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Polymorphism of diaclyglycerol acyltransferase 1 gene and its associations with milk traits in Bunaji, Rahaji and Bokoloji indigenous breeds of cattle in Nigeria

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ABSTRACT: The experiment was carried out to study the single nucleotide polymorphisms of diacylglycerol Acyltransferase 1 (DGAT1) gene and its association with milk traits among some selected indigenous breeds of cattle in Nigeria. Milk and blood samples were collected and analyzed from 60 lactating cows comprising 20 each of Rahaji, Bunaji (White Fulani) and Sokoto Gudali breeds of first parity and within their early lactation days (1-60). A reference sequel was downloaded from Ensembl while the DGAT1 sequences were cleaned and edited using Bioedit software. The DGAT1 sequences were aligned with reference sequences to identify the SNPs using clustal X hosted in Bioedit software. Genetic diversity indices were estimated using DNAsp software while allelic and genotypic frequencies were estimated by code written in R software. Discriminate principal component analysis (DAPC) was carried out in R software to classify the breeds based on the number of SNPs identified. The results of genetic diversity indices for three breeds of Nigerian Indigenous cattle revealed that Bokoloji, Rahaji and Bunaji had 4, 6 and 9 polymorphic informative sites respectively. The gene diversity showed Rahaji and Bunaji had 1.000 whereas Bokoloji had 0.900. The nucleotide diversity was low in Rahaji (0.0028) whereas high in Bunaji (0.0553) and Bokoloji (0.0167). White Fulani was found to have a higher variation of the DGAT1 gene among the breeds of cattle studied. It was recommended that further research should be carried out on gene expression analysis for milk traits to reveal other genes responsible for milk yield and composition.

Keywords: DGAT1, cattle, milk composition, gene polymorphism.

INTRODUCTION

Genetic polymorphism in a candidate gene can be tested for its association with economically important traits and can be used in marker-assisted selection under any breeding programme. The diacylglycerol acyltransferase 1 (DGAT 1) gene plays a key role in the synthesis of triacylglycerol. The K allele of the gene has a positive effect on the subcutaneous fat thickness in Nellore cattle (Curi *et al*., 2011), sirloin fat depth in Aberdeen Angus-sired beef cattle (Gill *et al*., 2009) and intramuscular fat content in Hungarian Angus bulls (Anton *et al*., 2011). In dairy sheep, the gene plays an essential role in milk fat metabolism, which is an interesting candidate gene for explaining the genetic variation in milk traits (Xu *et al*., 2008; Scata *et al*., 2009). The Diacylglycerol Acyltransferase 1 (DGAT1) gene is one of the functional candidate genes affecting milk composition traits (Kühn *et al*., 2004). The DGAT1 gene is positioned on the centromeric region of bovine chromosome 14 and spans 14,117 bp with 17 exons (Winter *et al*., 2002). The dinucleotide change (AA/GC) at positions 10433 and 10434 (rs AJ318490.1) in exon 8 leads to a non-conservative substitution of Lysine by Alanine at position

232 and has been shown to strongly affect milk yield and milk composition in Swedish Red Breed and Holstein cattle (Näslund *et al*., 2008), German Angeln dairy cattle (Sanders *et al*., 2006) and French dairy cattle (Gautier *et al*., 2007). The Lysine variant (K232) is associated with increased fat and protein contents, as well as fat yield while the Alanine variant (232A) is associated with increased milk and protein yields in cattle (Winter *et al*.,2002; Sanders *et al*.,2006; Rahmatulla *et al*., 2015). Therefore, this study aimed to evaluate the evolutionary pattern and genetic diversity of diacylglycerol acyltransferase 1 gene in bovine genome in some selected breeds of cattle in Nigeria as a baseline for improving their milk production.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in Danbatta Local Government, Kano State. Kano lies between longitude 9°30' and 12°30' North and latitude 9°30' and 8°42' East on an elevation of 468 m. It has a mean daily temperature range of 30 to 33°C and annual rainfall ranges between 787 and 960mm (KNARDA, 2001).

Experimental animals and management

A total number of sixty (60) clinically healthy lactating cows made up of twenty (20) each of Bunaji, Rahaji, and Bokoloji breeds of similar age at first parity at Audu Bako College of Agriculture farm were randomly selected and used for the experiment. The animals were managed under semi semi-extensive management system.

Blood collection

Samples of three (3 ml) of blood were collected from 60 animals (20 from each breed) in the morning (8-9 am) via the jugular vein with a 5 ml syringe gauge into a 5 ml Ethylenediaminetetraacetic acid (EDTA) vacutainer tube and stored in an ice block. Each tube was gently mixed by inversion, labelled with breed number and transferred to the laboratory for DNA extraction at -4°C (African Bioscience Laboratory (Ibadan, Nigeria).

Genomic DNA extraction

DNA extraction was carried out using ZR-96 Genomic DNA miniprep. Nanodrop 1000 spectrophotometer was used for the determination of DNA quality and quantity using a Roche DNA purification kit (Roche Diagnostics GmbH, Mannheim) according to the manufacturer's instructions (Bertani *et al*., 1999).

Polymerase chain reaction (PCR)

Cycling conditions were as follows: initial denaturation at 94°C for 4 minutes followed by 30 cycles at 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 2 minutes and a final volume contained 25 ng genomic DNA, 1 µM primers, 2 mM MgCl2, 1 U Platinum® Taq polymerase, and 1 x PCR buffer (Invitrogen Life Sciences, Dublin, Ireland). PCR products were purified and sequenced. The polymerase chain reaction was conducted in 10 µl volumes, each containing 100 ng of genomic DNA, 10 x PCR buffer (100 mMTris pH 8.9, 50 Mmkcl, 15 mMMgCL, 0.01% gelatin, 0.1% Triton X-100, 10 mg/ml BSA, 10 pmole of each primer, 40 µM of dNTPs and 0.5 unit TaqDNA poly, erase (Promega, USA).

Sequence analysis and genotyping

A reference sequence was downloaded from Ensembl while the DGAT1 sequences were cleaned and edited using Bioedit software. The DGAT1 sequences were aligned with reference sequences to identify the SNPs using the clustal X hosted in Bioedit software. Genetic diversity indices were estimated using DNAsp software while allelic and genotypic frequency were estimated by code written in R software. Discriminate principal component analysis (DAPC) was carried out in R software to classify the breeds based on the number of SNPs identified. Variant prediction effects on the SNPs were carried out using Ensembl. Preliminary analysis was carried out to correct significant phenotypic effects using the General Linear Model Procedure of SAS (2009). After adjustment of significant phenotypic effects of the DGAT1 gene, SNPs were associated with phenotypic data using one-way ANOVA of SAS (2009). Significant means were separated using the Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The results of genetic diversity indices for three breeds of Nigerian indigenous cattle are presented in Table 1. The results revealed that Bokoloji, Rahaji and Bunaji had 4, 6 and 9 polymorphic informative sites. The gene diversity showed that Rahaji and Bunaji had 1.000 whereas Bokoloji had 0.900. The nucleotide diversity was low in Rahaji (0.0028) whereas high in Bunaji (0.0553) and Bokoloji (0.0167). The average number of nucleotide differences revealed that Bunaji had the highest with 9.243 followed by Rahaji 6 and the least was Bokoloji 2. This implies that

Table 1. Genetic diversity indices for three breeds of Nigerian indigenous cattle.

Breed	N	TM	PIS	Hd	N	K
Bokoloji	5	5	4	0.900	0.0167	2
Rahaji	2	6	6	1.000	0.0028	6
Bunaji	7	20	9	1.000	0.0553	9.243
Overall	12	26	10	0.955	0.0403	14.439

N: Number of sequences, TM: Total number of mutations, PIS: Polymorphic informative sites, Hd: Haplotype (gene) diversity, N: nucleotide diversity, k: Average number of nucleotide differences.

Table 2. Average number of nucleotide differences between populations.

Breed	Bokoloji	Rahaji	Bunaji
Bokoloji	1	6.710	9.200
Rahaji	0.0034	1	6.671
Bunaji	0.0009	0.0597	1

Bunaji and Rahaji have great genetic diversity in the DGAT 1 gene, which could be explored as an insurance package against adverse environmental conditions due to diversity among environments, nutritional standards and challenges from infectious agents. These act as storehouses of genetic variation which form the basis for selection (Akinyemi and Salako, 2010), to be drawn upon in times of stressful environmental conditions such as high disease incidence (Yakubu, 2009) and erosion (Sandip, 2013). Thus, knowledge of the extent of this genetic diversity is essential for the genetic improvement of breeds and the development of appropriate breeding programs (Akinyemi, and Salako, 2010). Protein polymorphisms were the first molecular markers used in livestock (Hanotte and Jianlin, 2005). The structures of proteins serve as the carriers of essential substances within the organisms, as regulators of physiological relationships and as building block units for substances, cellular and organic structures (Das and Deb, 2008). Biochemical variants of proteins may present higher accuracy procedures for better measurement of genetic variation because of their polymorphism and simple mode of inheritance (Akinyemi and Salako, 2010). Bunaji breed has a total number of mutations of 20 while Rahaji and Bokoloji have 6 and 5, respectively. The high value of the total number of mutations by Bunaji is an indication that Bunaji cattle has the capacity to generate enough fitness that would facilitate adaptation in different management systems compared to Rahaji and Bokoloji breeds. This might be that the population of Rahaji and Bokoloji undergo bottleneck selection or are in existence as a result of resistance to disease or loss of their original purpose of existence (Dauda and Duwa, 2018).

The results of average number of nucleotide differences between populations are presented in Table 2 and Figure 1. The results show that the average number of nucleotide differences between the populations (upper diagonal) and average number of nucleotide substitution per site between breeds (lower diagonal) showing DGAT 1 gene divergence in the three breeds studied. The nucleotide diversity between Rahaji and Bunaji (0.0597) with nucleotide substitution per site (6.671) was the highest whereas, between Bunaji and Bokoloji was 0.0009 with nucleotide substitution per site of 9.200 was the lowest. The highest nucleotide diversity obtained between Rahaji and Bunaji could be due to low exchange of genetic material over time which might be as a result of distance. The lowest nucleotide diversity between Bunaji and Bokoloji cattle could be due to the exchange of genetic materials that had taken place over time between them which had reduced the nucleotide diversity that would have theoretically described their differences (Anya *et al*., 2018). This may be due to location proximity, whereby there was non-directional and uncontrolled crossbreeding among local populations. It could also be due to ethnic farming communities arising from selection induced by different ethnic cultural practices (Dauda *et al*., 2018). The degree of closeness may be characterized by a common breeding system and genetic migration between both populations. The implication of having the lowest nucleotide diversity between Bunaji and Bokoloji is that the breeds will have similar milk yield and composition traits. Thus, the average number of nucleotide differences per gene is a measure of genetic distance. It is theoretically obtained as a difference in allele frequencies for all loci in animal genome (Yunusa *et al*., 2013; Dauda *et al*., 2018). Nucleotide diversity/genetic distance is important in determining the hybrid vigour (heterosis) expected during crossbreeding (Yunusa *et al*., 2013). Since to exploit animal genetic resources, it is pertinent to have background knowledge of the amount of genetic variation that exists between and within the species (Dauda *et al*., 2018).

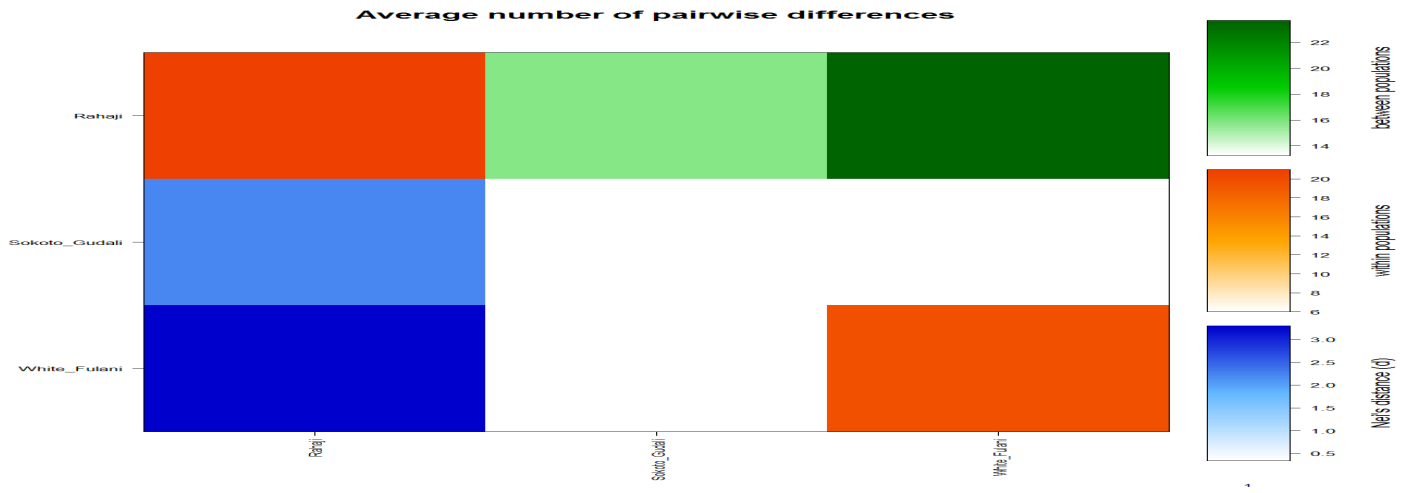

Figure 1. Average number of pairwise difference.

Table 3. Analysis of molecular analysis for three breeds of Nigerian cattle.

Level of variation	DF	Mean squares	Percentage variation
Among populations	2	1.036	13.05
Within population	9	6.900	86.95
Total	11	7.935	100

Overall F_{ST} for all the breeds: 0.1305.

The F_{st} values (0.02568 - 0.11682) observed in this research were higher compared to the report by Hoda *et al*. (2014) for the Capore breed of goat but different from -5.589 and -5.469 reported by Hoda *et al*. (2014) for Dukati and Hasi breeds of goats. The differences in F_{st} values could be due to differences in species. This implies that the measure of the difference in the allele frequency found in this study of the DGAT gene between the populations was higher. In these cases, large fragments of DNA may pass from one individual directly into the germline of another, perhaps transduced deliberately via a human transgenic manipulation (Mallet, 2001). If individual breeds are clustered into distinct populations, gene flow between populations can be described as the product of the effective population size (Ne) and the proportion of migrants per generation (m).

The average number of nucleotide substitutions per site between population (Dxy) in this study ranged from 0.03687 to 0.05978, the number of net nucleotide substitutions per site between population (Da) ranged from 0.00095 to 0.00698, number of mutational steps between haplotypes (Nst) ranged from 0.02497 to 0.12466 and average number of differences between two population (Kxy) ranged from 13.2 to 21.4. Gene flow estimates were high among all populations. This finding agrees with historical records of human immigration among countries or regions (Eaksittipong, 2017). This might be due to location proximity, whereby there was non-directional and uncontrolled crossbreeding among local populations. It could also be due to traditional farming communities arising from selection prompted by different cultural practices (Anya *et al*., 2018).

The results of molecular analysis for three breeds of Nigerian cattle are presented in Table 3. The results showed the percentage variation among the population was 13.05 whereas within the population was 86.95 with overall F_{ST} for all the breeds 0.1305. The high percentage variation within a population gives an indication of the variations present in a within population. DGAT1 gene having high variations advocates that the gene is heterogeneous in nature hence, it can be explored for genetic improvement through selection (Abbaya and Dauda, 2018). Therefore, the variations in this study that existed in the DGAT1 gene of the three breeds of cattle might be exploited for selection, improvement and conservation within the population since genetic resources depend on the knowledge of the variations of genes, which have played a very fundamental role in livestock production (Dauda, 2021). Crepaldi *et al.* (2001) reported that domestic animal diversity is critical for food security

Table 4. Single nucleotide polymorphism of DGAT 1 gene in three breeds of Nigerian cattle.

Hyplotype type	Haplotype members	Single nucleotide polymorphism	Position with respect to cattle genome	Breed containing the SNP
Hap1	R7MF_ MDGAT1	A > C	14_555681	S18MF_ MDGAT1, W8MF_ MDGAT1, W8MF_ MDGAT1, W8MF_ MDGAT1
Hap2	R10MF_ MDGAT1	A>G	14_555688	W7MF_ MDGAT1, S11MF_ MDGAT1
Hap3	S6MF_ MDGAT1	G>C	14_555693	W15MF_ MDGAT1, S9MF_ MDGAT1,
Hap4	S9MF_ MDGAT1, S11MF_ MDGAT1, W13MF_ MDGAT1	A>C	14_555698	R7MF_ MDGAT1, R10MF_ MDGAT1,
Hap5	S13MF_ MDGAT1	G>T	14_555701	S12MF_ MDGAT1, S13MF_ MDGAT1, S9MF_ MDGAT1, S11MF_ MDGAT1,
Hap6	S18MF_ MDGAT1			
Hap7	W7MF_ MDGAT1			
Hap8	W8MF_ MDGAT1			
Hap9	W14MF_ MDGAT1			
Hap10	W15MF_ MDGAT1,			

Adenine (A), guanine (G), thymine (T), or cytosine (C).

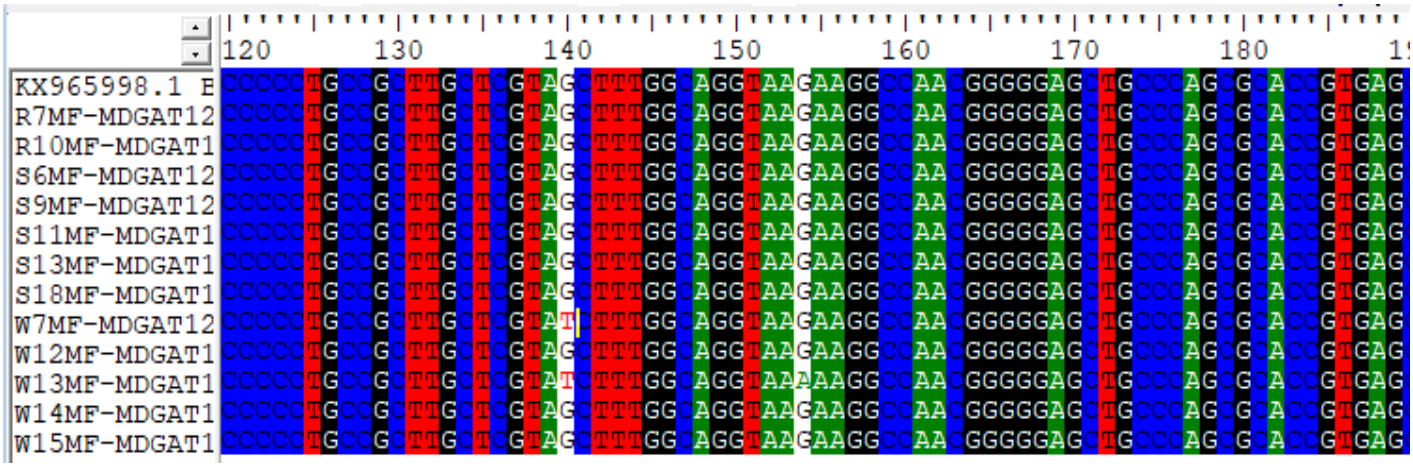

Figure 2. Positions of single nucleotide polymorphisms in DGAT1 gene of three breeds of Nigerian indigenous cattle using KX965990 sequence as reference.

and essential to meet the unpredictable future demands of population increase, climate change and more virulent disease pathogens. Thus, a genetic reservoir not only depends on the number of breeds but also on the genetic diversity within and between these breeds. The overall F_{st} for all the breeds in this study was within the range compared to F_{ST} values for mammals which generally range from 0 to 0.25. This indicates that the populations have shared genetic material through high levels of breeding.

The results of single nucleotide polymorphism (SNPs) of the DGAT 1 gene in three breeds of Nigerian cattle are presented in Table 4 and Figure 2. The results revealed ten (10) Hyplotypes in the three Nigerian cattle breeds (Rahaji, Bunaji and Bokoloji). This implies that there is a set of DGAT 1 DNA variations along a chromosome that tend to be inherited together. Since, a haplotype can refer to a combination of alleles in a single gene, or it could be alleles across multiple genes. Basically, it just means that these are variations in the DNA that are so close together that they tend not to recombine, and therefore tend to be passed down through the generations (Bailey-Wilson, 2021). The single nucleotide polymorphism and position

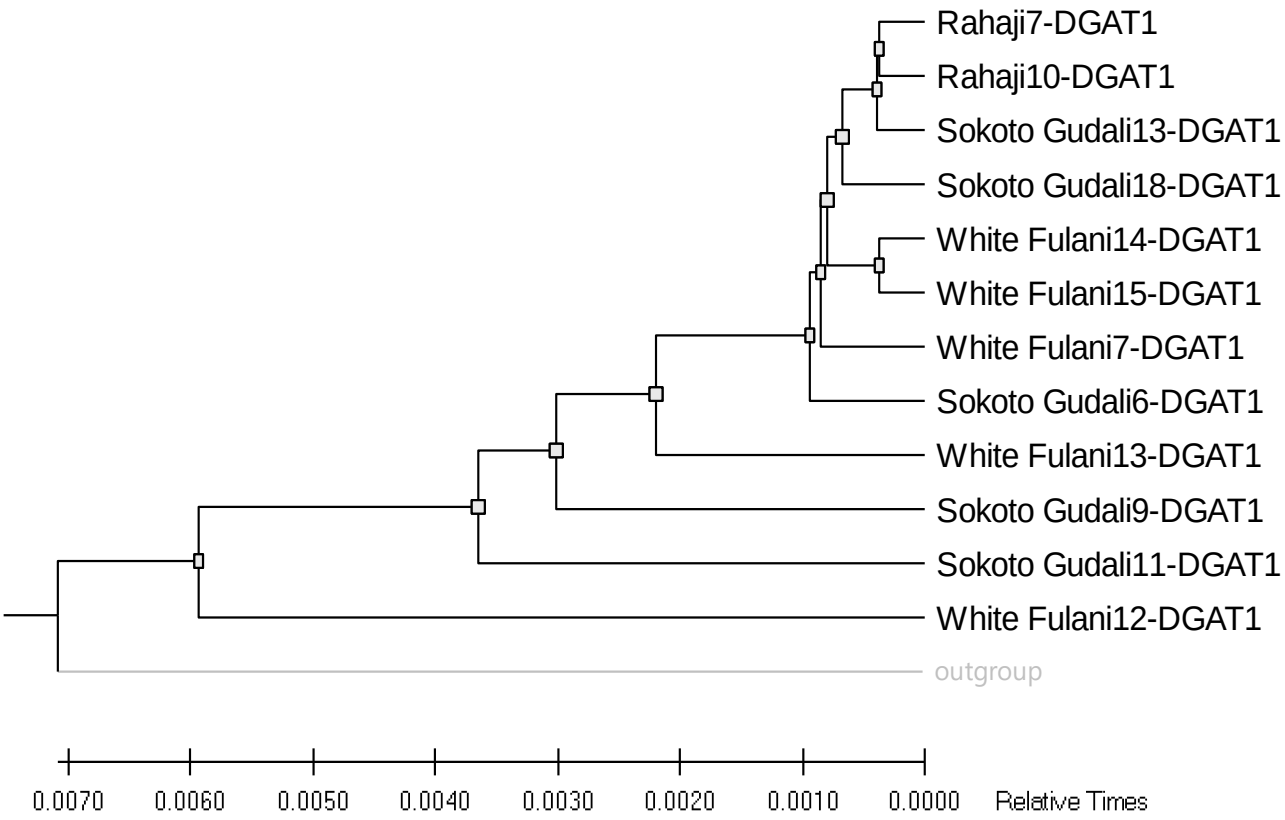

Figure 3. Evolutionary relationships of DGAT1 in three breeds of cattle.

with respect to cattle genome of hylotype 1, 2, 3, 4, and 5 are A > C, A>G, G>C, A>C, G>T and 14_555681, 14_555688, 14_555693, 14_555698, 14_555701 respectively, which in turn produced Single nucleotide polymorphism as follows S18MF_ MDGAT1, W8MF_ MDGAT1, W8MF_ MDGAT1, W8MF_ MDGAT1 (hyplotype 1), W7MF_ MDGAT1, S11MF_ MDGAT1 (hyplotype 2), W15MF_ MDGAT1, S9MF_ MDGAT1 (hyplotype 3), R7MF_ MDGAT1, R10MF_ MDGAT1 (hyplotype 4) and S12MF_ MDGAT1, S13MF_ MDGAT1, S9MF_ MDGAT1, S11MF_ MDGAT1 (hyplotype 5). The aforementioned (SNPs) observed in this study indicate the variation of a single base pair. Several genes localized within QTL are thought to influence important traits in dairy cattle. Single nucleotide polymorphisms (SNPs) are mostly used to genetically characterize such chromosomal regions.

Within and between populations, genetic variation occurs, resulting in polymorphisms that can be linked to a hereditary characteristic or a phenotype in the presence of an environmental stimulus (Hirschhorn and Daly, 2005). A single-nucleotide polymorphism (SNP) is a difference in the genetic code (i.e., polymorphic). SNPs are the most prevalent type of genetic variation and are often utilized to investigate genetic differences between and within populations. SNPs in the coding (exons), intergenic, and noncoding (introns) regions of the genome that may contribute to alterations in the genomic sequence (Dijk *et al*., 2014; Ahmad *et al.,* 2018).

Evolutionary relationships of DGAT1 in three breeds of cattle are presented in Figure 3. The evolutionary tree showed all three breeds intermingle and tend to cluster together. The genetic relationships of the cattle based on their sequences revealed by the phylogenetic tree were in accordance with the well-known evolutionary history of *Bovidae* subfamily speciation (Floudas, 2007). The implication of the similarities in the proteins is that any selection programmes designed for Milk yield and fat yield selection will be applicable for Rahaji, Bunaji and Bokoloji breeds. This is an evidence of trans-species evolution which might be attributed to the coding nature of the sequences (Dauda *et al*., 2018).

Conclusion

White Fulani was found to have a higher variation of the DGAT1 gene among the breeds of cattle studied. The evolutionary tree for the DGAT1 gene revealed that all the breeds interbred and tend to cluster together.

Recommendation

It was recommended that further research should be carried out on gene expression analysis for milk traits to reveal other genes responsible for milk yield and composition.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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