Original Research

Genetic Analysis of *DGAT1* Loci Related to Milk Production Traits in Native Sahiwal Cattle

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Abstract

A dinucleotide substitution in exon-8 of DGAT1 has been identified to be responsible for variation in milk parameters in many cattle breeds of the world. Therefore, the present study was conducted in Sahiwal cows to investigate the genetic variation in DGAT1 gene through RFLP marker and to observe their association with milk performance parameters. Genomic DNA was extracted whole blood of 70 unrelated milking Sahiwal cows through spin column method. The 411bp fragment of DGAT1 was amplified and digested overnight with Eae I restriction enzyme to explore the genetic variability in DGAT1 gene. The lactation records of selected Sahiwal cows were tested for significance of association with genetic marker information. All the analyzed samples showed only 'KK' genotype and the gene and genotype frequency was observed fixed. The study concluded that exon 8 of DGAT1 gene was monomorphic in Sahiwal cattle and no association could be established in Sahiwal cows between DGAT1 gene and milk parameters.

Key words: DGAT1 Gene, Milk, RFLP, Sahiwal Cattle

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Introduction

Increased milk production has emerged as one of the main dairy breeding goals (Meredith *et al*., 2012) throughout the world. The genetic improvement in milk performance traits of native breeds could increase their potential values and improve production levels. The effective genetic improvement requires genetic information about the genetic variability and their effects on milk production. The potential value of indigenous livestock breeds for milk production traits must be analyzed and conserved to maximize the milk production so that they can become a self-sustainable resource. Livestock selection for improved production of milk has also influenced the evolution of animal breeds (Beja-Pereira *et al*., 2003) on global basis. Sahiwal is one of the most renowned milch breeds (Tolenkhomba and Yadav, 2012) of India which

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is famous for higher milk production. Many synthetic breeds throughout the world have been developed from animals of Sahiwal breed (Rehman *et al*., 2008). The candidate gene, Diacylglycerol aminotransferase (*DGAT1*) located on the centromeric end of the bovine chromosome 14 in the QTL region (Farnir *et al*., 2002) is considered to be directly responsible for 50% variation in milk fat content and milk yield in dairy cattle (Banos *et al*., 2008) and has strong functional and positional role in milk traits (Furbass *et al*., 2006). The knock-out trial for *DGAT* gene has shown reduced or inhibited milk secretion in mouse lines (Smith *et al*., 2000). 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 and milk yield (Winter *et al*., 2002) in different bovine breeds (Sorensen *et al*., 2006). The polymorphic status of *DGAT1* gene and their association with milk yield and milk fat has been amply reported in exotic cattle, however, there have been scant studies in Indian cattle.

The identification of specific pattern of allele and genotypic frequencies in indigenous cattle breeds and their association with lactation performance may result in detection of causal factors responsible for variation in performance for milk production. 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. Therefore, the present investigation was undertaken to with an objective to estimate the gene and genotypic frequency of *DGAT1* gene in Sahiwal cattle and their association with milk performance traits.

Materials and Methods

Milking Sahiwal cows (N=75) with minimum of 120 days lactation and completed at least one parity were selected randomly from Livestock Research Station, Kodamdesar, Bikaner (Rajasthan). The biological material such as blood for analysis was collected from the same animals included in the study in accordance with the standard ethical procedures. The phenotypic information on different lactation parameters such as lactation yield, (LL), 305-day milk yield (305-DMY), daily milk yield (ADMY) and lactation length were collected from farm records and were standardized to reduce systematic errors and to make the data set uniform. Information on outliers animals and aberrant lactation was excluded from the present study. Animals with history of mastitis or dystokia were also excluded for the collection of biological material.

About 2ml of blood was taken from jugular vein in a sterile vacutainer tube containing Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant under strict aseptic conditions from all the selected animals of Sahiwal cattle. 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. The quality and quantity of extracted genomic DNA was assessed through Nano-drop

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spectrophotometer and 0.8% agarose electrophoresis. The well-established dinucleotide AA/GC substitution in exon 8 of *DGAT1* gene was selected as genetic marker for Sahiwal population study. Species specific primers were used to amplify the selected genomic regions of exon-8 of *DGAT1* gene 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.

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

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

The selected 411bp region of the cattle *DGAT1* gene was amplified by PCR in a reaction mixture containing 5X PCR buffer (5µl), 1.5mM $MgCl_2$ (3µl), 10 Mm dNTP's mix (1µl), forward primer 70pmol/µl (1µl), reverse primer 70 pmol/µl (1µl), genomic DNA 25 ng/µl (4µl), Taq DNA polymerase 5U/µl (0.2µl) and DNAase free water (10.8µl). 5% dimethyl sulphoxide (DMSO) was added to the amplification mixture for equal amplification of both alleles as suggested by Winter *et al*. (2002). The PCR programme used for the amplification of *DGAT1* gene is presented in Table 2.

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

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

The quality and size of the PCR amplicons for studied locus was assessed on 1.5% agarose gel containing ethidium bromide (1% solution) (Fig. 1). The 411-bp amplicons for the respective *DGAT1* gene were digested with 5U of *Eae* I (1µl) restriction enzyme in a 40µl reaction mixture containing 10X buffer (5µl) and nuclease free water (35µl). The reaction mixture was mixed properly through spinning and kept under water bath at 37ºC for overnight digestion. The different *DGAT1* genotypes were analyzed as per method of Winter *et al*. (2002) on 8% polyacrylamide gel. The results of electrophoretic separation were visualized and documented through GelDOC Unit after staining with ethidium bromide dye. The lactation records of

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selected Sahiwal cows were tested for significance of association with genetic marker information using least square method of SPSS *ver.* 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

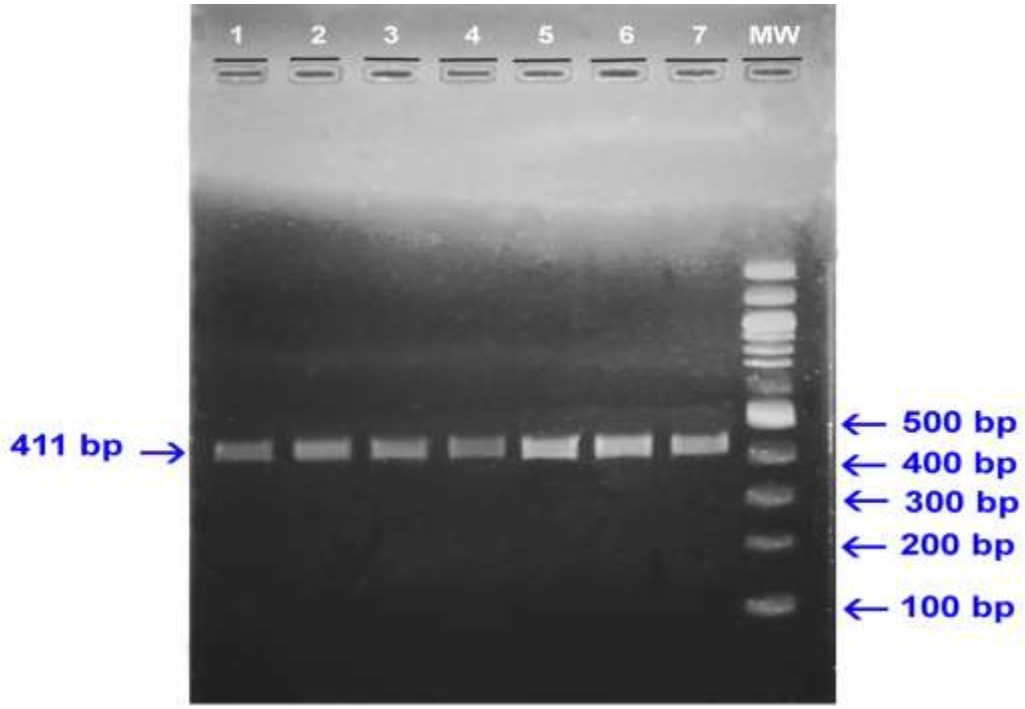

Fig.1: PCR amplicons of exon-8 of *DGAT1* gene (Lane 1- 7: PCR Amplicons of 411 bp, MW: Molecular weight marker)

Statistical analysis was carried out under single gene model to estimate the effect of genotype on the traits. The following univariate analysis of variance was carried out through general linear model (GLM) procedure to analyse the differences among genotypes:

$$X_{ij} = \mu + g_i + e_{ij}$$

Where-

X_{ij} = mean observed value of milk performance parameter; μ= general mean

gi= fixed effect of the i^{th} genotype ; e_{ij}= random error, $e_{ij} \sim NID(0, \sigma_e^2)$

Least squares means for each lactation traits and their corresponding standard errors were also computed.

Results and Discussion

The genetic variation in exon-8 region of *DGAT1* gene in Sahiwal cattle (*Bos indicus*) was reported in the present study. The RFLP analysis enables interpretation of genetic information on wider basis for the above mentioned QTL on BTA14 for GC/AA dinucleotide base pair substitution in *DGAT1* gene leading to non-conservative lysine to alanine amino acid substitution (*K232A*). The *in vitro* amplification of genomic DNA

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from all the samples revealed amplification band of 411bp of *DGAT1* exon-8 coding region using species specific oligonucleotide primers. The restriction digestion of PCR amplified products of *DGAT1* gene through *Eae* I restriction enzyme revealed the revealed the presence of only one undigested intact band of 411bp, representative of '*KK*' genotype in all the seventy five animals studied. None of the animal included in the study revealed polymorphism in the *DGAT1* gene of Sahiwal cattle. *Eae* I restriction enzyme digests the 'A' allele but not 'K' allele. The digestion of the 'A' allele produces two fragments of 208 and 203 bp. The present study provides evidence that mutation is not present at *Eae* I restricted site in *DGAT1 exon* 8 loci in Sahiwal cattle. The present study is in agreement with the findings of Kaupe *et al*. (2004) in Nellore cattle, Tantia *et al*. (2006) in Rathi, Sahiwal, Tharparkar, Deoni, Red Kandhari and Punganur cattle and Ganguly *et al.* (2013) in Sahiwal cattle for *DGAT1* locus. Most of the earlier studies which established the conserve nature of *DGAT1* gene in indigenous cattle were conducted on limited number of animals. The *DGAT1* allele '*A*' and genotype '*KA*' was reported to be most frequent in crossbred population (Molee *et al*., 2015).

The mean lactation performance of the selected milking animals for average for all parity for different lactation traits was analyzed and the breed wise results are displayed in Table 3. The average lactation yield, daily milk yield, 305 day milk yield and lactation length were observed to be 1441.80 kg, 1824.89 kg, 5.98 kg and 240 days, respectively.

Table 3: Lactation performance (LSM±SE) of studied Sahiwal animals

Total milk yield (kg)	305 day Milk Yield (kg)	Daily milk yield (kg)	Lactation length (days)
1441.80±52.68	1824.89±47.44	5.98±0.16	240±4.95

The nationwide higher number of graded animals in Sahiwal cattle population (77.62%) could be a contributing factor for low performance of Sahiwal animals (Anonymous, 2013). An association between genotype of *DGAT1* gene and milk performance could not be established for *K232A* polymorphism through RFLP analysis due to absence of alanine coding allele '*A*' in each of the studied breeds examined. The reports of Bennewitz *et al*. (2004) also indicated that *K232A* polymorphism of *DGAT1* is not sole responsible for all the genetic variation in QTL located at the centromeric end of chromosome 14 for milk related traits. The fix nature of '*K*' allele in indigenous cattle breeds of Rathi, Sahiwal and Kankrej reflects their exceptional adaptive value. The greater overall immunity of indigenous cattle against many diseases and their survival adaptation to coarse roughage with low requirement of green could be due to enhanced acyltransferase activity of *DGAT1* gene which catalyzes the synthesis of retinol esters and thus regulates the synthesis of Vitamin A or retinol. The significant and favourable association of *DGAT1* '*K*' allele with low somatic cell count in lactating cattle was reported by Manga and Riha (2011) that partially explains the

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exceptional genetic resistance of indigenous cattle against mastitis as the triglycerides are also one of the main constituent of cell membrane in immunocompetent cells (Liu *et al*., 2007).

Conclusion

The present study concluded that native Sahiwal cattle is lacks variation in exon 8 of DGAT1 gene through RFLP assay and are selected for fat enhancing lysine allele of DGAT1 gene and variation in other region of DGAT1 gene should be explored through alternate technique to observe their impact on milk performance parameters.

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References

1. Anonymous (2013). Ministry of Agriculture and Farmers Welfare, Department of Animal Husbandry, Dairying and Fisheries, Government of India. Krishi Bhawan, New Delhi.
2. Banos G, Woolliams JA, Woodward BW, Forbes AB and Coffey MP (2008). Impact of single nucleotide polymorphisms in leptin, leptin receptor, growth hormone receptor and diacylglycerol acyltransferase (*DGAT1*) gene loci on milk production, feed and body energy traits of UK dairy cows. Journal of Dairy Sciences. 91: 3190–3200. doi:10.3168/jds.2007-0930.
3. Beja-Pereira A, Luikart G, England PR, Bradley DG, Jann OC, Bertorelle G, Chamberlain AT, Nunes TP, Metodiev S, Ferrand N and Erhardt G (2003). Gene-culture coevolution between cattle milk protein genes and human lactase genes. Nature Genetics. 35: 311-313.
4. Bennewitz J, Reinsch N, Paul S, Looft C, Kaupe B, Weimann C, Erhardt G, Thaller G, Ku CH, Schwerin M, Thomsen H, Reinhardt F, Reents R and Kalm E (2004). The *DGAT1 K232A* mutation is not solely responsible for the milk production quantitative trait locus on the bovine chromosome 14. Journal of Dairy Sciences. 87: 431-442.
5. Farnir F, Grisart B, Coppieters W and Riquet J (2002). Simultaneous mining of linkage and LD to fine map QTL in outbreed half-sib pedigrees: revisiting the location of a QTL with major effect on milk production on bovine chromosome 14. Genetics. 161: 275-287.
6. Furbass R, Winter A, Fries F and Kuhn C (2006). Alleles of the bovine *DGAT1* variable number of tandem repeat associated with a milk fat QTL at chromosome 14 can stimulate gene expression. Physiological Genomics. 25: 116-120.
7. Ganguly I, Kumar S, Gaur GK, Singh U, Kumar A, Kumar S and Mann S (2013). *DGAT1* Polymorphism *K232A* in Sahiwal (Indian zebu) and Frieswal (*Holstein Friesian* X Sahiwal crossbred) cattle. Indian Journal of Animal Research. 47(4): 360-363.
8. Jalving R, Van't-Slot R and Van-Oost BA (2004). Chicken single nucleotide polymorphism identification and selection for genetic mapping. Poultry Science. 83: 1925-1931.
9. Kaupe B, Winter A, Fries R and Erhardt G (2004). *DGAT1* polymorphism in *Bos indicus* and *Bos taurus* cattle breeds. Animal Genetics. 71: 182-187.
10. Liu L, Zhang Y, Chen N, Shi X, Tsang B and YU YH (2007). Up regulation of myocellular *DGAT1* augments triglyceride synthesis in skeletal muscle and protects against fat-induced insulin resistance. Journal of Clinical Investigation. 117: 1679-1689.
11. Manga I and Riha H (2011). The *DGAT1* gene *K232A* mutation is associated with milk fat content, milk yield and milk somatic cell count in cattle. Journal of Animal Breeding. 54(3): 257-263.

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DOI 10.5455/ijlr.20170928050917

12. Meredith BK, Kearney FJ, Finlay EK, Bradley DG, Fahey AG, Berry DP and Lynn DJ (2012). Genome-wide associations for milk production and somatic cell score in Holstein-Friesian cattle in Ireland. BMC Genetics. 13: 21-32.
13. Molee A, Poompramun C and Mernkrathoke P (2015). Effect of casein genes- *beta-LGB*, *DGAT1*, *GH*, and *LHR* - on milk production and milk composition traits in crossbred Holsteins. Genetics and Molecular Research. 14(1): 2561-2571.
14. Rehman Z, Khan MS, Bhatti SA, Iqbal J and Iqbal A (2008). Factors affecting first lactation performance of Sahiwal cattle in Pakistan. Archive in Animal Breeding. 51: 305-317.
15. Sambrook J and Russell DW (2001). *Molecular cloning: A laboratory manual*. 3rd Edn. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.
16. Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, Sanan DA, Raber J, Eckel RH and Farese JRRV (2000). Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking *DGAT*. Nature Genetics. 25: 87-90.
17. Sorensen B, Kuhn C, Teuscher F, Schneider F, Weselake R and Wegner J (2006). Diacylglycerol acyltransferase (*DGAT*) activity in relation to muscle fat content and *DGAT1* genotype in two different breeds of *Bos taurus*. Journal of Animal Breeding. 49(4): 351-356.
18. Tantia MS, Vijh RK, Mishra BP, Mishra B and Kumar BST (2006). *DGAT1* and *ABCG2* polymorphism in Indian cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) breeds. BMC Veterinary Research. 2: 32.
19. Tolenkhomba TC and Yadav BR (2012). Effect of beta casein genotypes on milk production traits in Sahiwal cattle. Indian Journal of Dairy Science. 65(3): 225-228.
20. Winter A, Kramer W, Werner FA, Kollers S, Kata S, Durste-witz G, Buitkamp J, Womack JE, Thaller G and Fries R (2002). 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. Proceeding of National Academy of Sciences. USA. 99: 9300-9305.

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