Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(1): 1170-1173

Received: 06-11-2018
Accepted: 09-12-2018

Ramkaran
Department of Veterinary Physiology and Biochemistry, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India

Anita Ganguly
Department of Veterinary Physiology and Biochemistry, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India

Sandeep Gera
Department of Veterinary Physiology and Biochemistry, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India

Preeti
Department of Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India

Ankit Magotra
Department of Veterinary Animal Genetics and Breeding, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India

Sandeep Kumar
Department of Veterinary Physiology and Biochemistry, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India

Correspondence
Sandeep Kumar
Department of Veterinary Physiology and Biochemistry, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India

Diacylglycerol *O*- acyltransferase 1 (DGAT 1) polymorphism in Hardhenu cross bred cattle (Holstein Friesian x Sahiwal x Hariana breed)

Ramkaran, Anita Ganguly, Sandeep Gera, Preeti, Ankit Magotra and Sandeep Kumar

Abstract
DGAT 1 enzyme catalyses the final step of milk triglyceride biosynthesis. A lysine to alanine substitution (K232A) at nucleotide position 10433-10434 at DGAT 1 is significantly associated with milk production traits. This polymorphism may potentially be used as a marker in selection programmes to enhance the production potential and to accelerate the rate of genetic gain. In the present investigation, DGAT1 K232A polymorphism was analysed in crossbred Hardhenu (HF X SW X Haryana) cattle (n=50) by restriction digestion of a 413 bp PCR fragment by *Eae* I. An undigested fragment of 413 bp indicated the K allele whereas two co-migrated fragments of 210 and 203 bp length indicated the A allele. In Hardhenu cattle population genotype frequencies were 0.30 (KK) and 0.70 (AK). The frequency of K and A allele were 0.65 & 0.35. Over all 15 KK and 35 AK animals were recognised in Hardhenu cattle whereas no animal of AA genotype were observed. The overall $DGAT1^{K}$ allele frequency in crossbred cattle was 0.65.

Keywords: Cattle, DGAT 1, Hardhenu, PCR, polymorphism

Introduction
Diacylglycerol *O*-acyltransferase (DGAT1; EC 2.3.1.20) is a microsomal enzyme catalyzing the addition of fatty acyl Co A to 1, 2, diacylglycerol to yield CoA plus triglycerol and is important in lipogenesis in many tissues, including mammary gland (Cases *et al*., 1998) [1]. The DGAT1 gene is a positional candidate gene for milk fat percentage with K232A substitution associated with higher fat percentage in *Bos taurus* (Winter *et al.,* 2002; Grisart *et al.*, 2004; Kuhn *et al.*, 2004; Kaupe *et al.*, 2004) [18, 5, 10, 7].

Numerous mutations in the bovine DGAT1 gene have been reported. However, a nonconservative substitution of lysine by alanine (DGAT1 K232A) with AA → GC exchange identified at position 10433 and 10434 of exon VIII (Grisart *et al*., 2002) [4] has been shown to be significantly associated with milk production and composition traits. Moreover, the K allele has been reported to be linked with an increase in fat content, fat yield & protein content and a decrease in protein & milk yields. (Winter *et al*., 2002, Vanbergue *et al*., 2016) [18, 15]. The DGAT1A allele has been found to be fixed in some *Bos taurus* breeds like Jersey, Holstein Friesian whereas very high to almost fixation of DGAT1K allele has been observed in some *Bos indicus* breeds. (Kaupe *et al*., 2004; Tantia *et al*., 2006, Ganguly *et al*., 2013) [7, 14, 3]. Recently, Ganguly *et al*., 2013 [3] observed the frequency of K and A allele in crossbred Frieswal (HF X Sahiwal) population as 0.64 and 0.36, respectively.

Hardhenu, a new strain of crossbred cattle has been developed by Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar which contains 62.5% blood of Holstein Friesian and rest of the 37.5% of Sahiwal and Hariana breed. The average lactation yield of this strain has been reported to be about 3350 Kg with an average fat content of 3.80%. The peak milk yield recorded at Cattle Breeding Farm of LUVAS, Hisar for this crossbred cattle is about 37 Kg in a single day. The wet average of this herd is about 12.5 Kg and average calving interval of this strain is 13 months and top 30% cows are producing 25 Kg milk in a day. The specificity of this strain is that it has heat tolerance power and remains stable at 48 ºC. The second composition of this strain is Sahiwal, which is well known for good milk yield as well as tick resistance, heat-tolerance and resistance to both internal and external parasites. The third opus incorporated in Hardhenu is the local dual purpose Hariana cattle having high conception rate.

The Hardhenu (Holstein Friesian X Sahiwal X Hariana) is a novel cattle introduced by LUVAS, Hisar for commercial exploitation, providing high productivity of Holstein Friesian and low repeat breeding of Sahiwal with sustainability and survivability of the Hariana. Looking at the increasing trend of crossbred cattle population in India and considering the significant association DGAT1 polymorphism with milk performance traits, the present was undertaken with the objective of estimating the frequency of alleles, K and A of DGAT1 in Hardhenu cow.

Materials and methods

DNA Isolation: Genomic DNA was isolated from 10 ml of blood of 50 randomly selected Hardhenu cattle maintained at Animal farm of LUVAS, Hisar, Haryana by phenol-chloroform method following standard protocol (Sambrook and Russell, 2001) [11].

Evaluation of purity, quality and concentration of DNA

The purity of the genomic DNA was assessed by UV spectrophotometer by checking the optical density (OD) value at 260 and 280 nm, which indicated the amount of DNA and the amount of protein in a given sample, respectively. The samples having OD ratio (260 nm/ 280 nm) 1.7 to 1.9 were used for the experiment. The concentration of DNA was calculated by using the following formula: DNA concentration (µg/µl) = OD260 x (Dilution factor) x 50/ 1000. Genomic DNA quality was checked to ensure the presence of intact DNA without any shearing. Horizontal submarine agarose gel electrophoresis was preformed to check the quality of DNA. The genomic DNA samples having good quality DNA (intact bands without smearing in gel) were used for further analysis.

PCR-RFLP: The PCR reaction was carried out in 0.2 ml PCR tubes in a thermal cycler (Applied Biosystem, USA). PCR was carried out in a final reaction volume of 25 µl containing approximately 100 ng DNA template, 12.5 ml of 1X PCR master mix (Thermo Fisher Scientific), 0.5 µl (10 pM/ µl) of each primer**.** Primer sequences were as follows: forward - 5'-GCACCATCCTCTTCCTCAAG-3' and reverse 5'-GGAAGCGCTTTCGGATG-3'. PCR amplification protocol included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C (30 s), 59 °C (30 s) and 72 °C (45 s) and a final extension at 72 °C for 10 min. PCR amplification was confirmed by running 10 µl of PCR product mixed with 2 µl of 6X gel loading dye (Thermo Fisher Scientific) on 1.5% agarose gel (depending on the expected size of amplified product) at a constant voltage of 70 V for 30 minutes in 1X TBE buffer. Ethidium bromide (0.5 µg/ml) was added @ 5µl of 1% solution /100 ml of gel solution. The amplified product was visualized as a single compact fluorescent band of expected size under UV light and documented by gel documentation system (Alphalmager, USA). The PCR products (10 µl) were digested with restriction enzyme (*Eae* I) as per the manufacturer's protocol. Cleavage by *Eae* I is diagnostic for the alanine-bearing allele. The reaction mixture was centrifuged for few seconds for uniform mixing and then incubated at 37 °C for 12-16 hours.

Analysis of the PCR-RFLP products: The enzyme-digested products were mixed with 6X gel loading dye at the rate of 10:1 and loaded on the 2.5% agarose gel along with 50 bp marker (Fermentas). Electrophoresis was carried out at 60 V for 1 hour and the bands were visualized and documented using Gel Documentation System. The bands were analyzed by comparing with molecular size marker. Genotyping of DGAT1 loci was carried out according to the band pattern of respective genotypes. Further, to validate the genotyping made through PCR-RFLP, representative amplicons were sequenced directly using automated DNA sequencer by Sanger's di-deoxy chain termination method.

Calculation of gene and genotypic frequencies: Gene and genotypic frequencies were calculated as given by Falconer and Mackay (1996) [2].

$$\text{Genotype frequency} = \frac{\text{Total number of individuals of a particular genotype}}{\text{Total number of individuals of all genotypes}}$$

$$\text{Gene frequency} = \frac{(2D+H)}{2N}$$

Where D = No. of homozygotes of particular genotypes
H = No. of heterozygotes having that gene and N = Total no. of individuals.

Results and discussion

The present investigation was undertaken to explore the frequency of alleles, K and A of DGAT1 in Hardhenu cow (n=50). The yield of DNA extracted from 10ml of blood ranged from 377 µg to 1113 µg with a mean of 745 µg. The OD ratio was in the range of 1.7-1.9 indicating purity of the extracted DNA.

A PCR product of 413 bp was amplified from Exon 8 of DGAT1 gene (Fig. 1). The PCR-RFLP studies on DGAT1 gene in Hardhenu cattle, using *Eae* I revealed two alleles, arbitrarily designated as K and A. Cleavage by *Eae* I was diagnostic for the alanine-bearing allele. An undigested fragment of 413 bp indicated the K allele and two co-migrated fragments (210 and 203 bp) indicated the A allele (Fig. 2). In Hardhenu cattle population genotype frequencies were observed to be 0.30 (KK) and 0.70 (AK). The frequency of K and A allele were 0.65 & 0.35 respectively (Table 1). A total of 15 KK and 35 AK animals were identified in Hardhenu cattle whereas no animal of AA genotype were observed. The genotype (KK and AK) were further confirmed by sequencing of representative sample (Fig. 3).

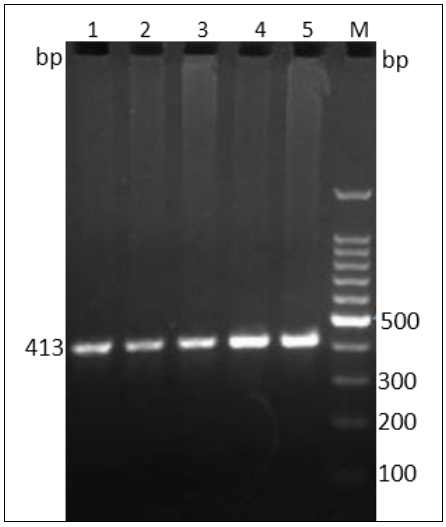

Fig 1: PCR products of DGAT1 gene. Lane 1-5: 413 bp amplified products; M: 100bp DNA ladder

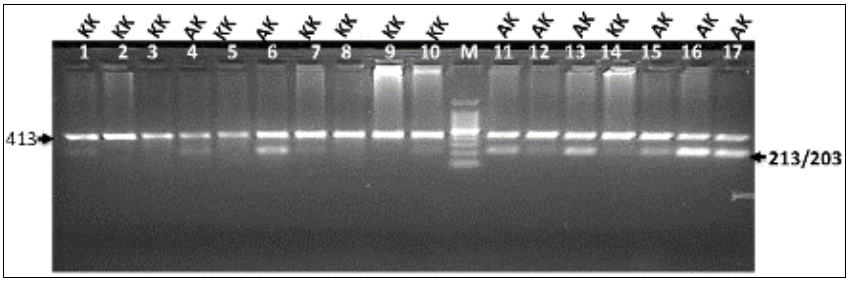

Fig 2. PCR-RFLP (*Eae* I) analysis of DGAT1 gene in Hardhenu cross bred cattle. KK- 413 bp; AK-413, 210 and 203 bp

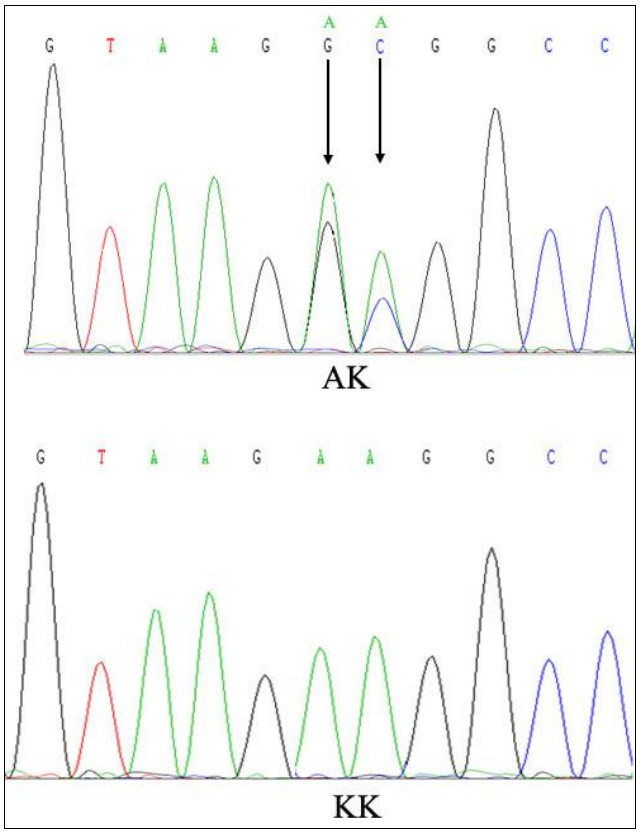

Fig 3: Chromatogram showing sequence confirmation of genotype AK and KK. Heterozygote (AK) is indicated by the presence of two peaks at SNP sites (arrow heads)

Table 1: Genotype and allele frequency of DGAT1 gene in Hardhenu crossbred cattle.

Breed	Genotype frequency			Total	Allele frequency	
	KK	AK	AA		K	A
Hardhenu crossbred cattle	0.30 (n=15)	0.70 (n=35)	0 (n=0)	50	0.65	0.35

DGAT1 gene encodes the Acyl Co A: Diacylglycerol O-acyltransferase (DGAT), a microsomal enzyme, which plays a central role in the metabolism of cellular glycolipids and triglycerol metabolism such as intestinal fat absorption, adipose tissue formation and lactation. DGAT catalyzes the final and the essential step in milk triglycerides synthesis (Cases *et al.,* 1998) [1]. Considering the importance of the DGAT1 gene for milk yield traits in general and milk fat content in particular, this study was carried out to identify different allelic variants in Hardhenu cattle. PCR-RFLP pattern in crossbred cattle showed two genotypes, AK and KK. The frequency of heterozygote was the highest (0.70), followed by those of lysine (0.30) homozygotes. The overall frequency of DGAT1^{K} allele in crossbred cattle was 0.65 (Table 1).

Studies on DGAT1 variants have also proved their strong association with milk production traits in many cattle breeds across the world (Grisart *et al.,* 2002; Winter *et al.,* 2002; Spelman *et al.,* 2002; Weller *et al.,* 2003) [4, 18, 13, 17]. The high frequencies of DGAT1 ^{K} (65% and above) allele were also reported in several *Bos taurus* cattle breeds like Holstein (Grisart *et al.,* 2002 and Spelman *et al*., 2002) [4, 13], Jersey and in German Angler (Spelman *et al*., 2002; Kaupe *et al*., 2007; Komisarek *et al*., 2004) [12, 7, 9] as well as in *Bos indicus* (*African zebu*) breeds like Anatolian Black (Winter *et al*., 2002) [18], Banyo Gudali and White Fulani (Houaga *et al*., 2017) [6]. DGAT1^{K} allele appears to be fixed in many *Bos indicus* breeds like Hariana, Tharparkar, Sahiwal and Nellore (Venkatachalapathy *et al*., 2014) [16]. The DGAT1^{A} allele was reported to be fixed in many *Bos taurus* breeds like Belgian Blue (beef), Hereford, Gelbvich, Pinzgauer, and Slovanian Syrmian (Winter *et al*., 2002 and Kaupe *et al*., 2004) [18, 7]. The high frequencies of DGAT1^{K} in *Bos taurus* dairy breeds (Holstein, Jersey and German Angler) may be due to constant selection for milk fat (Spelman *et al*., 2002 and Kaupe *et al*., 2004) [12, 7]. Ganguly *et al*. (2013) [3] observed the frequency of K and A allele in crossbred Frieswal (HF X Sahiwal) population as 0.64 and 0.36 respectively. Similar DGAT1 K and A allele frequencies were observed in the present study.

The DGAT1^{A} allele in Indian crossbred cattle has probably been introduced from *Bos taurus* through introgression and further selection for milk volume might have increased its frequency in the population. In spite of selective and heterozygote advantage of DGAT1^{A} allele, the frequency of DGAT1^{K} allele remained high (0.65) even after three decades of cross breeding. Similar situation was also reported in the New Zealand and Israel Holstein Population (Spelman *et al*., 2002 and Weller *et al*., 2004) [12, 17]. In well-defined Indian cattle breeds, DGAT1 K232A polymorphism may not be a suitable candidate for selection purpose due to very high frequency to almost fixed nature of K allele. However, in crossbred cattle population of India, the DGAT1 K232A polymorphism may serve as a candidate for future selection once association of K232A polymorphism with production traits is established.

Conclusion

It is concluded from the present investigation that Screening of DGAT1 K232A polymorphism in Hardhenu cattle (HF X SW X Haryana) population (n=50) revealed KK and AK genotype frequencies 0.30 and 0.70, respectively.) A total of 15 KK and 35 AK animals were identified in Hardhenu cattle whereas no animal of AA genotype were observed. The overall DGAT1^{K} allele frequency in crossbred cattle was 0.65. This preliminary information may further help in taking up association studies between DGAT1 polymorphisms and production traits in Hardhenu cattle.

References

1. Cases S, Smith SJ, Zheng YW, Myers HM, Lear SR, Sande E *et al*. Identification of a gene encoding an acyl CoA: diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. Proc. Natl. Acad. Sci. USA. 1998; 95:13018-13023.
2. Falconer DS, Mackay TFC. Introduction to Quantitative Genetics. (4th edn.), Longmans Green, Harlow, Essex, UK, 1996.
3. Ganguly I, Kumar S, Gaur GK, Singh U, Kumar A, Kumar S *et al.* DGAT 1 polymorphism K232A in Sahiwal (Indian zebu) and Frieswal (Holstein Friesian x Sahiwal crossbred) cattle. Indian J Anim Res. 2013; 12:195-198.
4. Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P *et al*. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Res. 2002; 12:222-231.
5. Grisart B, Farnir F, Karim L, Cambisano N, Kim JJ, Kvasz A *et al*. Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition. Proc. Natl. Acad. Sci. 2004; 101:2398-2403.
6. Houaga I, Muigai AWT, Kyallo M, Githae D, Youssao IAK, Stomeo F. Effect of breed and Diacylglycerol acyltransferase 1 gene polymorphism on milk production traits in Beninese White Fulani and Borgou cows. GJABG. 2017; 5(5):403-412.
7. Kaupe B, Winter A, Fries R, Erhardt G. DGAT1 polymorphism in *Bos indicus* and *Bos taurus* cattle breeds. Anim. Genet. 2004; 71:182-187.
8. Kaupe B, Brandt H, Prinzenberg EM, Erhardt G. Joint analysis of the influence of CYP11B1 and DGAT1 genetic variation on milk production, somatic cell score, conformation, reproduction, and productive lifespan in German Holstein cattle. J Anim. Sci. 2007; 85:11-21.
9. Komisarek J, Waskowicz K, Michalak A, Dorynek Z. Effects of DGAT1 variants on milk production traits in Jersey cattle. Anim. Sci. Papers and Rep. 2004; 22:307-313.
10. Kuhn C, Thaller G, Winter A, Bininda-Emonds ORP. Evidence for multiple alleles at the DGAT1 locus better explains a quantitative trait locus with major effect on milk fat content in Cattle. Genetics. 2004; 167:1873-1881.
11. Sambrook J, Russel DW. Molecular cloning: a laboratory manual. (3rd edn.), Cold Spring Harbor Laboratory press, New York, USA, 2001.
12. Spelman R, Georges M, Snell R. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Res. 2002; 12:222-231.
13. Spelman RJ, Ford CA, McElhinney P, Gregory GC. Characterization of the DGAT 1 gene in the New Zealand dairy population. J Dairy Sci. 2002; 85:3514-3517.
14. Tantia MS, Vijh RK, Mishra BP, Mishra B, Kumar STB, Sodhi M. DGAT1 and ABCG2 polymorphism in Indian cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) breeds. BMC Vet. Res. 2006; 2:32.
15. Vanbergue E, Peyraud JL, Guinard-Flament J, Charton C, Barbey S, Lefebvre R *et al*., Effects of DGAT1 K232A polymorphism and milking frequency on milk composition and spontaneous lipolysis in dairy cows. J Dairy Sci. 2016; 99(7):5739-5749.
16. Venkatachalapathy RT, Sharma A, Radha K. Polymorphism at DGAT1 locus in Indian buffalo, zebu and *Bos indicus x Bos taurus* cattle breeds. Vet. Sci. Res. J. 2014; 5(1-2):13-17.
17. Weller JI, Golik M, Seroussi E, Ezra E, Ron M. Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population. J Dairy Sci. 2003; 86:2219-2227.
18. Winter A, Krämer W, Werner FAO, Kollers S, Kata S, Durstewitz G *et al.*, Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl CoA: diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. Proc. Natl. Acad. Sci. USA. 2002; 99:9300-9305.