EFFECTS OF BOVINE *PPARGC1A* AND *LTF* GENE VARIANTS ON MILK YIELD AND COMPOSITION TRAITS IN HOLSTEIN-FRIESIAN AND JERSEY COWS

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ABSTRACT

In this study, the association of bovine *PPARGC1A* and *LTF* gene polymorphisms with milk production and composition was investigated in dairy cattle. A total of 200 Holstein and Jersey cows, 100 from each breed, were used in the study. Total milk yield, 305-day, and test-day milk yield records were recorded. Milk fat/protein yield and percentage were calculated. Lactation rank, calving season, and service period were also taken into account in the analyses. Genomic DNA was extracted from whole blood samples by the phenol-chloroform-isoamyl alcohol method. Genotyping was carried out by the PCR-RFLP method. In this context, two polymorphisms at *PPARGC1A* and *LTF* genes located in intron 9 and 6, respectively, were evaluated. Genotypic/allelic frequencies, compliance with Hardy-Weinberg Equilibrium, and population genetics parameters were calculated. The general linear model (GLM) procedure was used to reveal the individual or interaction effects of these genes on the studied traits. The *LTF*/*Eco*RI marker was significantly associated with the lactation milk yield, 305-d milk yield, and 305-d milk fat yield in Jersey cattle. Moreover, the *PPARGC1A*×*LTF* interaction affected the test-d milk yield, test-d protein yield, 305-d milk yield, and 305-d milk fat yield in Jersey cattle. The CCAA and TTAB genotypes were found to be desirable for milk yield and fat content in Jersey cattle. The *PPARGC1A*×*LTF* interaction was also significantly associated with the test-d protein yield in the entire study population. This study may provide important knowledge on the genetic markers affecting milk production and the selection strategies in dairy cattle.

Key words: cattle, SNP, *PPARGC1A*, *LTF*, dairy production.

INTRODUCTION

Most economically important traits in livestock are quantitative and complex traits that are genetically controlled by polygenic inheritance. In recent years there has been immense interest in analyzing these traits, especially, intending to estimate the breeding values of selection candidates (Schmid & Bennewitz 2017). Global population growth will soon lead to significant limitations on food resources. In this context, animal products will also be greatly affected by this situation. Increasing the production to be obtained from the individual animal is one of the most basic solutions to face these problems. Selection practices supported by recent molecular genetic techniques have enabled the selection of superior individuals to be performed more effectively and reliably than conventional approaches. In particular, the dairy industry has been quick to implement these technologies in routine breeding programs, allowing for further gains in the accuracy of breeding values, and the inclusion of novel functional traits (Fleming et al., 2018). Furthermore, new genotype-phenotype associations are still being defined in different dairy breeds.

Previous studies have shown that BTA6 harbors quantitative trait loci (QTL) highly associated with major milk production traits including milk yield, milk fat yield/percentage, and milk protein yield/percentage (Khatkar et al., 2004; Pasandideh et al., 2015). Therefore, this genomic region is decisive in the genetic evaluation of milk yield and content, and the relevant genes are candidates for new interactions. Bovine peroxisome proliferator-activated receptor-γ coactivator-1α (*PPARGC1A*) is located on chromosome 6 (43,380,463-43,501,184 reverse strand) (Ensembl genome browser, 2022) and it acts as a mediator for the expression of the genes related to adipogenesis, gluconeogenesis, and oxidative metabolism (Pasandideh et al., 2015). The protein product of this gene (PGC-1α) is an important activator of several different transcriptional coactivators which can interact with several mitochondrial genes in the nucleus, resulting in enhanced mitochondrial biogenesis (Eivers et al., 2012, Lin et al., 2005). It is also associated with the regulation of fatty acid oxidation (Zhang et al., 2006) and angiogenesis (Arany, 2008). The bovine lactoferrin (*LTF*) gene is a member of the transferrin gene family and it spans about 34.5 kb of genomic DNA located on BTA22 (52,952,571-52,986,619 forward strand) (Ensembl genome browser, 2022). Its 708 aa protein product has been shown to have antimicrobial and iron homeostasis properties (El-Domany et al., 2019). Polymorphisms in the *LTF* gene are associated with somatic cell score, lactogenesis, health traits, mammary development, and milk protein secretion (Ateya et al., 2016; El-Domany et al., 2019; Kaminski et al., 2006; Wojdak-Maksymiec et al., 2006; Zheng et al., 2005). Taken together, the nucleotide alterations in the *PPARGC1A* and *LTF* may influence many functional traits directly or indirectly. There have been numerous reports in the literature describing the relationship of the *PPARGC1A* and *LTF* genes with milk production traits in various cattle breeds but the results are mostly controversial. Therefore, the purpose of the present study was to comparatively evaluate the effects of *PPARGC1A* and *LTF* gene polymorphisms as well as the genotypic interactions on milk yield and content in Holstein-Friesian and Jersey dairy cattle.

MATERIALS AND METHODS

Animals, sampling, and the phenotyping

The investigated cattle were from two dairy cattle breeds with a total of 200 individuals including Jersey (n = 100) and Holstein-Friesian (n = 100). All animals were raised on two commercial dairy farms located in the Black Sea Region of Turkey. All cattle were subjected to similar feeding and management conditions. They were housed in free-stall barns and fed *ad libitum*. A dairy total mixed ration was formulated to meet NRC (2001) recommendations. The cows were milked three times a day. The milk yield of each cow was recorded daily in milking parlors equipped with electronic devices that automatically recorded the quantity of milk produced by every individual animal. In addition, milk samples were analyzed for milk fat and protein content. Thus, milk fat and milk protein yield and percentage were evaluated based on the entire lactation period, 305-d milk production, and the test day. Approximately 5 mL of blood was sampled from the jugular vein of each animal for genetic analysis under as aseptic conditions as possible.

DNA extraction and PCR-RFLP analysis

Genomic DNA was isolated from blood samples using a standard phenol-chloroform extraction protocol (Green & Sambrook, 2012). The quantification of genomic DNA concentration was assayed by NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Genotyping of the SNPs in the *PPARGC1A* and *LTF* genes was performed by the PCR-RFLP. In this context, we investigated the T>C SNP of the bovine *PPARGC1A* gene at position 1892 located in intron 9. The primers used to amplify the corresponding fragment of the *PPARGC1A* gene were as follows: Forward:

AGGTAAGATGCACGTTGGC and Reverse: CTGGTACTCCTCGTAGCTGTC. Next, we amplified a 301-bp fragment to analyze the SNP located in intron 6 of the *LTF* gene by using the following primer sequences: Forward: GCCTCATGACAACTCCCACAC and Reverse: CAGGTTGACACATCGGTTGAC. The PCR reactions were performed in a final volume of 25 µL containing 2.50 µL DNA sample (~60 ng genomic DNA) as a template, 12.50 µL PCR master mix 5× FIREPol® Master Mix (Solis BioDyne, Estonia), 1 µL (0.5 µM) of each primer, and 8 µL of nuclease-free water (Thermo Scientific). The PCR protocols were set based on the studies conducted by Pasandideh et al. (2015) and El-Domany et al. (2019) for the *PPARGC1A* and *LTF* genes, respectively, with some modifications. When the amplification was successfully performed, the PCR products were subjected to restriction enzyme digestion. In this respect, *Hae*III (New England BioLabs) and *Eco*RI (Thermo Scientific, #ER0221) enzymes were used for the *PPARGC1A* and *LTF*, respectively. The RFLP reaction mixture was carried out in 20 µL consisting of 15 µL PCR product, 1 µL restriction enzyme, 5 µL 10× buffer, and 8 µL nuclease-free water (Thermo Scientific). The digestion products were electrophoresed on a 3% (w/v) agarose gel. The electrophoresis patterns were visualized with a UV transilluminator (DNR-Minilumi, DNR Bio-Imaging Systems, Israel) and ethidium bromide (2 µg/mL) was used as a DNA-intercalating dye.

Statistical analysis

Gene and allelic frequencies in *PPARGC1A* and *LTF* loci were estimated using the equations described by (Falconer & Mackay, 1996). The compliance with Hardy-Weinberg equilibrium (HWE) was tested and the population genetic parameters, including gene heterozygosity (He), polymorphism information content (PIC), and the effective number of alleles (Ne) were estimated for both loci. Association analysis was performed using the least squares method of the general linear model (GLM) procedure using Minitab statistical software (Minitab, Pennsylvania, USA, v17.1.0) based on the following statistical model:

$$Y_{ijklm} = \mu + A_i + B_j + G_k + I_l + e_{ijklm}$$

where Y_{ijklm} is the trait measured, μ is the overall mean, A_i is the fixed effect of lactation rank, B_j is the fixed effect of calving season, G_k is the fixed effect of the genotype, I_l is the genotypic interactions, and e_{ijklm} is the random error. The genotype-phenotype comparisons were performed based on both breed-specific and the entire population. When the association was evaluated in the entire animal material, the fixed effect of the farm was included in the statistical model. Tukey's test was used as a post hoc comparison.

RESULTS AND DISCUSSION

Regulation of milk production is a highly complex biological process involving many mechanisms and pathways. Thus, many genes directly or indirectly contribute to this process. To date, numerous genotypic components of variance have been associated with milk production traits in dairy cattle. In particular, some genes are effective in very different biological pathways and play an important role in the diversity of different traits due to their pleiotropic nature. For instance, the *PPARGC1A* gene has a wide phenotypic effect from adipogenesis to oxidative energy metabolism (Pasandideh et al., 2015). Similarly, the *LTF* gene is involved in many different biological processes, such as lactogenesis, iron metabolism, and health properties (El-Domany et al., 2019). Surprisingly, novel associations of variation in these genes with different phenotypic traits have been identified, especially in dairy cattle. In this study, we evaluated the association of *PPARGC1A* and *LTF* gene polymorphisms with milk yield and content in Holstein and Jersey cows. The electropherograms of PCR-RFLP analysis are presented in Figure 1A-D. Although the BB genotype was not observed in the *LTF* gene,

there was an adequate genotypic distribution that allowed us to make the necessary evaluations in the association analysis. In the *PPARGC1A* gene, we observed all three genotypes and relatively a balanced genotypic distribution in both breed-specific and the entire study population that resulted in compatibility with the HWE (Table 1). In all observations, the predominant genotype was the heterozygotes while the TT genotype had the lowest frequency. The balanced condition in the genotypic distributions for the *PPARGC1A c.1892T>C* polymorphism led to a high genetic variation for this genetic marker and hence the estimation of the desired population genetic parameters, as shown in Table 1. Concerning the *LTF* marker, the heterozygous genotype was also predominant but the absence of the BB genotype resulted in an unbalanced genotypic distribution and a deviation from the HWE (Table 2). The A allele had a very high frequency in both the Holstein and Jersey breeds. This, unsurprisingly, led to the estimation of lower population genetic parameters for the *LTF* compared to the *PPARGC1A* gene. From a population genetics perspective, highly variable genotypic distributions of the same genetic markers among different populations and deviations from HWE are typical characteristics of dairy herds. One of the main reasons for this situation is the intense selection of dairy cattle populations, especially for the Holstein-Friesians. Using artificial insemination with a few sires producing a large number of daughters results in increased inbreeding levels and population stratification (Ardicli et al., 2019b; Lacorte et al., 2006). Here, it should be noted that we observed a moderate genetic diversity for the selected markers in this study. Especially, for the *PPARGC1A* marker, population genetic parameters of He>0.49; Ne>1.96; PIC>0.36 were estimated for all breed groups. Based on the classification suggested by Botstein et al. (1980), PIC values can be denoted using three levels of informativeness as follows: PIC>0.50 (high polymorphism), 0.25<PIC<0.50 (moderate polymorphism), and PIC<0.25 (low polymorphism). Accordingly, both markers studied in the present study are moderately informative for both breed-specific determination and the entire experimental population (Tables 1 and 2). Evaluation of population genetic parameters is a very important task in genetic studies because these indices express population structure defined by genetic variation of a particular gene or genes. To give an example, the low heterozygosity values indicate that inbreeding may be a potential problem at the herd level, and therefore pedigree data should be taken into account in detail. Moreover, these parameters give important clues about the effectiveness of the selected genetic markers in the studied population and how descriptive they are for the population. (Ardicli et al., 2019a). Taken together, in this study, admissible levels of population genetic parameters were observed in Holstein and Jersey cows.

In the present study, we performed a comparative assessment of the effects of *PPARGC1A* and *LTF* gene polymorphisms on milk yield and content. In this respect, we performed the association analysis for Holstein and Jersey breeds separately. Next, we analyzed the genotypic effects in the entire study population. On the other hand, genotype effects were evaluated based on both individual genetic markers and *PPARGC1A* × *LTF* interaction. Results revealed that the *PPARGC1A* and *LTF* marker effects were insignificant in the Holstein breed (Table 3). There was no significant association between the *PPARGC1A* × *LTF* and any of the phenotypic traits as well (Table 4). But, a tendency ($P<0.1$) was observed for the association of *PPARGC1A* c.1892T>C polymorphism with 305-d milk yield and 305-d milk protein yield. The TT animals seemed to have a higher mean for these traits (Table 3). Concerning the Jersey cows, there were no significant effects of the *PPARGC1A* c.1892T>C polymorphism on any of the traits analyzed (Table 5). Similarly, Kowalewska-Łuczak et al. (2010) showed that there were no statistically significant associations between the individual genotypes of both SNPs and milk traits in the Jersey breed. On the contrary, Schennink et al. (2009) found a significant association between *PPARGC1A* c.1892T>C and milk fat composition in Dutch Holstein-Friesian cattle. Pasandideh et al. (2015) reported that *PPARGC1A* c.1892T>C significantly affected milk fat content adjusted for two milkings per day, estimated breeding value for milk,

estimated breeding value for milk fat content, milk protein yield adjusted for 305 days, and milk protein yield adjusted for mature body weight in Iranian Holsteins. Weikard et al. (2005) found a significant association between the *PPARGC1A* c.1892T>C and milk fat yield in a major dairy cattle population. These researchers have also indicated that the *PPARGC1A* gene could be involved in genetic variation underlying the QTL for milk fat synthesis on BTA6. Although not statistically confirmed, a similar relationship was observed in the Jersey breed in the present study. As shown in Table 5, the CC genotype produced a remarkably higher fat yield (305-d) compared to alternative genotypes (P=0.078). *PPARGC1A* has a key function in activating many nuclear hormone receptors and transcription factors regulating energy homeostasis. Moreover, this gene mediates the expression of genes involved in adaptive thermogenesis, oxidative metabolism, adipogenesis, and gluconeogenesis (Weikard et al., 2005). Hence, it is quite possible to find novel associations between the *PPARGC1A* gene variants and milk production traits. Bovine *LTF* is a valuable genetic marker for health traits in Holstein cattle such as mastitis tolerance/susceptibility (Ateya et al., 2016). The variation in this gene has been also associated with milk, fat, protein yields and milk fat, protein concentration (O'Halloran et al., 2009), total milk production (Maletić et al., 2013), and milk fat percentage (Asadollahpour Nanaei et al., 2016). In this study, we found that *LTF*/*Eco*RI polymorphism significantly affected the lactation milk yield, 305-d milk yield, and 305-d milk fat yield (P<0.05) in Jersey cattle. The ANOVA results indicated that the heterozygous genotype is preferable regarding these traits. The heterozygous animals were characterized by +802 kg lactation milk yield, +513 kg 305-d milk yield, and 22.54 kg 305-d milk fat yield higher means compared to the AA animals (Table 5). Supportively, El-Domany et al. (2019) reported that *LTF*/*Eco*RI polymorphism was significantly associated with the order of lactation, days in milk, dry period, level of production, 305-d milk yield, and daily milk yield (P<0.05). As in our study, these researchers have found that the heterozygous genotype is preferable in terms of these characteristics. It is worth noting here that the BB genotype was absent in both Holstein and Jersey breeds. Previous studies have also indicated that the BB genotype of the *LTF*/*Eco*RI polymorphism is not observed in Holstein-Friesians (Asadollahpour Nanaei et al., 2016; El-Domany et al., 2019) with some exceptions (Wojdak-Maksymiec et al., 2006). Hence, further studies with larger dairy cattle populations are needed to confirm the significant associations observed in this study and to obtain novel associations.

In general, association studies in livestock focus on each locus separately in most QTL studies, and therefore these studies do not account for the interaction between different loci (Ardicli et al., 2019b). But these interactions may account for differences in genotype responses across populations and genetic backgrounds (Tambasco et al., 2003). From a broader perspective, it would be more accurate to explain the variation in a trait using multiple genetic markers rather than being determined by a single individual genetic marker. We report here some novel associations between the *PPARGC1A* × *LTF* and milk production traits. In this context, this genotypic interaction significantly affected the test-d milk yield, test-d protein yield, 305-d milk yield, and 305-d milk fat yield (P<0.05) in Jersey cattle. The ANOVA results indicated that the CCAA genotype of the *PPARGC1A* × *LTF* interaction was characterized by higher milk yield and content compared to the alternative combined genotypes (Table 6). Although the individual effects of the markers on any of the traits were not significant (Table 7), the *PPARGC1A* × *LTF* interaction was significantly associated with the test-d protein yield in the entire study population. The CCAA and TTAB genotypes seemed to have better protein yield compared to other genotypes (Table 8). On the other hand, the *PPARGC1A* × *LTF* interaction showed tendencies through lactation milk yield (P=0.067) and 305-d milk fat yield (P=0.091) in Jersey cattle (Table 6) and test-d milk yield (P=0.051) in the entire population (Table 8).

In the present study, some of the previously reported associations were confirmed, and furthermore, novel associations have been shown in dairy cattle. The selected SNPs are located in the intronic regions. It is important to note that alterations in the introns play important roles in mRNA stability and alternative splicing. Thus, they can influence phenotypic variation indisputably (Le Hir et al., 2003). The main limitation of this study is the limited sample size. However, this is not a genotypic frequency scanning study nor a routine association study. We have tried to compare the effectiveness of the *PPARGC1A* and *LTF* markers as well as the *PPARGC1A* × *LTF* interaction in Holstein and Jersey breeds by using cows with similar days in milk and dry periods. Hence, the significant associations reported here may be useful for further analysis.

CONCLUSIONS

This paper focuses on the effects of the SNPs located in the bovine *PPARGC1A* and *LTF* genes on milk yield and content in dairy cattle. The *LTF*/*Eco*RI marker was significantly associated with the lactation milk yield, 305-d milk yield, and 305-d milk fat yield in Jersey cattle. Moreover, the *PPARGC1A* × *LTF* interaction affected the test-d milk yield, test-d protein yield, 305-d milk yield, and 305-d milk fat yield ($P<0.05$) in Jersey cattle. The *PPARGC1A* × *LTF* interaction was also significantly associated with the test-d protein yield in the entire study population. This study clearly shows that the *PPARGC1A* × *LTF* interaction may be a decisive marker to evaluate milk yield and content in dairy cattle even if the individual effects of genes are not so remarkable. The CCAA and TTAB genotypes deserve attention when evaluating the milk yield and fat content in Jersey cattle. Although genetic selection has gradually evolved from marker-assisted selection (MAS) applications, where a limited number of genetic markers are evaluated, to genome-based technologies, the identification of new genotype-phenotype relationships is still important on a herd basis. To provide successful MAS strategies, the interpretation of novel associations between the genetic markers and economic traits in animal material of interest is very important because most traits are rather complex than expected. New genetic associations will enable the development of different perspectives and increase accuracy in dairy cattle selection.

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DNA samples were re-extracted from the blood samples obtained within the scope of the ethical committee (decision number: 2010/6-05), by the Local Ethical Committee for Animal Experiments at Namik Kemal University.

REFERENCES

Arany, Z. (2008). PGC-1 coactivators and skeletal muscle adaptations in health and disease. *Current opinion in genetics & development*, 18, 426-434.

Ardicli, S., H. Samli, B. Soyudal, D. Dincel & F. Balci (2019a). Evaluation of candidate gene effects and environmental factors on reproductive performance of Holstein cows. *South African Journal of Animal Science*, 49, 379-374.

Ardicli, S., H. Samli, B. Vatansever, B. Soyudal, D. Dincel & F. Balci (2019b). Comprehensive assessment of candidate genes associated with fattening performance in Holstein–Friesian bulls. *Archives Animal Breeding*, 62, 9-32.

Asadollahpour Nanaei, H., S. Ansari Mahyari & M. Edriss (2016). Single nucleotide polymorphism of the lactoferrin gene and its association with milk production and reproduction traits in Iranian Holstein cattle. *Journal of Livestock Science and Technologies*, 4, 71-76.

Ateya, A., Y. El-Seady, S. Atwa, B. Merghani & N. Sayed (2016). Novel single nucleotide polymorphisms in lactoferrin gene and their association with mastitis susceptibility in Holstein cattle. *Genetika*, 48, 199-210.

Botstein, D., R. L. White, M. Skolnick & R. W. Davis (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American journal of human genetics*, 32, 314.
Eivers, S., B. McGivney, J. Gu, D. MacHugh, L. Katz & E. Hill (2012). PGC-1α encoded by the PPARGC1A gene regulates oxidative energy metabolism in equine skeletal muscle during exercise. *Animal genetics*, 43, 153-162.
El-Domany, W. B., H. A. Radwan, A. I. Ateya, H. H. Ramadan, B. H. Marghani & S. M. Nasr (2019). Genetic Polymorphisms in LTF/EcoRI and TLR4/AluI loci as candidates for milk and reproductive performance assessment in Holstein cattle. *Reproduction in Domestic Animals*, 54, 678-686.
Ensembl Genome Browser (2022). https://www.ensembl.org/index.html last access: 18.10.2022.
Falconer, D. S. & T. F. C. Mackay. (1996). Introduction to quantitative genetics. Harlow, England: Pearson Education Ltd.
Fleming, A., E. A. Abdalla, C. Maltecca & C. F. Baes (2018). Invited review: Reproductive and genomic technologies to optimize breeding strategies for genetic progress in dairy cattle. *Archives Animal Breeding*, 61, 43-57.
Green M & Sambrook, J. (2012). Isolation of high-molecular-weight DNA from mammalian cells using proteinase K and phenol. In Molecular Cloning: A Laboratory Manual, 47-48. Cold Spring Harbor, New York, USA: Cold Spring Harbor Laboratory Press.
Kaminski, S., K. Oleński, P. Brym, T. Malewski & A. Sazanov (2006). Single nucleotide polymorphism in the promoter region of the lactoferrin gene and its associations with milk performance traits in Polish Holstein-Friesian cows. *Russian journal of genetics*, 42, 924-927.
Khatkar, M. S., P. C. Thomson, I. Tammen & H. W. Raadsma (2004). Quantitative trait loci mapping in dairy cattle: review and meta-analysis. *Genetics Selection Evolution*, 36, 163-190.
Kowalewska-Łuczak, I., H. Kulig & M. Kmieć (2010) Associations between the bovine PPARGC1A gene and milk production traits. *Czech Journal of Animal Science*, 55, 195-199.
Lacorte, G., M. Machado, M. Martinez, A. Campos, R. Maciel, R. Verneque, R. Teodoro, M. Peixoto, M. Carvalho & C. Fonseca (2006). DGAT1 K232A polymorphism in Brazilian cattle breeds. *Genetics and Molecular Research*, 5, 475-482.
Le Hir, H., A. Nott & M. J. Moore (2003). How introns influence and enhance eukaryotic gene expression. *Trends in biochemical sciences*, 28, 215-220.
Lin, J., C. Handschin & B. M. Spiegelman (2005). Metabolic control through the PGC-1 family of transcription coactivators. *Cell metabolism*, 1, 361-370.
Maletić, M., S. Kanjac, N. Đelić, N. Lakić, M. Pavlović, S. Nedić & Z. Stanimirović (2013) Analysis of lactoferin gene polymophism and its association to milk quality and mammary gland health in Holstein-Friesian cows. *Acta Veterinaria-Beograd*, 63, 487-498.
NRC (2001). Nutrient Requirements of Dairy Cattle. Washington, DC., USA: Natl. Acad. Press.
O'Halloran, F., B. Bahar, F. Buckley, O. O'Sullivan, T. Sweeney & L. Giblin (2009) Characterisation of single nucleotide polymorphisms identified in the bovine lactoferrin gene sequences across a range of dairy cow breeds. *Biochimie*, 91, 68-75.
Pasandideh, M., M. Mohammadabadi, A. Esmailizadeh & A. Tarang (2015). Association of bovine PPARGC1A and OPN genes with milk production and composition in Holstein cattle. *Czech Journal of Animal Science*, 60, 97-104.
Schennink, A., H. Bovenhuis, K. M. Léon-Kloosterziel, J. A. Van Arendonk & M. H. Visker (2009). Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. *Animal Genetics*, 40, 909-916.
Schmid, M. & J. Bennewitz (2017). Invited review: Genome-wide association analysis for quantitative traits in livestock–a selective review of statistical models and experimental designs. *Archives Animal Breeding*, 60, 335-346.

Tambasco, D., C. Paz, M. Tambasco-Studart, A. Pereira, M. Alencar, A. Freitas, L. Coutinho, I. Packer & L. d. A. Regitano (2003). Candidate genes for growth traits in beef cattle crosses Bos taurus× Bos indicus. *Journal of Animal Breeding and Genetics*, 120, 51-56.

Weikard, R., C. Kühn, T. Goldammer, G. Freyer & M. Schwerin (2005). The bovine PPARGC1A gene: molecular characterization and association of an SNP with variation of milk fat synthesis. *Physiological Genomics*, 21, 1-13.

Wojdak-Maksymiec, K., M. Kmiec & J. Ziemak (2006). Associations between bovine lactoferrin gene polymorphism and somatic cell count in milk. *Veterinarni Medicina Praha*, 51, 14.

Zhang, Y., K. Ma, P. Sadana, F. Chowdhury, S. Gaillard, F. Wang, D. P. McDonnell, T. G. Unterman, M. B. Elam & E. A. Park (2006). Estrogen-related receptors stimulate pyruvate dehydrogenase kinase isoform 4 gene expression. *Journal of Biological Chemistry*, 281, 39897-39906.

Zheng, J., J. L. Ather, T. S. Sonstegard & D. E. Kerr (2005). Characterization of the infection-responsive bovine lactoferrin promoter. *Gene*, 353, 107-117.

Table 1. Gene frequency, population genetic indices, and HWE test results for the bovine *PPARGC1A* genotypes in the studied population

Breed	*n*	Gene frequency			Allele frequency		He	Ne	PIC	HWE test
		CC	CT	TT	C	T				
Holstein	100	0.22	0.59	0.19	0.52	0.48	0.4992	1.9968	0.3746	*P*>0.05
Jersey	100	0.33	0.47	0.20	0.57	0.43	0.4902	1.9616	0.3701	*P*>0.05
Total	200	0.28	0.53	0.19	0.54	0.46	0.4968	1.9873	0.3734	*P*>0.05

He: heterozygosity; Ne: the effective number of alleles; PIC: polymorphism information content; HWE: Hardy-Weinberg Equilibrium

Table 2. Gene frequency, population genetic indices, and HWE test results for the bovine *LTF* genotypes in the studied population

Breed	*n*	Genotype frequency			Allele frequency		He	Ne	PIC	HWE
		AA	AB	BB	A	B				
Holstein	100	0.24	0.76	0	0.62	0.38	0.4712	1.8911	0.3602	*P*<0.01
Jersey	100	0.35	0.65	0	0.67	0.33	0.4422	1.7928	0.3444	*P*<0.001
Total	200	0.30	0.70	0	0.65	0.35	0.4550	1.8349	0.3515	*P*<0.001

He: heterozygosity; Ne: the effective number of alleles; PIC: polymorphism information content; HWE: Hardy-Weinberg Equilibrium
P<0.01, *P*<0.001 not consistent with the HWE

Table 3. Least square means for the *PPARGC1A* and *LTF* genotype effects on milk production traits in the Holstein-Friesian breed (*n*=100).

Trait	*PPARGC1A*			*LTF*	
	CC	CT	TT	AA	AB
Lactation milk yield, kg	9704±1525	8511±1408	9374±1597	9341±1535	9052±1377
Test-d milk yield, kg	23.91±2.05	23.22±1.89	23.81±2.15	23.04±2.06	24.25±1.85
305-d milk yield, kg	10086±730	9485±674	10346±764	10101±735	9843±659
305-d milk fat yield, kg	383.51±32.50	358.20±30.00	384.92±34.01	383.50±32.71	367.60±29.32
305-d milk protein yield, kg	320.74±22.72	300.80±20.94	327.50±23.71	322.50±22.80	310.21±20.50
Test-d milk fat yield, kg	0.91±0.08	0.88±0.07	0.89±0.08	0.88±0.08	0.91±0.07
Test-d milk protein yield, kg	0.76±0.06	0.74±0.06	0.76±0.07	0.74±0.06	0.77±0.06
Milk fat content, %	3.83±0.26	3.85±0.24	3.79±0.28	3.88±0.27	3.76±0.24
Milk protein content, %	3.19±0.08	3.19±0.08	3.19±0.08	3.23±0.09	3.16±0.08

Table 4. Least square means for the *PPARGC1A* × *LTF* genotype effects on milk production traits in the Holstein-Friesian breed (*n*=100).

Genotype	Lactation milk yield	Test-day milk yield	305-d milk yield	305-d milk fat yield	305-d milk protein yield	Test-d milk fat yield	Test-d protein yield	Milk fat content	Milk protein content
CCAA	10230±2020	23.64±2.72	10335±967	391.10±43.00	330.40±30.00	0.89±0.10	0.76±0.08	3.79±0.36	3.23±0.11
CCAB	9179±1371	24.17±1.84	9837±656	376.00±29.20	310.90±20.41	0.93±0.07	0.77±0.06	3.88±0.24	3.17±0.08
CTAA	8087±1499	22.43±2.02	9461±718	362.60±31.90	300.20±22.30	0.86±0.08	0.71±0.07	3.95±0.26	3.19±0.09
CTAB	8934±1462	24.01±1.97	9508±700	353.80±31.10	301.50±21.70	0.91±0.07	0.77±0.06	3.75±0.25	3.19±0.09
TTAA	9707±1914	23.05±2.57	10507±916	396.70±40.70	336.90±28.40	0.88±0.09	0.74±0.08	3.91±0.33	3.27±0.11
TTAB	9042±1605	24.56±2.16	10184±768	373.10±34.20	318.10±23.90	0.89±0.08	0.77±0.07	3.66±0.28	3.12±0.09

Table 5. Least square means for the *PPARGC1A* and *LTF* genotype effects on milk production traits in Jersey breed (*n*=100).

Trait	*PPARGC1A*			*LTF*	
	CC	CT	TT	AA	AB
Lactation milk yield, kg	6258±31	5717±285	5753±401	5508±313^{b}	6310±274^{a}
Test-d milk yield, kg	15.62±0.70	14.93±0.64	14.91±0.90	14.64±0.70	15.67±0.62
305-d milk yield, kg	5092±232	4819±212	4796±298	4646±233^{b}	5159±203^{a}
305-d milk fat yield, kg	258.20±11.10	244.70±10.10	239.50±14.20	236.20±11.10^{b}	258.74±9.76^{a}
305-d milk protein yield, kg	171.91±7.99	162.57±7.29	165.60±10.30	159.22±8.02	174.13±7.01
Test-d milk fat yield, kg	0.79±0.22	0.74±0.21	0.96±0.29	0.71±0.23	0.95±0.20
Test-d milk protein yield, kg	0.53±0.03	0.51±0.03	0.52±0.03	0.51±0.03	0.53±0.02
Milk fat content, %	5.09±1.04	5.01±0.95	5.97±1.33	4.89±1.04	5.82±0.91
Milk protein content, %	3.38±0.06	3.39±0.05	3.46±0.08	3.44±0.06	3.38±0.06

$^{a, b}$Different letters within a column indicate significant differences (P<0.05).

Table 6. Least square means for the *PPARGC1A* × *LTF* genotype effects on milk production traits in Jersey breed (*n*=100).

Genotype	Lactation milk yield	Test-day milk yield	305-d milk yield	305-d milk fat yield	305-d milk protein yield	Test-d milk fat yield	Test-d protein yield	Milk fat content	Milk protein content
CCAA	6365±416	16.50±0.94^{a}	5237±309^{a}	269.70±14.80^{a}	175.70±10.70	0.88±0.30	0.55±0.04^{a}	5.33±1.39	3.35±0.08
CCAB	6151±380	14.74±0.85^{ab}	4947±282^{ab}	246.60±13.70^{ab}	168.13±9.73	0.70±0.27	0.50±0.03^{b}	4.84±1.27	3.41±0.07
CTAA	5113±421	13.74±0.95^{b}	4414±313^{b}	221.70±15.00^{b}	147.80±10.80	0.71±0.30	0.46±0.03^{b}	5.09±1.40	3.36±0.08
CTAB	6320±298	16.12±0.67^{a}	5223±222^{a}	267.70±10.60^{a}	177.33±7.63	0.77±0.22	0.55±0.02^{a}	4.93±0.99	3.41±0.06
TTAA	5047±606	13.67±1.36^{b}	4286±450^{b}	217.20±21.50^{b}	154.20±15.50	0.53±0.44	0.49±0.05^{b}	4.23±2.02	3.60±0.12
TTAB	6459±464	16.15±1.04^{a}	5306±345^{a}	261.80±16.50^{a}	176.90±11.90	1.38±0.33	0.54±0.04^{a}	7.70±1.54	3.32±0.09

$^{a, b}$Different letters within a column indicate significant differences (P<0.05).

Table 7. Least square means for the *PPARGC1A* and *LTF* genotype effects on milk production traits in the entire studied population (*n*=200).

Trait	*PPARGC1A*			*LTF*	
	CC	CT	TT	AA	AB
Lactation milk yield, kg	8257±544	7506±461	7943±566	7762±512	8042±459
Test-d milk yield, kg	19.29±0.84	18.42±0.71	18.77±0.88	18.25±0.79	19.40±0.71
305-d milk yield, kg	6890±301	6419±255	6809±313	6625±283	6787±254
305-d milk fat yield, kg	310.50±13.80	290.30±11.70	299.70±14.40	298.40±13.00	302.00±11.70
305-d milk protein yield, kg	225.20±9.68	210.30±8.21	224.40±10.10	218.43±9.12	221.51±8.18
Test-d milk fat yield, kg	0.87±0.17	0.83±0.14	0.93±0.17	0.80±0.16	0.95±0.14
Test-d milk protein yield, kg	0.63±0.03	0.61±0.02	0.62±0.03	0.61±0.03	0.64±0.02
Milk fat content, %	4.64±0.76	4.66±0.65	5.05±0.79	4.54±0.72	5.03±0.64
Milk protein content, %	3.30±0.05	3.31±0.04	3.35±0.05	3.34±0.05	3.29±0.04

Table 8. Least square means for the *PPARGC1A* × *LTF* genotype effects on milk production traits in the entire studied population (*n*=200).

Genotype	Lactation milk yield	Test-day milk yield	305-d milk yield	305-d milk fat yield	305-d milk protein yield	Test-d milk fat yield	Test-d protein yield	Milk fat content	Milk protein content
CCAA	8471±720	19.74±1.11	7029±398	321.00±18.30	229.50±12.80	0.90±0.22	0.65±0.036^{a}	4.70±1.01	3.28±0.07
CCAB	8044±543	18.84±0.84	6752±300	300.00±13.80	220.90±9.68	0.83±0.17	0.62±0.03^{ab}	4.57±0.76	3.31±0.06
CTAA	7015±570	17.38±0.88	6261±315	282.11±14.50	204.20±10.10	0.78±0.17	0.57±0.03^{b}	4.67±0.80	3.29±0.05
CTAB	7996±478	19.47±0.74	6577±264	298.60±12.20	216.38±8.51	0.88±0.15	0.64±0.02^{ab}	4.65±0.67	3.32±0.05
TTAA	7799±773	17.63±1.20	6585±427	292.10±19.70	221.60±13.80	0.71±0.24	0.60±0.04^{ab}	4.25±1.08	3.45±0.07
TTAB	8087±622	19.90±0.96	7034±344	307.30±15.80	227.30±11.10	1.15±0.19	0.65±0.03^{a}	5.86±0.87	3.25±0.06

$^{a, b}$Different letters within a column indicate significant difference (P<0.05).

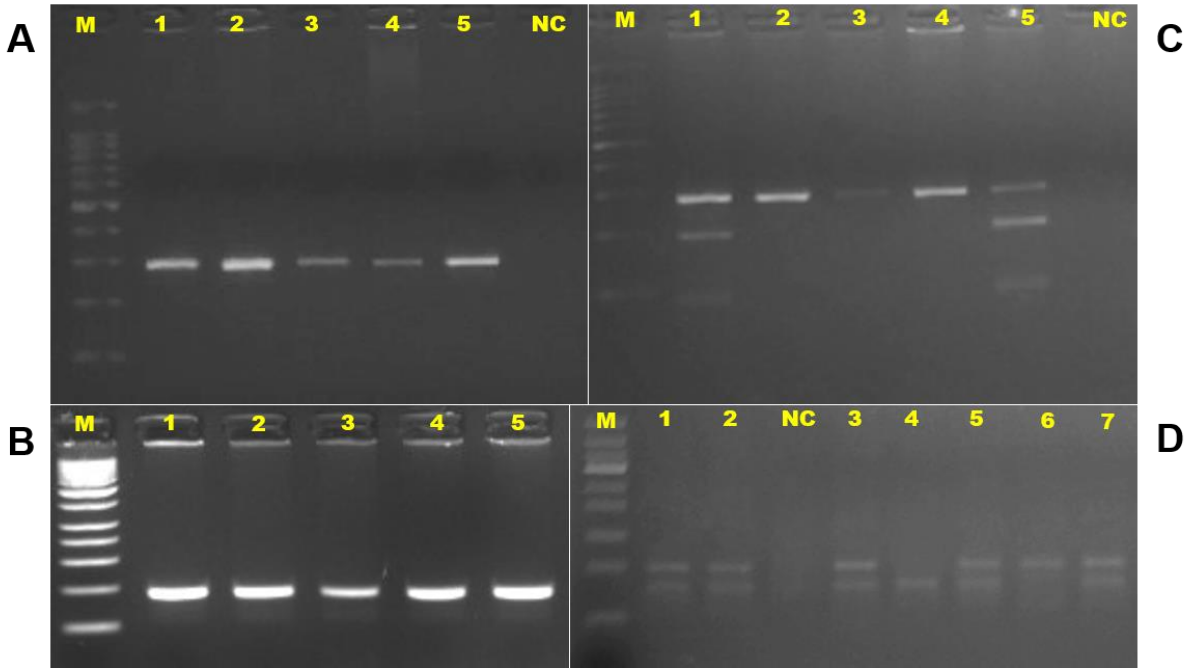


Figure 1. The electropherograms of the PCR-RFLP analysis in the present study. (A) The electrophoresis pattern of PCR amplification (301 bp) for the bovine *LTF* gene. (B) The electrophoresis pattern of PCR amplification (195 bp) for the bovine *PPARGC1A* gene. (C) The electrophoresis pattern of restriction enzyme digestion of the *LTF*/*Eco*RI polymorphism. Lines 1 and 5: AB; Lines 2, 3, and 4: AA (AA: 301 bp and AB: 301 bp, 201 bp, and 100 bp, BB genotype is absent). (D) The electrophoresis pattern of restriction enzyme digestion of the *PPARGC1A*/*Hae*III polymorphism. Lines 1, 2, 3, 5, and 7: CT; Line 4: CC; Line 6: TT (TT: 195 bp; CT: 195 bp, 163 bp, and 32 bp; CC: 163 bp and 32 bp). M: Marker; NC: Negative control.