hypothesis. A polymorphism association study evaluated breeding values (BV) for 305-day milk (BV-M), fat (BV-F), and protein (BV-P) production, employing the allele substitution model using a sample of 139 cows belonged to 27 full and half-sib

 

families of a MOET (multiple ovulation and embryo transfer) selection nucleus. Association was found between the *LGB* polymorphism and BV-M, BV-F and BV-P. Animals with *LGB* AA genotype have, on average, higher BV when compared to animals with *LGB* AB and BB genotypes (277.85 kg for BV-M, 12.09 kg for BV-F and 9.33 kg for BV-P). These findings contribute to a better understanding on the influence of these polymorphisms on milk production traits in Guzerá cattle.

**Key words:** Molecular markers; PCR-RFLP; Dairy cattle; *Bos indicus*; QTL

## INTRODUCTION

In the last decades, milk production has become one of the most important activities in terms of generating wealth and employment, with Brazil being the fourth largest milk world producer with a production of 25.4 billion tons in 2020 (USDA, 2021). Management and breeding procedures as well as biotechnology application has increased efficiency in livestock production (El-Aziz et al., 2016). Phenotypes of economic interest such as fattening performance, and milk and composition production are largely influenced by several genes of small effects, which presents candidates to assist selection for these traits (Ardicli et al., 2019).

Genetic polymorphisms have brought major contributions to characterization of domestic breeds, tracking evolutionary history of populations and ascertainment of differences between cattle breeds. Knowledge of genetic variability is also crucial for genetic conservation programs (Machugh et al. 1994; Ahmed et al., 2017). Furthermore, some polymorphisms in genes that code milk proteins and proteohormones have been associated to economically important traits for industrial processability of milk (Russo and Mariani, 1978; Aleandri et al., 1992; Caroli et al., 2009; Ardicli et al., 2019).

Part of variation in milk yield and composition is underlined by the polymorphisms found in genes encoding milk proteins and hormonal machinery involved in secretion of milk (Lemay et al., 2009). *CSN3*, *LGB* and *PRL* genes have polymorphisms associated with milk production, composition and quality, which are traits selected in breeding programs worldwide (Dekkers, 2004).

Main milk proteins are caseins alpha-s1, beta, alpha-s2 and kappa-casein and among whey proteins, alpha-lactoalbumin and beta-lactoglobulin (Ahmed et al., 2017). Fifty-four variants in milk proteins encoding genes have already been described in bovines (Caroli et al., 2009). Two variants in κ-casein (*CSN3*) gene localized of the BTA chromosome 6 (*CSN3* alleles A and B) differ by a double substitution involving a Thr136Ile and an Ala148Asp (Mercier et al., 1973). Another important polymorphism is present in β-lactoglobulin (*LGB*) gene mapped on BTA chromosome 11. Most common forms are *LGB* A and *LGB* B, differing each other by Asp64Gly and Val118Ala double substitution (Farrell et al., 2004). This substitution result in specific physicochemical characteristics as well as in expression of caseins levels in milk, being higher in variant *LGB* A (Aschaffenburg and Drewry, 1957). *CSN3* and *LGB* genes are located in quantitative trait loci (QLTs) for milk quality and production traits, including composition (Lemay et al., 2009; Olsen et al., 2016).

 

In *Bos taurus*, presence of *CSN3* B or *LGB* B allele has been associated with an increased quantity and concentration of milk proteins and curd firmness, as well as with a reduction of clotting time, resulting in higher cheese yield (Jakob and Puhan, 1995; Horne et al., 1996; Lodes et al., 1996; Fitzgerald and Hill, 1997). In the other hand, *LGB* AA genotype is correlated with increase in milk production and protein content, and reduction in caseins concentration (Ng-Kwai-Hang et al., 1984; Aleandri et al., 1992; Bovenhuis et al., 1992; Ardicli et al., 2019). *LGB* BB genotype is associated with higher milk heat stability and increased amounts of casein, dry matter, and fat retention in cheese cloth, resulting in a higher cheese yield (Aleandri et al., 1992; Bovenhuis et al., 1992; Molina et al., 2006).

Genes encoding hormones participate in metabolism regulation, so it is related to animal performance. Prolactin gene (*PRL*) belongs to a gene family that co-evolved with growth hormone and chorionic somatotrophin genes, localized on BTA chromosome 23 (Niall et al., 1971; Alipanah et al., 2007). Disruption of the *PRL* gene have showed the role of this hormone in mammary gland development, lactogenesis and gene expression of milk proteins (Horseman et al., 1997). Hence, *PRL* gene is a QTL for milk production traits (Brym et al., 2005). Thyroglobulin is a glycoprotein precursor for thyroid hormones T3 (triiodothyronine) and T4 (thyroxine). Thyroglobulin (*TG*) gene maps on centromeric region of BTA chromosome 14, linked to *DGAT1* gene, a strong QTL for milk production traits (Riquet et al., 1999; Li et al., 2020).

A silent transition A→G at codon 103 (exon 3) of *PRL* gene produces *PRL* A and B alleles respectively, which were associated with milk production traits (Lewin et al., 1992; Chung et al., 1996). Besides that, *PRL* AA genotype reduces production of milk fat and increases total milk production in *B. taurus* animals (Dybus et al., 2005; Miceikienè et al., 2006; Ghasemi et al., 2009). A SNP C→T in the 5'-UTR (untranslated region) of bovine *TG* gene was associated with marbling in beef cattle (Barendse, 1999; Thaller et al., 2003). Regarding to association of this SNP with production traits in dairy cattle, only one study was conducted in *B. taurus* (Khatib et al., 2007). However, these authors did not find a significant association between *TG* gene polymorphism and milk production and composition in Holstein animals. There are some studies associating genetic markers with temperament and production traits in dairy indicine breeds and only few studies involving Guzerá cattle (Santos et al., 2017; Rosse et al., 2017; Paiva et al., 2020).

Guzerá is a dual-purpose (milk and beef production) cattle adapted to tropical climatic conditions (Peixoto et al., 2021). These animals are rustic, tolerant to heat stress, and resistant to some diseases, endo and ectoparasites (Winkler and Penna, 1992; Penna et al., 2005; Peixoto et al., 2010). Due to its adaptability to environmental adversity as well as its productive potential for dual-purpose, in 1991, this breed was listed in FAO as a genetic resource to be preserved (FAO, 1995).

In 1994, it was deployed in Brazil the National Program for the Improvement of Guzerá Dairy Cattle (PNMGuL). The main objective of the PNMGuL is to promote the genetic evaluations using modern methodologies for the analysis of phenotypic and molecular data. Genetic evaluations are based on data from the progeny test and MOET selection nucleus schemes (Penna et al., 1998; Bruneli et al., 2020). However, marked-assisted and genomic selection could also be an auxiliar strategy in the Guzerá breeding program, contributing to the increase accuracy and, consequently, genetic gain in dairy farms (Ardicli et al., 2019).

Therefore, the aim of this work was to characterize the genetic polymorphisms in *CSN3, LGB, PRL* and *TG* genes and try to associate these polymorphisms with variations in milk production and composition traits. These associations may provide information about the perspective of the development of marker-assisted strategies for the conservation and improvement of the Guzerá breed.

## MATERIAL AND METHODS

### Animal samples

This study used blood samples from 260 animals, representing around 10% of the animals in each of the 15 major Guzerá herds located in Minas Gerais state, in southeastern Brazil. Among the herds sampled, some herds exploit Guzerá animals for dual-purpose (dairy and beef), using proven sires from both breeding programs. Other herds use Guzera strictly for beef purposes and others for milk production purposes. Samples of blood and semen were collected for DNA extraction and genotyped for polymorphisms of *CSN3*, *LGB*, *PRL,* and *TG* genes. From this total, 139 cows belonged to 27 full and half-sib families of MOET selection nucleus. Multiple ovulation and embryo transfer (MOET) nucleus were proposed by Nicholas and Smith (1983) and were implemented in several selection programs. In MOET's nucleus schemes, families are produced yearly and sib families are raised under uniform management conditions in the same farm. Those schemes allow for an increase in the rate of genetic progress, although there is a possibility of an increase in the inbreeding coefficient. In this sense, an open MOET's selection nucleus was created in 1994 in Guzerá cattle (Penna et al., 1998) as the first MOET's selection nucleus in Brazil. First lactation phenotypes were obtained from the database of the National Program for the Improvement of the Guzerá Dairy Cattle coordinated by Embrapa Gado de Leite and Centro Brasileiro de Melhoramento Genético do Guzerá (CBMG) (Bruneli et al., 2020).

Records of 305-day milk, fat, and protein yield from progeny test and MOET nucleus are yearly and jointly evaluated to estimate individual breeding values (BVs) which were used in this study. These BV estimates were obtained from data of first lactations of full and half-sib Guzera´ cows born and raised in the MOET's nucleus scheme, simultaneously recorded nucleus in the same environmental and management conditions each year, through dairy control and data were adjusted for a lactation of 305 days. These animals belong in the dataset of the progeny test of Guzera bulls (Peixoto et al., 2006). BVs are calculated using the restricted maximum likelihood method and fitting an animal model by the algorithms available in MTDFReML (Boldman et al., 1995). The statistical model for estimation of breeding values included the fixed effects of herd-calving year, calving season, age at calving as a covariate, and the random effects of permanent environment, animal, and residual by solving a mixed model equation, including a relationship matrix.

### Molecular procedures

Genomic DNA was extracted by the proteinase-K:phenol-chloroform method (Sambrook and Russell, 2001) and proteinase K/salting out protocol (Miller et at., 1988) from semen and blood samples, respectively. Genotyping for polymorphisms in *CSN3*, *LGB*, *PRL* and *TG* genes were performed by PCR-RFLP (polymerase chain reaction and

 

restriction fragment length polymorphism). Primers used were: *CSN3* F (5′-TGT GCT GAG TAG GTA TCC TAG TTA TGG-3′) and *CSN3* R ( 5′-GCG TTG TCT TCT TTG ATG TCT CCT TAG-3′); *LGB* F (5' TGT GCT GGA CAC CGA CTA CAA AAA G 3') and *LGB* R (5' GCT CCC GGT ATA TGA CCA CCC TCT 3'); *PRL* F (5' CGA GTC CTT ATG AGC TTG ATT CTT 3') and *PRL* R (5' GCC TTC CAG AAG TCG TTT GTT TTC 3'); *TG* F (5' GGG GAT GAC TAC GAG TAT GAC TG 3') and *TG* R (5' GTG AAA ATCT TGT GGA GGC TGT A 3'), respectively to amplify a portion of *CNS3*, *LGB*, *PRL* and *TG* genes, containing each of evaluated polymorphisms. Primer sequences were checked for specificity through research conducted on BLAST from NCBI's GenBank database. Then, PCR products were digested using *Hinf*I (Invitrogen, Carlsbad, CA) to *CSN3*, *Hae*III (Invitrogen, Carlsbad, CA) to *LGB*, *Rsa*I (Invitrogen, Carlsbad, CA) to *PRL* and *Psu*I (Promega, Madison, WI, USA) to *TG* by incubation at 37º C over night, according to manufacturer's recommendations. Primer concentrations and thermocycling conditions for PCR amplification of gene polymorphisms *CSN3*, *LGB*, *PRL* and *TG* were performed, respectively, according to published procedures for Barroso et al., (1998), Medrano and Cordova (1990), Mitra et al., (1995) and Barendse (1999) respectively, with modifications. Amplifications were carried out in a final volume of 25 µL with IV-B PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 0.1% Triton X-100, 1.75 mM MgCl2), 0.2 mM each dNTP (Invitrogen, Carlsbad, CA), 8% dimethyl sulfoxide (J. T. Baker, Xalostoc, Edo. De Mexico, Mexico), 1 U of Taq DNA polymerase (Phoneutria Biotecnologia and Serviços, Belo Horizonte, MG, Brazil) and 100 ng of genomic DNA for all the polymorphisms that were genotyped. Reactions were undertaken using a PxE 0.2 Thermal Cycler (Electron Corporation, Waltham, MA). The PCR-RFLP products were resolved by electrophoresis on 8% polyacrylamide gels and visualized by silver and ethidium bromide stained. Allele sizes were estimated by comparison with a ΦX174 RF DNA/*Hae*III Fragments Ladder (Invitrogen) and identified in accordance with the expected pattern for each polymorphism genotyping.

## Evaluation of genotypic and allelic frequency of polymorphisms

Firstly, the allele and genotype frequencies were calculated for the full sample (260 animals). Allelic and genotypic distribution were calculated for each polymorphism by the standard procedure (Falconer and Mackay, 1996). Deviations of Hardy-Weinberg equilibrium (HWE) was tested for all alleles using GENEPOP software v1.32 statistics package (Raymond and Rousset, 1995; Yeh et al., 2000). Population genetics indices, including homozygosity (Ho) and heterozygosity (He), were calculated, based on the allele frequency of each polymorphism using formulas described by Botstein et al., (1980).

## Association study statistics

In order to access possible associations of the *CSN3, LGB, PRL* and *TG* genes polymorphisms with milk production traits, data on BV for 305-day milk production (BV-M), fat (BV-F), and protein production (BV-P) were used. All BV estimates were expressed in kg. For the association study, the variance analysis only regarded BVs and molecular data of 139 cows from 27 full and half-sib sire families born, managed and controlled until the end of the first lactation in the open MOET selection nucleus scheme, and with at least three

daughters. This dataset was defined regarding that the design of MOET selection nucleus allowed each contemporary families to be raised and produce in the same environmental and management conditions. Therefore, it may have minimized the environmental deviations and increased the power to detect gene effects. Association analysis of the genetic polymorphisms was estimated using an allele substitution model (Rosendo et al., 2012) using the following equation (Eq. 1):

$$Yij = u+Si+Mj(Si)+eij \quad \text{(Eq. 1)}$$

where *Yij* is the BV of each daughter with marker genotype *j*, *Si* is the fixed effect of sire *i*, *Mj(Si)* is the allele substitution effect nested into sire *i*, being *j* the number of + alleles (0, 1 and 2) of the marker and *eij* is the residual term. The mixed procedure (PROC MIXED) available in SAS® was used for statistical analysis (SAS, 2003). Effects of the polymorphisms for each gene were determined in separate analyzes. Statistical significance level in F-test used in all analyzes was P value ≤ 0.05.

## RESULTS

Allelic and genotypic frequencies observed for each locus, as well as P values for HWE test are summarized in Table 1. The A allele frequencies were 0.83, 0.18 and 0.25 for, respectively, *CSN3*, *LGB* and *PRL* genes, while the least frequent allele was the T allele of *TG* gene with a frequency of 0.09. Non-significant differences from HWE expectancies were observed for all loci analyzed (P > 0.05).

**Table 1.** *CSN3, LGB, PRL,* and *TG* allelic and genotypic frequencies, observed (HO) and expected (HE) heterozygosity, P values for Hardy-Weinberg equilibrium test for 260 Guzerá animals under selection for milk production traits.

| Gene | Allele frequency | | Genotype frequency | | | HO | HE | P *value* |
|---|---|---|---|---|---|---|---|---|
| | A | B | AA | AB | BB | | | |
| ***CSN3*** | 0.83 | 0.17 | 0.7000 | 0.2615 | 0.0385 | 0.2615 | 0.2817 | 0.2709 |
| ***LGB*** | 0.18 | 0.82 | 0.0192 | 0.3308 | 0.6500 | 0.3308 | 0.3016 | 0.1469 |
| ***PRL*** | 0.25 | 0.75 | 0.0615 | 0.3808 | 0.5577 | 0.3808 | 0.3776 | 1.0000 |
| | T | C | TT | TC | CC | | | |
| ***TG*** | 0.09 | 0.91 | 0.0154 | 0.15 | 0.7483 | 0.15 | 0.1647 | 0.1375 |

Results of association studies are summarized in Table 2. Significant associations (P < 0.05) with BV-M, BV-F and BV-P were observed only for polymorphism in LGB gene. Average BV-M, BV-F and BV-P for each genotype at each marker are shown in Table 3.

Animals with *LGB* AA genotype presented higher BV for all parameters when compared to those with *LGB* BB and *LGB* AB genotypes, with differences of 277.85 kg in BV-M, 12.09 kg in BV-F, and 9.33 kg in BV-P. Finally, for polymorphisms in *CSN3*, *PRL* and *TG* genes no significant (P > 0.05) associations were found between these genes and variation observed in BV-M, BV-F and BV-P for the sampled animals.

 

**Table 2.** Variance analysis of the milk yield and composition breeding values to test the *CSN3*, *LGB*, *PRL* and *TG* polymorphisms effects from 139 Guzerá MOET nucleus progenies selected for dairy purposes.

| Source | Genes | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *CSN3* | | | *LGB* | | | *PRL* | | | *TG* | | |
| | DF[d] | F[e] | P value[g] | DF[d] | F[e] | P value[g] | DF[d] | F[e] | P value[g] | DF[d] | F[e] | P value[g] |
| BV-M[a] | 2 | 0.53 | 0.592 | 2 | 4.22 | 0.0167* | 2 | 0.23 | 0.796 | 2 | 0.26 | 0.61 |
| BV-F[b] | 2 | 0.49 | 0.613 | 2 | 4.25 | 0.0160* | 2 | 0.02 | 0.976 | 2 | 0.24 | 0.628 |
| BV-P[c] | 2 | 0.3 | 0.737 | 2 | 4.3 | 0.0155* | 2 | 0.09 | 0.915 | 2 | 0.39 | 0.53 |

[a] breeding value for 305-days milk yield (kg); [b] breeding value for 305-days fat yield (kg); [c] breeding value for 305-days protein yield (kg); [d] degrees of freedom; [e] F statistic; *significance level (P < 0.05).

**Table 3.** Means of breeding values for 305-days milk (BV-M), fat (BV-F) and protein (BV-P) yield (kg) by genotype group of the *CSN3, LGB, PRL* and *TG* genes with their respective standard deviations (SD: ±) from 139 Guzerá MOET nucleus progenies selected for dairy purposes.

| | ***CSN3*** | | |
|---|---|---|---|
| | **AA** | **AB** | **BB** |
| **Trait** | ***n*=96** | ***n*=43** | ***n*=2** |
| BV-M (±) SD | 398.3±266.2 | 408.1±261.2 | 210.9±252.3 |
| BV-F (±) SD | 18.10±11.31 | 17.82±11.07 | 10.28±11.27 |
| BV-P (±) SD | 13.35±8.53 | 13.65±8.27 | 8.86±6.36 |
| | ***LGB*** | | |
| | ***n*=5** | ***n*=46** | ***n*=89** |
| BV-M (±) SD | 667.4±383.2 | 388.6±248.6 | 390.5±260.6 |
| BV-F (±) SD | 29.61±13.65 | 17.54±11.21 | 17.51±10.87 |
| BV-P (±) SD | 22.34±10.36 | 12.80±8.27 | 13.23±8.25 |
| | ***PRL*** | | |
| | ***n*=10** | ***n*=50** | ***n*=81** |
| BV-M (±) SD | 419.2±311.4 | 379.9±220.3 | 407.7±284.1 |
| BV-F (±) SD | 18.00±13.76 | 17.63±9.62 | 18.06±11.88 |
| BV-P (±) SD | 14.05±10.51 | 13.08±6.92 | 13.48±9.08 |
| | ***TG*** | | |
| | **TT** | **TC** | **CC** |
| | ***n*=1** | ***n*=22** | ***n*=118** |
| BV-M (±) SD | 250.9 | 419.9±241.3 | 396.0±269.3 |
| BV-F (±) SD | 12.89 | 18.76±9.84 | 17.79±11.50 |
| BV-P (±) SD | 10.26 | 14.22±7.54 | 13.25±8.64 |

## DISCUSSION

Recently, association studies have focused on genome wide (GWAS) to put forward many new candidate genes for milk production and composition, animal welfare and behavior in selection assistance (Fang et al., 2017; dos Santos et al., 2017; Iung et al., 2019; Cai et al., 2020). However, many of the candidate genes detected in GWAS studies have not yet been confirmed as much as the physiological mechanisms on milk metabolism are still unclear (Yue et al., 2017; Liu et al., 2020). Functional studies based on animal or cellular models have been only seldomly reported as well (Zhu and Zhao, 2007; Lin et al., 2019; Bordbar et al., 2020). This scene reinforces the importance of carrying out association studies of single polymorphisms in candidate genes with interest traits.

In this study *CSN3*, *LGB*, *TG* and *PRL* genes were chosen based on their biological role in mammary gland metabolism as well as based on the evidence of association found in

other breeds as indicated in several previous studies (Ardicli et al., 2019). So, this was the first study to show the association between polymorphisms in milk proteins and protohormones genes with milk composition and production traits in Guzerá cattle.

Some studies investigated allelic and genotypic frequencies for *CSN3*, *LGB*, *TG* and *PRL* genes polymorphisms in *B. indicus* (indicine) breeds. Indicine breeds typically have lower frequencies (<30%) for *CSN3* B allele than those observed in *B. taurus* (taurine) *breeds*. *CSN3* B allele frequencies (0.38 and 0.65) were, respectively, reported in Czech Fleckvieh (Kučerová et al., 2006) and Jersey cattle (Shetty et al., 2006). Allelic frequency for *CSN3* gene reported previously by Azevedo et al. (2009) from a sample of Guzerá cattle is similar to those described here. In Guzerá cattle, lower frequencies for *CSN3* B allele (<10%) have been described by Silva and Del Lama (1996) and Kemenes et al., (1999). The highest frequency described for the *CNS3* B allele in Guzerá cattle was 0.31, however only 26 individuals were genotyped (Dadhich et al., 2006). On the other hand, a study evaluating the variability of the polymorphism located in the *CSN2* gene revealed that animals of the Guzerá animals have high A2 allele (0.97) and A2A2 genotype (0.93) frequencies (Rangel et al., 2017). A2A2 milk has been correlated with lower rates of allergy development caused by milk consumption.

For *LGB* gene polymorphism, frequency of B allele, ranging from 0.36 (Dadhich et al., 2006) to 0.84 (Lin et al., 1986), were described in Gyr and Ayrshire breed, respectively. In this research, the highest frequency (0,82) of *LGB* B allele in relation to others described in the literature for Guzerá cattle (0.66-0.79) (Silva and Del Lama, 1996; Kemenes et al., 1999; Dadhich et al., 2006). There is a wide variation in allele frequencies for the *PRL* gene polymorphism. Probably, the difference in population structure, genetic distance, and herds sampled among different breeds, populations and herds, as well as differences in the trajectory of artificial selection among them, may explain the differences in allelic and genotypic frequencies found for *LGB* and *PRL* genes.

This study was the first to evaluate the *TG* gene polymorphism in the Guzerá cattle. The frequency of the *TG* T allele has been reported as lower than the frequency of the *TG* C allele in *Bos taurus* (Thaller et al., 2003; Moore et al., 2003), in an industrial cross with different contributions from *B. taurus* and *B. indicus*, and absent in a study carried out in Nellore animals (Fortes et al., 2009). In this study, the *TG* T allele, in the Guzerá cattle, was found with a low frequency of 0.09. The *TG* T allele has been identified as favorable for intramuscular fat deposition (Barendse et al. 2004), although its possible effect on milk production and composition has not been clarified so far. The low frequency of the *TG* T allele in the only study of association with milk production is suggested as a possible explanation for a lack of association detection (Khatib et al., 2007), which may also explain the lack of association detection of this marker in this study. The rate of genetic gain due to direct selection of an allele depends on its initial frequency, therefore, it is an important aspect in defining molecular markers for use in breeding programs (Falconer, 1981). If the favorable allele is the most frequent, the possibility of genetic gain is limited (Ron and Weller, 2007). Therefore, if in the future the *TG* T allele has favorable associations with the characteristics of milk or meat production in the Guzerá cattle, the possibility of genetic gain with selection of this marker will be promising. Even though some association studies have failed to detect the effect of this polymorphism on milk production and meat marbling traits, this polymorphism is available on the GeneSTAR Quality Grade commercial panel

 

(Genetic Solutions / Bovigen Pty. Ltd.), which is a panel used to perform the molecular value predictions for beef feed efficiency, marbling and tenderness.

There were no significant differences between observed genotypic frequencies and those expected in HWE for all polymorphisms evaluated here. It has been proposed that adherence to HWE test should be performed for candidate genes to reduce false positive findings in association studies of complex traits (Ardicli et al., 2019). If a process of stratification of population is happening, spurious associations can be found between marker and quantitative traits (Deng et al., 2001). Therefore, adherence to HWE increases the reliability of the results on association. The absence of significant deviations from expected by HWE can be considered an indicator that artificial selection, to date, may still not been causing disequilibrium in genotypic and allelic frequencies for the polymorphisms analyzed.

MOET selection nucleus scheme has been implanted for the Brazilian Guzerá is succeeding. These schemes produce full and half-sib sire families, whose production data are used in the genetic evaluation of animals, shortening generation interval and accelerating genetic gain per generation (Strathie and McGuirk, 1995; Penna et al., 1998; Bouquet et al., 2015; Granleese et al., 2015). In the MOET scheme, families are produced yearly, and the sib families are at the same age and reared under uniform management conditions, which reduces the physiological and environmental deviations on the phenotypic variation. It is the main reason for these analyzes be able to detect significance of some effects in this study.

Aiming to increase the power of detection one algorithm using full and half-sib sire families information was proposed by Le Roy et al., (1998). A simulation study confirmed the efficacy of the MOET nucleus family structure to detect association between genetic polymorphisms and milk production traits in MOET nucleus (Peixoto et al., 2009). Therefore, detection of association is possible in populations with conditions similar to those of the MOET nucleus, but with designed family structure, even if a small number of individuals were genotyped (Rosendo et al., 2012; Wakchaure et al., 2015).

In this study, only *LGB* gene polymorphism was significantly associated ($P \leq 0.05$) with BV for milk production and composition. An association between molecular and BV data with $P < 0.0255$ can be considered strong (Zepeda-Batista et al., 2017). *LGB* B allele is the most common among indicine breeds, while in taurine breeds this tendency is not frequently observed (Ahmed et al., 2017). Animals with *LGB* AA genotype present higher average for BV-F (12.09 kg), BV-P (9.33 kg) and BV-M (277.85 kg) when compared with the average BV of *LGB* BB and AB animals. These same trends were found in other studies (Aleandri et al., 1992; Bovenhuis et al., 1992; Kaminski et al., 2002; Zepeda-Batista et al., 2017). However, previous studies have described that the presence of *LGB* BB genotype has been associated with higher production and favorable effect of the fat production (Pupková, 1980; McLean et al., 1984; Ng-Kwai-Hang et al., 1986; Aleandri et al., 1992; Bovenhuis et al., 1992; Hill, 1993). On the other hand, other studies found non-significant association between polymorphism in *LGB* gene with BV for milk production in *B. taurus* animals (Neubauerová, 2001; Kučerová et al., 2006).

In Holstein-Frisian breed, a study evaluated relationship between *LGB* gene polymorphism and weight gain. Animals carrying *LGB* AA genotype presented average values of daily weight gain higher than *LGB* AB and BB animals (Ardicli et al., 2019). Interestingly, the frequency of the *LGB* AA genotypes observed was lower than the

frequency of the *LGB* AB and BB genotypes, a trend also observed in this study. Due to association with milk production data, reported here for the first time in Guzerá cattle, and to the possibility of a relationship with daily weight gain, *LGB* gene polymorphism, especially the *LGB* AA genotype, represents a molecular marker to be considered in the programs of improvement of the Guzerá cattle, due to its dual-purpose (milk and meat production).

Non-significant associations were found ($P > 0.05$) between polymorphisms on *CNS3*, *PRL* and *TG* genes and BV for milk production in this study. Neubauerová (2001) also did not find associations between *CSN3* genotypes and BV related to parameters of milk production. However, it was observed in the current study that average BV of animals with *CSN3* AA and AB genotypes are larger than those observed in animals *CSN3* BB (192.3 kg for BV-M, 7.68 for BV-F and 4.64 to BV-P). This trend was also found by Kučerová et al., (2005) and Kaminski et al., (2002), that showed an increased BV for milk production associated with the *CNS3* AA genotype compared to *CNS3* AB and *CNS3* BB animals in Czech Fleckvieh breed. Therefore, it seems that *CSN3* AA genotype tends to increase parameters related to milk production (Neubauerová, 2001). This finding suggests a possible dominance action of *CNS3* A over *CNS3* B allele with respect to BV for milk production evaluated here, which deserves to be further investigated.

Results of this study about the association between *PRL* gene and milk production traits are similar to those of Chrenek et al. (1999), who examined the influence of these polymorphisms in Brown Swiss cattle and found no significant differences between cows with different genotypes, though the authors did not point out possible reasons for the lack of detection of association. Divergently, some researchers reported so far, the *PRL* AA genotype has been associated with higher milk production in Black and White Holstein, Jersey and Montebeliard cattle (Brym et al., 2005; Ghasemi et al., 2009; Dybus et al., 2005). On the other hand, Sacravarty et al. (2008) reported that the best genotype for milk production was *PRL* BB in Guzerá cows from second to fourth lactation in a study carried out in India with 51 cows using Harvey's mixed model.

Association between polymorphisms in candidate genes and milk production traits has been reported, although the results are divergent (Golijow et al., 1999; Zepeda-Batista et al., 2017). This divergence has been attributed to several factors that affect milk production and composition. The milk sampling station, for example, interferes with protein concentration and pH, and variations between 3.2-6.0% (protein concentration) and 2.0-5.6% (pH) have been observed throughout the different seasons of the year (Bernabucci et al., 2015). Additionally, genotypic associations of some genes with milk production traits in different breeds can produce varied results due to their genetic background, and age of animals. Reasons for lack of agreement in this type of study may include many other aspects: relation between genotype and environment, that can interfere with the manifestation of a polygenic characteristic and, statistical bies, e.g. small sample sizes, which result in false positive or negative results, and, consequently, significant under or over estimation of the effects of polymorphisms (Beavis, 1994). In this context, by assuming an additive model, as that used in this study, the analyses can impair the detection of more complex or other mechanisms of intra-gene action. In addition, in an attempt to increase the accuracy of effect detection, the survey of the existence of gene interactions, such as pleiotropy, epistasis and linkage disequilibrium, should be taken into account when

 

different combinations of polymorphisms are evaluated in association studies (Hayes et al., 2005; Carvalho et al., 2012; Ardicli et al., 2019).

Failure to find association between polymorphisms described in *CSN3*, *TG* and *PRL* genes and milk production traits, do not invalidate these genes as molecular markers for assisted-selection in Guzerá cattle. Many markers in literature were developed and validated for *B. taurus* animals, so their genetic variability and effects on phenotype can be quite different in *B. indicus* (The Bovine HapMap Genome Consortium, 2009). Milk proteins genes have been examined in different *B. indicus* breeds and six new polymorphisms have been identified in indicine breeds that at first sight are segregating exclusively in them, indicating a high level of genetic variation in these loci in *B. indicus* (Ahmed et al., 2017). The evaluation of other regions of *CSN3*, *PRL* and *TG* genes can discover new potential molecular markers for milk production traits, as described in the 3'UTR region of the *DGAT1* gene in Guzerá cattle (Rosse et al., 2014).

Over the past 10 years, an increase in the adoption of genomic assessments has been observed in dairy populations around the world (Boison et al., 2017). Well-established conventional genetic screening systems have provided the solid foundation for successful genomic selection in developed countries (Mrode et al., 2019). However, there is a paucity of knowledge about the potential for genomic selection in *Bos indicus* dairy cattle populations, as well as species-specific or cattle-specific marker panels. The association between polymorphism in *LGB* gene and milk production data in Guzerá cattle detected in this study needs to be confirmed with a large sample and functional experiments, such as gene expression studies. Therefore, the results of this work show that polymorphism in the *LGB* gene is a candidate to be evaluated in the construction of molecular marker panels aimed at genomic evaluation in herds of Indian breeds selected for milk production.

## ACKNOWLEDGMENTS

The authors thank the care and advisement of Professor Vânia Maldini Penna and Professor Cleusa Graça da Fonseca for the support throughout this study, farmers represented by the Centro Brasileiro de Melhoramento Genético do Guzerá (CBMG2) for the collaboration, and farm workers for assisting biological material collection. R.S.S. and M.G.C.D.P. were supported by FAPEMIG fellowships. Financial support: FAPEMIG, CNPq, PRONEX/FAPEMIG, EMBRAPA, FINEP.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

Ahmed AS, Rahmatalla S, Bortfeldt R, Arends D, et al. (2017). Milk protein polymorphisms and casein haplotypes in Butana cattle. *J. Appl. Genet.* 58: 261-271.

Aleandri R, Buttazzoni LG, Schneider JC, Caroli A, et al. (1992). The Effects of Milk Protein Polymorphisms on Milk Components and Cheese-Producing Ability. *J. Dairy Sci.* 73(2): 241-255.

Alipanah M, Kalashnikova L and Rodionov G (2007). Association of prolactin gene variants with milk production traits in Russian Red Pied cattle. *Iran. J. Biotechnol.* 5(3): 158-161.

Ardicli S, Samli H, Vatansever B, Soyudal B, et al. (2019). Comprehensive assessment of candidate genes associated with fattening performance in Holstein–Friesian bulls. *Arch. Anim. Breed.* 62: 9-32.

Aschaffenburg R and Drewry J (1957). Genetics of the ß-lactoglobulins of cow's milk. *Nature*. 180: 376-378.
Azevedo ALS, Nascimento CS, Steinberg RS, Carvalho MRS, et al. (2008). Genetic polymorphism of the kappa-casein gene in Brazilian cattle. *Genet. Mol. Res.* 7: 623-630.
Barendse WJ (1999). Assessing lipid metabolism. Patent, International Publication Number: WO 99/23248. World International Property Organization.
Barendse WJ, Bunch R, Thomas M, Armitage S, et al. (2004). The TG5 thyroglobulin gene test for a marbling quantitative trait loci evaluated in feedlot cattle. *Austr. J. Exp. Agricult*. 44: 669-674.
Barroso ADS, Dunner S and Cañón J (1998). Technical note: detection of bovine Kappa-casein Variants A, B, C and E by means of Polymerase Chain Reaction- Single Strand Conformation polymorphism (PCR-SSCP). *J. Anim. Sci.* 76: 1535-1538.
Beavis WD (1994). The power and deceit of QTL experiments: Lessons from comparative QTL studies. Pages 250–266 in Proc. 49th Annu. Corn and Sorghum Res. Conf., Am. Seed Trade Assoc., Alexandria, VA.
Bernabucci U, Basiricò L, Morera P, Dipasquale D, et al. (2015). Effect of summer season on milk protein fractions in Holstein cows. *J. Dairy Sci.* 98(3): 1815-27.
Boison SA, Utsunomiya ATH, Santos DJA, Neves HHR, et al. (2017). Accuracy of genomic predictions in Gyr (*Bos indicus*) dairy cattle. *J. Dairy Sci.* 100(7): 5479-5490
Boldman KG, Kriese LA and Van Vleck LD (1995). A manual for use of MTDFREML. A set of programs to obtain estimates of variances and covariances [DRAFT], Department of agriculture, Agricultural Research Service, Beltsville, U.S.
Bordbar F, Jensen J, Du M, Abied A, et al. (2020). Identification and validation of a novel candidate gene regulating net meat weight in Simmental beef cattle based on imputed next-generation sequencing. *Cell Prolif.* 53: e12870.
Botstein D, White RL, Skolnick M and Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331.
Bouquet A, Sørensen AC and Juga J (2015). Genomic selection strategies to optimize the use of multiple ovulation and embryo transfer schemes in dairy cattle breeding programs, *Livest. Sci.* 174: 18-25.
Bovenhuis H, Van Arendonk JAM and Korver S (1992). Associations Between Milk Protein Polymorphisms and Milk Production Traits. *J. Dairy Sci.* 75: 2549-59.
Bruneli FAT, Peixoto MGCD, Santana Junior ML, Pereira RJ, et al. (2020). Programa Nacional de Melhoramento do Guzerá para Leite: resultados do Teste de Progênie, do Programa de Melhoramento Genético de Zebuínos da ABCZ e do Núcleo MOET (Documento 249). Embrapa Gado de Leite, Juiz de Fora, MG, Brasil.
Brym P, Kamiñski S and Wójcik E (2005). Nucleotide sequence polymorphism within exon 4 of the bovine prolactin gene and its associations with milk performance traits. *J. Appl. Genet.* 45(2): 179- 185.
Cai Z, Dusza M, Guldbrandtsen B, Lund MS, et al. (2020). Distinguishing pleiotropy from linked QTL between milk production traits and mastitis resistance in Nordic Holstein cattle. *Genet. Sel. Evol.* 52: 19.
Caroli AM, Chessa S and Erhardt GJ (2009). Invited review: milk protein polymorphisms in cattle: effect on animal breeding and human nutrition. *J. Dairy Sci.* 92: 5335-5352.
Carvalho TDD, Siqueira F, Torres Junior RADA, Medeiros SRD, et al. (2012). Association of polymorphisms in the leptin and thyroglobulin genes with meat quality and carcass traits in beef cattle. *Rev. Bras. de Zootec.* 41: 2162-2168.
Chrenek P, Huba J, Oravcova M, Hetenyi L, et al. (1999). Genotypes of bGH and b PRL genes in relationships to milk production. In: 50th Annual Meeting of the EAAP Zuerich Book of Abstracts.
Chung ER, Rhim TJ and Han SK (1996). Associations between PCR-RFLP markers of growth hormone and prolactin genes and production traits in dairy cattle. *Korean J. Anim. Sci.* 38: 321-336.
Dadhich S, Patel RK, Soni KJ, Singh KM, et al. (2006). Estimation of allelic frequency of κ-casein and β-lactoglobulin genes in *Bos indicus* cattle breeds. *Int. J. Cow Sci.* 2: 48-51.
Dekkers JCM (2004). Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. *J. Anim. Sci.* 82: 313-328.
Deng H, Chen W and Recker RR (2001). Population Admixture: Detection by Hardy-Weinberg Test and Its Quantitative Effects on Linkage-Disequilibrium Methods for Localizing Genes Underlying Complex Traits. *Genetics*. 157: 885-897.
Dos Santos FC, Peixoto MGCD, Fonseca PAS, Pires MFA, et al. (2017). Identification of Candidate Genes for Reactivity in Guzerá (*Bos indicus*) Cattle: A Genome-Wide Association Study. *PLoS ONE.* 12(1): e0169163.
Dybus A, Grzesiak W, Kamieniecki H, Szatkowska I, et al. (2005). Association of genetic variants of bovine prolactin with milk production traits of Black-and-White and Jersey cattle. *Archiv. Tierzucht.* 48: 149-156.
El-Aziz AHA, Mahrous UE, Kamel SZ and Sabek AA (2016). Factors Influencing *in vitro* Production of Bovine Embryos: A Review. *Asian J. Anim. Vet. Adv.* 11: 737-756.
Falconer DS (1981). Introduction to Quantitative Genetics, 2nd ed. Logman Inc. New York
Food and Agriculture Organization of the United Nations (1995). World Watch List for Domestic Animal Diversity. 2ed. FAO, Rome, Italy.
Falconer DS and Mackay TFC (1996). Introduction to quantitative genetics, Pearson Education Ltd, Harlow, England.

Fang L, Sahana G, Su G, YuY, et al. (2017). Integrating Sequence-based GWAS and RNA-Seq Provides Novel Insights into the Genetic Basis of Mastitis and Milk Production in Dairy Cattle. *Sci. Rep.* 7: 45560.

Farrell HM, Jimenez-Flores JR, Bleck GT, Brown EM, et al. (2004). Nomenclature of the Proteins of Cows' Milk-Sixth Revision. *J. Dairy Sci.* 87: 1641-1674.

Fitzgerald R and Hill J (1997). The relationship between milk protein polymorphism and the manufacture and functionality of dairy products. Pages 355-371 In: Milk Protein Polymorphism III. (Ed. Creamer, L. K), Publisher: Brussels: International Dairy Federation.

Fortes MRS, Curi RA, Chardulo LAL, Silveira AC, et al. (2009). Bovine gene polymorphisms related to fat deposition and meat tenderness. *Genet. Mol. Biol.* 32(1): 75-82.

Ghasemi N, Zadehrahmani M, Rahimi G and Hafezian SH (2009). Associations between prolactin gene polymorphism and milk production in montebeliard cows. *Int. J. Genet. Mol. Biol.* 1: 48-51.

Golijow CD, Giovambattista G, Rípoli MV, Dulout FN et al. (1999). Genetic variability and population structure in loci related to milk production traits in native argentine creole and commercial argentine holstein cattle. *Genet. Mol. Biol.* 22(3): 395-398.

Granleese T, Clark SA, Swan AA and van der Werf JHJ (2015). Increased genetic gains in sheep, beef and dairy breeding programs from using female reproductive technologies combined with optimal contribution selection and genomic breeding values. *Genet. Sel. Evol.* 47: 70.

Hayes B, Moen T and Goddard M (2005). Dissection of genetics of complex traits and progress in aquaculture and livestock species. *AgBiotechNet.* 7: 1-10.

Hill JP (1993). The relationship between ß-lactoglobulin phenotypes and milk composition in New Zealand dairy cattle. *J. Dairy Sci.* 76: 281-286.

Horne DS, Banks MJ and Muir DD (1997). Milk protein polymorphism. Genetic polymorphism of bovine κ-casein: Effects on renneting and cheese yield. Proceedings of the IDF Seminar Held in Palmerston North, New Zealand, 161-181.

Horseman ND, Zhao W, Montecino-Rodriguez E, Tanaka M, et al. (1997). Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the Prolactin gene. *EMBO Journal.* 16: 6926-6935.

Iung LHS, Petrini J, Ramírez-Díaz J, Salvian M, et al. (2019). Genome-wide association study for milk production traits in a Brazilian Holstein population. *J. Dairy Sci.* 102(6): 5305-5314.

Jakob E and Puhan Z (1995). Implications of genetic polymorphism of milk proteins on production and processing of milk. *IDF Bulletin.* 304: 2-25.

Kemenes PA, Regitano LCA, Rosa AJM, Packer IU, et al. (1999). κ-casein, β-lactoglobulin and growth hormone allele frequencies and genetic distances in Nelore, Gyr, Guzerá, Caracu, Charolais, Canchim and Santa Gertrudis cattle. *Genet. Mol. Biol.* 22(4): 539-541.

Kaminski S, Rymkiewicz-Schymczyk J, Wojcik E and Rusc A (2002). Associations between bovine milk protein genotypes and haplotypes and the breeding value of Polish Black-and-White bulls. *J. Anim. Feed Sci.* 11: 205-221.

Khatib H, Zaitoun I, Chang YM, Maltecca C and Boettcher P (2007). Evaluation of association between polymorphism within the thyroglobulin gene and milk production traits in dairy cattle. *J. Anim. Breed. Genet.* 124: 26-28.

Kučerová J, Němcová E, Štípková M, Jandurová O, et al. (2005). The association between CSN3 genotypes and milk production parameters in Czech Pied Cattle. In: 56th Ann. Meet. EAAP Uppsala, Sweden.

Kučerová J, Matějíček A, Jandurová OM, Sorensen P, et al. (2006). Milk protein genes CSN1S1, CSN2, CSN3, LGB and their relation to genetic values of milk production parameters in Czech Fleckvieh. *Czech J. Anim. Sci.* 51: 241-247.

Le Roy P, Elsen JM, Boichard D, Mangin B, et al. (1998). An algorithm for QTL detection in mixture of full and half sib families. In: 6th World Cong. *Genet. Appl. Livest. Prod.* 26: 257-260.

Lemay DG, Lynn DJ, Martin WF, Neville MC, et al. (2009). The bovine lactation genome: insights into the evolution of mammalian milk. *Genome Biol.* 10(4)R43: 1-18.

Lewin HA, Schmitt K, Hubert R, Vanelk MJT et al. (1992). Glose linkage between bovine prolactin and BoLA-DRB3 genes: Genetics mapping in cattle by single sperm typing. *Genomics.* 13: 44-48.

Li F, Cai C, Qu K, Liu J, et al. (2020). *DGAT1* K232A polymorphism is associated with milk production traits in Chinese cattle. *Anim. Biotechnol.* 32(4): 427-431.

Lin CY, McAllister AJ, Ng-Kwai-Hang KF and Hayes JF (1986). Effects of milk protein loci on first lactation production in dairy cattle. *J. Dairy Sci.* 69: 704-712.

Liu L, Zhou J, Chen CJ, Zhang J, et al. (2020). GWAS-Based Identification of New Loci for Milk Yield, Fat, and Protein in Holstein Cattle. *Animals.* 10(11): 2048.

Lin S, Zhang H, Hou Y, Liu L, et al. (2019). SNV discovery and functional candidate gene identification for milk composition based on whole genome resequencing of Holstein bulls with extremely high and low breeding values. *PLoS ONE.* 14(8): e0220629.

Lodes A, Buchberger J, Krause I, Aumann J, et al. (1996). The influence of genetic variants of milk protein on the compositional and technological properties of milk. *Milchwissenschaft.* 51: 543-547.

Machugh DE, Loftus RT, Bradley DG, Sharp PM, et al. (1994). Microsatellite DNA variation within and among European cattle breeds. *Proceedings of the Royal Society of London, Series B*. 256: 25-31.

McLean DM, Graham ER, Ponzoni RW and McKenzie H (1984). Effects of milk protein genetic variants on milk yield and composition. *J. Dairy Res*. 51: 531-46.

Medrano JF and Corcodova EA (1990). Polymerase chain reaction amplification of bovine b-lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. *Anim. Biotechnol.* 1: 73-77.

Mercier J, Brignon CG and Ribadeau-Dumas B (1973). Structure primarie de la caseine -bovine B. Sequence complete. *Eur. J. Biochem*. 35: 222-235.

Miceikienė I, Peciulaitienė N, Baltrėnaitė L, Skinkytė R, et al. (2006). Association of cattle genetic markers with performance traits. *Biologija*. 1: 24-29.

Miller SA, Dykes DD and Polesky HF (1988). A simple salting out procedure for extracting DNA from nucleated cells. *Nucleic Acids Res*. 16: 1215.

Mitra A, Schlee P, Balakrishnan CR and Pirchner F (1995). Polymorphisms at growth-hormone and prolactin loci in Indian cattle and buffalo. *J. Anim. Breed. Genet.* 112: 71-74.

Molina LH, Benavides T, Brito C, Carrillo B, et al. (2006) Relationship between A and B variants of k-casein and b-lactoglobulin and coagulation properties of milk. *Int. J. Dairy Technol.* 59: 188-191.

Moore SS, Li C, Basarab J, Snelling WM, Kneeland J, et al. (2003). Fine mapping of quantitative trait loci and assessment of positional candidate genes for backfat on bovine chromosome 14 in a commercial line of *Bos taurus*. J. *Anim. Sci*. 81: 1919-1925.

Mrode R, Ojango JMK, Okeyo AM and Mwacharo JM (2019). Genomic Selection and Use of Molecular Tools in Breeding Programs for Indigenous and Crossbred Cattle in Developing Countries: Current Status and Future Prospects. *Front. Genet.* 9: 694.

Neubauerová V (2001). Detection of genetic markers and their use in cattle and other hoofed animals. Thesis. ZF JU České Budějovice.

Ng-Kwai-Hang KF, Hayes JF, Moxley JE and Monardes HG (1984). Association of genetic variants of casein and milk serum proteins milk, fat, and protein production in dairy cattle. *J. Dairy Sci*. 67: 835-840.

Ng-Kwai-Hang KF, Hayes JF, Moxley FE and Monardes HG (1986). Relationships between milk protein polymorphisms and major milk constituents in Holstein-Friesian cows. *J. Dairy Sci*. 69: 22-26.

Niall HD, Hogan ML, Sayer R, Rosenblum IY, et al. (1971). Sequences of pituitary and placental lactogenic and growth hormones: Evolution from primordial peptid by gene reduplication. *PNAS*. 68: 866-869.

Nicholas FW and Smith C (1983). Increased rates of genetic change in dairy cattle by embryo transfer and splitting. *Anim. Prod*. 36: 341-353.

Olsen HG, Knutsen TM, Lewandowska-Sabat AM, Grove H, et al. (2016). Fine mapping of a QTL on bovine chromosome 6 using imputed full sequence data suggests a key role for the group-specific component (GC) gene in clinical mastitis and milk production. *Genet. Sel. Evol.* 48(1): 79.

Paiva JT, Peixoto MGCD, Bruneli FAT, Alvarenga AB, et al. (2020). Genetic parameters, genome-wide association and gene networks for milk and reproductive traits in Guzerá cattle. *Livest. Sci.* 242: 104273.

Peixoto MGCD, Verneque RS, Teodoro RL, Penna VM, et al. (2006). Genetic trend for Milk yield in Guzerat herds participating in progeny testing and MOET nucleus schemes. *Genet. Mol. Res*. 5(3): 454-465.

Peixoto MGCD, Martinez ML, Teodoro RL, Machado MA, et al. (2009). Detecção de QTL em dados de famílias estruturadas como as de um núcleo MOET por meio do método da regressão. *Arq. Bras. Med. Vet. Zootec.* 61(4): 941-948.

Peixoto MGCD, Poggian CF, Verneque RS, Egito AA, et al. (2010). Genetic basis and inbreeding in the Brazilian Guzerá (*Bos indicus*) subpopulation selected for milk production. *Livest. Sci.* 131(2-3): 168-174.

Peixoto MGCD, Carvalho MRS, Egito AA, Steinberg RS, et al. (2021). Genetic Diversity and Population Genetic Structure of a Guzerá (*Bos indicus*) Meta-Population. *Animals*. 11(4): 1125.

Penna VM, Madalena FE and Alvim MTT (1998). Open MOET nucleus of selection in Guzerá. In: World Congress on Genetics Applied to Livestock Production (WCGALP), 6, Australia. Vol. 27. WCGALP, 439.

Penna VM, Melo VJM, Teodoro RL and Peixoto MGCD (2005). Aspectos técnicos, econômicos, sociais e ambientais da atividade leiteira. 1 ed. Juiz de Fora, Embrapa Gado de Leite.

Pupková GV (1980). Milk protein polymorphism and milk production of Estonian Black Pied cows. *Dairy Science*. 45: 6620.

Rangel A, Zaros LG, Lima TC, Borba L, et al. (2017). Polymorphism in the Beta Casein Gene and analysis of milk characteristics in Gir and Guzerá dairy cattle. *Genet. Mol. Res.* 16(2): gmr16029592.

Raymond M and Rousset F (1995). Genepop (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* 86: 248-249.

Riquet J, Coppieters W, Cambisano N, Arranz J, et al. (1999). Fine-mapping of quantitative trait loci by identity by descent in outbred populations: application to milk production in dairy cattle. *PNAS*. 96: 9252-9257.

Ron M and Weller JI (2007). From QTL to QTN identification in livestock - winning by points rather than knock-out: a review. *Anim. Genet.* 38: 429-439.

Rosendo A, Iannuccelli N, Gilbert H, Riquet J, et al. (2012). Microsatellite mapping of quantitative trait loci affecting female reproductive tract characteristics in Meishan × Large White $F_2$ pigs. *J. Anim. Sci.* 90(1): 37-44.

Rosse IC, Steinberg RS, Coimbra RS, Peixoto MGCD, et al. (2014). Novel SNPs and INDEL polymorphisms in the 3-UTR of DGAT1 gene: in silico analyses and a possible association. *Mol. Biol. Rep.* 42: 1573-4978.

Rosse IC, Assis JG, Oliveira FS, Leite LR, et al. (2017). Whole genome sequencing of Guzerá cattle reveals genetic variants in candidate genes for production, disease resistance, and heat tolerance. *Mamm. Genome*. 28: 66-80.

Russo V and Mariani P (1978). Polimorfismo delle proteine del latte e relazioni ma varianti genetiche e caratteristiche di interesse zootecnico tecnologico e caseario. *Rivista di zootecnia e veterinária.* 5: 289.

Sacravarty G, Vadodaria VP, Joshi CG, Brahmkshtri BP, et al. (2008). Prolactin Gene Polymorphism and its Association With Economic Traits in Kankrej Cattle. *Ind. J. Dairy Sci*. 61(4): 273-275.

Sambrook J and Russel DW (2001). Molecular Cloning. 3rd ed. 3 vol. Cold Spring Harbor Laboratory Press, New York.

SAS Institute Inc. (2003) SAS/STAT User's Guide: Version 9. 5th edn. SAS Institute Inc., Cary, North Carolina.

Shetty S, Patel RK, Soni KJ, Singh KM, et al. (2006). Allelic frequency of κ-casein and β-lactoglobulin in Jersey cattle. *Indian J. Anim. Res.* 15: 15-21.

Silva IT and Del Lama MA (1996). Milk protein polymorphisms in Brazilian Zebu cattle. *Braz. J. Genet.* 20: 625-630.

Strathie RJ and McGuirk BJ (1995). Developments with MOET dairy breeding scheme. *Br. Cattle Breed. Club. Dig.* 50: 9-15.

Thaller G, Kuhn C, Winter A, Ewald G, et al. (2003). DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Anim. Genet.* 34: 354-357.

The Bovine HapMap Consortium (2009). Genome-Wide Survey of SNP Variation Uncovers the Genetic Structure of Cattle Breeds. *Science.* 324: 528-532.

United States Department of Agriculture (USDA) 2021. Available in <https://www.fas.usda.gov/data/brazil-dairy-and-products-annual-7#:~:text=Post%20forecasts%20milk%20production%20in,a%20major%20recession%20in%20Brazil.> last accessed on March 17, 2021.

Wakchaure R, Ganguly S, Praveen P, Kumar A, et al. (2015). Marker Assisted Selection (MAS) in Animal Breeding: A Review. *J. Drug Metab. Toxicol* 6(5): 1-4.

Winkler R and Penna VM (1992). O Guzerá. *Informativo Agropecuário.* 16: 10-14.

Yeh FC, Yang RC, Boyle TB, Ye Z, et al. (2000). POPGENE, the user-friendly shareware for population genetic analysis, Molecular biology and biotechnology centre, University of Alberta, Alberta, Canada.

Yue SJ, Zhao YQ, Gu XR, Yin B, et al. (2017). A genome-wide association study suggests new candidate genes for milk production traits in Chinese Holstein cattle. *Anim. Genet.* 48: 677-681.

Zepeda-Batista JL, Saavedra-Jiménez LA, Ruíz-Flores A, Núñez-Domínguez R, et al. (2017). Potential influence of κ-casein and β-lactoglobulin genes in genetic association studies of milk quality traits. *Asian-Australas. J. Anim. Sci.* 30(12): 1684-1688.

Zhu M and Zhao S (2007). Candidate gene identification approach: progress and challenges. *Int. J. Biol. Sci.* 3(7): 420-427.