Fixation of K allele in K232A polymorphism of DGAT1 gene in Sahiwal and Hariana cattle

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ABSTRACT

Diacylglycerol O-acyltransferase 1 (DGAT1) is one of the key enzymes in controlling the rate of triglyceride synthesis in adipocytes, it has been studied as a candidate for association with the milk fat content in cattle. In the present investigation, K232A polymorphism in exon 8 region of DGAT1 gene has been studied using *Eae*I/PCR-RFLP assay in Sahiwal and Hariana cattle breeds. The restriction digestion of the 491 bp PCR product showed the presence of KK genotype with a genotypic frequency of 1.0. The KA and AA genotypes were not observed in the screened samples. The allelic frequency of DGAT1 K allele was calculated as 1.0 and that of DGAT1 A allele was zero. The present study revealed monomorphic nature of DGAT1 gene in the screened samples of Sahiwal and Hariana breed.

Key words: DGAT1 gene, polymorphism, Hariana, Sahiwal, EaeI/PCR-RFLP

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INTRODUCTION

Diacylgycerol O-acyltransferase 1 (DGAT1) plays a central role in formation of lipid in different tissues of biological body and metabolism of cellular glycerolipids. It catalyzes the final step in triacylglycerol (TAG) biosynthesis by converting diacylgycerol (DAG) and fatty acyl-coenzyme A (CoA) into triacylglycero1 (Wang et al. 2007). The DGAT1 gene is characterized in cattle, buffalo, pig, monkey, human, mice and rat. The DGAT1 gene is 1470 bp in size encoding for 489 amino acid precursors, comprising of 17 exons with a molecular mass of ~55 KDa. Grisart et al. (2002), Winter et al. (2002) and Weller et al. (2003) identified a polymorphism in exon 8 of the DGAT1 gene in Bos taurus, AA → GC exchange resulting in a non-conservative substitution of amino acid 232 Lysine (K) → Alanine (A). This polymorphism has been associated with increased fat yield, fat and protein percentage as well as decrease in milk production and protein content. Therefore, DGAT1 is considered a strong candidate gene for improving milk fat contents and its composition.

Several polymorphism studies of DGAT1 gene and its association with milk yield and composition have been observed in the exotic cattle (Bos taurus) including Holstein-Friesian, Fleckvieh Jersey and German cattle breeds (Grisart et al. 2001; Spelman et al. 2002; Winter et al. 2002; Thaller et al. 2003 and Tabaran et al. 2015). Considering limited study in Indian cattle (Bos indicus) breeds (Tantia et al. 2006; Ganguly et al. 2013), the present study was undertaken to investigate the status of K232A polymorphism and allele frequency of DGAT1 gene in Indian Sahiwal and Hariana cattle.

MATERIALS AND METHODS

Location and animal source

The study was undertaken at Instructional Livestock Farm Complex (ILFC) and Department of Animal Genetics and Breeding, DUVASU, Mathura (UP). A total of 100 animals of Sahiwal (n = 50) and Hariana (n = 50) cattle were used for the present study. The animals were randomly selected and were reared under standard management conditions.

Sampling and analytical methods

Three ml of blood was collected in EDTA containing vacutainer tubes. The genomic DNA was isolated by whole blood DNA extraction kit (Genei-Merck, India). The quality of DNA was checked by using 0.7% agarose electrophoresis and the quantity of DNA was

estimated by spectrophotometer.

The PCR was performed by using Forward Primer: '5'-CAC CAT CCT CTT CCT CAA G-'3' and Reverse Primer: '5'-AAG GAA GCA AGC GGA CAG-'3' reported by Winter et al. (2002). The PCR reaction contained 25µl reaction mixture that included 10µmol of each primer, 100mM dNTP, 1X Taq buffer (with 2.0 mM MgCl2), 1.0 units Taq polymerase and 50 ng of genomic DNA as template. The PCR was performed with denaturation at 94oC for 5 minutes followed by 30 cycles each of 94oC for 30 seconds, 58oC for 30 seconds, 72oC for 40 seconds and then a final step at 72oC for 5 minutes. The products were analyzed by 1.0 % agarose gel electrophoresis.

The amplified products were digested by *Eae*I restriction enzyme for PCR-RFLP assay. 10 µl of amplified product was digested with 10 units of *Eae*I enzyme for 5 hours at 37ºC in water bath. The digested products were detected by electrophoresis in 2% agarose gel in 1X TBE buffer and eithidium bromide (10 mg/µl).

Statistical analysis

The data was generated by estimating the frequencies of different amplified products. The allelic frequency and genotypic frequencies of DGAT1 gene was estimated by standard procedure (Falconer and Mackay, 1996).

RESULTS AND DISCUSSION

The PCR of screened samples revealed 491 bp product by performing agarose gel electrophoresis. The *Eae*I/PCR-RFLP assay of the 491 bp PCR product showed the presence of KK genotype with a genotypic frequency of 100% (Figure 1). KA and AA genotype were not found in the screened samples and had zero genotypic frequency. The KK genotypic frequency were found almost similar to finding of Kaupe et al. (2004) in Nellore (99%), white Fulani (92%); Lacorte et al. (2006) in Brazilian breeds Nellore (100%), Guzerat (100%), Red Sindhi (95%), Gyr (94%) and Ganguly et al. (2013) in Sahiwal (96%) cattle breed.

In the present study, the allelic frequency of DGAT1 K allele was calculated as 1.0 and that of DGAT1 A allele was zero. The results were almost similar to the reports of Kaupe et al. (2004); Lacorte et al. (2006)

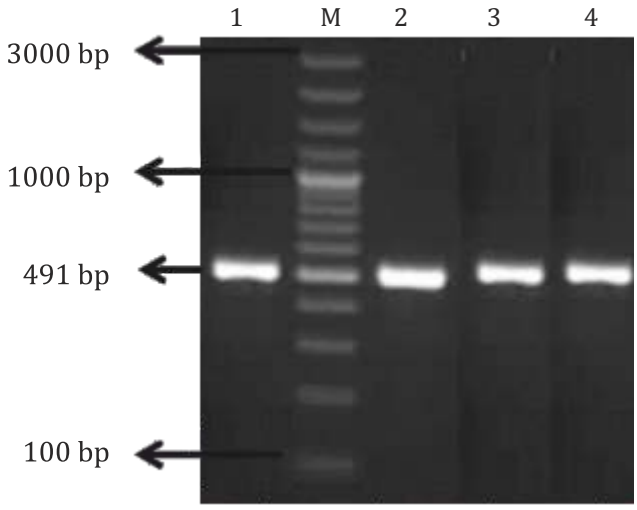

Figure 1. DGAT/*Eae*I PCR-RFLP assay showing genotype pattern in 2.0% agarose gel; Lane 1: Undigested PCR product, 2: Marker (100 bp ladder), 2, 3, 4 : KK genotype (491 bp only).

and Ganguly et al. (2013) as they obtained very high allelic frequency of DGAT1 K allele (0.97) in different cattle breeds. Tantia et al. (2006) also reported fixed DGAT1 K allele in six cattle (Bos indicus) and five buffalo (Bubalus bubalis) breeds of India. In contrast, in several studies the frequency of K allele was ranged from 0.27 to 0.65 in Holestein firisian cattle (Nowacka-Woszuk et al. 2008). There are several reports indicating that DGAT1 K allele is significantly associated with high fat yield, fat and protein percentage as well as decrease in milk production and protein content (Tabaran et al. 2015).

In the present study, we observed absence of K232A polymorphism of exon 8 region of DGAT1 gene in Hariana and Sahiwal cattle, consequently we could not establish any association between genotype and milk production trait because in these cattle DGAT1 K allele were found fixed. Further investigations in large population of these cattle may be useful for studying the status of this allele/SNP in order to exploit it for marker assisted selection for milk traits in cattle.

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