TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44: 695-701

doi:10.3906/vet-1812-4

Effect of Osteopontin gene variants on milk production traits in Holstein Friesian crossbred cattle of Kerala

Francis Anand LALI[1,*], Kulangara ANILKUMAR[1], Thazhathuveetil ARAVINDAKSHAN[2]

[1]Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Thrissur, Kerala, India

[2]Centre for Advanced studies in Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Thrissur, Kerala, India

Received: 01.12.2018 • **Accepted/Published Online:** 04.04.2020 • **Final Version:** 02.06.2020

Abstract: Osteopontin gene (*OPN*) is located in the quantitative trait loci (QTL) for milk production traits in bovine chromosome 6. In the present study the impact of cytosine to thymine transition in the intron-4 of *OPN* gene (g.8514C > T) on milk production traits was analysed in the Holstein Friesian crossbred cattle of Kerala. Genomic DNA was isolated and a fragment of 290 bp enclosing the polymorphic site was amplified and genotyped by restriction fragment length polymorphism (RFLP) using endonuclease, *BSeN*1. The genetic variants were distributed according to Hardy-Weinberg equilibrium. The T allele was found to be the major one (T/0.76, C/0.24) and the genotype frequencies were TT/0.60, CT/0.32, and CC/0.08. The amplicons of genotypes were sequenced by sangers dideoxy termination technique and confirmed the mutation. Association study using the General Linear Model-Analysis of Variance (GLM-ANOVA) considering marker, season of calving, parity and herd as fixed factors and dairy trait as dependent variable revealed that none of the yield traits (305 day milk yield, peak yield, fat yield, protein yield, solids not fat yield, lactose yield, daily milk yield) or composition traits (fat percent, protein percent, solids not fat percent and lactose percent) of milk production analysed were significantly differed between CT and TT genotyped animals. The *OPN* gene polymorphism (g.8514C > T) can be suggested for marker assisted selection (MAS) for future breeding programmes for dairy cattle only after extensive association studies.

Key words: Osteopontin gene, milk production traits, single nucleotide polymorphism, RFLP, Holstein Friesian crossbred cattle of Kerala

1. Introduction

Dairy farming acts as the heart and soul for the socioeconomic development of millions of rural households in India. Even though the production potential of present cattle population was improved by decades of traditional selection procedures, the domestic production is inadequate to cope up with the increasing demands of milk and milk products in the country. It is already proved that incorporation of molecular information in the selection process (MAS/genomic selection) can dramatically accelerate genetic improvement of dairy cattle [1]. Among the molecular markers single nucleotide polymorphisms (SNPs) are the best genetic markers for the improvement of an economic trait [2].

A few of these markers directly affect the functional traits which are referred to as direct markers, present within the candidate genes [3]. Majority of SNPs affecting production traits, are explained as indirect markers since they are located in the vicinity of chromosomal regions affecting the economic traits. Indirect markers are present near QTL and the genotypes of the markers can explain the economic trait as they are seen linked to the QTL. By genetic recombination during gamete formation the chance of occurrence of markers and QTL together will change with respect to the linkage disequilibrium (LD) between the marker and the QTL. Therefore, the association results of indirect markers are dependable only in local populations and it may change after a few generations [4]. Whereas the effect of genetic variations in candidate genes will remain constant since it acts through the gene function.

According to cattle quantitative trait loci data base 'cattle QTLdb', bovine chromosome 6 (BTA-6) possess the highest number (12306) of QTLs in autosomes. The studies conducted by Olsen et al. [5] explained the importance of 420 Kb region on BTA-6 harbouring *PPARGC1A*, *PKD2*, *OPN*, and *ABCG2* genes for milk production traits in cattle. Osteopontin is a highly phosphorylated acidic glycoprotein found in different tissues and secretions [6] with highest concentrations in milk [7] encoded by

* Correspondence: lalimagnus@gmail.com

OPN gene consisting of 7 exons. The genetic variations of *OPN* gene influence the amount of OPN secreted in milk throughout lactation [8] and in lactation persistency [9]. It is involved in the involution of mammary gland in mice [10].

The cattle population in Kerala, the southern state of India possesses more than 90% crossbred animals developed by decades of crossbreeding and selection strategies since 1960 to merge the disease resistance, heat tolerance, and adaptability of native cattle and milk production potential and early sexual maturity of exotic cattle [11]. The exotic breeds such as Brown Swiss, Holstein Friesian, and Jersey were used for crossbreeding programmes in Kerala [12]. The current breeding policy recommends Holstein Friesian and Jersey crossbred bulls for crossbreeding of existing cattle population through artificial insemination. The dairy farmers in the state consider high yielding Holstein Friesian crossbreds as preferable genetic group compared with Jersey crossbreds. It is worth to note that the Food Safety and Standards Authority of India fixed the minimum content of fat and solids not fat in milk as 3.2 and 8.3 percentages, respectively. The yield and composition traits of milk production require ceaseless improvement for the sustainability of dairy sector. The present study is concentrated on depicting the influence of *OPN* or Secreted Phosphoprotein 1 (*SPP1*) gene on milk yield and composition traits in Holstein Friesian crossbreds in Kerala.

2. Materials and methods

2.1. Animals

The influence of single nucleotide polymorphism in the Osteopontin gene on milk production traits was investigated in a total of 144 Holstein Friesian crossbreds calved during the period from October 2012 to October 2013. These animals were maintained in University Livestock Farm, Mannuthy (104) and Cattle Breeding Farm, Thumburmuzhi (40) of Kerala Veterinary and Animal Sciences University under similar feeding and managemental practices.

2.2. Sample collection

Representative morning and evening milk samples (30 mL) were collected separately at the time of milking once in a month for 10 months of lactation from all animals identified for the present study. A total of 2880 samples were collected and refrigerated till the analysis of milk components started.

Animal number, date of birth, date of calving, parity and dates of milk collection were recorded along with test day morning and evening milk fat, protein, solids not fat and lactose percent. Morning and evening test day milk yield of all animals under study were recorded monthly for 10 months of lactation. The milk recording registers were perused to find out the lactation length and peak milk yield (PKY) of lactation of animals in the present study and recorded along with test day milk yield.

2.3. Milk production traits

Milk yield for 305 days of lactation (MY305d) was calculated from test day milk yield employing test interval method as described in ICAR Guidelines for computing accumulated lactation yield [13]. Milk fat (FP), protein (PP), solids not fat (SNFP), and lactose (LACP) percent were analysed and recorded for every 10 months morning and evening test day milk samples using milk analyser (MRC-scientific instruments). Lactation yields of fat (FY), protein (PY), solids not fat (SNFY), and lactose (LACY) were estimated by test interval method as aforementioned procedure. The composition of milk components (FP, PP, SNFP, and LACP) were calculated as the ratio of respective components to MY305d.

2.4. Effects of nongenetic factors on milk production traits

The nongenetic factors: season, parity, and herd were considered in the model while studying the effect of genotypes in the present study. Lactation seasons were calculated by partitioning the year into 3 seasons: from October to January (postmonsoon), February to May (summer), and June to September (monsoon) and using the calving date as a reference [14]. The milk production data of parity 1 to 7 was included in the present analysis.

2.5. Single nucleotide polymorphism analysis

From each animal, 5 mL of blood was collected from the jugular vein using Ethylene Diamine Tetra Acetic acid (EDTA) coated vacutainers. The samples were brought to the laboratory at 4 °C, temperature being maintained with the aid of ice packs and stored at –20 °C until needed for DNA extraction. The genomic DNA was isolated by standard phenol chloroform extraction procedure [15]. The *OPN* gene fragment enclosing cytosine to thymine transition (g.8514C > T) in the intron-4 (GenBank accession number ID: AY878328.1.) was amplified using custom synthesised forward (5′GCAAATCAGAAGTGTGATAGAC3′) and reverse (5′CAAGCCAAACGTATGAGTT 3′) primers [16]. Optimization of PCR was carried out with final concentration of 200 µ*M* dNTPs, magnesium chloride 1.5 m*M*, primers 5 pmol and *Taq* DNA polymerase 0.5 U in 15 µL reaction with initial denaturation 94 °C for 4 min, denaturation 94 °C for 45 s, primer annealing 55–65 °C for 30 s, primer extension 72 °C for 45 s, and final extension 72 °C for 4 min (Biorad T100). The amplification was checked by agarose gel electrophoresis and the product size was confirmed using 50 bp DNA ladder as DNA size marker. Four micro-litre of amplified product was digested with 5 U of restriction endonuclease, *BSeN*1 at 37 °C for 2 h in thermal cycler (Biorad T100). The restricted fragments

were analysed using 2% agarose gel electrophoresis in 1X Tris-Borate-EDTA buffer. The restriction patterns were visualized by gel documentation system. The genotype and allele frequencies were estimated by direct counting method [17].

The forward and reverse strands of PCR products were sequenced by Sanger's Dideoxy sequencing protocol (SciGenom Labs, Cochin) and analysed with the aid of bioinformatics tools such as Sequence Manipulation Suit (SMS), EMBOSS, and Basic Local Alignment Search Tool (BLAST) of National Centre for Biotechnology Information (NCBI) site (http://www.ncbi.nlm.nih.gov/BLAST).

2.6. Association analysis

The impact of single nucleotide polymorphism (g.8514C > T) on milk production traits was analysed by General Linear Model-Analysis of Variance (GLM-ANOVA) considering genotype, season of calving, parity and herd as fixed factors and dairy trait as dependent variable (SPSS 21.0). The nongenetic factors: season, parity, and herd were considered in the statistical analysis. The main effects of fixed factors included in the model were studied. The model was

$$Y^{ijklm} = \mu + G_i + S_j + P_k + H_l + e_{ijklm}$$

Where,

Y_{ijklm} – dairy trait of m^{th} cow, of i^{th} genotype, j^{th} season, k^{th} parity, and l^{st} herd

μ- overall mean of dairy trait

G_i- effect of genotype i: TT, CT (i = 1 or 2)

S_j – effect of season j: postmonsoon, summer, monsoon (j =1, 2 or 3)

P_k – effect of parity k (k = 1 to 7)

H_l – effect of herd l (l = 1 or 2)

e_{ijklm}- random error

3. Results

Visualization of gene fragments by 2% agarose gel electrophoresis revealed 2 alleles T and C with bands of 290 bp and 200 bp and 90 bp, respectively (Figure 1). The genotype (TT/0.60, CT/0.32, CC/0.08) and allele (T/0.76, C/0.24) frequencies of g.8514C > T polymorphism of crossbred cattle are presented in the Table 1.

The sequencing of osteopontin variants confirmed the mutation in Holstein Friesian crossbred cattle of Kerala. The sequences showed 100% similarity with Genbank reference sequences (ID: AY878328.1.) by BLAST analysis Figure 2. The chromatograms of CT and TT genotypes of g.8514C > T polymorphism are depicted in Figure 3.

Analysis of effect of polymorphism with different yield and composition traits of milk production could not reveal any significant changes with respect to g.8514C > T genotypes. Mean values for milk production traits for TT and CT genotyped animals are presented in Table 2.

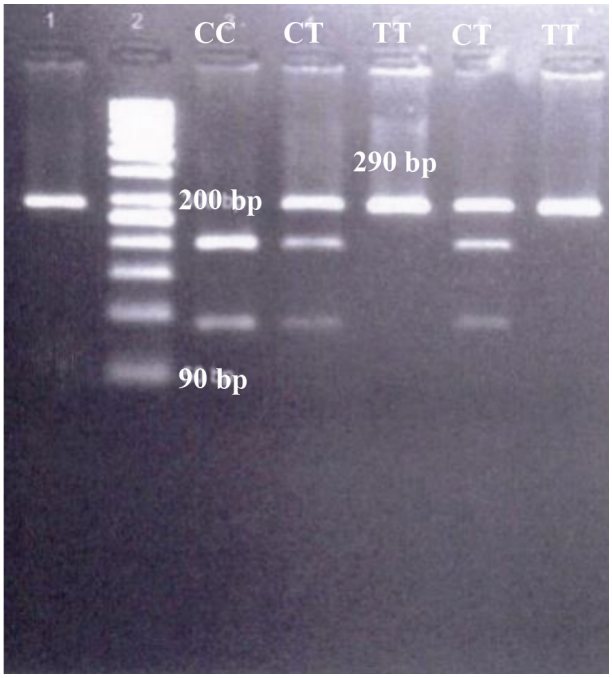

Figure 1. RFLP pattern of g.8514C>T polymorphism in intron-4 of Osteopontin gene for Holstein Friesian crossbred cattle of Kerala. Lane 1-PCR product (290 bp), Lane 2-50 bp DNA ladder, Lane 3-Genotype CC (200 and 90 bp), Lane 4, 6-Genotype CT (290, 200 and 90 bp), Lane 5, 7-Genotype TT (290 bp).

Table 1. Genotype and allele frequencies of g.8514C > T polymorphism in Osteopontin gene in Holstein Friesian crossbred cattle of Kerala.

Genotype frequencies			Allele frequencies		Chi-square test for HW-equilibrium
			Major allele	Minor allele	P-value
TT 0.60	CT 0.32	CC 0.08	T 0.76	C 0.24	0.23

4. Discussion

The main goal of dairy cattle genomic research is to identify genes underlying the variation of milk production traits that can be functional in breeding programs. The SNPs showing significant association with milk components would afford a main opportunity for Marker-Assisted Selection (MAS) programmes in livestock [18]. A study of single nucleotide polymorphisms and their effects on milk production are economically desirable for most selection decisions in dairy cattle breeding programs, through which rates of genetic gain could be promoted by direct selection on the alleles [19].

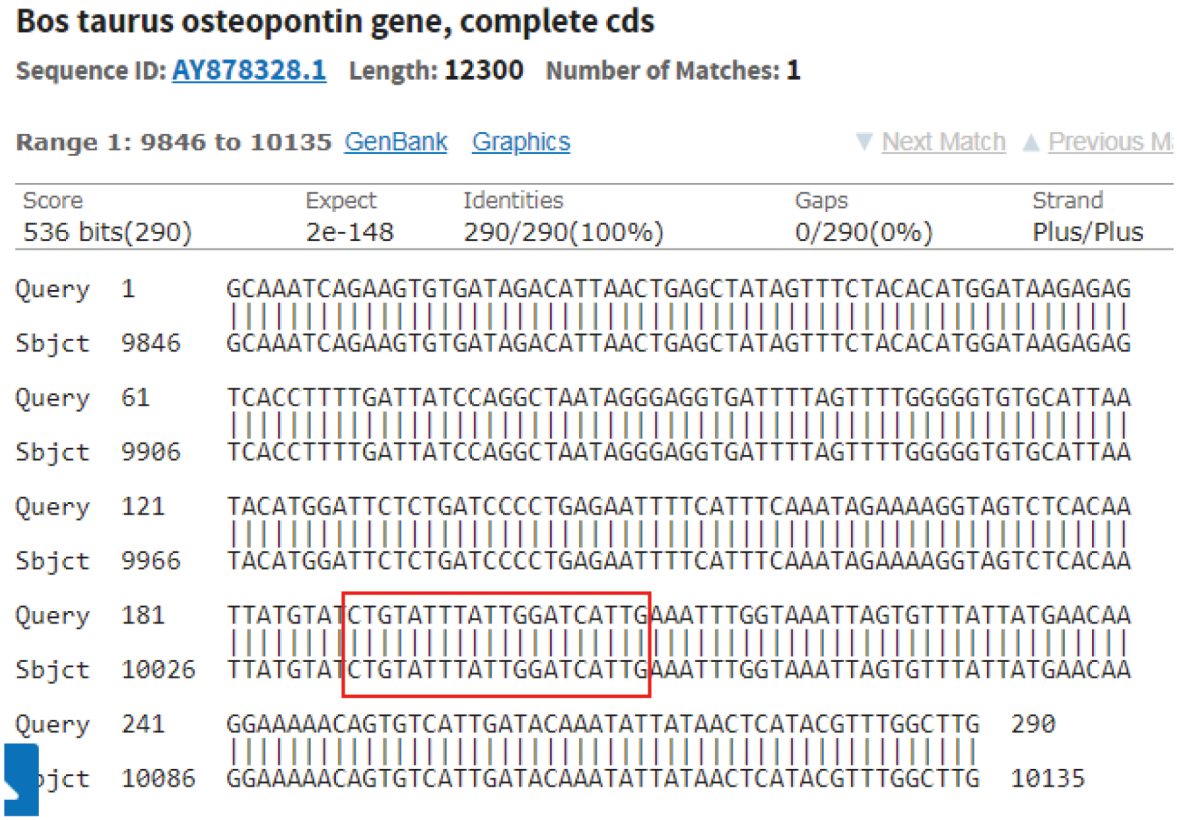

Figure 2. BLAST result of Osteopontin gene fragment (290 bp) of TT genotyped Holstein Friesian crossbred cattle of Kerala with reference sequence (ID: AY878328.1.). Red coloured region indicates g.8514C > T polymorphism site in intron-4 of Osteopontin gene.

4.1. Single nucleotide polymorphism analysis

The results of g.8514C > T polymorphism analysis in fourth intron of *OPN* gene (T/0.76, C/0.24) were consistent with other studies that reported T allele as major allele in Czech Fleckvieh (T/0.82, C/0.18) cattle [20] and Peranakan Ongole (T/0.83, C/0.17) cattle [21]. Further studies on genetic polymorphisms of *OPN* genes in Jersey cattle reported that C allele as the minor allele with frequency 0.22 [22]. In contrast with the present results, White et al. [23] reported that T allele was present at 13.1% and 13.5% in 2 independent populations (GPE8 and GPE7) of US beef cattle. In another study, the C and T allele frequencies of Osteopontin gene were reported as 49% and 51%, respectively in Holstein population [16]. The genotype frequencies observed in the present study (TT/0.60, CT/0.32, CC/0.08) were in line with results (TT/0.53, CT/0.39, CC/0.08) obtained in Girolando cattle [24]. The distribution of g.8514C > T variants in Holstein Friesian crossbred cattle of Kerala was in accordance with Hardy-Weinberg expectations and it eliminates the chance of indirect selection and population stratification.

4.2. Association analysis

The animals with CC genotypes were very limited in the present study and so the association analysis was done with other 2 genotypes. Association analysis by GLM - ANOVA considering genotype, season of calving, parity and herd as fixed factors and milk production trait as dependent variable explained that g.8514C > T polymorphism has not been significantly associated with milk production traits in Holstein Friesian crossbreds of Kerala. In agreement with the present results, Łuczak and Hanna [22] and Kulaj et al. [25] also could not derive any significant results for g.8514C > T polymorphism with regard to milk composition traits in Jersey cattle. In contrast, the influence of C allele on protein content was explained in CDDR Holstein [16,18] and Iranian Holstein [20,26,27] populations where significantly higher protein percent was noticed in CC genotyped animals. In the present study, no significant association was found out for FP (P = 0.71). However, the previous association studies in cattle [16,18, 26] explained that C allele was associated with higher milk fat percentage. Any reports regarding the effect of g.8514C > T polymorphism with SNFP or LACP in milk were found out to compare with the present study.

In the present study, the yield traits (MY305d, FY, PY, SNFY, and LACY) were also not found to be associated with the g.8514C > T polymorphism in Holstein Friesian crossbreds. Similarly, Boleckova et al. [20] also could not

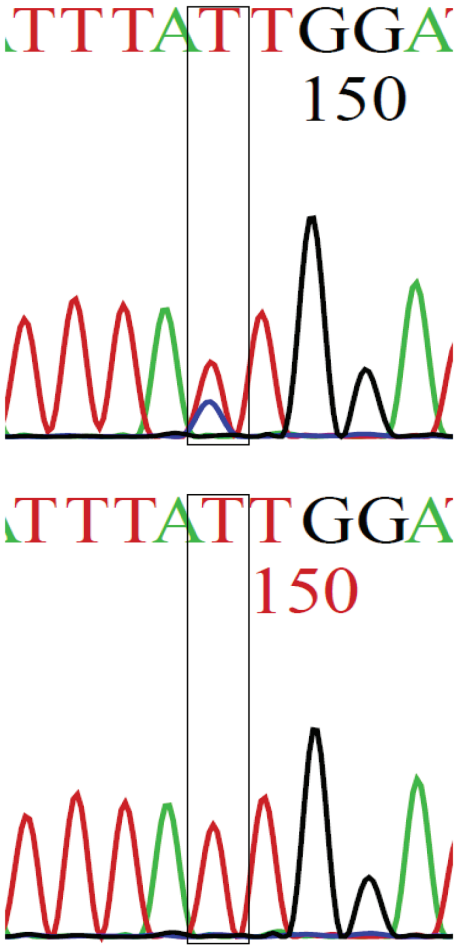

Figure 3. Chromatogram of g.8514C > T polymorphism in intron-4 of Osteopontin gene for Holstein Friesian crossbred cattle of Kerala a) Chromatogram of CT genotype showing double peaks b) Chromatogram of TT genotype.

find any significant effect for milk yield and fat yield in case of *OPN* gene variants in Czech Fleckvieh cows. However, the present observations are against the reports of lower milk yield associated with C allele compared with T allele [18,25,26,27].

The occurrences of population substructure, high selection pressure or presence of null alleles have indicated as the reasons for inconsistent results for association analysis [28]. It is worth bearing in mind that, polygenic traits like milk production may vary with respect to breed or even populations of the same breed [29]. Further, an adequate association study, also depends on nonallelic interactions and linkage [30].

Suchit et al. [31] reviewed that alternative splicing and posttranslational modifications derive different isoforms for osteopontin and it has several biological functions including milk production. Single nucleotide

Table 2. Effect of g.8514C > T polymorphism in Osteopontin gene on milk production traits [MY305d (305 day milk yield), PKY (peak yield), FY (fat yield), PY (protein yield), SNFY (solids not fat yield), LACY (lactose yield), DMY (daily milk yield), FP (fat per cent), PP (protein percent), SNFP (solids not fat percent) and LACP (lactose percent)] in Holstein Friesian crossbred cattle of Kerala. Milk production traits are not significantly differed between genotypes (P ≥ 0.05).

Trait	Genotypes (mean ± SE)		P-value
	TT	CT	
MY305d (kg)	2357.60 ± 90.12	2327.01 ± 94.92	0.78
PKY (kg)	12.73 ± 0.50	12.90 ± 0.52	0.77
FY (kg)	92.23 ± 3.13	91.96 ± 3.30	0.83
PY (kg)	75.21 ± 4.04	74.36 ± 4.16	0.91
SNFY (kg)	186.05 ± 10.9	184.47 ± 11.2	0.82
LACY (kg)	102.27 ± 5.92	101.53 ± 6.08	0.78
DMY (kg)	8.41 ± 0.26	8.06 ± 0.27	0.76
FP (%)	3.96 ± 0.06	3.99 ± 0.06	0.71
PP (%)	3.18 ± 0.04	3.21 ± 0.04	0.49
SNFP (%)	7.91 ± 0.04	7.92 ± 0.05	0.81
LACP (%)	4.35 ± 0.03	4.34 ± 0.03	0.93

polymorphisms located in splicing sites had more effect than all other SNPs [32], since it can produce splice variants. Dudemaine et al. [8] explained that genetic variations in *OPN* gene influence the amount of osteopontin secreted in milk throughout lactation. In order to reveal the actual mechanism behind the influence of osteopontin on milk composition, the study has to be extended to upstream and downstream regions of the gene.

The number of animals bearing C allele in the studied population is remaining as a limitation in the present investigation, and it is reflected in the association analysis. To confirm the present association results in Holstein Friesian crossbred cattle of Kerala, an extended study in a large population has to be undertaken.

In conclusion, the present study investigated the involvement of g.8514C > T polymorphism in the milk production traits in Holstein Friesian crossbreds of Kerala. The conflicting association results between the present and the previous studies necessitate a detailed investigation in a larger population before suggesting g.8514C > T polymorphism as a molecular marker for milk production traits in crossbred cattle of Kerala. The advent of molecular tools coupled with automated high-throughput assays explained the importance of single nucleotide polymorphisms (SNPs) as targets to improve milk production. The identification of SNPs which are in

LD with QTL affecting milk production is a prerequisite to implement genomic selection in the crossbred cattle population of Kerala.

Acknowledgements
The authors are very thankful to the Dean of College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala for providing all necessary facilities and financial support for the research.

Conflict of Interest
The authors declare that there is no conflict of interest.

References

1. Meuwissen THE, Hayes BJ, Goddard ME. Prediction of total genetic value using genome-wide dense marker maps. Genetics 2001; 157: 1819-1829.
2. Blasco A, Toro MA. A short critical history of the application of genomics to animal breeding. Livestock Science 2014; 166: 4-9.
3. Dekkers JCM. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. Journal of Animal Science 2004; 82: 313-328.
4. Schmid M, Bennewitz J. Invited review: Genome-wide association analysis for quantitative traits in livestock–a selective review of statistical models and experimental designs. Archives Animal Breeding 2017; 60: 335-346.
5. Olsen HG, Lien S, Gautier M, Nilsen H, Roseth A et al. Mapping of a milk production quantitative trait locus to a 420-kb region on bovine chromosome 6. Genetics 2005; 169: 275-283.
6. Sodek J, Ganss B, McKee MD. Osteopontin. Critical Reviews in Oral Biology and Medicine 2000; 11: 279-303.
7. Schack L, Lange A, Kelsen J, Agnholt J, Christensen B et al. Considerable variation in the concentration of osteopontin in human milk, bovine milk, and infant formulas. Journal of Dairy Science 2009; 92: 5378-5385.
8. Dudemaine PL, Thibault C, Alain K, Bissonnette N. Genetic variations in the *SPP1* promoter affect gene expression and the level of osteopontin secretion into bovine milk. Animal Genetics 2014; 45: 629-640.
9. Bissonnette N. Short communication: Genetic effect of variations in the osteopontin gene (*SPP1*) with lactation persistency in dairy cattle. Journal of Dairy Science 2018; 101: 456-461.
10. Prokesch A, Smorlesi A, Perugini J, Manieri M, Ciarmela P et al. Molecular aspects of adipoepithelial trans-differentiation in mouse mammary gland. Stem Cells 2014; 32: 2756-2766.
11. Lali FA, Anilkumar K. Crossbreeding of cattle population in India. Journal of Indian Veterinary Association 2019; 17: 16-24.
12. Lali FA, Bindu KA. Microsatellite BM1500 polymorphism and milk production traits in Vechur and crossbred cattle of Kerala. Veterinarski Arhiv 2011; 81: 35-42.
13. The International Committee for Animal Recording. The Global Standard for Livestock Data Procedure 2, Section 2 ICAR Guidelines for Computing Accumulated Lactation Yield. Rome, Italy: ICAR; 2020.
14. Joseph BA. Assessment and alleviation of environmental stress on productive performance of rabbits. PhD, Kerala Veterinary and Animal Sciences University, Pookode, India, 2011.
15. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. 3rd ed. New York, NY, USA: Cold Spring Harbor Laboratory Press; 2001.
16. Leonard S, Khatib H, Schutzkus V, Chang YM, Maltecca C. Effects of the osteopontin gene variants on milk production traits in dairy cattle. Journal of Dairy Science 2005; 88: 4083-4086.
17. Falconer DS, Mackay TFC. Introduction to Quantitative Genetics. 4th ed. Essex, UK: Longmans Green; 1996.
18. Khatib H, Zaitoun I, Wiebelhaus-Finger J, Chang YM, Rosa GJM. The effect of bovine *PPARGC1A* and *OPN* genes with milk composition in two independent Holstein cattle populations. Journal of Dairy Science 2007; 90: 2966-2970.
19. Mousavizadeh SA, Salehi A, Aminafshar M, Sayyadnejad MB Nazemshirazi MH. Novel SNPs of the *ABCG2* gene and their associations with milk production traits in Iranian Holstein bulls. Journal of Agricultural Science and Technology 2013; 15: 1145-1151.
20. Boleckova J, Matejickova J, Stipkova M, Kyselova J, Barton L. The effect of five polymorphisms with milk production traits in Czech Fleckvieh cattle. Czech Journal of Animal Science 2012; 57: 45-53.
21. Anggraeni A, Talib C, Asmarasari SA, Herawati T, Andreas E. Genetic polymorphisms of *IGF1, GH*, and *OPN* genes in crosses Peranakan Ongole cattle based on birth type in Central Java. Indonesian Journal of Animal and Veterinary Sciences 2017; 22: 165-172.
22. Łuczak IK, Hanna K. Genetic polymorphisms of *FAM13A1, OPN, LAP3*, and *HCAP-G* genes in Jersey cattle. Turkish Journal of Veterinary and Animal Sciences 2013; 37: 631-635.
23. White SN, Casas E, Allan MF, Keele JW, Snelling WM et al. Beef cattle evaluation of six DNA markers developed for milk production traits reveals an Osteopontin polymorphism is associated with post weaning growth. Journal of Animal Science 2007; 85: 1-10.
24. Mello F, Cobuci JA, Martins MF. Effect of the polymorphism g.8514C>T in the Osteopontin gene (*SPP1*) with milk yield in the dairy cattle breed Girolando. Animal Genetics 2012; 34: 67-69.

25. Kułaj D, Pokorska J, Ochrem A, Dusza M, Makulska J. Effects of the c.8514C>T polymorphism in the osteopontin gene (*OPN*) on milk production, milk composition and disease susceptibility in Holstein-Friesian cattle. Italian Journal of Animal Science 2019; 18: 546-553.

26. Salehi A, Khadijeh N, Mahdi A, Mohammad BS, Rohoallah S. The effect of bovine osteopontin (*OPN*) gene with milk production traits in Iranian Holstein bulls. Iranian Journal of Biotechnology 2015; 13: 1092-1097.

27. Pasandideh M, Mohammadabadi MR, Esmailizadeh AK, Tarang A. Effect of bovine *PPARGC1A* and *OPN* genes with milk production and composition in Holstein cattle. Czech Journal of Animal Science 2015; 60: 97-104.

28. Lacorte G, Machado M, Martinez M, Campos A, Maciel R et al. DGAT1 K232A polymorphism in Brazilian cattle breeds. Genetics and Molecular Research 2006; 5: 475-482.

29. Braunschweig M, Hagger C, Stranzinger G, Puhan Z. Associations between casein haplotypes and milk production traits of Swiss Brown cattle. Journal of Dairy Science 2000; 83: 1387-1395.

30. Boettcher P, Caroli A, Stella A, Chessa S, Budelli E et al. Effects of casein haplotypes on milk production traits in Italian Holstein and Brown Swiss cattle. Journal of Dairy Science 2004; 87: 4311-4317.

31. Suchit K, Anupama M, Sunil K, Kumar YA, Kumar NA. Role of Osteopontin in bovines and their association with different traits: A review. International Journal of Genetics 2018; 10: 370-372.

32. Wang G, Vander Walt JM, Mayhew G, Li YJ, Zuchner S et al. Variation in the miRNA-433 binding site of *FGF20* confers risk for Parkinson disease by overexpression of α-synuclein. American Journal of Human Genetics 2008; 82: 283-289.