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Polymorphism Analysis of DGAT1 Gene and Novel Association Studies with Milk Production Traits in Domestic Dromedary Camel in India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Several polymorphisms related to different gene loci have been proven to affect milk production traits and milk composition in camel. The current investigation was carried out in order to determine the genetic polymorphism of the DGAT1 gene and its association with milk production traits in camels. The SNP analysis focused on a 773 bp long fragment of the DGAT1 gene that was amplified using specific primer pairs through PCR. The Novel dromedary sequencing results revealed partial exon-6,10; complete exon-7, 8, and 9 as well as complete intron-6, 7, 8, and 9 in

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the 773 bp DNA fragment. A single nucleotide variation was identified at position 463 C>T of exon-8 in both Bikaneri and Kachchhi breeds. Association studies of genotypes of DGAT1 gene (CC and CT) with milk production and composition traits were examined using GLM in SPSS. The effect of breed on lactation yield and lactation length was found non-significant (P>0.05) and the effect of breed on peak yield was found highly significant (P≤0.01) and on average daily milk yield was found significant (P≤0.05). Effect of genotype, year of calving, age of calving and parity on lactation length, lactation yield, peak yield and average daily milk yield were found non-significant (P>0.05).

Keywords: DGAT1 gene; camel; dromedary; milk production traits; polymorphism; association study' SNP' indian camel' MAS.

ABBREVIATIONS

PCR : Polymerase chain reactions
DGAT1 : Diacylglycerol acyltransferases
GLM : Generalized linear model
SNP : Single nucleotide polymorphism
NCBI : National Centre for Biotechnology Information

1. INTRODUCTION

Camels in India have several benefits for agriculture. They are uniquely adapted to hot and arid environments, making them well-suited for the desert ecosystem [1]. Camels are primarily used for carting, draft, and transportation, but they also provide secondary benefits such as milk and hair production [2]. Camel milk has distinct nutritional properties, including low fat content and high levels of unsaturated and long-chain fatty acids. It also contains proteins rich in lactoferrin and lysozymes, as well as higher percentages of total salts, protective proteins, and vitamin C [3]. Additionally, she camel has a longer lactation period and camel milk can be processed into various products like fermented milk, soft cheese, and flavoured milk [4]. These advantages make camels a valuable asset for agriculture in India, particularly in arid and semi-arid regions of thar desert of Rajasthan state. However, camel farming in India faces several challenges for agricultural purposes. The traditional use of camels as draught animals has decreased due to the availability of cheaper mechanical machineries [3]. Additionally, the transition from traditional extensive forms of camel farming to modern semi-intensive or intensive forms may impact the perception of camel farming as an environmentally sustainable production system [5]. To maintain a sustainable development approach, camel stakeholders need to balance the camel demography with the environmental carrying capacity, preserve camel diversity, develop alternative feeding systems to preserve water resources in desert areas, promote high-value products, control health constraints, and respect the social role of camels [6]. These challenges highlight the need for innovative approaches to sustain camel agriculture in India. Advanced molecular biology techniques can work like a tool to increase the production and value of camel milk. With the remarkable progress in the field of molecular genetics technology, researchers have been able to achieve a significant breakthrough in the identification of molecular markers.

1.1 Marker Assisted Selection

Marker-assisted selection (MAS) in milk production in livestock offers several benefits. MAS allows for the identification of associations between DNA markers and productive potential, such as milk yield and composition [7]. Traditional marker-assisted selection methods were limited by the complexity of traits and the small effects of individual genes [8]. Simulation studies have shown that breeding schemes using genetic marker information can significantly improve true breeding values and genetic superiority in dairy animals [9]. Additionally, the use of high-throughput marker systems can increase the efficiency and flexibility of breeding programs. Overall, MAS has the potential to enhance milk production and improve breeding outcomes in livestock.

Marker-assisted selection (MAS) in milk production in livestock involves the use of genetic markers associated with specific genes. Several candidate genes have been identified for their associations with milk production traits. These genes include ABCG2, CD44, and SPP1, [10] ACADVL and IRF6, [11] and various genes identified through genome-wide association studies (GWAS) and RNA-sequencing [11]. These genes have been found to have significant genetic effects on milk yield, milk fat percentage, milk protein percentage and other milk production traits in dairy cattle.

Marker assisted selection (MAS) in camel milk involves the identification of genetic polymorphisms in various genes. One such gene is the K-CN gene, which has been studied for its polymorphisms and their effects on milk traits and properties [12]. Another gene involved in MAS is the β-casein (CSN2) gene, which has a specific SNP (2126 A/G) that has been associated with milk composition traits in Maghrebi camels [13]. Additionally, the alpha-lactalbumin gene has been studied for its genetic polymorphisms and their relationship with milk protein concentration in Maghrebi camels. Furthermore, a gene associated with milk β-lactoglobulin (BLG) content has been identified, with a specific SNP (G78A) being associated with lower levels of milk BLG content [14]. Cruz et al. [15] conducted a genome-wide association study and found that the DGAT1 gene accounts for most of the variability in milk fatty acid composition in Holstein cattle. Overall, this gene and its polymorphisms can play a crucial role in marker assisted selection for improving camel milk traits.

1.2 Camel DGAT1

The DGAT1 gene in dromedary camels from North Africa, as indicated by Elbers et al. (2019), spans a length of approximately 9327 bp. This gene is comprised of 16 exons and 15 introns, as stated in the GenBank entry KAB1258102.1. However, there is currently no available information regarding the molecular structure of the DGAT1 gene in Indian dromedary camels. The previously mentioned gene structure was obtained from a specific region (bases 41215260 to 41224586) on chromosome 25, utilizing whole genome Illumina assemblies or shotgun sequencing (CamDro3) of the North African dromedary Camel. Consequently, this study was initiated to provide the initial characterization of the DGAT1 gene in Indian dromedary camels.

2. MATERIALS AND METHODS

The animals for the present investigation were selected from National Research Centre on Camel (ICAR-NRCC), Bikaner, Rajasthan, India. Blood of 21 Bikaneri and 24 Kachchhi Camels were collected for the association studies. Different traits like Identification number, Date of Birth, Date of calving, Date of drying, Parity, Lactation length, Lactation yield and Peak yield of total 45 she camel were extracted from the records maintained at camel farm house, ICAR-NRCC, Bikaner. PCR amplification of 773 bp DGAT1 gene fragment was executed using standard PCR protocols by using primers designed with the help of Primer-BLAST system of NCBI [16] and were synthesized from Eurofins genomics. Primers (listed in the Table 1) were mapped out using Primer-BLAST system of NCBI [16]. The standardized PCR programme used for best amplification of the desired fragments is given in Table 2. The amplified products were visualized, documented by agarose gel electrophoresis.

For each positive PCR product, 40 µl sample was sent for sequencing to GeneOmbio technologies Pvt. Ltd. For the statistical analysis gene (allele) and genotypic frequencies were calculated with the help of DGAT1 gene sequencing results and GenAlEx 6.5 software as per Falconer and Mackay [18], which was based upon the Hardy-Weinberg equilibrium laws.

Table 1. DGAT1 Primer sequences and Expected fragment size for the selected genomic region of DGAT1 DNA

Primer Sequence (5'-3')	Expected Size
F- GCCTTCCTGCTTGAGTCCAT **R- CCACTTGGAGCTGGGTAAGG**	**773 bp**

Table 2. Protocol of PCR by DGAT1 Primers

	Step	Temp. ^{0}C	Time	No. of Cycle
1.	**Initial denaturation**	95	5 min	1
2.	**Denaturation**	95	60 sec	
3.	**Annealing**	57	45 sec	34
4.	**Extension**	72	45 sec	
5.	**Final Extension**	72	10 min	1

Relationship of each dependent variable with the two genotypic patterns of DGAT1 gene in Bikaneri and Kachchhi camel were analysed separately for the investigation of Novel association between Camel DGAT1 SNP and milk production traits. Statistical analysis was performed by using SPSS (IBM® SPSS® software platform, 2005). Analysis of variance (ANOVA) of linear model for dependent variable was applied as $Y_{ijklmn} = \mu + G_i + S_j + H_k + P_l + Q_m + e_{ijklmn}$. Where, Y_{ijklmn} = observed value of the trait, μ = overall mean, G_i = fixed effect of i^{th} genotype (i = 1,2), S_j = fixed effect of j^{th} age at calving (j = 1,2,3), H_k= fixed effect of k^{th} breed (k=1,2), P_l = fixed effect of l^{th} parity (l=1,2,3,4), Q_m = fixed effect of m^{th} year of calving (1,2,3), e_{ijklmn} = random error.

3. RESULTS AND DISCUSSION

Followed investigation steps were performed for association study between polymorphism in Camel DGAT1 gene and different milk production traits:

3.1 PCR Amplification of DGAT1 Gene by DGAT1 Primer

The genomic DNA was isolated with DNA isolation kit of Thermo scientific™. The isolated, purified DNA was processed for PCR by using primer pairs is shown in Table 2. Clears bands of DNA with high concentration can be seen between 700 to 1000 bp bands of marker or ladder (Fig 1). After bidirectional sequencing (Forward and reverse) of PCR products and its visualization using Codon Code aligner software, 773 base pair DGAT1 gene fragment, its genetic variants were identified. No SNP was detected at K232A site. It can be due to fixed allele. Another novel SNP was detected in last codon of exon-8 at 463rd base in DNA fragment of 773 bp. Other SNPs, rather than the DGAT1 K232A were also found by Schennink [17] in many species like cattle, buffalo, goat and sheep.

3.2 Allele, Genotypic Frequency of Novel SNP in DGAT1 gene

The gene (allele) and genotypic frequencies were calculated as per Falconer and Mackay [18] which is based upon the Hardy-Weinberg equilibrium laws. Numbers of animals having different allelic variants were calculated by DNA sequencing results (in Codon Code aligner software). Gene and genotype frequencies were calculated for the 463rd base site of exon-8 in DNA fragment of 773 bp. The allele and genotypic frequencies are shown in Table 3 and Table 4, respectively.

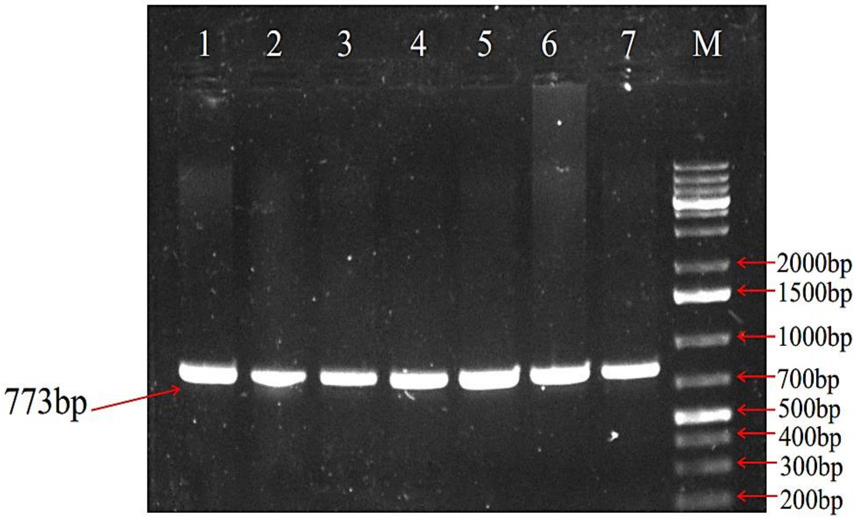

Fig 1. Diacylglycerol Acyltransferase (DGAT1) gene resolved on 1.0% agarose gel M marker 1k bp plus DNA ladder (M), 1 to 7 are DGAT1 gene product

Table 3. Allele frequencies of C and T in Bikaneri and Kachchhi camel

Allele	Bikaneri (B)	Kachchhi (K)
C	0.786	0.942
T	0.214	0.058

Table 4. Genotypic frequencies of CC and CT in Bikaneri and Kachchhi camel

Genotype	Bikaneri (B)	Kachchhi (K)
CC	**0.643**	**0.885**
CT	**0.357**	**0.115**

Table 5. Mean±SE and effect of various factor on milk production traits

	No. of animal	Lacation Yeild (kg)	Lactation length(days)	Peak Yeild(kg)	Av.Daily Milk Yeild (kg)
Mean	45	1497.36±146.60	380.42±22.03	7.12±0.36	3.72±0.23
Breed		**NS**	**NS**	**	*
Bikaneri	21	1219.67±195.73	356.67±34.55	6.07±0.45	3.20±0.25
Kachchhi	24	1740.35±206.02	401.21±28.15	8.04±0.49	4.17±0.35
Genotype		**NS**	**NS**	**NS**	**NS**
CC	35	1551.89±180.54	379.03±25.39	7.39±0.45	3.82±0.29
CT	10	1306.51±191.38	385.30 ± 46.37	6.19 ± 0.27	3.37± 0.16
R2		0.217	0.132	0.43	0.413
Year of calving		**NS**	**NS**	**NS**	**NS**
2018	13	1294.30±185.74	384.77±43.44	6.32±0.54	3.18±0.34
2019	15	1585.63±276.42	350.73±30.25	7.72±0.76	4.25±0.45
2020	17	15.74.76±273.61	403.29±40.77	7.20±0.55	3.66±0.36
Age of Calving		**NS**	**NS**	**NS**	**NS**
upto 6 year	10	1453.45±398.64	408.30±38.71	6.80±0.69	3.34±0.40
6-10 year	15	1599.55±280.73	383.60±34.65	7.40±0.84	3.94±0.051
>10 year	20	1442.69±221.68	364.10±38.41	7.07±0.42	3.74±0.26
Parity		**NS**	**NS**	**NS**	**NS**
1	8	1005.70±47.60	355.50±31.59	5.78±0.59	2.76±0.26
2	18	1613.79±195.17	420.39±33.28	7.15±0.54	3.69±0.30
3	8	1524.16±305.33	378.13±59.43	7.30±0.75	3.95±0.55
4 & above	11	1644.95±447.59	334.82±53.23	7.91±0.96	4.29±0.63

*NS = non-significant (p value >0.05); * = significant (p value ≤ 0.05), ** = highly significant (p value ≤0.01)*

3.3 Association of Breed, Genotype, Year of Calving Age of Calving, Parity with Milk Production Traits

Association studies between CC and CT genotypes of DGAT1 gene and milk traits were examined using GLM in SPSS (IBM® SPSS® software platform, 772005). Lactation length (LL), lactation yield (LY), average daily milk yield (AvDM) and peak yield (PY) were studied. The effect of breed on lactation yield and lactation length was found non-significant (P>0.05) and effect of breed on peak yield was found highly significant (P≤0.01) and on average daily milk yield was found significant (P≤0.05). Effect of genotype, year of calving, age of calving and parity on lactation length, lactation yield, peak yield and average daily milk yield were found non-significant (P>0.05) as shown in Table 5. Association studies between novel CC, CT genotypes of camel DGAT1 gene and milk traits have not been cited yet. Whereas, investigation of Zayeed et al. [19] on lactating camels, mentioned high variations in milk traits due to many factors such as breed, lactation stage, parity number etc. Musaad et al. [20] found significant effect (P≤0.05) of breed of camels on Milk yield. Mehaia et al. [21] reported significant effect (P≤0.05) of breed on milk composition traits in camel.

4. CONCLUSION

The aim of this investigation was to examine the DGAT1 gene in dromedary camels in order to determine genetic polymorphism and assess its association with milk production traits. Through PCR amplification, sequencing, and bioinformatics analysis, a single nucleotide variation was identified at position 463 C>T of exon-8 in both Bikaneri and Kachchhi breeds, and association studies were conducted using GLM in SPSS software platform. The study found that the breed had no significant effect on lactation yield and lactation length, but had a highly significant effect on peak yield and a significant effect on average daily milk yield, while genotype, year of calving, age of calving, and parity had no significant effect on any of the milk production traits.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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