



The Impact of *DGAT1* K232A Mutation on Milk Production and Quality Traits in Vechur and Crossbred Cattle of Kerala, India

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ABSTRACT

Background: A dinucleotide substitution in exon 8 of the gene coding for acyl CoA: diacylglycerol acyltransferase 1 (DGAT1), which changes the amino acid sequence from lysine to alanine (p.Lys232Ala) in the mature protein, was found to have a significant effect on milk production and quality traits in certain cattle breeds. The present study was conducted on Vechur and Crossbred cattle of Kerala to evaluate the genetic diversity and effect of K232A polymorphism of *diacylglycerol O-acyltransferase (DGAT1)* gene on milk production and quality traits (305-day milk yield, peak yield, average daily yield, fat per cent and SNF per cent).

Methods: A total of 60 Vechur and 180 crossbred cows were genotyped using High resolution melt (HRM) curve analysis. Association analysis was carried out using fixed general linear model (GLM) of SPSS V.26.

Result: Monomorphic KK genotype was observed in Vechur cattle. The frequencies of genotypes for KK, KA and AA genotype were estimated as 0.55, 0.28 and 0.17 in crossbred cattle. The K allele was found to be fixed in Vechur cattle population. In the population under study, the allele K exhibited a higher prevalence compared to allele A, with a frequency of 0.69 as opposed to 0.31. The genotypes of K232A had significant ($p \leq 0.01$) effect on 305-day milk yield, peak yield, average daily yield and fat per cent in crossbred animals. The KK and KA genotypes showed significantly superior values for fat per cent ($p \leq 0.01$) compared to AA genotypes. The AA genotype showed significantly superior values for 305-day milk yield, peak yield and average daily yield compared to KK and KA genotypes. Hence, the findings of this study indicate that the *DGAT1* gene exhibits significant potential as a candidate gene influencing both milk production and quality attributes in crossbred cattle of Kerala.

Key words: Crossbred, DGAT, Milk, Polymorphism, Vechur.

INTRODUCTION

Bovine milk is one of the primary foods recommended for healthful human consumption. With a diverse composition, it contains essential macro and micronutrients for our health and growth, due to their contribution of essential amino acids and fatty acids, milk protein and milk fat (Gibson, 2011). The importance of milk production traits cannot be overstated in livestock production and the economy it supports (Caroli *et al.*, 2010; An *et al.*, 2012). Improving milk production has been a constant concern; thus, identifying candidate genes for enhanced milk production traits. To enhance productivity, the livestock sector has implemented a breeding programme that focuses on selecting and utilising animals with superior milk production attributes. Identifying and validating genetic markers linked to milk production traits is a vital step in establishing a marker-assisted selection (MAS) approach. Therefore, the achievement of enhanced productivity through genetic selection is a prevalent objective in many animal breeding programmes on a global scale (Meredith *et al.*, 2012; Narayana *et al.*, 2017; Heimes *et al.*, 2019). Vechur cattle milk is distinguished from crossbred cattle milk by its superior digestibility, enhanced buffering capacity and notable therapeutic properties. Comparative studies by Annie *et al.*, (2025) have demonstrated that Vechur milk possesses a significantly higher total antioxidant capacity than crossbred cow milk, suggesting a greater therapeutic value.

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One of the most important enzymes in triglyceride metabolism is diacylglycerol O-acyltransferase (DGAT1), which catalyses both principal triglyceride synthesis pathways (Lehner and Kuksis, 1996; Farese *et al.*, 2000). The *DGAT1* gene encodes this enzyme, which is situated on chromosome 14 and consists of 17 exons. It has been recognised as a potential marker for the quantitative trait loci (QTLs) associated with milk production and quality in cattle (Riquet *et al.*, 1999; Farnir *et al.*, 2002). The DGAT1

plays a crucial role in a wide range of physiological processes, such as lipoprotein assembly, adipose tissue formation, intestinal fat absorption and lactation. Additionally, *DGAT1* is responsible for the metabolism of triacylglycerol in higher eukaryotes (Cases *et al.*, 1998).

There have been numerous documented mutations in the bovine *DGAT1* gene. In a study conducted by Grisart *et al.* (2002), a noteworthy correlation was found between a specific genetic mutation (*DGAT1* K232A) and various aspects of milk production and composition. This alteration involved an exchange of codons from AA to GC between positions 10433 and 10434 of exon 8. Furthermore, it has been consistently shown that the presence of the K allele is associated with elevated levels of fat content and fat yield, as well as increased protein content. Conversely, there is a negative correlation between the K allele and protein and milk yields (Coppieters *et al.*, 1998; Grisart *et al.*, 2002; Winter *et al.*, 2002). In a study conducted by Szyda and Komisarek (2007), it was found that the polymorphism in the *DGAT1* gene had a significant additive effect on fat, milk and protein yields compared to various polymorphisms. The current study aimed to determine the frequencies of the K and A alleles in Vechur and Crossbred cattle of Kerala and their association with milk production traits in Crossbred cattle.

MATERIALS AND METHODS

Experimental animals

The present study was carried out during the academic year 2022-2023 at the Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. All experimental procedures were conducted in accordance with guidelines and regulations of institute ethics committee of Kerala Veterinary and Animal Sciences University, Kerala, India. For polymorphism study a total of 60 adult Vechur and 180 crossbred cows were sampled. Vechur cows were randomly selected from the Vechur Conservation Centre, Mannuthy, Thrissur, Kerala, India. For association analysis with milk production traits, samples and data were collected from 180 crossbred cattle maintained at University Livestock Farm, Mannuthy and Cattle Breeding Farm, Thumburmuzhy, Thrissur, Kerala, India.

Sample and data collection

Peripheral whole blood samples (2 mL) were collected from jugular veins into tubes containing K3-EDTA as an anticoagulant and stored at 4°C. Genomic DNA was extracted using phenol chloroform method from collected blood samples (Sambrook and Russell, 2001). The concentration, purity and quality of DNA were checked by NanoDrop spectrophotometer (Thermo Scientific, USA). DNA samples having good quality, purity and integrity were used for further study. Representative milk samples from 180 crossbred cattle during morning and evening were collected once in a month for ten months and brought to

laboratory at refrigerated conditions. Data on test day milk yield and average daily milk yield were collected from farm records. The samples were tested for test day fat per cent and test day SNF per cent using automatic milk analyser (MRC instruments). Estimation of 305-day milk yield and corresponding milk parameters was carried out using test interval method (TIM) method according to guidelines by International Committee for Animal Recording (ICAR, 2020).

High resolution melt (HRM) curve analysis

The population was screened for this SNP by High resolution melt (HRM) curve analysis. The conditions for HRM were optimised initially by conducting conventional PCR at a temperature range of 58°C to 65°C, based on which an HRM protocol was designed, for screening the target population for the double nucleotide substitution. Primers were custom designed to amplify a short fragment (177 bp) with the polymorphism from the *Bos taurus* Genome downloaded from NCBI (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/002/263/795/GCF_002263795.1_ARS-UCD1.2/GCF_002263795.1_ARS-UCD1.2_genomic.fna.gz) using Primer 3 (V.0.4.1) web application. The sequences of forward and reverse primers were 5'-CTTGCTCGTAGCTTTGGCAGG-3' and 5'-CGAAGAGGAAGTAGTAGAGATC-3' respectively. The PCR was performed in 25 µL reaction volume with 50 ng genomic DNA, 12.5 µL DNA Emerald Amp® GT PCR Master mix (Takara) and 10 pM of each primer (Sigma-Aldrich). Thermal cycling parameters of 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 58-65°C for 30 s, 72°C 30 s and 72°C for 5 min. The annealing temperature was standardised to 60°C.

DNA samples obtained from Vechur and Crossbred Cattle were screened for the double nucleotide substitution located in exon 8 of *DGAT1*, by using a novel High-resolution melt (HRM) curve analysis protocol. Reactions were carried out in Eco Real-Time PCR System (Illumina) using Eco 48-well plates which were sealed by Eco adhesive seals. One of the homozygous samples confirmed by the agarose gel electrophoresis and sequencing was taken as the reference sample. Reaction was conducted using Sso Fast EVA green super mix, primers and template DNA. Thermal cycling parameters of 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 60°C for 30 s, 72°C 30 s and 72°C for 5 min. Followed by melt curve analysis. Representative PCR products showing different melt curve were selected for each fragment and sequenced using respective forward and reverse primers to detect the variations, if any, at the nucleotide level. Sequencing was performed by automated sequencer using Sanger's dideoxy chain termination method at Agri Genome Labs Pvt. Ltd. Cochin.

Association analysis

The association of genotypes of *DGAT1* K232A polymorphism with milk production traits such as 305-day milk yield, peak yield, average daily yield, fat per cent and SNF per cent were analysed using fixed general linear model (GLM) of SPSS V.26. Post hoc test (Duncan's multiple

range tests) was used to identify homogeneous subsets. The general linear model used for milk production traits was:

$$Y_{ijklm} = \mu + H_i + S_j + P_k + G_l + e_{ijklm}$$

Where,

Y_{ijklm} = Trait of m^{th} cow in i^{th} herd, j^{th} season, k^{th} parity and belonging to l^{th} genotype.

μ = Population mean of trait.

H_i = Effect of i^{th} herd ($i = 1$ or 2).

S_j = Effect of j^{th} season ($j = 1$ to 3).

P_k = Effect of k^{th} parity ($k = 1$ to 4).

G_l = Effect of l^{th} genotype ($l = 1$ or 2).

e_{ijklm} = Random error.

RESULTS AND DISCUSSION

A total of 60 Vechur and 180 crossbred cows were analysed for *DGAT1* K232A gene polymorphism. Genotyping was performed using the HRM analysis method. The 177 bp long fragment of exon 8 of *DGAT1* which spanned the dinucleotide substitution was amplified under gradient

PCR for standardising the conditions for HRM analysis. An AA to GC dinucleotide substitution located in exon 8 of the bovine *DGAT1* gene that replaces lysine (K) by alanine (A) in encoded protein (K232A polymorphism) was found at NC_037341.1:611019, 611020 positions respectively. The K232A polymorphism of Vechur and crossbred cattle was analysed using a novel HRM protocol.

The amplicon was 179 bp long fragment, which gave three pronounced melt curves indicating three different genotypes in the target population (Fig 1 and 2). The representative amplicons exhibiting different melt curves were sequenced to confirm the genotype as KK (Tm 91.5°C), KA (Tm 87.8°C) and AA (Tm 91.1°C) as depicted in Fig 3. The genotype and allele frequencies are mentioned in Table 1. The dinucleotide polymorphism GC/AA (K232A) was confirmed by chromatogram analysis. Monomorphic KK genotype was observed in Vechur cattle. The genotypes frequencies were as follows: homozygous genotype KK (55%), heterozygous genotype KA (28.33%) and homozygous genotype AA (16.67%). The K allele was found to be fixed in Vechur cattle population. The same trend was

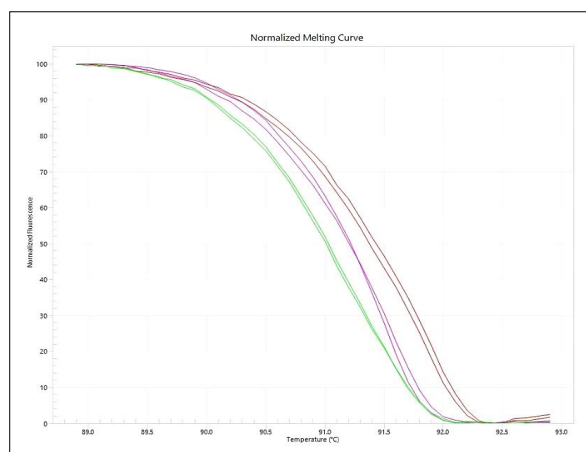


Fig 1: Normalised melt curve of HRM analysis, depicting homozygote KK genotype (red), homozygote AA (pink) and heterozygote KA (green).

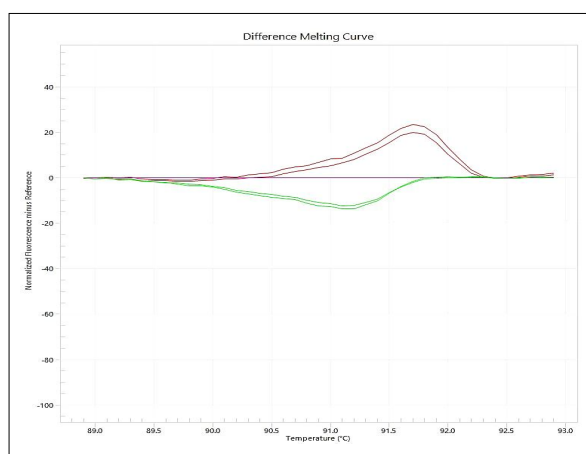


Fig 2: Difference melt curve of HRM analysis, depicting homozygote KK genotype (red), homozygote AA (pink) as reference and heterozygote KA (green).

also observed in indigenous cattle in earlier studies (Venkatachalapathy *et al.*, 2014). Analysis of the *DGAT1* region in Rath, Sahiwal, Deoni, Punganur, Tharparkar and Red Kandhari revealed the presence of the fixed *DGAT1*K allele. In the studied population of crossbred cattle, allele K had a predominance frequency of 69% over allele A with a frequency of 31%. According to Dudášová *et al.* (2021), the most common genotype in Holstein cattle was homozygous AA (80.88%), followed by heterozygous AK (16.91%) and homozygous KK (2.21%). In Romanian Holstein, Tăbăran *et al.* (2015) observed a predominance of the KK genotype (0.553), a lower frequency of the AK genotype (0.359) and the lowest frequency of the AA genotype (0.088). (Ganguly *et al.*, 2013) reported that although all the three genotypes were observed in Frieswal cattle, however, no animal of AA genotype was found in Sahiwal cattle and only four animals were of heterozygous genotype (AK). Due to the very high frequency to nearly fixed nature of the K allele in Sahiwal and other well-defined Indian cattle varieties, the *DGAT1* K232A polymorphism may not be suitable for selection purposes in indigenous cattle.

The dinucleotide polymorphism GC/AA resulted in an amino acid change from lysine to alanine. The effect of amino acid substitution on the protein function was predicted by PolyPhen-2 tool, which indicated that amino acid change at position 232 of *DGAT1* protein (p.Lys232Ala) was possibly benign with a score of 0.003 (Fig 4).

The genotypes of K232A had highly significant ($p \leq 0.01$) influence on 305-day milk yield, peak yield, average daily yield and fat per cent in crossbred animals. KK and KA genotypes showed highly significantly superior values for fat per cent ($p \leq 0.01$) compared to AA genotypes. The AA genotype showed significantly higher 305-day milk yield, peak yield and average daily yield compared to KK and KA genotypes. Table 2 presents the means with standard error for milk production traits of various genotypes of the K232A polymorphism in the *DGAT1* gene. Similar studies reported that the K allele was associated with decreased milk and protein yields and increased fat yield (Bovenhuis *et al.*, 2015; Bobbo *et al.*, 2018) as well as fat and protein percentage (Tomka *et al.*, 2016; Bobbo *et al.*, 2018). Furthermore, it has been consistently shown that the presence of the K allele is associated with elevated levels of fat content and fat yield, as well as increased protein content. Conversely, there is a negative correlation between the K allele and protein and milk yields (Coppieters *et al.*, 1998; Grisart *et al.*, 2002; Winter *et al.*, 2002). In a study conducted by Berry *et al.* (2010), it was discovered that the K allele of *DGAT1* K232A is strongly linked to increased milk fat content, milk protein content and milk fat yield. On the other hand, it is also associated with lower milk and protein yield in Danish dairy cattle.

A recent study examined how the lactation stage interacts with different genotypes in 1,800 Dutch Holstein-

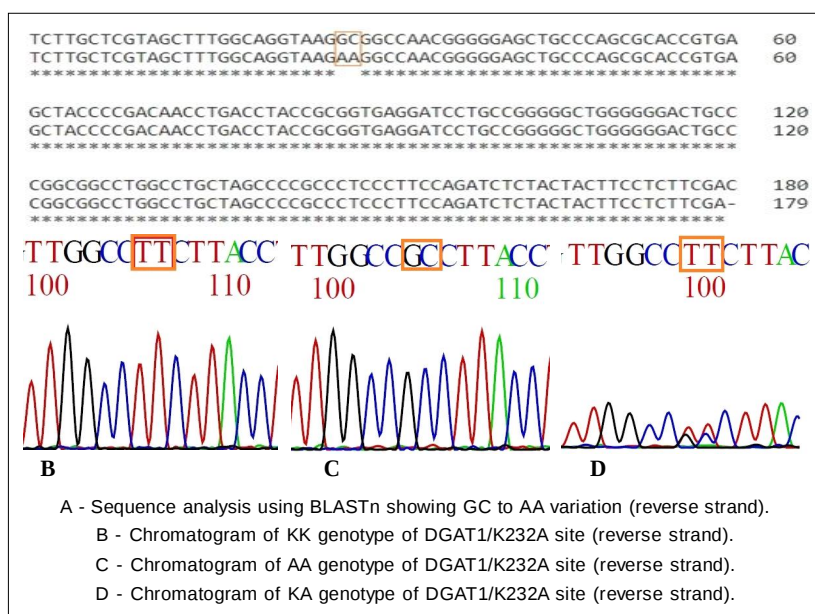


Fig 3: Sequence analysis and chromatogram of *DGAT1* gene comprising K232A polymorphism.

Table 1: Genotype and allele frequencies.

Gene	SNP	Genotype frequencies			Allele frequencies	
<i>DGAT1</i>	p.K232A	KK/0.55	KA/0.28	AA/0.17	K/0.69	A/0.31

Figures in parenthesis represent number of observations.

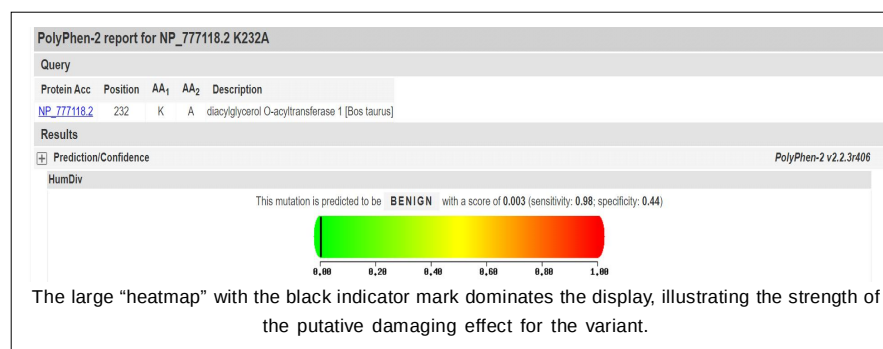


Fig 4: Results of the PolyPhen-2 analysis for single variant query (p.Lys232 Ala).

Table 2: Effect of K232A polymorphism in *DGAT1* gene on milk production traits in crossbred cattle of Kerala.

Trait	Mean $\pm$ SE			p-value
	KK (99)	KA (51)	AA (30)	
305-day milk yield (kg)	2688.28 $\pm$ 85.61 ^b	2667.65 $\pm$ 119.28 ^b	2994.25 $\pm$ 155.52 ^a	p $\leq$ 0.01
Peak yield (kg)	12.72 $\pm$ 0.35 ^b	12.61 $\pm$ 0.49 ^b	13.64 $\pm$ 0.64 ^a	p $\leq$ 0.01
Average daily yield (kg)	8.81 $\pm$ 0.28 ^b	8.75 $\pm$ 0.39 ^b	9.82 $\pm$ 0.51 ^a	p $\leq$ 0.01
Fat per cent	4.09 $\pm$ 0.04 ^a	4.02 $\pm$ 0.05 ^a	3.61 $\pm$ 0.07 ^b	p $\leq$ 0.01
SNF per cent	7.83 $\pm$ 0.03	7.75 $\pm$ 0.04	7.69 $\pm$ 0.05	p>0.05

Means with different superscripts in the same row differ significantly; Figures in parenthesis represent number of observations.

Friesian cows. The study focused on seven milk production traits, including milk yield, lactose yield, lactose content, fat yield, fat content, protein yield and somatic cell score. It has been reported that the effect of *DGAT1* K232A on milk production varies with the stage of lactation (Lu *et al.*, 2020). Lu *et al.* (2015) investigated the influence of the *DGAT1* K232A polymorphism on the milk metabolome and proteome of two groups of cows (AA and KK genotype cows). In comparison to milk from cows with AA genotypes, milk from cows with KK genotypes had lower levels of uridine diphosphate (UDP)-linked sugar, creatine and citrate, while showing higher levels of choline, carnitine, sphingomyelin and stomatin. Thus, the cows with variations in *DGAT1* polymorphism exhibited alterations in milk metabolome and proteome, shedding light on the underlying mechanism of how *DGAT1* K232A polymorphism affects milk production characteristics. Gurcan *et al.* (2018) reported that the genotypic frequency of the KA genotype was considerably higher than that of the homozygous genotypes across all populations studied, including Holstein, Jersey and indigenous breeds such as Native Black, East Anatolian Red and Grey Steppe cattle in Turkey. In a recent study by Mahmoudi and Rashidi (2023), they conducted a meta-analysis of pooled data to examine the relationship between the K232A polymorphism of the *DGAT1* gene and milk yield and composition. It was found that the K allele of the K232A polymorphism had a notable impact on boosting the fat and protein contents in cattle milk, especially when two copies of this allele were inherited together. Conversely, the A allele of the K232A polymorphism had detrimental effects on these traits.

CONCLUSION

The findings derived in our study show that p.Lys232Ala polymorphism is associated with milk production and quality traits in crossbred cattle. The K allele was found to be fixed in Vechur cattle population. In the crossbred cattle population under study, the allele K exhibited a higher prevalence compared to allele A. The KK and KA genotypes showed significantly superior values for fat per cent compared to AA genotypes. The AA genotype showed significantly superior values for 305-day milk yield, peak yield and average daily yield compared to KK and KA genotypes. Therefore, we can conclude that the *DGAT1* p.Lys232Ala polymorphism may be utilised in cattle breeding programmes in Kerala by selecting favourable alleles based on the breeding program's objective. This cannot be accomplished in Vechur cattle because of the lack of this polymorphism in *DGAT1* gene.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there is no actual or potential conflict of interest that could inappropriately influence in this work.

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