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Original scientific paper

EVALUATION OF DGAT1-EXON 8 K232A SUBSTITUTION IN GIR AND KANKREJ (*Bos indicus*), INDIAN ORIGIN CATTLE AND ITS ASSOCIATION WITH MILK PRODUCTION TRAITS

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The aim of the present study was to screen the genotype profile of bovine DGAT1 gene among Gir and Kankrej cattle in Gujarat, India. Total number of 200 Gir cattle and 100 Kankrej cattle were evaluated for *Cfr*I-RFLP based genotyping of DGAT1 gene. We observed that only two genotypes (KK and KA) exist among the studied population with the alleles frequency of 0.925 (n=170) and 0.075 (n=30), respectively for K and A in Gir cattle as well as 0.895 (n=79) and 0.105 (n=21) respectively in Kankrej cattle. The overall calculated allele frequency for K and A was 0.915 and 0.085, respectively in both zebu (*Bos indicus*) cattle. Association of genotypes with milk production traits revealed that KA had significant ($P<0.05$) effect on total milk yield, as compared to KK in Gir and Kankrej cattle breed. Similarly, KK genotype had significant ($P<0.05$) effect on fat percentage, as compared to KA in Gir and Kankrej.

Keywords: DGAT1-exon 8, Polymorphism, *Bos indicus*, PCR-RFLP, Milk production traits

INTRODUCTION

In the last two decades due to advances in molecular biology tool particularly in gene mapping research has been open the path for the discovery of many polymorphic sites throughout the bovine genome that can serve as genetic markers for selection criteria in breeding programme. In dairy cattle, among many different candidates, diacylglycerol acyltransferase 1 (DGAT1) gene

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is considered to be a very strong positional candidate gene for fat percent of milk (GRISART *et al*., 2002). DGAT1 gene is located on the centromeric end of the bovine chromosome 14 (COPPIETERS *et al*., 1998).

DGAT1 gene encodes an integral microsomal enzyme acyl-CoA: diacylglycerol-acyltransferase which catalyses last step in triglyceride synthesis that play important role in many physiological processes involving triacylglycerol metabolism such as lactation, intestinal fat absorption, lipoprotein assembly and adipose tissue formation (CASES *et al*., 1998; LEHNER *et al*., 1996). Bovine Milk fat is mostly composed of triglycerides which accounts for 98% of the total fat by weight, so this is a key enzyme for milk triacylglycerol synthesis in the mammary gland (MARSHALL *et al*., 1977)

The polymorphic site in DGAT1 gene for *Cfr*I restriction endonuclease, present in the exon 8 and QTL (Quantitative trait loci) variation which cause the substitution of lysine with alanine and exchange at position 232 (K232A) of the predicted protein (GRISART *et al*., 2002). K allele of the DGAT1 gene increases fat yield and fat percentage while A allele of this gene increases milk and protein yields (THALLER *et al*., 2003). SMITH *et al*., 2000 found that DGAT1 knockout mice were not able to secrete milk, most likely due to impaired or deficient triglyceride synthesis in the mammary gland. DGAT1 gene polymorphism studies in *Bos indicus* and *Bos taurus* breeds showed that K allele is a wild type and the A allele is mutant, substitution probably occurred after the separation of *Bos indicus* and *Bos taurus* (KAUPE *et al*., 2004). Very limited work has been done in India on genotyping and / or its association with milk production traits in zebu cattle and buffalo (PATEL *et al*., 2009; PATEL *et al*., 2012; TANTIA *et al*., 2006; INDRAJIT *et al*., 2013). To the best of our knowledge this is a first report in Gir and Kankrej zebu cattle (*Bos indicus*) for genotyping of DGAT1 and its association with milk production trait.

Based on these considerations, present study aimed to find the allele and genotype frequency of the DGAT1 K232A and estimate the effects of the two DGAT1 variants on milk production traits in the Indian breeds Gir and Kankrej.

MATERIALS AND METHODS

Samples: The Blood samples were collected from the jugular vein of cattle into Potassium EDTA containing vacutainer (BD vacutainer) and transported to the laboratory in ice box at 4°C. They were stored at -20°C until the genomic DNA extraction. The blood samples collected from 178 Gir and 97 Kankrej (in Kankrej 28 male and 69 female) cattle. They are randomly selected from the herds, maintained at Kankrej cattle breeding farm Thara and Mandvi of Gujarat Livestock Development Board (GLDB), Livestock Research Station, College of Veterinary Science and Animal Husbandry cattle farm (AAU), Anand, private cattle farm of Anand and Kheda district of Gujarat. The Gir (22) and Kankrej (3) cattle semen straw were received from Amul Research and Development Association (ARDA), Ode farm and Sabarmati Ashram Gaushala (SAG), Bidaj farm.

DNA isolation: The genomic DNA was isolated from whole blood samples collected from 178 Gir and 97 Kankrej cattle as well as frozen semen straws of 22 Gir and 3 Kankrej. DNA was isolated by standard organic extraction method (SAMBROOK *et al*., 1989). The quality of isolated genomic DNA were checked by 0.8% agarose gel electrophoresis techniques while quantity of isolated genomic DNA were checked by Nano drop spectrophotometer.

PCR-RFLP analysis: Using polymerase chain reaction (PCR) the 411 bp fragment of exon 8 in bovine DGAT1 gene was amplified. Polymerase chain reactions (PCR) were performed

in a 25 µl volume using Fermantas PCR mix (contained 1X PCR buffer with 1.5 Mm $Mgcl_2$, 1 Unit of *Taq* DNA Polymerase, 0.4 mM dNTPs, 100 ng genomic DNA as a template, 10 pM each of sense and antisense primer (Sigma) and sterilized distilled water to make a final volume of 25 µl. Primer sequences were : F 5´- GCA CCA TCC TCT TCC TCA AG 3', R 5´- GGA AGC GCT TTC GGA TG 3' (KAUPE *et al*., 2004). The PCR reaction contains the following steps: Pre denaturation for 5 minute at 94ºC followed by 32 cycles of 94ºC for 30 seconds, 60ºC for 1 minute, 72ºC for 1 minute and final extension for 10 minutes at 72ºC. The amplified 411 bp PCR product of DGAT1 gene was separated on 1.5% agarose gel electrophoresis for 60 min and stained with ethidium bromide, visualized and photographed under UV transilluminator (Labnet). For genotyping, 10 µl of the PCR product was digested with 2 U of *Cfr*I restriction endonucleases 1X buffer and make it 25 µl with distilled water and incubated for two hours at 37°C. The digested DNA fragments were separated on 3 % agarose gel electrophoresis and viewed under UV transilluminator after staining with ethidium bromide.

Statistical analysis: Genotyping and Allele frequency of the polymorphic (K232A) DGAT1 gene was calculated by simple direct allele counting according to the Hardy-Weinberg equilibrium. Chi-square test was used to determine the possible deviations of genotype frequencies from expectation (FALCONER *et al*., 1996). The allele frequencies (1) and expected genotype frequencies (2) were calculated by using formula 1 and 2 respectively.

$p = (2KK + KA) / 2n$; $q = (2AA + KA) / 2n$ (1)

$KK = np^2$; $AA = nq^2$ and $KA = 2n \times p \times q$ (2)

Where p and q are the frequencies of K and A allele in observed population; KK, KA and AA are the number of animals with these genotypes; n is the total number of animals.

The effect of DGAT1 genotype on milk production traits in standard length of lactation and fat percentage were analysed and the significant differences were determined by one-way analysis of variance (ANOVA) using the Real Statistics Resource Pack software (Release 4.3) (ZAIONTZ 2015). The relationship between genotypes and traits was considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

In the presented study, 411 bp fragment of DGAT1 gene was amplified and digested with *Cfr*I restriction enzyme. The *Cfr*I restriction enzyme digestion products were separated by electrophoresis on 3% agarose gel. In the DGAT1 gene, point mutation from lysine to alanine (K232A), which results in two alleles K and A. The allele that has lysine at position 10433 or 10434 was denoted as the K allele. The mutation from lysine to alanine cause the formation of a restriction site for *Cfr*I restriction enzyme and this allele was denoted as A allele.

Three different genotypes were expected with the *Cfr*I digestion of DGAT1 gene. As a result of the digestion, if both of the alleles of the DGAT1 genes were no restriction sites for *Cfr*I restriction enzyme, only 411-bp long band was expected. These cattle were genotyped as KK. If just one of the gene copy had restriction site then three fragments (411 bp, 208 bp and 203 bp) were expected. These cattle were genotyped as KA. In case presence of restriction sites on both copies of DGAT1 gene, it was expected to observe only 208 bp and 203 bp bands and cattle exhibiting these two bands were genotyped as AA genotype. However, there is only 5 bp size difference between 208 bp and 203 bp, these two DNA fragments (208 bp and 203 bp) were visualized as single band on 3% agarose gel (Figure 1).

The Hardy Weinberg equilibrium (HWE) was investigated with chi-square test (FALCONER *et al*., 1996), observed and expected genotypes, P value and the allele frequencies of DGAT1 gene obtained by PCR-RFLP analyses are summarized in Table 1. The value of chi-square in this study was 2.59, lower than the critical value of 5.99, which means that the population under study is in Hardy Weinberg equilibrium, P value is 0.27 the result is not significant at ($p<0.05$). The deviation from HWE was not detected in the Gir and Kankrej breeds, for the DGAT1 gene. Two genotypes (KK and KA) and two alleles (lysine K and alanine A) were obtained. The lysine allele was found predominant in this study. The gene frequencies of K (0.915) and A (0.085) alleles, were found in the studied *Bos indicus* cattle. These showed that the frequency of the K allele in *Bos indicus* cattle population was higher than A allele. This result is similar with other *Bos indicus* cattle populations, had reported fixed DGAT1 K allele 0.99 in Nellore cattle (KAUPE *et al*., 2004), 0.960 in Sahiwal an Indian zebu (INDRAJIT *et al*., 2013), 0.850‑ 0.990 in Nellore (SOUZA *et al*., 2010). Our present studies of DGAT1 gene K allele (0.915) and A allele (0.085) frequencies of Gir and Kankrej cattle are also comparable with the frequencies of A (0.59) and K allele (0.41) in Indian Holstein cattle, this indicates that K allele are highly conserved between the zebu and Indian Holstein cattle (PATEL *et al*., 2009).

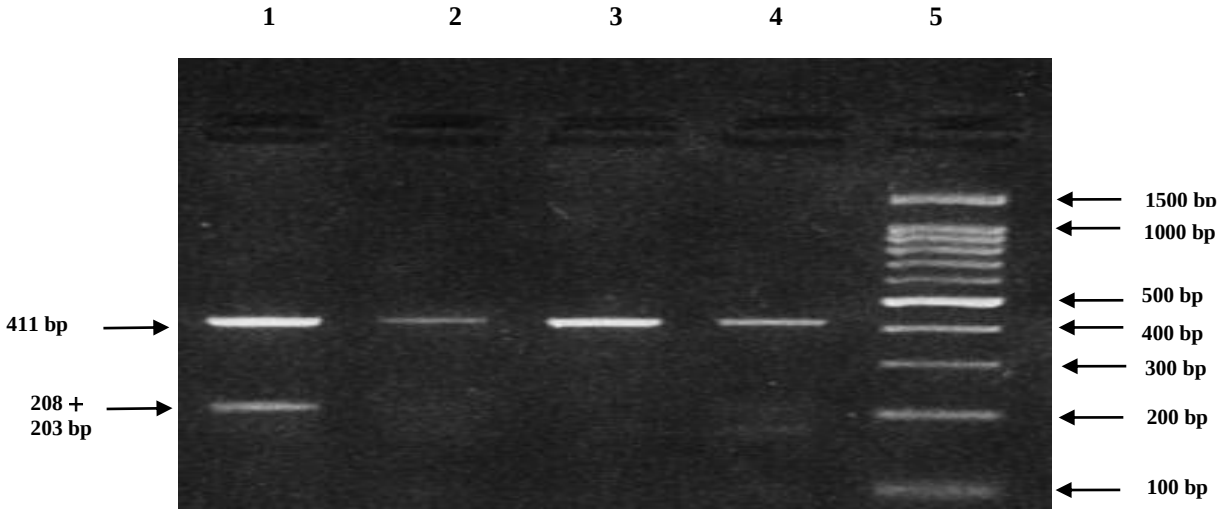

Figure 1: Representative results of PCR–RFLP analysis of DGAT1 gene on 3% agarose gel 1: KA genotype (411 bp, 208 bp and 203 bp), 2 and 3: KK genotype (411 bp), 4: Undigested PCR product (411 bp), 5: 100 bp DNA ladder.

TANTIA *et al*., 2006 found absence of DGAT1 A allele in six cattle and five buffalo breeds of India, LACORTA *et al*., 2006 also confirmed that absence of A allele in Nellore and Guzerat cattle and low frequency 0.04 in Gyr and 0.025 in Redsindhi cattle of Brazil. In the present study, similar result of very high frequency (0.915) of K allele towards fixation was observed in gir and kankrej (*Bos indicus*) breed. SZYDA and KOMISAREK (2007) analysed an effect of nine SNPs in LEP, LEPR, BTN1A1 and DGAT1 genes on milk production traits and found

that DGAT1 K232A polymorphism had a much larger positive effect on fat, protein and milk yields than the other polymorphisms studied.

Table 1. Allele frequencies and result of chi-square test for comparison of proportions between gir and kankrej cattle

			DGAT1 genotype			Alleles Frequencies		χ^2 Test	P value
Cattle	N	∑	KK	KA	KK	K	A		
Gir	200	Observed	170	30	00	0.925	0.075	1.41	0.49 ^{NS}
		Expected	171.25	27.25	1.125				(df = 2)
Kankrej	100	Observed	79	21	00	0.895	0.105	2.44	0.29 ^{NS}
		Expected	80.10	18.80	1.10				(df = 2)
Total	300	Observed	249	51	00	0.915	0.085	2.59	0.27 ^{NS}
		Expected	251.16	46.67	2.17				(df = 2)

NS - non-significant, (P>0.05), df: degree of freedom

The means values and standard deviation of milk and fat yield in standard length of lactation was showed in Table 2. Based on the statistical analysis DGAT1 polymorphism had significant effect on evaluated milk production and fat percentage. The present study showed that animals with KA genotype had a significantly (P<0.05) higher total milk yield, than those with genotype KK in both Gir and Kankrej cattle. Similarly, significant (P<0.05) differences between the DGAT1 genotypes KK and KA were obtained for fat percentage in the Gir and Kankrej cattle, KK genotype cattle produce higher fat percentage than KA genotype cattle.

Table 2. Means and standard deviations of milk production traits in Gir and Kankrej cattle of different DGAT1 genotypes

			Traits (means ±SD)	
Breed	**Genotypes**	**N**	**Mean milk yield (litres per 305 days)**	**Fat content%**
Gir	KK	170	3938.53 ± 590.46[a]	4.28 ± 0.28a
	KA	30	4443.33 ± 294.48a	4.04 ± 0.18a
Kankrej	KK	79	1830.88 ± 154.82a	4.63 ± 0.23a
	KA	21	2072.38 ± 229.36a	4.15 ± 0.16a

a : significant at p < 0.05

There are several studies which investigated the relationship between DGAT1 genotypes and milk productivity parameters in dairy cattle, among them Fleckvieh, German breed (THALLER *et al*., 2003), Dutch, New Zealand Holstein-Friesians breed (GIRISART *et al*., 2002), Polish breed (SZYDA *et al*., 2007) and found significant association between DGAT1 genotypes and milk production traits in cattle. THALLER *et al*., 2003 also found that genotype KK showed higher milk fat yield compared to other genotypes.

CONCLUSION

The most frequent genotype in Gir and Kankrej cattle population was KK. Similarly, allele K was most frequent than allele A in the present study. Based on the statistical analysis, KA genotypes may have greater influence on average milk yield (P<0.05) among Gir and Kankrej cattle. Our findings revealed that significant difference was also found among cows with different DGAT1

genotypes (KK and KA) in terms of average fat percentage ($P<0.05$). These values confirm that DGAT1 gene plays the main role in phenotypic variation of traits in question making the variant in the gene, a valuable tool for gene-assisted selection to improve the milk production traits.

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EVALUACIJA DGAT1-EXON 8 K232A SUBSTITUCIJE KOD GIR I KANKREJ (*Bos indicus*), GOVEDA IDIJSKOG POREKLA I NJIHOV UTICAJ NA PROIZVODNE OSOBINE MLEKA

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Izvod

Cilj ovog rada bio je da se uradi skrining profila bovin DGAT1 gena kod Gir i Kankrej goveda u Gujaratu, Indija. Ukupno 200 Gir i 100 Kankrej goveda je ocenjeno na osnovu *Cfr*I-RFLP kod DGAT1 gena. Zaključeno je da je samo kod dva genotipa (KK i KA) utvrđena frekvencija od 0.925 (n=170) i 0.075 (n=30), za K, odnosno A alel, kod Gir goveda, kao i 0.895 (n=79) i 0.105 (n=21) kod Kankrej goveda. Ukupna frekvencija za K, odnosno A alel bila je 0.915 i 0.085, kod *Bos indicus* goveda. Međuzavisnost svojstava ukazala je da genotip KA ima značajan efekat ($P<0.05$) na ukupan prinos mleka, u odnosu na KK kod Gir i Kankrej goveda. Slično tome, KK genotip je imao značajan ($P<0.05$) efekat na sadržaj masnoće u odnosu na KA kod Gir i Kankrej goveda.

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