MOLECULAR CHARACTERIZATION OF *DGAT1* GENE IN SAHIWAL CATTLE

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ABSTRACT

Diacylglycerol aminotransferase (*DGAT1*) gene present in the QTL region is one of the potential candidate genes that have significant effect on milk fat content in dairy cattle. An alanine to lysine substitution (*K232A*) due to dinucleotide substitution in exon-8 of *DGAT1* has been identified to be responsible for milk fat variation in different bovine breeds. The present study was carried out in Sahiwal milking cows to characterize the *DGAT1* gene through polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP) genotyping. Whole blood sample (3 ml) was collected from 70 unrelated milking Sahiwal cattle maintained at Livestock Research Station, Kodamdesar, Bikaner, Rajasthan in an aseptic manner. Genomic DNA was extracted from whole blood through spin column method. The quality and concentration of extracted DNA was checked on 0.8% agarose gel and Nano Drop spectrophotometer, respectively. The exon 8 region of *DGAT1* gene was amplified using species specific primers (GenBank Accession No. AJ318490). The amplified 411bp region of *DGAT1* gene was digested overnight with 5U of *Eae* I restriction enzyme in a mixture containing 10X buffer and nuclease free water. The genetic variability in exon 8 of *DGAT1* gene was assessed on 8% polyacrylamide gel electrophoresis. The frequency of different alleles and genotypes of *DGAT1* gene were estimated through standard procedure of Falcon and Mackay (1998). All the analyzed samples showed 'KK' genotype with single fragment of 411bp and the gene and genotype frequency were observed fixed. The study concluded that exon 8 of *DGAT1* gene was monomorphic in Sahiwal cattle for *K232A* region and other single nucleotide polymorphisms (SNP's) of the region should be explored to detect individual variation in milk fat yield.

Key words: Sahiwal cattle, milk fat, *DGAT1* gene, RFLP

Introduction

Milk contains almost all the nutrients vital for life with biological value greater than 94%. Newer breeding goals especially on milk composition traits have recently been identified following the demands of a healthier human diet (Krovvidi *et al*., 2013). Indigenous cattle breeds are known for their higher fat yield than exotic cattle (Soni *et al*., 2016). Sahiwal, one of the most renowned milch breeds (Tolenkhomba and Yadav, 2012) of India, constitutes about 3.23% of total indigenous cattle population (Anonymous, 2013). The effective genetic improvement requires genetic information about the genetic variability and their effects on milk production. The incorporation of genotypic information as fixed factor in estimation of breeding value through traditional methods can significantly improve selection responses (Naqvi, 2007). The candidate genes such as diacylglycerol aminotransferase (*DGAT1*) gene (Winter *et al*., 2002) is a potential gene that was identified to have significant effect on milk composition traits in dairy cattle (Komisarek and Dorynek, 2009) due to their location in the (QTL) region. *DGAT1* gene is located on the centromeric end of the bovine chromosome 14 in the QTL region and is considered to be directly responsible for 50% variation in milk fat content and milk yield in dairy cattle (Banos *et al*., 2008). A di-nucleotide polymorphism (GC/AA) in exon eight of *DGAT1* gene harbours a non-conservative lysine to alanine substitution (*K232A*) with profound effect on milk fat content (Winter *et al*., 2002) and fat deposition in different bovine breeds (Sorensen *et al*., 2006). The polymorphic status of *DGAT1* gene has been amply reported in exotic cattle; however, there have been scant studies in Indian cattle.

Restriction fragment length polymorphisms (RFLP's) is one of the most abundant type ideal polymorphic genetic markers for the detection of genetic variation in polygenic traits (Jalving *et al*., 2004) and detects single nucleotide polymorphisms (SNPs) located at restriction site, easy to conduct and less cumbersome. The identification of specific pattern of allele and genotypic frequencies in indigenous cattle breeds may result in detection of causal factors responsible for variation in performance for milk production. In addition, few researches have been conducted for the exploration of genetic variation in *DGAT1* gene in indigenous breeds of Sahiwal. Therefore, the present investigation was undertaken with the objectives to study the genetic variability and to estimate the gene and genotypic frequencies of *DGAT1* gene in Sahiwal cattle.

Materials and Methods

An overall 75 milking Sahiwal cows were selected randomly for the present experimental trials from pure breeding population of Sahiwal maintained at Livestock Research Station, Kodamdesar, Bikaner (Rajasthan). About 2 ml of blood was taken from jugular vein of each selected animal in a ethylene diamine tetra acetic acid (EDTA) containing sterile vacutainer tube under strict aseptic conditions. Samples were refrigerated at 4ºC and preserved until processing. Genomic DNA from the whole blood sample was extracted through spin column method as per standard method (Sambrook and Russell, 2001) under manufacturer's protocols with slight modification through Genomic DNA Isolation Kit supplied by Himedia Pvt Ltd. The purity (OD ratio 260/280) and

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concentration (ng/µl) of extracted genomic DNA was determined by Nano-drop spectrophotometer. The quality of genomic DNA was assessed through 0.8% agarose electrophoresis to detect fragmentation or shearing if any. Only the good quality genomic DNA was allowed to be processed further.

A pair of species specific primers was used to amplify the selected genomic regions of exon-8 of *DGAT1* gene were constructed on the basis of available sequences of *DGATI* gene in NCBI GenBank database. The sequences of primers, the accession number of reference sequence and expected fragment length of different selected region are represented in Table 1. The selected 411bp region of the cattle *DGAT1* gene spanning parts of exon 7 and exon 9 (including intron 7, exon 8 and intron 8 entirely) was amplified by PCR in a final reaction volume of 25 µl. 5% dimethyl sulphoxide (DMSO) was added to the amplification mixture for equal amplification of both alleles as suggested by Winter *et al*. (2002). PCR reaction mixture used for amplification of genomic DNA was 5X PCR buffer (5 µl), 1.5 mM $MgCl_2$ (3 µl), 10 Mm dNTP's mix (1 µl), forward primer 70 pmol/ìl (1 µl), reverse primer 70 pmol/µl (1 µl), genomic DNA 25 ng/µl (4 µl), Taq DNA polymerase 5 U/µl (0.2 µl) and DNAase free water (10.8 µl). The gradient PCR programme was used to find out the appropriate annealing temperature that was further used for the amplification of all samples. The reactions were carried out in programmable thermocycler (Chino Scientific Gradient Thermocycler). The PCR programme used for the amplification of *DGAT1* gene is presented in Table 2. The quality and size of the PCR amplicons for different studied locus were assessed on 1.5% agarose gel containing ethidium bromide dye.

The dinucleotide polymorphism (GC/AA) responsible for alanine to lysine (*K232A*) polymorphism in *DGAT1* gene was detected with the use of restriction endonuclease enzyme, *Eae* I having restriction site of Y^GGCC_R. The 411-bp amplicons for the respective *DGAT1* gene were digested with 5U of *Eae* I (1 µl) restriction enzyme in a 40 ìl reaction mixture containing 10X buffer (5 µl) and nuclease free water (35 µl). The reaction mixture was mixed properly through spinning and kept under water bath at 37ºC for overnight digestion. The different *DGAT1* genotypes were analyzed on 8% polyacrylamide gel. The results of electrophoretic separation were visualized and documented through GelDOC Unit after staining with ethidium bromide dye. The frequency of different alleles and genotypes of *DGAT1* gene were estimated through standard procedure of Falconer and Mackay (1996).

Results and Discussion

The RFLP analysis of *DGAT1* gene through *Eae* I restriction enzyme revealed the presence of single genotypic pattern '*KK*' in all the 75 studied animals of Sahiwal breed and no animal of either '*KA*' or '*AA*' genotype was observed in any of the studied breed (Fig. 4.15). The *Eae* I restriction enzyme digests the '*A*' allele but not '*K*' allele required for alanine and lysine production, respectively. Only one undigested intact band of 411-bp, representative of '*KK*' genotype, was observed (Fig. 1). Thus, the allelic frequency of allele '*K*' was found to be fixed in Indian dairy cattle breed of Sahiwal with complete absence of *DGAT1* '*A*' allele.

The fix nature of allele '*K*' was initially identified by Kaupe *et al*. (2004) in Nellore cattle. An analogous finding about

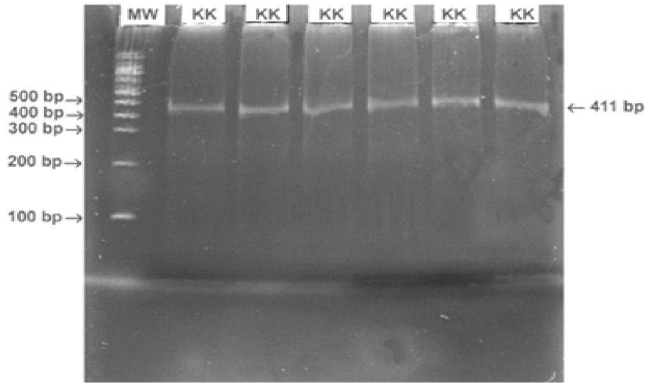

Fig.1: RFLP genotypes of *DGAT1* gene on 8% polyacrylamide gel

fixed nature of '*K*' allele was reported in 20 animals of six Indian dairy cattle breeds of Rathi, Sahiwal, Tharparkar, Deoni, Red Kandhari and Punganur by Tantia *et al*. (2006). Most of the earlier studies which established the conserve nature of *DGAT1* gene in indigenous cattle were conducted on limited number of animals. In this context, the present study is supposed to be one of the first studies conducted on large number of milking animals to establish the fix nature of *DGAT1* gene in indigenous cattle breed of Sahiwal. The fixation of allele '*K*' in Sahiwal cattle could be due to effect of genetic drift acting upon the population during evolution as well as by the founder effect. The absence of genetic diversity at GC/AA position of *DGAT1* gene most likely reflects the selective breeding being carried out in indigenous breeds for increase milk fat content (Kaupe *et al*., 2003). Likewise, in contrast to the present findings, Simmental breed was traditionally selected for '*A*' variant of *DGAT1* gene (Scotti *et al.,* 2010). Previous studies in many exotic cattle breeds throughout the world have reported differences in breed composition for '*K*' and '*A*' allele with wide interval frequency in *DGAT1* gene (Mashhadi *et al*., 2012 and Dokso *et al*., 2015). The *DGAT1* allele '*A*' and genotype '*KA*' was reported to be most frequent in crossbred population (Molee *et al*., 2015). In addition, considering the fixed nature of *DGAT1* '*K*' allele in Indian breeds, the frequency of allele '*K*' could be modulated in future selection programmes to optimize the milk yield potential of Indian dairy cows. Moreover, it has been shown that '*K*' allele is related to an increase in saturated and decrease of unsaturated fatty acids in milk which may impose a negative effect on human health (Schennink *et al.,* 2007). Thus fatty acid profile of milk could be altered through selective breeding in near future to realize the concept of designer milk (Demeter *et al*., 2009). Therefore, the present study concluded that the estimation of frequency of '*A*' allele in indigenous cattle breeds could be utilized as an indicator test for level of cross breeding in the population (Souza *et al*., 2014).

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Table 1: Primer sequences and expected fragment size of PCR product of *DGAT1* gene

Selected Region	Primer Sequences	GenBank Accession No.	Expected Fragment Length	References
DGAT1 exon-8 region	Forward (5'-GCACCATCCTCTTCCTCAAG-3') Reverse (GGAAGCGCTTTCGGATG-3')	AJ318490	411-bp	Winter *et al*. (2002)

Table 2: PCR programming for amplification of exon-8 of *DGAT1* gene

Steps	Temperature	Time	No. of Cycle
I. Initial Denaturation	95°C	5 min.	1 cycle
II. Cycle (i) Denaturation (ii) Annealing (iii) Synthesis	 95°C 52°C 72°C	 1 min. 30 sec. 1 min.	40 cycles
III. Final extension	72°C	10 min.	1 cycle
IV. Hold	4°C	5 min	1 cycle

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