

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(7): 645-649
www.biochemjournal.com
Received: 08-04-2026
Accepted: 14-05-2026

GC Thorat

M.V.Sc Scholar, Department of
Animal Genetics and Breeding,
Mumbai Veterinary College,
Mumbai, Maharashtra, India

SJ Komatwar

Associate Professor,
Department of Animal
Genetics and Breeding,
Mumbai Veterinary College,
Mumbai, Maharashtra, India

MP Sawane

Professor, Department of
Animal Genetics and Breeding,
Mumbai Veterinary College,
Mumbai, Maharashtra, India

VD Pawar

Associate Professor,
Department of Animal
Genetics and Breeding,
Mumbai Veterinary College,
Mumbai, Maharashtra, India

RR Pharande

Associate Professor,
Department of Veterinary
Microbiology, Mumbai
Veterinary College, Mumbai,
Maharashtra, India

SH Dalvi

Professor, Department of
Veterinary Biochemistry,
Mumbai Veterinary College,
Mumbai, Maharashtra, India

Corresponding Author:**SJ Komatwar**

Associate Professor,
Department of Animal
Genetics and Breeding,
Mumbai Veterinary College,
Mumbai, Maharashtra, India

Genetic effect of leptin gene variants on milk traits in Dangi cattle

GC Thorat, SJ Komatwar, MP Sawane, VD Pawar, RR Pharande and SH Dalvi

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7i.9098>

Abstract

The bovine Leptin gene is recognized as an important candidate gene influencing milk yield and milk composition traits in cattle. The primary objective of this study was to detect genetic polymorphisms within exon-2, intron-2 and exon-3 of the bovine Leptin gene and to assess their association with milk production traits in Dangi cattle. Total sixty lactating Dangi cows were selected from the Kotul, Akole, and Sangamner areas of Ahilyanagar district of Maharashtra. Genomic DNA was isolated from blood samples, DNA concentration were 50 ng/ul suitable for molecular analysis, with purity ratios (OD 260/280) within the optimal range of 1.8 to 2.0. The PCR-RFLP technique was utilized to screen the polymorphism. The specific DNA fragments corresponding to exon-2(330bp), intron-2 (422bp), and exon-3(538bp) of the Leptin gene were successfully amplified in Dangi cattle using locus-specific primers. The analysis of intron-2 using the *Sau3AI* restriction enzyme revealed a polymorphic pattern, identifying two distinct genotypes AA and AB within the population, with the allelic frequency for the 'A' allele calculated as 0.92, while the 'B' allele frequency was 0.08. Conversely, the exon-2 and exon-3 regions were found to be monomorphic, with the fixation of a single allele (frequency = 1.00) in the studied population. The milk composition traits were recorded average values of 4.278±0.138% for milk fat, 8.943±0.073% for solids-not-fat (SNF), 3.283±0.028% for protein, and 4.873±0.051% for lactose. The statistical analysis revealed no significant association between the polymorphic intron-2 genotypes and the milk production traits in studied Dangi cow population. To validate these findings, future research may incorporate a larger sample size and potentially explore other candidate genes or promoter regions to better understand the genetic architecture of milk traits in Dangi cattle.

Keywords: Dangi cattle, leptin gene, genetic polymorphism

Introduction

The domestication of livestock through artificial selection has significantly expanded genetic and phenotypic diversity. In India, where approximately 70% of the population resides in rural areas, the livestock sector is a cornerstone of agricultural economics, rural income, and food security. According to national census data, India's livestock population stands at 536.76 million, with cattle comprising 193.46 million (142.11 million indigenous and 51.36 million crossbred) and buffaloes representing 109.85 million. India is the world's leading milk producer, contributing 24.76% (239.30 million tonnes) to global production. To enhance native dairy productivity, the Department of Animal Husbandry and Dairying (DAHD) utilizes digital frameworks like the e-Gopala application and breeding initiatives such as the Rashtriya Gokul Mission.

Molecular breeding programs leverage genetic markers, including RFLPs, microsatellites, and SNPs—to optimize marker-assisted selection (MAS) and map quantitative trait loci (QTL). India recognizes 53 distinct cattle breeds, with Maharashtra possessing seven native breeds: Deoni, Gaolao, Khillar, Red Kandhari, Dangi, Kokan Kapilla, and Kathani (ICAR-NBAGR, 2025 report). The Dangi breed is a hardy draft animal native to the heavy rainfall zones of Maharashtra's Nashik district and Gujarat's Dangas district. Protected by an oily skin secretion, they are optimized for field traction, though individual cows can yield 3 to 4 kg of milk per day under favourable management (Janmeda *et al.*, 2013) [4].

Among candidate milk production genes (*ABCG2*, *BoLA-DRB3*, *DGAT1*, *STAT5A*, etc.), the Leptin (*LEP*) gene stands out as a key physiological regulator. Mapping to bovine chromosome 4q32, this three-exon gene produces a 16 kDa hormone that coordinates

metabolic pathways, energy balance, immune function, reproduction, and lactogenesis. This study systematically evaluates the *Leptin* gene in Dangi cattle to fulfil two primary objectives: To detect genetic polymorphisms within Exon-2, Intron-2, and Exon-3 regions using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and to statistically evaluate the association between identified leptin genotypes and economic milk traits within the studied population (Ogorevc *et al.*, 2009) [12].

Materials and Methods

Experimental Animals and Sample Collection

The field trial comprised 60 lactating Dangi cows maintained by local farmers across three distinct geographic zones within the Ahilyanagar district of Maharashtra: Kotul, Akole, and Sangamner. To ensure continuous milk availability for the six-month evaluation window, enrolment criteria restricted the cohort to cows in early lactation (3-4 months post-partum). From each animal, a 5 ml blood sample was drawn aseptically from the jugular vein into specialized EDTA-coated vacuum tubes. These samples were immediately transferred into automated cold chains and moved to the Genetic Investigation Laboratory (GIL), Department of Animal Genetics and Breeding, Mumbai Veterinary College, where they were kept at 4°C until molecular processing. Fortnightly milk sampling (10 ml) was carried out over a six-month lactation phase, with immediate compositional analysis for fat, protein, solids-not-fat (SNF), and lactose percentages performed using a calibrated LACTOSCAN automated milk analyzer. Concurrently, baseline 5 ml jugular blood samples were drawn into sterile EDTA-coated vacutainers and kept at 4°C

until molecular profiling.

Genomic Isolation and Quantification

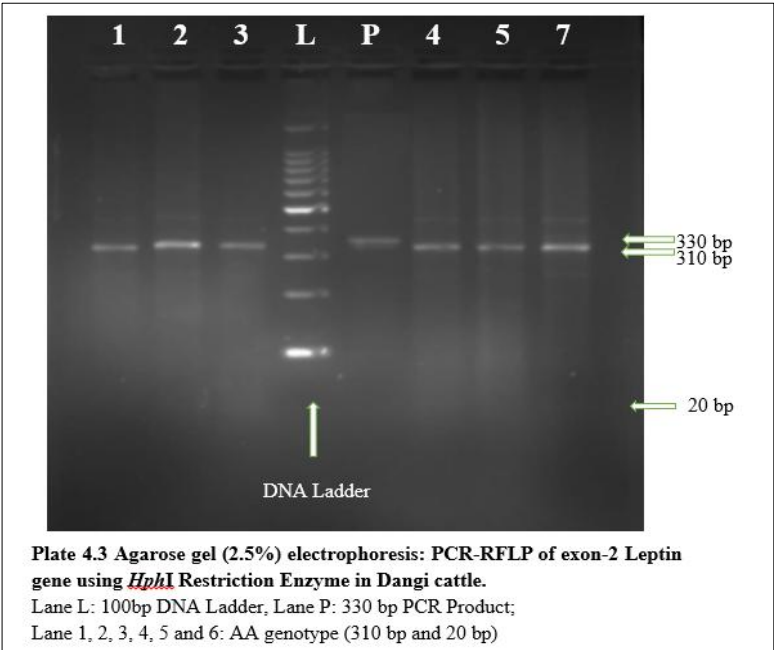
Total genomic DNA was extracted from white blood cell fractions using a modified Phenol-Chloroform-Isoamyl Alcohol (P:C:I) methodology. The isolated DNA pellets were resuspended in sterile double-distilled water. Nucleic acid purity and concentration levels were determined using a NanoDrop ND-2000 Spectrophotometer, selecting extracts that displayed an optimal OD {260/280} ratio between 1.8 and 2.0. Structural integrity was verified via horizontal 0.8% agarose gel electrophoresis.

Amplification and RFLP Genotyping

Locus-specific oligo primers were used to amplify target fragments within a pre-programmed thermal cycler: The thermocycling protocols were tailored for each distinct domain as: Exon-2: Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 45 sec; followed by a final extension at 72°C for 5 min. Intron-2: Initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min; followed by a final extension at 72°C for 15 min. Exon-3: Initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 95°C for 45 sec, annealing at 55°C for 45 sec, and extension at 72°C for 45 sec; followed by a final extension at 72°C for 10 min. Following successful amplification, the PCR products were subjected to overnight restriction digestion using specific endonucleases like Exon-2 amplicons were incubated with *Hph* I at 37°C, Intron-2 amplicons were incubated with *Sau*3AI at 37°C, Exon-3 amplicons were incubated with *Hinf*I at 37°C.

Table 1: Oligonucleotide Primer Sequences and Predicted Product Mass

Locus Identified	Primer Directives	Nucleotide Sequence Sequence (5'→3')	Amplicon Size (bp)	Primary Reference Source
Exon-2	Forward (F)	5'-GGGAAGGGCAGAAAGATAG-3'	330 bp	Haegeman <i>et al.</i> (2000)
	Reverse (R)	5'-TGGCAGACTGTTGAGGATC-3'		
Intron-2	Forward (F)	5'-TGGAGTGGCTTGTTATTTTCTTCT-3'	422 bp	Liefers <i>et al.</i> (2002) [8]
	Reverse (R)	5'-GTCCCCGCTTCTGGCTACCTAACT-3'		
Exon-3	Forward (F)	5'-GGGAAGGGCAGAAAGATAGG-3'	538 bp	Jhala <i>et al.</i> (2011) [5]
	Reverse (R)	5'-GCCGCAACATGTCC7GTAGT-3'		



The digested DNA fragments were separated on a 2.5% agarose gel alongside a 100 bp DNA step ladder, visualized under UV transillumination, and archived using a digital gel documentation system.

Statistical Modeling

Allelic profiles and genotypic distributions were calculated using direct allele-counting methods. The relationship between segregating intronic genotypes and individual milk parameters was analyzed using a two-sample t-test.

Results and Discussion

Exon-2 Locus

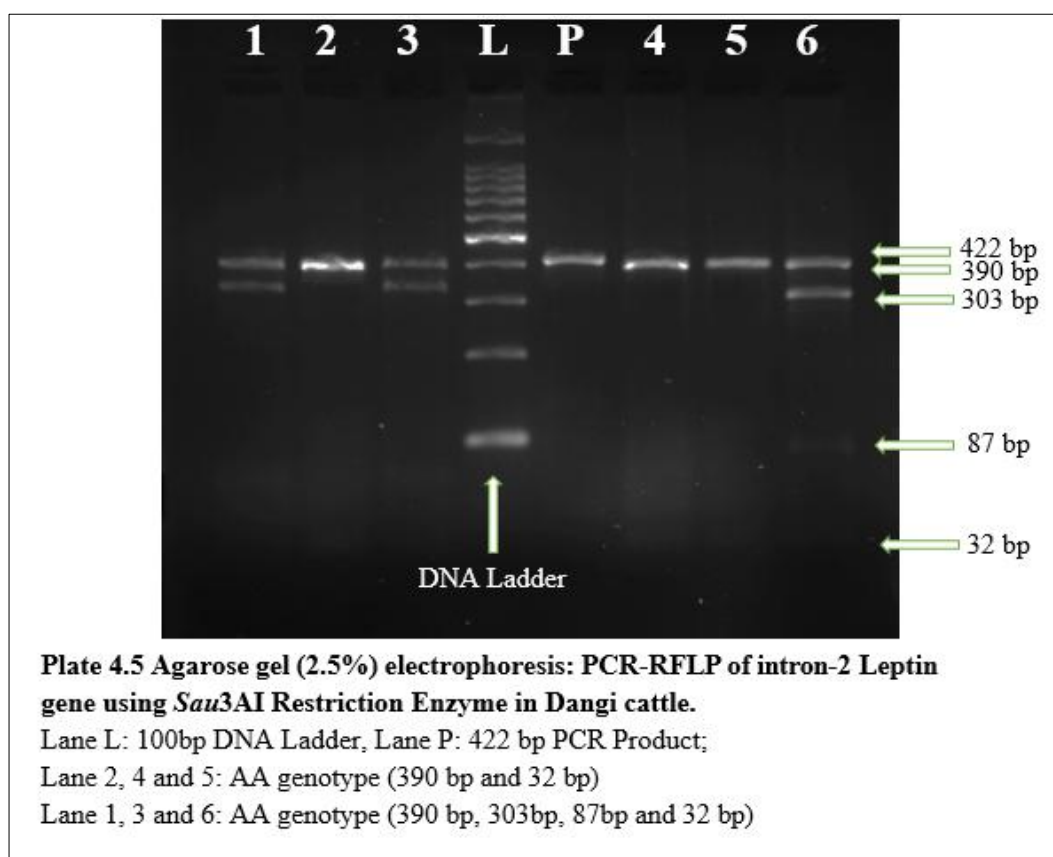
Incubation with the *Hph* I enzyme generated a completely uniform restriction pattern across all 60 samples, resolving every individual into two fragments of 310 bp and 20 bp. No alternative heterozygous ('AB') or homozygous ('BB') patterns were found, indicating a monomorphic status with a fixed 'AA' genotype and 'A' allele frequency of 1.00. This matches findings in indigenous Sahiwal and Rathhi herds, confirming that coding loops are highly conserved among purebred *Bos indicus* lines (Dandapat *et al.*, 2009; Chouhan

& Pannu, 2021) [3, 2]. This stands in contrast to the high genotypic diversity reported in commercial *Bos taurus* dairy herds (Tomka *et al.*, 2016) [14].

Intron-2 Locus

Digestion with *Sau* 3AI revealed a polymorphic pattern inside the Dangi cohort, yielding two distinct configurations: Homozygous AA Genotype: Produced fragments of 390 bp and 32 bp, dominating the cohort at a frequency of 83.33% (N=50), heterozygous AB Genotype: Yielded four distinct fragments at 390 bp, 303 bp, 87 bp, and 32 bp, appearing in 16.67% (N=10) of the cattle, homozygous BB Genotype was entirely lacking from the population.

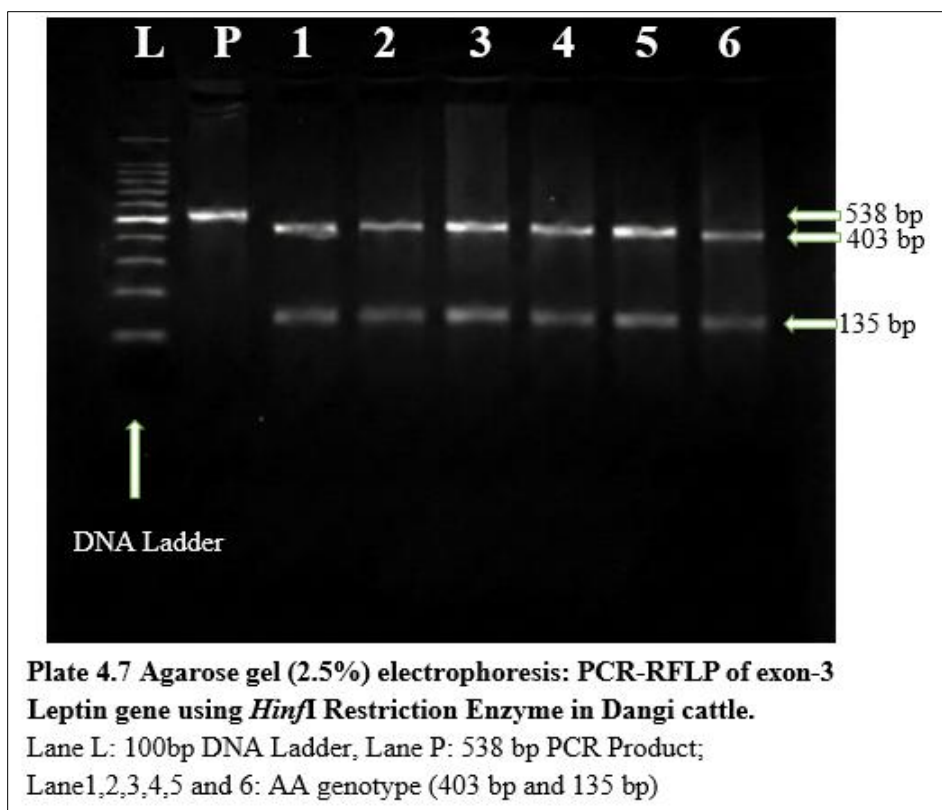
The calculated allelic frequencies were 0.92 for the major 'A' allele and 0.08 for the minor 'B' allele. This skewed distribution mirrors patterns found in autochthonous Busha lines and native Turkish breeds, where the 'A' variant is preferentially selected in native cattle adapted to local environments (Maletic *et al.*, 2019; Oztabak *et al.*, 2010) [9, 13].



Exon-3 Locus

Enzymatic digestion using *Hinf* I cleaved the 538 bp segment into identical bands of 403 bp and 135 bp for all 60 animals. This confirms a monomorphic 'AA' genotype with an 'A' allele frequency of 1.00. This matches findings in Gir

cattle and Mehsana buffaloes (Jhala *et al.*, 2011) [5], but differs from exotic *Bos taurus* lines like the Jersey, where the alternative 'B' variant occurs regularly (Choudhary *et al.*, 2005) [1].



Milk Traits and Association Analysis

The baseline values for milk constituents across the 60 Dangi cows were $4.278 \pm 0.138\%$ for milk fat, $8.943 \pm 0.073\%$ for solids-not-fat (SNF), $3.283 \pm 0.028\%$ for protein, and $4.873 \pm 0.051\%$ for lactose. These baseline values align closely with performance averages reported for this breed because the Exon-2 and Exon-3 loops showed nothing sequence variation, statistical association tests were restricted to the Intron-2 domain.

Dangi Milk Yield Components across Intron-2 Genotypes

Parameter	Intron-2 genotypes		p-value
	AA (n=50)	AB (n=10)	
Fat%	4.32 ± 0.16	4.07 ± 0.22	0.504
SNF%	8.98 ± 0.07	8.76 ± 0.26	0.258
Protein%	3.29 ± 0.03	3.23 ± 0.08	0.363
Lactose%	4.88 ± 0.06	4.83 ± 0.13	0.693

The two-sample t-test confirmed that the Intron-2 genotypes have no statistically significant effect on milk parameters in this population ($p > 0.05$). This indicates that the *Sau* 3AI intronic polymorphism operates primarily as a neutral genomic landmark rather than a direct performance modulator, consistent with reports in Jersey, Pinzgau, and Holstein Friesian lines (Kulig *et al.*, 2009; Moravcikova *et al.*, 2012; Kiber & Aytekin, 2023) [7, 10, 6]. This contrasts with findings in commercial dairy herds, where intensive selection for milk volume has linked these markers to significant performance changes (Liefers *et al.*, 2002; Moussavi *et al.*, 2006) [8, 11].

Conclusion

Molecular analysis of the *Leptin* gene in 60 lactating Dangi cows revealed that the coding loops within Exon-2 and Exon-3 are monomorphic, with the major 'A' allele fixed at a

frequency of 1.00. This indicates high structural stability and breed purity. The non-coding Intron-2 locus is actively segregating into 'AA' (83.33%) and 'AB' (16.67%) configurations, with allelic frequencies of 0.92 for 'A' and 0.08 for 'B'. However, statistical testing confirmed that this intronic variation does not significantly influence milk fat, SNF, protein, or lactose parameters ($p > 0.05$). Future research should focus on larger cohorts and upstream promoter element sequencing to further characterize the genetic architecture of milk production traits in this native draft breed.

Declarations

Data Availability Statement The raw data supporting the conclusions of this study will be made available by the authors upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

Funding this research was funded by the Maharashtra Animal and Fishery Sciences University, Nagpur.

Author Contributions

G. C. Thorat: Investigation, Methodology, Data curation, Formal analysis, and Writing - original draft.

S. J. Komatwar: Conceptualization, Supervision, Methodology, Funding acquisition, and Writing - review and editing.

M. P. Sawane: Validation, Methodology, and Writing - review and editing.

V. D. Pawar: Resources, Data curation, and molecular assay support.

R. R. Pharande: Formal analysis and experimental validation.

S. H. Dalvi: Resources, and Writing - review and editing.

All authors have read and agreed to the published version of the manuscript.

Acknowledgments: The authors thank the Associate Dean, Mumbai Veterinary College, Mumbai, India for providing the necessary facilities for this study. The authors are also grateful to the advisory committee members for their technical support during the research.

References

1. Choudhary V, Kumar P, Bhattacharya TK, Bhushan B, Sharma A. DNA polymorphism of leptin gene in *Bos indicus* and *Bos taurus* cattle. *Genetics and Molecular Biology*. 2005;28(4):740-742.
2. Chouhan H, Pannu U. Identification of polymorphism in LEP gene by single strand conformation polymorphism (SSCP) in Rathi cattle. *International Journal of Current Microbiology and Applied Sciences*. 2021;10(2):1834-1840.
3. Dandapat A, Kumar D, Ghosh AK, Banarjee D. Association of leptin gene polymorphism with growth, milk production and reproduction traits in Sahiwal and crossbred cattle. *Indian Journal of Animal Sciences*. 2009;79(9):892-896.
4. Janmeda M, Pandya G, Dangar N. Dangi Cattle - A boon for hilly tract farmers. *The Indian Cow*. 2013;7-9.
5. Jhala NB, Vataliya PH, Rank DN, Ramani UV. Leptin gene exon-3 polymorphism in Gir cattle and Mehsana buffalo. *Animal Science Reporter*. 2011;5(3):91-94.
6. Kibar M, Aytekin I. Lack of evidence for association between the leptin/Sau3AI gene and milk yield traits in Holstein Friesian dairy cattle. *Journal of Dairy Research*. 2023;90(4):339-342.
7. Kulig H, Kmiec M, Luczak IK, Andziak G. Effect of leptin gene polymorphisms on milk production traits of Jersey cows. *Turkish Journal of Veterinary and Animal Sciences*. 2009;33(2):143-146.
8. Liefers SC, Te Pas MFW, Veerkamp RF, Vander Lende T. Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake and fertility in Holstein Heifers. *Journal of Dairy Science*. 2002;85:1633-1638.
9. Maletic M, Paprikic N, Lazarevic M, Hodzic A, Davidovic V, Stanisic L, *et al*. Insight in leptin gene polymorphism and impact on milk traits in autochthonous Busha cattle. *Acta Veterinaria-Beograd*. 2019;69(2):153-163.
10. Moravcikova N, Trakovicka A, Hazuchova E, Bujko J, Kasarda R. Associations between polymorphisms in the leptin gene and milk production traits in Pinzgau and Slovak Spotted cattle. *Acta agriculturae Slovenica*. 2012;(Suplement 3):259-263.
11. Moussavi AH, Ahouei M, Nassiry MR, Javadmanesh A. Association of leptin polymorphism with production, reproduction and plasma glucose level in Iranian Holstein cows. *Asian-Australasian Journal of Animal Sciences*. 2006;19(5):627-631.
12. Ogorevc J, Kunej T, Razpet A, Dove P. Database of cattle candidate genes and genetic markers for milk production and mastitis. *Animal Genetics*. 2009;40(6):832-851.
13. Oztabak K, Ny T, Un C, Mengi A, Karadag O, Soysal D. Leptin gene polymorphisms in native Turkish cattle breeds. *Kafkas Universitesi Veteriner Fakultesi Dergisi*. 2010;16(6):951-956.
14. Tomka J, Vasickova K, Oravcova M, Bauer M, Huba J, Vasicek D, *et al*. Effects of polymorphisms in DGAT1 and LEP genes on milk traits in Holstein primiparous cows. *Mljekarstvo: Dairy Experts Journal*. 2016;66(2):122-128.
15. Zhang Y, Proenca R, Maffel M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse (*Mus musculus*) obese gene and its human homologue. *Nature*. 1994;372:425-432.