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Study on DGAT1 Gene Polymorphism in Surti and Banni Buffaloes By PCR-RFLP

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Abstract

This investigation was undertaken with the objective to study DGAT1 gene exon 8 polymorphism using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in 53 Surti and 56 Banni buffaloes. The restriction digestion of 412 bp product with *AluI* revealed only one genotype AA in both Surti and Banni buffaloes. The frequencies of allele A were observed as 0.55 and 0.54 in Surti and Banni buffaloes, respectively on restriction digestion with *HincII*. The restriction digestion of amplified product with *HphI* revealed two fragments. The frequency of allele A were 0.71 and 0.36 in Surti and Banni buffaloes, respectively. We found that the 412 bp DGAT1 gene fragment was fairly polymorphic with *HincII* and *HphI* restriction enzymes, while monomorphic with *AluI* restriction enzyme in both buffalo populations studied.

Keywords: Banni, DGAT1, PCR-RFLP, polymorphism, Surti

Introduction

DGAT1 is a microsomal enzyme catalysing the addition of fatty acyl Co-A to 1, 2, diacylglycerol to yield Co-A plus triglycerol and it plays central role in physiological processes involving cellular glycerolipids and triacylglycerol metabolism such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation and lactation (Cases *et al.,* 1998). Additionally, there are also reports of effect of DGAT1 on fertility and spontaneous oxidize flavour (SOF) (Demeter *et al.,* 2009; Juhlin *et al.,* 2012). DGAT1 gene is localized on centromeric region of 14th bovine chromosome. A span 14117 bp consists of 17 exons (Grisart *et al*., 2004). Several workers have independently reported the association of K232A (Lysine to alanine) substitution in exon 8 of DGAT1 gene with milk production traits especially milk yield and fat % in different breeds of *Bos taurus* (Winter *et al.* 2002; Grisart *et al.,* 2004; Nowacka-Woszuk *et al*., 2008).

The PCR-RFLP is one of the most commonly used techniques for tracing the inheritance of genes and gene markers. RFLP can detect a single nucleotide polymorphism (SNP) that eliminates a restriction site in a strand of DNA (PCR fragments) on digestion with restriction enzyme. If a restriction site is present, the DNA strand is cleaved, resulting in the strand decreasing in size and thus showing up as two or more different bands on a gel. Differences in the length of the fragments generated as a result of mutation like insertions, deletions and base substitutions can be detected using PCR-RFLP (Liu and Cordes, 2004).

Buffalo is considered as a major dairy animal in South Asian countries and also contributes towards meat production as well as draught power (Tanpure *et al*., 2012). Interestingly, buffalo milk accounts for the largest share (51%) of the total milk produced in India. Buffalo play an important role in the rural economy of developing countries like India. In addition to the utility of these animals for milk and meat production, their adaptation to natural conditions and tolerance to diseases make them economically advantageous (Aytekin *et al*., 2011). Surti and Banni buffaloes are two breeds of Gujarat that are distinct and different in their origin and many other aspects so the polymorphism of DGAT1 gene in Surti and Banni buffalo was found necessary to explore. The aim of present study was to investigate distribution of the allelic and genotypic frequencies of this gene in Surti and Banni buffaloes.

Materials and Methods

Resource population: The study was conducted on 53 Surti buffaloes maintained at Livestock Research Station of the University at Navsari and 56 Banni buffaloes maintained at GLDB farm, Bhuj, Gujarat. The study was duly approved by Institutional Animal Ethics Committee of the College. About 5-7 ml of the blood was collected from each animal from the jugular vein in sterile vacutainer (Greiner, Bio-one) containing 0.5 M EDTA (pH 8.0) solution as an anti-coagulant under sterile conditions and stored in refrigerator.

DNA extraction: Genomic DNA was isolated from whole blood samples of 53 Surti and 56 Banni buffaloes according to John *et al*. (1991) with necessary modifications. Purity and concentration of genomic DNA were estimated by Nanodrop 2000_c spectrophotometric reading at OD_{260} and OD_{280}. The quality of genomic DNA was checked by running the samples in 0.8 % submarine agarose gel electrophoresis at 80 V for 60 minutes.

PCR – RFLP: From the purified genomic DNA, a 412 bp region spanning over a part of exon 2 and exon 9 of the DGAT1 gene was amplified using a set of primers (Table 1). PCR was carried out for both the fragments in a final reaction volume of 25 µl consisting 12.5 µl of 2X PCR assay buffer containing 4.0 mM $MgCl_2$, 0.05 unit/µl of Taq DNA polymerase, 200 µM dNTPs mix, 1 µl of each primer containing 10 pmol/µl, 3 µl of genomic DNA (30 ng/µl) and 7.5 µl nuclease free deionized water.

Table 1: Primer sequences used to amplify fragment of DGAT1 gene

Primer sequence	Tm (°C)	Fragment size	Reference
Forward 5'-GCACCATCCTCTTCCTCAAG-3'	60.0	412 bp	Cardoso *et al*., 2011
Reverse 5'-GGAAGCGCTTTCGGATG-3'	61.4		

The amplification of the 412 bp region was carried out using a thermal cycler, pre-programmed with initial denaturation for 10 min at 95°C followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 60°C for 45 s and extension at 72°C for 60 s; and a final extension at 72°C for 10 min. The amplified fragments were separated on a 2.0 % agarose gel at a constant voltage 80 V for 60 min in 0.5X TBE buffer and documented using gel documentation system.

The 412 bp PCR products were digested with *AluI, HincII* and *HphI* restriction enzymes (Table 2). The PCR products were incubated and inactivated at 37°C and 80°C for 25 and 20 minutes, respectively for restriction digestion with *AluI* while these samples were incubated and inactivated at 37°C and 65°C for 25 and 20 minutes, respectively for restriction digestion with *HincII* and *HphI*.

The digested PCR products were run on 2.5 % agarose gel at a constant voltage 80 V for 90 to 120 min in 0.5X TBE buffer. The restriction patterns were visualized under UV light and photographed by gel documentation system. Determination of genotype frequencies and χ^2 test were carried out using POP Gene 1.31 software.

Table 2: Restriction enzymes and their restriction sites

Restriction enzymes	Restriction site
AluI	5' A G ↓ C T 3' 3' T C ↑ G A 5'
HincII	5' G T Y ↓ R A C 3' 3' C A R ↑ Y T G 5'
HphI	5' G G T G A N8 ↓3' 3' C C A C T N7 ↑ 5'

Results and Discussion

The DNA extraction by phenol chloroform method showed a good DNA quality by spectrometry and electrophoresis of PCR products resulted in bright single compact band of 412 bp. Restriction on digestion with *AluI* patterns revealed the presence of five restriction sites on both the strands expected to results into six fragments 167, 137, 40, 30, 19 and 19 bp. Expected third to sixth fragments (40, 30, 19 and 19) could not be resolved properly using agarose gel electrophoresis due to its small bp difference and small size. The only one genotype AA was found in both the breeds of buffaloes (Plate 1) and therefore the allelic frequency of 'A' and 'B' were 1.00 and 0.00, respectively as shown in Table 3.

Table 3: Genotypic and allelic frequencies for *DGAT1* locus in Surti and Banni buffaloes with *AluI*

Buffalo	Locus	Genotype	Observed number of genotype	Genotype frequency	Allele frequency	
					A	B
Surti	DGAT1 412	AA	53	1	1.0	0.0
		AB	0	0		
		BB	0	0		
Banni	DGAT1 412	AA	56	1	1.0	0.0
		AB	0	0		
		BB	0	0		

Table 4: Genotypic and allelic frequencies for *DGAT1* locus in Surti and Banni buffaloes with *HincII* and *HphI*

Buffalo	Restriction Enzyme	Genotype	Observed number of genotype	Expected number of genotype	Genotype frequency	Allele frequency		Chi square test
						A	B	
Surti	*HincII*	AA	5	16.03	0.09	0.55	0.45	P<0.001
		AB	48	26.24	0.91			
		BB	0	10.73	0.00			
	HphI	AA	34	26.72	0.64	0.71	0.29	P<0.01
		AB	7	21.82	0.13			
		BB	12	4.46	0.23			
Banni	*HincII*	AA	4	16.33	0.07	0.54	0.46	P<0.001
		AB	52	27.82	0.93			
		BB	0	11.85	0.00			
	HphI	AA	19	7.26	0.34	0.36	0.64	P<0.001
		AB	2	25.80	0.03			
		BB	35	22.94	0.63			

The restriction digestion of the 412 bp amplicons of DGAT1 gene in Surti and Banni buffaloes with *HincII* revealed two genotypes for the locus (AA: 372 and 40 bp bands; AB: 412, 372 and 40 bp bands, Plate 2 and 3). The frequency of genotype AA and AB were observed as 0.09 and 0.91 in Surti and 0.07 and 0.93 in Banni buffaloes, respectively (Table 4). The frequency of allele A was observed as 0.55 and 0.54 and in Surti and Banni buffalo and frequency of allele B was observed as 0.45 and 0.46 in Surti and Banni buffalo, respectively.

Restriction enzyme *HphI* was used to digest the 412 bp amplicons of DNA of Surti and Banni buffaloes. PCR-RFLP results revealed two alleles (A and B) and three genotypes for the locus (AA: 139, 129, 98 and 46 bp bands; BB: 175, 139 and 98 bp bands; AB: 175, 139, 129, 98 and 46 bp bands, Plate 4 and 5). In the present study the genotypic frequencies of AA, AB and BB were observed as 0.64, 0.13, 0.23 in Surti and 0.34, 0.03, 0.63 in Banni buffaloes, respectively (Table 4). The frequency of allele A and B were observed as 0.71 and 0.29 in Surti buffalo and 0.36 and 0.64 in Banni buffalo, respectively.

Ozdil and Ilhan (2012) investigated the genetic differentiation of three indigenous Anatolian buffalo populations in the 8th exon of DGAT1 gene. *AluI, HincII* and *HphI* restriction enzymes out of 14 enzymes were found to be polymorphic. In the present study our findings conflict with their findings as we found monomorphic pattern with *AluI*. Similar finding of polymorphism of DGAT1 exon 8 was recorded in the present study with *HincII* and *HphI* restriction enzymes. The monomorphic pattern found on restriction digestion with *Alu*I is in agreement with the findings of Tantia *et al*. (2006) who reported

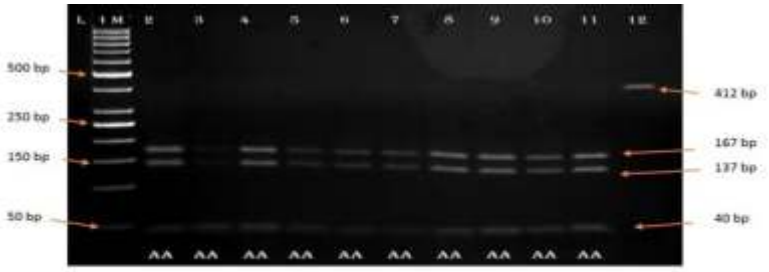

Plate 1. RE (*AluI*) digestion of DGAT1 PCR products of Surti and Banni buffaloes.
Lane: L 1 - 50 bp ladder, 2 to 6 – RFLP products of Surti buffaloes, 7 to 11 – RFLP products of Banni buffaloes, 12 – undigested PCR product.

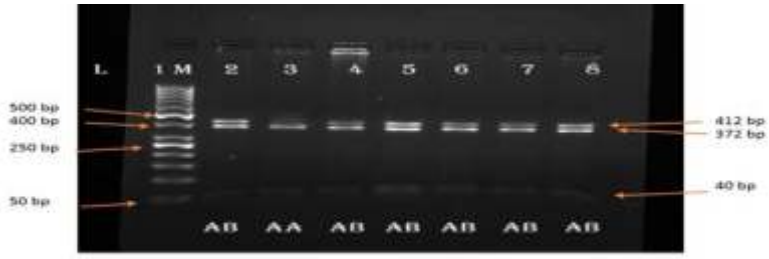

Plate 2. RE (*HincII*) digestion of *DGAT1* PCR products of Surti buffaloes
Lane: L 1 - 50 bp ladder, 2 to 8 – RFLP product of Surti buffaloes

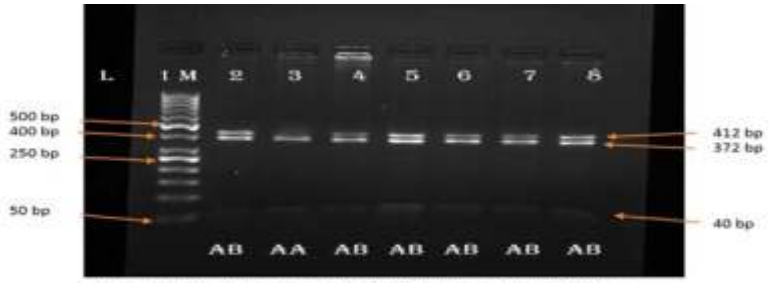

Plate 3. RE (*HincII*) digestion of *DGAT1* PCR products of Banni buffaloes.
Lane: L 1 - 50 bp ladder, 2 to 8 – RFLP product of Banni buffaloes

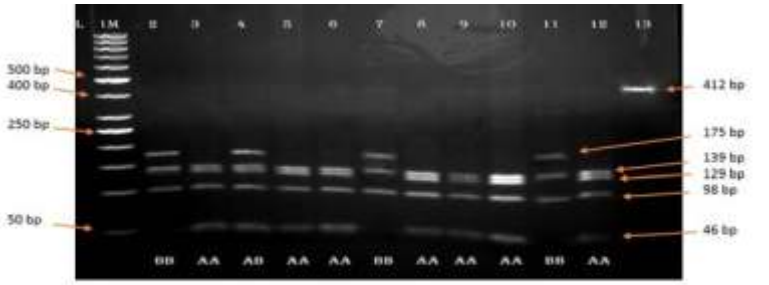

Plate 4. RE (*HphI*) digestion of *DGAT1* PCR products of Surti buffaloes.
Lane: L 1 - 50 bp ladder, 2 to 12 – RFLP products of Surti buffaloes, 13 – Undigested PCR product

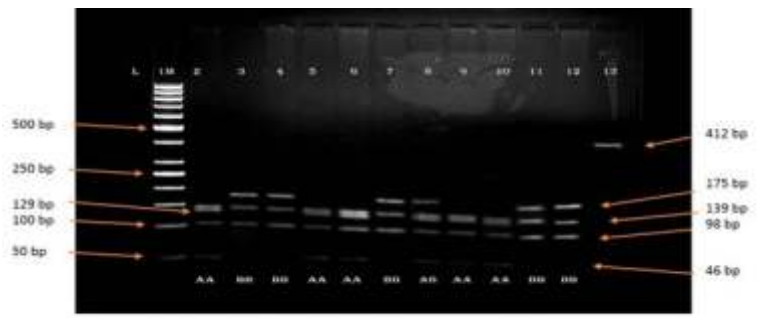

Plate 5. RE (*HphI*) digestion of *DGAT1* PCR products of Banni buffaloes.
Lane: L 1 - 50 bp ladder, 2 to 12 – RFLP products of Banni buffaloes, 13 – Undigested PCR product.

fixed DGAT1 K allele in six breeds of cattle (*Bos indicus,* viz. Sahiwal, Rathi, Deoni, Tharparkar, Red Kandhari and Punganur) and five buffalo breeds (*Bubalus bubalis,* viz. Murrah, Jaffarabadi, Surti, Mehsana and Bhadawari) of India with *CfrI* restriction enzyme, while it conflicts with our findings with *HincII* and *HphI* restriction enzymes. Our findings with *HincII* and *HphI* are similar and with *AluI* restriction enzymes conflicts with Raut *et al*. (2012) who examined polymorphism in the region spanning exon 7 to exon 9 of the DGAT1 gene in Murrah and Pandharpuri buffaloes. Three alleles (A, B and C) and four novel SNPs were identified in the buffalo DGAT1 gene. Heydarian *et al*. (2014) analyzed buffaloes from 5 provinces of Iran. Three PCR-SSCP patterns were found. Their results were similar to our findings with *HincII* and *HphI,* but in contrast with *AluI* restriction enzyme. Venkatachalapathy *et al.* (2014) observed monomorphic KK genotype in buffalo and Indian zebu cattle breeds with *CfrI* restriction enzyme that is similar to our findings with *AluI*. Komisarek *et al*. (2004), Pareek *et al.* (2005), Manga and Riha (2011), Leskova *et al*. (2013), Dokso *et al.* (2015) and Tabaran *et al.* (2015) found polymorphism in several exotic breeds of cattle using *CfrI* restriction enzyme which concur to our findings with *HincII* and *HphI* restriction enzymes.

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Conflict of Interest: All authors declare no conflict of interest.

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