VETERINARY SCIENCE RESEARCH JOURNAL
Volume 5 | Issue 1&2 | Apr. & Oct., 2014 | 13-17
DOI : 10.15740/HAS/VSRJ/5.1and2/13-17
e ISSN-2230-942X | Visit us - *www.researchjournal.co.in*

RESEARCH ARTICLE

Polymorphism at DGAT1 locus in Indian buffalo, zebu and *Bos indicus x Bos taurus* cattle breeds

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Abstract : An investigation was carried out to detect variable positions DGAT locus of Indian cattle and buffalo breeds. DNA samples were collected from 540 animals belonging to four breeds of riverine buffalo (*Bubalus bubalis*), three breeds of Indian zebu cattle (*Bos indicus*) and three synthetic cattle breeds (*Bos indicus* x *Bos taurus*). PCR-RFLP with *Cfr I* restriction enzyme was performed to detect polymorphism in the 411 bp fragment covering partial exon-7 to partial exon-9 region of the DGAT1 gene. Monomophic KK genotype was observed in buffalo and Indian zebu cattle breeds. Three genotypes, KK, KA and KA were observed in synthetic cattle breeds (*Bos indicus* x *Bos taurus*) with highest frequency of KA. The frequency of K (lysine) allele were 0.74, 0.72 and 0.69 in Frisiana, Frieswal and Sunandini cattle respectively. Sequence analysis revealed double nucleotide substitution at 202nd position of the fragment from AA to GC, which corresponds to the 14th position of the exon 8 with amino acid substitution of hydrophobic alanine (A) to positively charged lysine (K) in the peptide sequence. These results provide an opportunity to validate its association with milk yield traits in synthetic cattle breeds for utilizing in selection programmes.

Key words : DGAT1 gene, Polymorphism, Cattle, Buffalo and RFLP

How to cite this paper : Venkatachalapathy, R. Thirupathy, Sharma, Arjava and Radha, K. (2014). Polymorphism at DGAT1 locus in Indian buffalo, zebu and *Bos indicus x Bos taurus* cattle breeds. *Vet. Sci. Res. J.*, **5**(1&2) : 13-17.

Paper History : Received : 12.08.2014; Revised : 23.08.2014; Accepted : 09.09.2014

INTRODUCTION

Diacylglycerol O-acyltransferase 1 (DGAT1) belongs to a gene family of three known members, the other two represent ACAT1 (Acyl-CoA:cholesterol acyltransferase 1) and ACAT2 (Acyl-CoA:cholesterol acyltransferase 2). DGAT1 was initially mapped to human chromosome 8q and in mice on 15q by FISH analysis (Cases *et al*., 1998). Several genome scans in cattle (Coppieters *et al.,* 1998; Heyen *et al.,* 1999; Ashwell *et al.,* 2001) revealed a putative QTL on the centromeric end of bovine chromosome 14 with a strong effect on milk fat yield and percentage, as well as for milk yield and milk composition. Subsequently, the chromosome segment harboring the QTL was fine mapped to less than 9.5 cM (5 cM) flanked by the closest non-identical markers ILSTS039 and BULGE004 (Riquet *et al.,* 1999; Jeon *et al.,* 1999; Nezer *et al.*, 1999). The interval was refined to a 3 cM segment on the centromere, flanked by the markers BULGE09 and BULGE11 (Looft *et al.,* 2001; Farnir *et al.,* 2002). The comparative positional cloning led to the identification of DGAT1 as candidate gene within the region of a QTL on bovine chromosome 14 for milk fat percentage and other milk yield traits (Grisart *et al*., 2002, Girsart *et al.*, 2004, Thaller *et al.,* 2003 and Bennewitz *et al.*, 2004). Expression of mRNA and activity of DGAT1 were ubiquitous in mouse and human tissues, with the highest levels in mammary gland, liver, small intestine, and adipose tissue (Cases *et al.*, 1998; Farese *et al.*, 2000; Smith *et al.,* 2000). DGAT1 gene encodes the Acyl CoA:Diacylglycerol O- Acyltransferase (DGAT), an integral membrane bound protein in plays a fundamental role in the metabolism of cellular glycerolipids. DGAT catalyses the only committed final step in the triglyceride synthesis and presumed to be rate-limiting in lipid metabolism (Cases *et al*., 1998; 2001). It is an important microsomal enzyme in higher

eukaryotes for physiological processes involving triacylglycerol metabolism such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation and lactation. Surprisingly, DGAT1 knockout mice were not able to secrete milk, most likely because of deficient triglyceride synthesis in the mammary gland (Smith *et al.,* 2000). Thus, both functional and positional data made DGAT1, a promising candidate gene for milk production traits in bovines. Therefore, the present study was carried out to identify different allelic variants of DGAT1 gene in Indian cattle and buffalo breeds.

RESEARCH METHODOLOGY

DNA samples were collected from 540 animals belonging to four breeds of riverine buffalo (*Bubalus bubalis*), three breeds of Indian zebu cattle (*Bos indicus)* and three synthetic cattle breeds (*Bos indicus* x *Bos taurus*) from government farms situated in different geographical locations of the country. Genotyping was carried out by PCR- RFLP to find out different allelic variants in DGAT1 locus.

Collection of blood samples :

10 ml of venous blood was collected under sterile conditions, from the jugular vein of animal in a 15ml polypropylene centrifuge tube containing 0.5ml of 2.7% EDTA solution as anticoagulant. The tube was tightly capped and shaken gently to facilitate thorough mixing of blood with the anticoagulant. Blood samples were transported to the laboratory in an icebox containing ice packs and stored in the refrigerator at –20^{0}C till the isolation of DNA.

DNA preparation :

Genomic DNA was isolated from the frozen blood samples using phenol-chloroform extraction method (Sambrook and Russell, 2001) and samples were checked for its quality, purity and concentration.

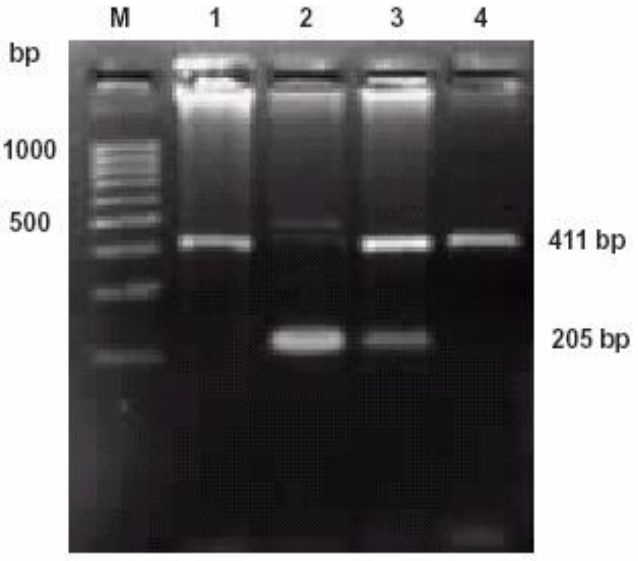
PCR :

PCR reaction was performed in a thermal cycler (PTC 200, MJ Research Ltd., San Francisco, USA). A 411 bp fragment containing partial exon-7 to partial exon-9 of the bovine DGAT1 gene that contained K232A substitution was amplified. PCR reactions were carried out in a 25 µl volume using 50ng of genomic DNA, 1x PCR buffer containing 1.5mM- $MgCl_2$, 0.2mM-dNTP, 0.5pm of each primer, 5% DMSO and 0.5 U Hotstar *Taq polymerase* (QUIAGEN, Hilden, Germany). Addition of 5% DMSO was facilitated the proper amplification of GC rich regions of DGAT1 gene. Primer sequences were: forward 5' – GCACCATCCTCTTCCTCAAG-3' and reverse 5'-GGAAGCGCTTTCGGATG-3'. PCR condition included 15 min at 95^{0}C, 35 cycles of 60 s at 94^{0}C, 60 s at 62^{0}C, 60 s at 72^{0}C and a final 10 min extension at 72^{0}C. Amplified PCR products were loaded into the wells of agarose gel with a standard 100 bp DNA ladder (GeneRuler, MBI Fermentas, Germany) as a marker to check the size of the fragment. Electrophoresis was carried out @ 6 volts/cm in 1X TBE buffer. Gels were stained with ethidium bromide and visualized under UV light. Representative samples from different genotypes were sequenced to find out the nucleotide differences between different alleles using automated sequencer (ABI prism) by Sanger's dideoxy chain termination method.

RESULTS AND DISCUSSION

PCR-RFLP typing with *cfr I* revealed three different genotypes (Fig. 1), KK (Lysine homozygote), KA (Lysine alanine heterozygote) and AA (alanine homozygote). The genotypic and allelic frequencies observed in different breeds of cattle are presented in the Table 1.

The *Cfr I* restriction enzyme digestion revealed an undigested single band with size of 411 bp in three Indian zebu cattle and buffalo breeds and denoted as $DGAT1^{KK}$ genotype (lysine) and the population was found to be monomorphic with respect to cfrI restriction site. This showed the fixation of $DGAT1^{K}$ (lysine) allele in the Indian zebu cattle buffalo population. The same trend was also observed in buffaloes in earlier studies (Winter *et al*., 2002). The present investigation further confirms the fixation of $DGAT1^{K}$ allele



Lane M : 100 bp DNA ladder
Lane 1 & 4 : 411 bp undigested PCR product
Lane 2 : AA genotype
L:ane 3 : AB genotype

Fig. 1 : Cfr I digestion pattern of 411 bp fragment of DGAT1 gene in crossbred cattle and buffalo

Table 1: Genotype and allelic frequencies of 411bp fragment of DGAT1 gene in cattle and buffalo breeds based on PCR-RFLP/ cfr I

Breed	Genotypic frequency			Allelic frequency	
	KK	KA	AA	K	A
Bos indicus x Bos Taurus					
Frisiana (42) Hariana x HF	0.43 (15)	0.54 (19)	0.03 (1)	0.74	0.26
Frieswal (40) Sahiwal x HF-	0.50 (10)	0.45 (9)	0.05 (1)	0.72	0.28
Sunandini (112) Local x Brown Swiss-	0.38 (8)	0.60 (13)	0.02	0.69	0.31
Overall (182)	0.44 (33)	0.52 (42)	0.04 (2)	0.70	0.30
Cattle (Bos indicus)					
Hariana, Sahiwal and Nimari (40 each)	1.0	0.0	0.0	1.0	0.0
Buffalo (Bubalus bubalis)					
Bhadawari (44), Mehsana (71), Murrah (57) and Surti (56)	1.0	0.0	0.0	1.0	0.0

Numbers in the parentheses denote the sample size

in buffaloes on the basis of large sample size from four different breeds. The fixed lysine allele has also been reported in Yak and many African as well as Indian Zebu breeds (Winter *et al*., 2002; Kaupe *et al*., 2004).

Lysine variant appears to be the likely ancestral form of DGAT1 gene. A constant appreciation of milk fat as source of energy in human nutrition over the years might have led to the selection for this trait and resulted in the fixation of $DGAT1^K$ allele, which proved to be efficient version of DGAT1 gene for fat synthesis (Grisart *et al*., 2002). The presence of lysine allele in buffaloes and Indian zebu cattle breeds might be one of the reasons behind the high fat content of milk.

PCR-RFLP pattern in *Bos tarus* x *Bos indicus* crossbred cattle showed three genotypes, KK, KA and AA. The frequency of heterozygote was the highest (0.52), followed by those of lysine (0.44) and alanine (0.04) homozygotes. The overall frequency of $DGAT1^K$ allele in crossbred cattle was 0.70.

The high frequencies of $DGAT1^K$ allele with 0.65 and above were also reported in several *Bos taurus* cattle breeds like Holstein (Grisart *et al*., 2002 and Spelman *et al*., 2002), Jersey and in German Angler (Spelman *et al*., 2002; Kaupe *et al*., 2004; Komisarek *et al*., 2004) as well as in *Bos indicus* (*African zebu*) breeds like Anatolian Black (Winter *et al*., 2002), Banyo Gudali and White Fulani (Kaupe *et al*., 2004). $DGAT1^K$ allele appears to be fixed in many *Bos indicus* breeds also like Hariana, Tharparkar, Sahiwal and Nellore. The $DGAT1^A$ allele was reported to be fixed in many *Bos taurus* breeds like Belgian Blue (beef), Hereford, Gelbvieh, Pinzgauer, and Slovanian Syrmian (Winter *et al.,* 2002 and Kaupe *et al.,* 2004). 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).

The $DGAT1^A$ allele in Indian crossbred cattle was probably 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 (0.70) 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). The $DGAT1^{AA}$ genotype (alanine homozygote) were noticed in two animals out of 76 crossbred screened in the present investigation, which further suggest possible introgression of $DGAT1^A$ allele from *Bos taurus* breed.

The sequencing of different alleles showed a double nucleotide substitution from GC to AA at 203rd and 204th position of 411bp fragment, which corresponds to 14th and 15th position of exon 8. This base substitution has caused amino acid change from positively charged lysine (K) to hydrophobic alanine (A) in the peptide sequence.

The alignment of derived amino acid sequences from various species showed the conserved lysine residue in the corresponding position among all examined mammals (human, mouse, rat, pig, sheep, buffalo and bison), with the exception of *Cercopithecus aethiops* (African green monkey), where it is nevertheless replaced by a positively charged arginine, demonstrating its functional importance and evolutionary conservation (Venkatachalapathy *et al*., 2008). Hence, the direct effect of substitution of lysine to alanine seems plausible on DGAT1 activity. However, $DGAT1^K$ appears to be an ancestral allele and K232A substitution might have occurred most likely after separation of the *Bos indicus* and *Bos taurus* lineages over 200000 years ago (Kuhn *et al*., 1994; Grisart *et al*., 2002). The increase in frequency of $DGAT1^A$ presumably occurred because of crossbreeding and selection for milk volume. The lysine to alanine substitution possibly also resulted to a lower energy drain on the cow leading to increased fertility (Lucy *et al*., 1992 and Kaupe *et al*, 2004).

Conclusion :

An investigation was carried out to detect variable positions DGAT locus of Indian cattle and buffalo breeds using PCR-RFLP. The $DGAT1^K$ (lysine) allele was found to be fixed in Indian zebu cattle and buffalo breeds. Three genotypes,

KK, KA and KA were observed in synthetic cattle breeds (*Bos indicus x Bos taurus*) with highest frequency of KA. The frequency of K (lysine) allele were 0.74, 0.72 and 0.69 in Frisiana, Frieswal and Sunandini cattle breeds respectively. The frequency of native K allele was predominant in synthetic breeds and results provide an opportunity to validate its association with milk yield traits in synthetic cattle breeds for utilizing in selection programmes.

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