DIACYLGLYCEROL-O-TRANSFERASE 1 (*DGAT1*) GENE AND MILK PRODUCTION TRAITS IN CROSSBRED CATTLE OF KERALA

Lali F. A.[1] and Anilkumar K.[2]
Centre for Advanced Studies in Animal Genetics and Breeding,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur, 680 651.

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Abstract

In the present study, the effect of di-nucleotide substitution (GC to AA) resulting in the amino acid change from lysine to alanine at 232nd position (K232A) of *DGAT1* protein was investigated in crossbred cattle (*Bos taurus* x *Bos indicus*) of Kerala. The gene (K/0.70 and A/0.30) and genotype frequencies (KK/0.52, KA/0.37 and AA/0.11) for K232A polymorphism were noted in the crossbred cattle population. Association study revealed that the KK genotyped individuals had significantly higher content of milk fat/AFP ($p \leq 0.01$), protein/MPPe ($p \leq 0.05$) and solids not fat/SNFPe ($p \leq 0.05$) than KA and AA genotyped animals. The results of the present study strongly recommend K232A polymorphism as a promising tool for future selection strategies of crossbred cattle of Kerala.

Key Words: *DGAT*1 gene, Dairy traits, Crossbred cattle, Selection

The dairy cattle genomic research is mainly aimed at identifying genes underlying the variation of milk production traits. The single nucleotide polymorphisms (SNPs) showing significant association with milk components would afford a main opportunity for Marker-Assisted Selection (MAS) programs in livestock (Khatib *et al.,* 2007) including dairy cattle. Mapping studies performed by Winter *et al*. (2002) placed *DGAT1* close to the region of a quantitative trait locus (QTL) for variation in milk fat content on bovine chromosome 14. Thaller *et al***.** (2003) explained the effects of a non-conservative di-nucleotide substitution (GC/AA) resulting in the amino acid change from lysine to alanine at 232nd position (K232A) of *DGAT1* protein. According to the genbank accession no. AY065621.1, the aforementioned polymorphism is located at 15th and 16th base pair of exon-8 of *DGAT1* gene of bovine genome. It became a functional candidate gene for lactation traits after studies confirmed the positive effect of the lysine variant on milk fat content. Grisart *et al.* (2004) constructed a high-density single nucleotide polymorphism map of the 3.8-centimorgan *BULGE30–BULGE9* interval containing the QTL and illustrated the influence of *DGAT1* gene on milk fat content. Expression study revealed that the K-allele was associated with increased production of triglycerides.

A study of single nucleotide polymorphisms and their effects on milk production are economically desirable for most selection decisions in dairy cattle breeding programs, through which rates of genetic gain could be promoted by direct selection on the alleles (Mousavizadeh *et al*., 2013). The present study was designed to find out the distribution of *DGAT/* K232A polymorphism and its effect on dairy traits in crossbred cattle of Kerala.

1. *Assistant Professor. e-mail : 1 lalimagnus@gmail.com*
2. *Professor*

Materials and methods

The present study on single nucleotide polymorphisms associated with milk production traits was conducted in a total of 144 crossbred cattle calved in the period from October 2012 to October 2013 maintained in University Livestock Farm, Mannuthy (104) and Cattle Breeding Farm, Thumburmuzhi (40).

***Dairy traits*:** Representative morning and evening milk samples (30 ml) were collected separately at the time of milking once in a month for ten months of lactation from all animals identified for the present study. The samples were kept at 4ºC till further analysis. The milk recording registers were perused to find date of birth, parity, lactation length (LL) peak milk yield (PKY) and test day milk yield of animals in the present study. The milk fat per cent was estimated for twelve samples by Gerber method as described in IS: 1224 (1977) part one and using milk analyser (MRC Scientific instruments) and proved that fat per cent was not differed significantly with the method of analysis. The fat, protein, solids not fat and lactose per cents of morning and evening milk samples were analyzed separately using milk analyzer. Milk yield for 305 days of lactation (MY305d) was calculated as total of monthly milk yield obtained by multiplying test day milk yield (morning yield + evening yield) with number of days in a month (30). The average daily milk yield (DMY) was calculated as MY305d/ LL. The test day fat yield was estimated by adding morning (morning MY/morning FP) and evening (evening MY/evening FP) fat yields. The lactation yield of fat was calculated as additive total of monthly yields (test day FY×30) of fat. The lactation yields of protein, solids not fat and lactose were also estimated by the same procedure. The per cent of each milk component was estimated by dividing the yield of each one with test day milk yield (test day fat yield/ test day milk yield) for every month. Further, for association study aggregate per cents of milk constituents in early (1^{st}, 2^{nd} & 3^{rd}), mid (4^{th}, 5^{th} & 6^{th}) and late (7^{th}, 8^{th} & 9^{th}) lactation were calculated as an average of per cents of respective months. The average per cents of fat (AFP), protein (APP), solids not fat (SNFP) and lactose (LACP) were also estimated by dividing 305 day milk yield with total yields of respective components.

***Single nucleotide polymorphism analysis*:** From each animal, 5 ml of blood was collected and DNA was isolated by phenol-chloroform extraction procedure (Sambrook and Russell, 2001). The concentration and purity of stock DNA sample were assessed by nanodrop spectrophotometry. Template DNA for PCR was prepared by diluting the DNA stock solution with TE buffer to a concentration of 50 ng/µl.

Using polymerase chain reaction 413 bp fragment of *DGAT1* gene encompassing the sequences of exon-8 was amplified using specific primers (Forward: 5′GCACCATCCTCTTCCTCAAG3′ and Reverse: 5′GGAAGCGCTTTCGGATG3′) as described by Thaller *et al*. (2003) in thermal cycler (Biorad T100™, USA) with a final concentration of 200 µ*M* dNTPs, Magnesium chloride 1.25 m*M*, primers 6 pmols each and *Taq* DNA polymerase 0.5 U in 15 µl reaction. The cycling conditions include initial denaturation 94 ºC for 4 min, denaturation 94 ºC for 45 s, primer annealing 60.0 ºC for 30 s, primer extension 72 ºC for 45 s and final extension 72 ºC for 4 min. The amplification was checked by 2% agarose gel in 1X Tris Borate EDTA (TBE) buffer and the product size was confirmed using 50 bp DNA ladder as DNA size marker. The amplicons (1.5 µl) were digested by incubating it with restriction enzyme, *Eae*I (2U) at 37 °C for 90 min. The RFLP patterns were visualized by separating the fragments in 8% PAGE followed by gel documentation. The genotypes were identified and the different genotypes were coded and recorded against phenotypic data for the traits under study. The genotypic frequencies were calculated by direct counting method (Falconer and Mackay, 1996). The distribution of single nucleotide polymorphisms in crossbred cattle was tested for Hardy-Weinberg equilibrium by *Chi* square test of significance (Snedecor and Cochran, 1994).

Association analysis : The effect of *DGAT1*/ K232A polymorphisms on milk production traits: 305 day milk yield (MY305d), peak yield (PKY), daily milk yield (DMY), fat yield (FY), protein yield (PY), SNF yield (SNFY), lactose yield (LACY) and fat (MFP), protein (MPP), SNFP and lactose (LACP) per cents for early, mid and late lactation were determined by General Linear Model- Univariate in SPSS 21.0

considering trait as the dependent variable and non- genetic factors (season and parity) and SNP as fixed factors. The main effects of fixed factors included in the model were studied along with Post-Hoc analysis. Lactation seasons were calculated by partitioning the year into three seasons: from October to January (post-monsoon), February to May (summer) and June to September (monsoon) and using calving date as a reference (Joseph, 2011). The model was Where, Y_{ijkl} – dairy trait of l^{th} cow, of i^{th} genotype j^{th} season and k^{th} parity, μ- overall mean of dairy trait, G_i- effect of genotype i (i= 1, 2 or 3), S_j – effect of season (j=1, 2 and 3), P_k – effect of parity **l** (k= 1 to 7) and e_{ijkl}- random error.

Results

The polymorphic region of exon 8 of *DGAT1* gene was amplified using custom synthesized primers to produce an amplicon of size 413 bp. Analysis of the dinucleotide polymorphism GC/AA (K232A) resulting in an amino acid change from lysine to arginine revealed all the three restriction digestion patterns (Fig. 1) in crossbred cattle population of Kerala. The sizes of alleles were 413 bp and 203 bp and 210 bp and frequencies obtained were 0.70 and 0.30 for K and A alleles, respectively. The distribution of genotypes of K232A polymorphism was according to Hardy-Weinberg equilibrium (p=0.27) and the genotype frequencies were 0.52, 0.37 and 0.11 for KK, KA, AA, respectively.

The effect of K232A polymorphism on dairy traits was investigated by GLM-univariate ANOVA (Table 1) and found that KK genotype had significantly higher ($p<0.01$) total fat per cent (4.25±0.05). The increased per cent of protein (2.93±0.02) and SNF (7.99±0.04) in milk of early lactation for KK genotype (lysine variant) was also statistically significant by Post Hoc analysis. The average values for all other traits studied were not significantly different for K232A variants.

Discussion

The frequency of K allele (lysine variant) of *DGAT1*/K232A polymorphism recorded in the present study (K/0.70, A/0.30) was in between the frequencies noted for purebred *Bos taurus* and *Bos indicus* cattle breeds in the literature. The pattern of distribution of K232A polymorphism was examined by Kaupe *et al*. (2004) in 38 different exotic and indigenous cattle breeds and reported the fixation of A allele in *Bos taurus* breeds and fixation of K allele in *Bos indicus* breeds. Moreover, the absence of A allele in Nellore and Guzerat cattle, while a very low frequency in Gir and Red Sindhi was observed by Lacorta *et al*. (2006). Contrary to the present results, Gautier *et al*. (2007) reported K allele as the minor allele in Holstein (0.37), Normande (0.13) and Montbeliarde breed (0.04).

The frequencies of *DGAT1*/K232A genotypes (KK/0.52, KA/0.37, AA/0.11) in crossbred cattle population in the present study were in H-W equilibrium. Genotype frequencies of K232A dinucleotide polymorphism in Holstein cows (KK:0.25, KA:0.45, AA:0.30) were reported by Banos *et al.* (2008) while that of Nellore (KK:0.94, KA:0.05, AA:0.01) by Souza *et al*. (2010).

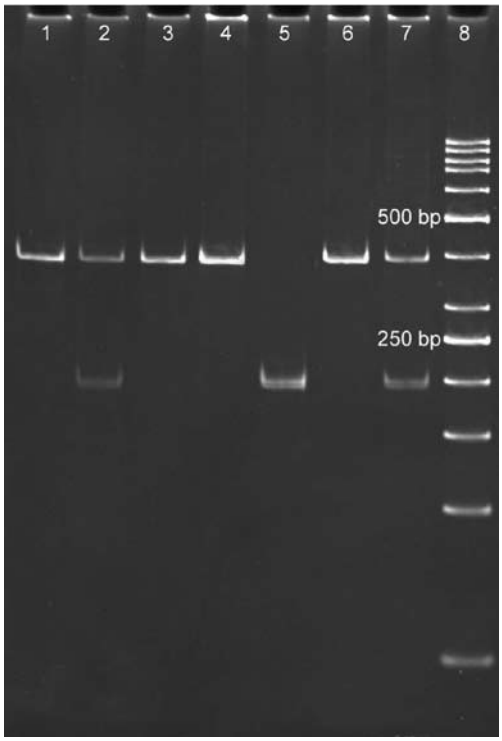

Fig. 1. RFLP pattern of *DGAT1*/K232A polymorphism on 8 % PAGE for crossbred cattle of Kerala Lane 1 - PCR product (413 bp); Lane 2, 7- Genotype KA (413, 203 and 210 bp); Lane 3, 4, 6 - Genotype KK (413 bp); Lane 5- Genotype AA (203 and 210 bp); Lane 8-50 bp DNA ladder

The association analysis confirmed the favorable effect of K allele of DGAT1/K232A polymorphism on milk composition traits in crossbred cattle of Kerala. Significant increase in the content of milk fat/AFP (p≤0.01), protein/MPPe (p≤0.05) and solids not fat/SNFPe (p≤0.05) was noticed in KK genotyped animals compared with KA and AA genotyped animals. The above results are in agreement with the findings of Berry *et al.* (2010) and Kaupe *et al.* (2007) regarding the fat and protein per cent.

Contrary to the present observation about lack of influence of *DGAT1*/K232A polymorphism on yield traits of milk production, Kaupe *et al*. (2007) and Berry *et al*. (2010) found significant reduction in lactation milk yield (-258 kg and -77 kg) and protein yield (-3.8 kg and -0.99) and increment in fat yield (+10.8 kg and 4.22 kg). Kadlecova *et al*. (2014) reported that AA homozygotes of *DGAT1*/K232A polymorphism were characterized by the highest average daily milk yield (P<0.05) that was higher by 1.29 kg than individuals with AK genotype and by 1.81 kg compared to KK homozygotes.

The present results regarding the *DGAT1*/K232A polymorphism indicated that the favourable allele (K allele) concerning to the milk component traits was retained as major one in crossbred cattle of the State, even though crossbreeding is practicing since decades with exotic breeds with low frequency of K allele. The di-nucleotide polymorphism, K232A can be recommended as a potential

Table 1. Effect of *DGAT1*/K232A polymorphism on dairy traits in crossbred cattle

Trait	Genotypes		
	KK	KA	AA
PKY(kg)	12.59±0.35	12.81±0.60	15.65±1.13
MY305d (kg)	2208.98±87.63	2188.74±127.84	2473.22±309.48
LL (days)	274.62±6.25	285.08±6.25	299.55±3.75
DMY(kg)	8.38±0.42	7.86±0.53	8.28±1.03
FY(kg)	81.82±3.64	87.90±4.83	99.69±10.67
AFP (%)**	4.25±0.05^{a}	4.01±0.07^{b}	3.96±0.11^{b}
PY(kg)	63.35±3.30	65.44±2.78	70.01±7.03
APP (%)	2.89±0.01	2.90±0.01	2.94±0.03
SNFY (kg)	172.97±9.02	178.54±7.56	191.48±19.33
SNFP (%)	7.89±0.04	7.93±0.03	8.03±0.08
LACY(kg)	94.84±4.13	98.14±10.54	105.02±3.19
LACP (%)	4.34±0.02	4.36±0.02	4.40±0.05
MFPe (%)	3.93±0.07	3.96±0.08	3.62±0.16
MFPm (%)	4.17±0.08	4.18±0.08	4.23±0.17
MFPl (%)	4.43±0.08	4.51±0.13	4.3±0.17
MPPe (%)*	2.93±0.02^{a}	2.88±0.02^{b}	2.84±0.01^{b}
MPPm (%)	2.91±0.02	2.89±0.02	2.91±0.03
MPPl (%)	2.91±0.02	2.92±0.02	2.92±0.03
SNFPe (%)*	7.99±0.04^{a}	7.86±0.04^{b}	7.75±0.04^{b}
SNFPm (%)	7.97±0.05	7.87±0.06	8.00±0.07
SNFPl (%)	7.91±0.06	7.93±0.06	8.02±0.07
LACPe (%)	4.38±0.02	4.32±0.02	4.26±0.02
LACPm (%)	4.38±0.03	4.34±0.03	4.40±0.04
LACPl (%)	4.34±0.03	4.34±0.04	4.37±0.03

Means having different superscripts differed significantly within a row
** p≤0.01, * p≤0.05

marker to improve milk fat content through marker-assisted selection (MAS) programmes in crossbred cattle population of the State.

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