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GENETIC PROFILING OF K232A MUTATION OF DGAT1 GENE IN MURRAH BUFFALO

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ABSTRACT

The aim of this study was to characterize and validate the candidate K232A in in Murrah buffalo's Diacylglycerol O-acyltransferase1 (DGAT1) gene. The DGAT1 gene has been identified as a possible candidate gene for milk fat. EaeI restriction enzyme was used to digest a 413 bp PCR product that covered the DGAT1 gene's exon 7, intron 8, exon 9, and partial exon 9 regions. This allowed us to screen for the reported mutation. Within our resource population, a monomorphic banding pattern with genotype KK was discovered. Additionally, sequencing was done to evaluate and validate the screening mutation in a specific region's nucleotide sequence. The outcome suggests that the Murrah buffalo has a substantially conserved sequence.

Keywords: *Bubalus bubalis*, buffaloes, DGAT1, mutation, candidate gene, Murrah

INTRODUCTION

Murrah is one of the best water buffalo breeds and the most widely distributed breed in the world, found in Bulgaria, South America, and all over Asia (Borghese, 2005). It is also known as Black Gold. Buffalo milks comparatively having higher fat percentage than cow milk. The average fat content of buffalo milk ranges from 7% to 9% (Rosati *et al*., 2002; Tonhati *et al*., 2000). For farmers point of in developing country like India, fat % is one of the very important marketing parameters because sale of milk based on the percentage of fat in milk, therefore, higher milk fat yield fetches better economic returns (Kumar *et al*., 2015). With the highest genetic potential for producing milk fat yield, the Murrah buffalo breed is the most significant. The average milk yield per lactation is 1,500 to 2,500 kg, with an average fat percentage of 7.3 (Borghese, 2005). In comparison to Bos Taurus, the native breeds of buffalo and dairy cow yield less but are generally higher in fat. Numerous candidate genotypes and haplotypes underlying QTLs (Quantitative Trait Loci) for

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different economic traits have been investigated recently in biotechnology and related fields (Tantia *et al*., 2006). A significant association has been witnessed by many researchers worldwide across different cattle breeds between the fat percentage and the K232A mutation. Specifically, higher levels of milk fat are associated with lysine at amino acid position 232, whereas lower levels of milk fat are associated with alanine at this same position (Berry *et al*., 2010; Grisart *et al*., 2002; Grisart *et al*., 2004; Gautier *et al*., 2007; Ganguli *et al*., 2013; Heydarian *et al*., 2014; Kaupe *et al*., 2004; Kuhn *et al*., 2004; Mishra *et al*., 2007; Ripoli *et al*., 2006; Raut *et al*., 2012; Sara *et al*., 2015; Spelman *et al*., 2002; Tantia *et al*., 2006; Thaller *et al*., 2003; Winter *et al*., 2002; Weller *et al*., 2003; Yuan *et al*., 2007; Gothwal *et al*., 2023). According to recent studies, this substitution occurs frequently in different breeds of cattle, ranging from extremely low frequency to fixation in breeds of cattle related to the *Bos indicus* (Tantia *et al*., 2006; Mishra *et al*., 2007; Ganguli *et al*., 2013). In light of the significance of the K232A mutation, the current study aimed to investigate K232A polymorphism in our resource population.

MATERIALS AND METHOD

Collection of Blood and Isolation of DNA

Blood samples were taken randomly for the current investigation from fifty lactating Murrah buffaloes housed at LUVAS Farm in Hisar, Haryana, India. Phenol chloroform method was used to extract genomic DNA from blood (Sambrook and Russell, 2001). A UV spectrophotometer (Spectro star nano BMGLABTECH) was used to assess the quality and the quantity of the extracted genomic DNA.

PCR amplification

The target area (exon 7, intron 8, exon 8, intron 9, and partial exon 9) of the DGAT1 gene was amplified using the reported set of primers F: GCACCATCCTCTTCCTCAAG and R: GGAAGCGCTTTCGGATG (Ganguly *et al*., 2013). PCR amplification was performed in a total volume of 25 µl using 100 ng of DNA template, 2X PCR buffer (pH 8.5), 50 units/ml of Taq pol, 400 uM dNTPs of each type, and 3 mM $Mgcl_2$. Initial denaturation at 94°C for 5 minutes was followed by 35 cycles of 94°C for 30 seconds, annealing at 59°C for 30 seconds, initial extension at 72°C for 45 seconds, and final extension at 72°C for 10 minutes in a thermal cycler (T-100 Bio Radd). The PCR amplified product was separated on 1.5% agrose gel with 0.5 µg/ml Ethidium bromide and photographed with a Gel Doc EZ Imager gel documentation system.

PCR-RFLP to screen candidate K232A polymorphism

The genetic profiling of our resource population for reported candidate mutation i.e. K232A were genotyped by PCR RFLP. With a total mixture of 20 ul (enzyme 0.25 µl, PCR amplified product 10 µl, buffer 2 µl, water 7.75 µl), the PCR products (10 µl) were digested with 2U EaeI restriction enzymes at 37°C for 16 h. The digested PCR product was mixed with 6 X gel loading dye and loaded on the 2.5% agarose gel along with 50 bp ladder (fermantas). The PCR amplified products were sent to Animal Biotechnology Department, LUVAS, Hisar for sequencing in both direction for characterization and genotype conformation of amplified region.

RESULT AND DISCUSSION

The targeted K232A polymorphism of the DGAT1 gene in Murrah buffalo was genotyped using PCR, RFLP, and DNA sequencing methods. The PCR products were visualised using a gel documentation system after being electrophoresed in 1% agarose gel at 70 V for 45 minutes with a molecular marker (50 bp). Figure 1 displays the amplified 413 bp PCR product. The The 413 bp amplified PCR product targets the K232A polymorphism found at the g. 6962 to 6963 loci in Buffalo (Yuan *et al*., 2007; Tantia *et al*., 2006; Mishra *et al*., 2007; Raut *et al*., 2012). According to Heydarian *et al*. (2014), the buffalo DGAT1 gene codes for 489 amino acids, which is similar to sequences above the K232A position and encodes "AA," which in cattle and buffalo leads to the K allele or lysine. Venkatachalapathy *et al*. (2014) reported similar results, focusing on this polymorphism in an amplified PCR product of 411 bp in several breeds of cattle (Hariana, Sahiwal, Nimari, and indigenous crossbred cattle) and buffalo (Bhadawari, Mehsana, Murrah, Surti). The target region in Jersey, Iraninan Buffalo, and Sudanese dairy cattle was also described by Komisarek *et al*. (2004); Heydarian *et al*. (2014); Sara *et al*. (2015), respectively. Many researchers amplified the K232A mutation in exon 8 of the DGAT 1 gene in various breeds of cattle and buffalo globally (Komisarek *et al*., 2004; Pareek *et al*., 2005; Strzalkowska *et al*., 2005; Heydarian *et al*., 2014; Sara *et al*., 2015) as this is one of the candidate gene significantly associated with milk traits.

K232A polymorphism and genotyping

After being digested by the restriction enzyme EaeI, the PCR product of 413 bp targeting the K232A polymorphism of the DGAT1 gene exhibited monomorphic pattern only KK genotype (Figure 2) in our population under study (Table 1), suggesting that only allele K is fixed at specific loci in Murrah buffalo. These mutations were validated by the use of sequencing technology. The chromatograph that displays the KK genotype is shown in Figure 3. This result is approximately in accordance with research conducted in several indigenous cattle (Sahiwal, Rathi, Deoni, Tharparkar, Red Karndhari, Panganur) and buffalo (Murrah, Jaffarabadi, Surti, Mehsana, and Bhadawari) breeds by Kaur (2015); Tantia *et al*. (2006); Ganguli *et al*. (2013). Similar findings were reported by Venkatachalapathy *et al*. (2014) for fixation of the K gene in indigenous Zebu (Hariana, Sahiwal, and Nimari cattle) and buffalo breeds (Bhadawari, Mehsana, Murrah, Surti). Previous research by Winter *et al*. (2002) also reported same trend in buffaloes.

Because of the agroclimatic conditions in northern India and the desire for a diet high in fat, selection for this trait may have led to the fixation of the K allele. The presence of K allele in Murrah buffalo might be one of the reasons behind the high fat content of milk.

CONCLUSION

The targeted loci of DGAT1 gene revealed highly conserved region in Murrah buffalos. All animals screened under study were found to be monomorphic revealed KK genotype indicating the fixation of K allele that might be due to natural selection on targeted loci. Since present study has conducted on relatively small sample size, further studies on large sample size needs to be warranted.

Table 1. Genotype and allele frequency of DGAT1 gene in Murrah buffalo.

Breed	Genotype frequency			Total	Allele frequency	
	KK	AK	AA		K	A
Murrah	1.00 (50)	0	0	50	1.00	-

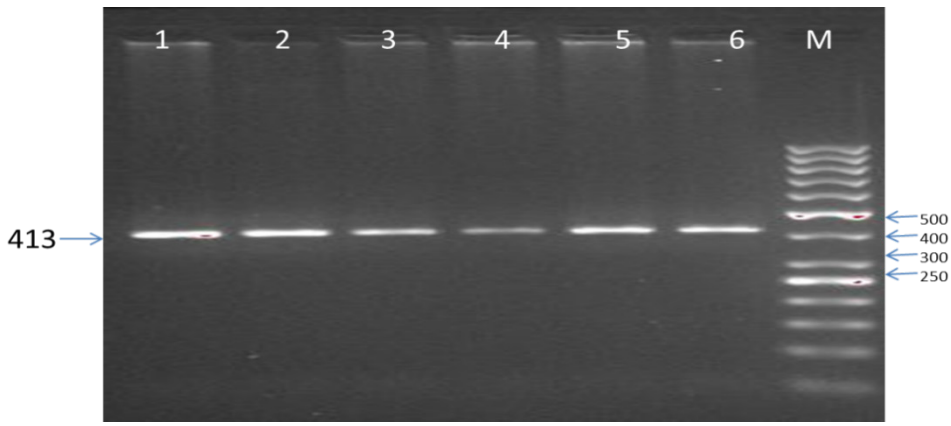


Figure 1. PCR amplified product of DGAT1 gene in Murrah buffalo.
Lane 1-6: Murrahbuffalo PCR product (413 bp). M 50 bp Marker.

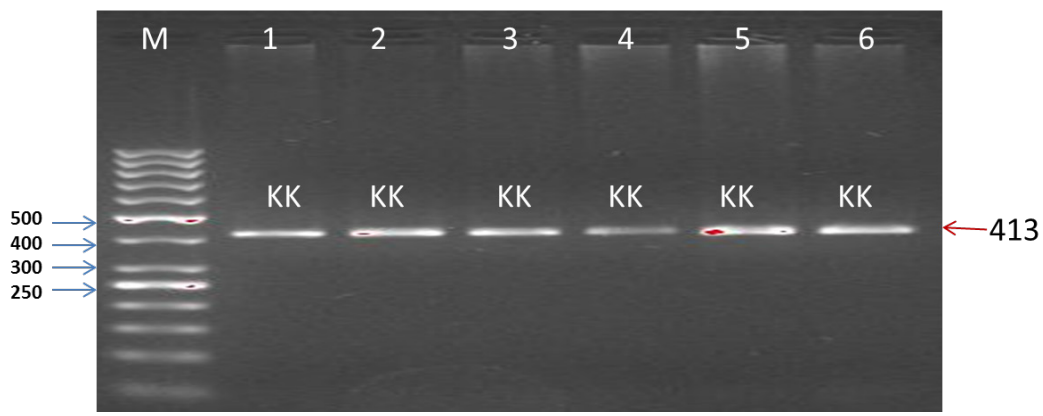
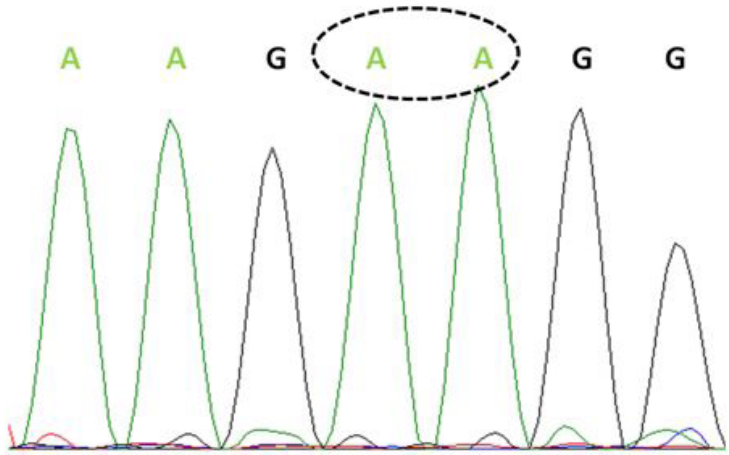


Figure 2. PCR RFLP genotyping K232A SNP using Eae I restrictionenzymein Murrah buffalo.
Lane 1-6: 413 bp KK genotype (monomorphic); M 50 bp Marker.



Nucleotide	Amino acid
AA	Lysine

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