BIO Web of Conferences **85**, 01071 (2024) https://doi.org/10.1051/bioconf/20248501071
I-CRAFT-2023

Investigation of genotype and allele distribution of ppargc1a-t19c locus in holstein cattle

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Abstract. This study aimed to investigate the genotype and allele distribution of the PPARGC1A-T19C locus in Holstein cattle. The PPARGC1A gene plays a crucial role in significant metabolic processes such as adaptive thermogenesis, gluconeogenesis, skeletal muscle fiber type switching, mitochondrial biogenesis, and adipogenesis. Additionally, it holds a key role in the activation of many hormone receptors and transcription factors, thereby emerging as a potential candidate gene for the milk traits of cattle. In this study, genotyping was performed using the PCR-RFLP method on 50 Holstein animal samples. The 205 bp PCR product was digested with the BsuRI restriction enzyme to reveal genotypic differentiation. Two different genotypes, TC and CC, were identified. The genotype frequencies were found to be 0.30 (TC) and 0.70 (CC). Allele frequencies were determined as 0.15 (T) and 0.85 (C). The Chi-square test showed that the PPARGC1A-T19C locus distribution was in Hardy-Weinberg equilibrium ($p>0.05$). In conclusion, these findings can be utilized to understand intra-breed genetic diversity and develop more effective selection strategies in the genetic improvement programs of the breed.

1 Introduction

Milk, a whitish liquid nourishment, is produced by the mammary glands of mammals shortly after giving birth. It is characterized by its distinctive flavor, aroma, and texture and is packed with essential nutrients in a well-balanced mix. Due to its rich nutritional content, milk serves as a fundamental food source, fulfilling the dietary needs of all mammalian species [1, 2]. While plant and animal-derived foods provide necessary nutrients in diverse ratios, milk offers certain nutrients, like proteins, minerals, and vitamins, crucial during growth stages in more abundant quantities than other foods. The milk constituents can be categorized into five primary groups: lactose (milk sugar), fats, proteins, minerals, and vitamins. Notably, several nutrients, such as lactose and casein, are exclusive to milk. An average liter of milk comprises around 36 grams of fat, 38 grams of protein, 52 grams of carbohydrates, and 7 grams of minerals, in addition to water and fat-soluble vitamins [3]. Recognized as a staple by dieticians, milk is a valuable source of calcium, phosphorus, and riboflavin (Vitamin B2). It also encompasses life-essential amino and fatty acids. Some components, including lactose, milk fat, casein, lactalbumin, and lactoglobulin, are unique to milk. Notably, cow's milk dominates the global milk market, contributing to 78.3% of total production [4]. Over the past three decades, advances in molecular biology have opened up novel avenues for selection and genetic advancement in livestock.

DNA markers, which highlight differences in nucleotide sequences across varied genotypes, are now commonly used for individual identification, establishing parentage, and managing genetic diseases. However, their primary potential lies in genotypic selection techniques, like marker-assisted selection (MAS), by pinpointing quantitative trait loci (QTL). Challenges that traditional breeding methods failed to address can now be tackled using these molecular markers [5, 6]. At present, several pairs of genes have a significant impact on milk characteristics. Essential genes in this context include PPARGC1A, DGAT1, Pit1, K-casein, beta-lactoglobulin, and OPN. The PPARGC1A gene, located on chromosome 6 (BTA6) in cattle, is identified as a significant marker for milk fat yield due to its specific chromosomal location. This gene is involved in the processes of energy, fat, and glucose metabolism within cells. Structured with 13 exons spanning 6,261 base pairs, the PPARGC1A gene exhibits varying expression levels across tissues.

Moreover, studies have indicated that the cDNA and protein sequences of the bovine PPARGC1A gene share a 92–95% similarity with their corresponding orthologous genes (genes that are structurally and functionally alike across different species) in humans, rats, and mice [6]. This investigation seeks to understand the genetic variability and prevalence of specific genotypes and alleles at the PPARGC1A-T19C locus within the Holstein breed.

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2 Material and method

2.1 Material

Fifty Holstein cow samples were collected from the Research and Application Farm of Çukurova University in Adana, Turkey. The cows were fed a concentrated feed and forage diet ad libitum. Blood was drawn from the cows' jugular veins for DNA extraction. DNA was isolated using a slightly modified salting-out method [7] and stored at 20°C. The quantity of DNA was measured using a spectrophotometer. The samples were then preserved at 20°C for subsequent analyses.

2.2 Method

2.2.1 Amplification of PCR products and genotyping

This study utilized the primers and PCR protocols described by [8]. The PCR reaction was carried out in a 20 μL mixture containing 5 μL (50 ng) DNA, 5 μL PCR Master Mix (Thermo Scientific, USA), 0.5 μL of each primer (forward and reverse), and 9 μL distilled water. *BsuRI* FastDigest restriction enzymes (Thermo Scientific, USA) were then used to digest the PCR products at 37°C for 5-15 minutes. The digestion reaction included: 8 μL PCR products, 4 μL distilled water, 2 μL 10X buffer, and 1 μL restriction enzymes. The digested fragments were separated on a 3% agarose gel stained with ethidium bromide. Fragment lengths were determined using an imaging system.

3 Results

The 205 bp fragment of the PPARGC1A-T19C locus was amplified by PCR and subsequently digested with the *BsuRI* enzyme. This revealed two genotypes, TC and CC. The genotype frequencies were estimated at 0.30 for TC and 0.70 for CC. Allele frequencies were calculated as 0.15 for the T allele and 0.85 for the C allele. A Chi-square test for Hardy-Weinberg equilibrium showed that the genotype distribution was in equilibrium ($p>0.05$), indicating the frequencies were consistent with expected values under random mating. These results provide insights into genetic variation at the PPARGC1A-T19C locus within the studied Holstein cattle population.

4 Discussion

Comparing our results with earlier research provides some interesting insights. Khatib et al. (2007) [9] reported genotype frequencies for the PPARGC1A-1892C/T SNP in Holstein cattle of 33.1% for CC, a value slightly higher than our observed frequency for the CC genotype at the PPARGC1A-T19C locus. Their reported frequency for the CT genotype, 65.0%, is significantly higher than our observed frequency for the TC genotype, which was 30%. Komisarek and Dorynek (2009) [8] studied the same locus in Polish Holstein-Friesian bulls and found a C allele frequency of 0.73, slightly lower than our observed frequency of 0.85 for the C allele. Their reported T allele frequency, 0.27, is higher than our observed frequency of 0.15 for the T allele. Piatkowska and Luczak (2019) [10] provided data on two different SNPs within the PPARGC1A gene in the Salers cattle breed. Though their study focused on different loci than our research, it is noteworthy that the C allele frequencies they observed (0.881 and 0.850) for their two SNPs are comparable to our observed C allele frequency of 0.85. Hassanein et al. (2016) [11] studied the PPARGC1A-T19C locus in Egyptian cattle, the same locus we examined. Their reported C allele frequency, 0.83, is very close to our result of 0.85. Their T allele frequency, 0.17, is also close to our observed frequency of 0.15 for the T allele. This suggests that the allele frequencies at the PPARGC1A-T19C locus might be conserved across different cattle populations.

5 Conclusion

This research sheds light on the genotype and allele distribution of the PPARGC1A-T19C locus within Holstein cattle, a crucial gene associated with numerous metabolic processes and potentially linked to milk traits in cattle. The identification of two distinct genotypes, TC and CC, with their respective frequencies, provides valuable insight into the genetic makeup of this breed. Furthermore, the observation that the locus distribution adheres to the Hardy-Weinberg equilibrium suggests that the population is under random mating without evidence for significant selection, genetic drift, mutation, or migration affecting this locus. These findings hold potential implications for understanding genetic diversity in the studied sample. Most importantly, the data garnered from this study can pave the way for formulating more precise and effective selection strategies and enhancing genetic improvement programs. As the demand for optimized cattle breeding grows, such genetic information becomes paramount in making informed decisions in animal husbandry and genetics.

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