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# Nucleotide sequence variation(s) in Bovine Diacyl-Glycerol Acyl -coA Acyltransferase 1 (DGAT1) Gene Across Nigerian Cattle Breeds

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## ABSTRACT

The DGAT1 gene expresses a protein which is responsible in the production of triglycerides. Triglycerides, being the key components of fat are synthesised by the linking of diacylglycerol to long chain fatty acyl CoAs. This process is aided by a minimum of two enzymes at least. One of which, is encoded by DGAT1. In this study, the polymerase chain reaction (PCR) along with single-strand conformation polymorphism (SSCP) analysis was used to reveal genetic variations in bovine DGAT1 gene in 154 cattle, belonging to two breeds farmed in Nigeria. These included the White Fulani and Sokoto Gudali cattle breeds. Sequence analysis of the exon 8 region of bovine DGAT1 gene revealed the pK232A variation in the White Fulani cattle breed. This has been previously described in other cattle of *Bos taurus* origin in Europe. The presence of this common variant in different cattle breeds irrespective of location may indicate a relationship from an evolutionary standpoint, in the same way the differences within a breed might be explained by either selection pressure or random genetic drift. Since this variation was found to increase milk fat in cattle of European origin, it is likely that it may be controlling the same metabolic process in the White Fulani cattle breed. However, further research need to be carried out to ascertain this.

**Keywords:** Nucleotide sequence variation, DGAT1 gene, Nigerian Cattle Breeds.

## INTRODUCTION

Diacyl-glycerol acyl -coAacyl_transferase 1 (DGAT1) is one enzyme that serves as the catalyst in the last stage during the synthesis of triglyceride (Mayorek et al. 1989). The DGAT1 gene expresses an enzyme which is mainly saddled with the responsibility of synthesising triglycerides. Triglycerides, being the main constituents of fat are synthesised when diacylglycerol become bounded to long chain fatty acyl CoAs. This process is catalyzed by a minimum of two enzymes, and one of which is encoded by DGAT1 (Cases *et al.,* 2001 and Winter *et al.,* 2002). Following the observation that lactation impairment in female mice were missing the two copies of DGAT, most likely as a result of stoppage in the synthesis of triglyceride in the mammary gland, the DGAT1 gene was put forth as a credible candidate gene for milk production traits (Smith *et al.,* 2000 and Winter *et al.,* 2002).

Sequence variation in DGAT1 has been described, and a well-studied polymorphism results in the substitution of lysine (k) with alanine (A) at position 232 of the amino acid sequence (known as k232A). This was first described by Grisart et al., (2002), where it was associated with various milk traits. In New Zealand (NZ), Spelman et al., (2002) revealed that the mean variant substitution effects where 2-3 kg of protein and 120 -130 L of milk for both the jersey and Holstein Friesian breeds, with a substitution effect of 6kg of milk fat for Holstein -Friesians and 3kg for jersey cows. However, none of this study was carried out on the indigenous cattle breeds of Nigeria.

The Nigerian cattle breeds are of *Bos indicus* origin, belonging to the cattle group called

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Zebu, which majorly consists of the dual purpose White Fulani (WF), Red Bororo (RB) and Sokoto Gudali (SG) breeds predominantly kept for milk and meat purposes. These cattle breeds are known to possess a thoracic hump that is fatty on their shoulders, and have a large dewlap and are acclimatized to tropical environments (Mattioli *et al.,* 2000).The White Fulani cattle, otherwise called the Bunaji breed is a vital component of Nigeria's livestock industry. They are widely distributed across the country, playing a significant role in milk and meat production. The breed's heat tolerance and adaptability to tropical environments make it an essential asset for smallholder farmers (Mattioli *et al.,* 2000). The SG breed has several coat colors, but the most commonly seen is the black and white colors with light underside. They have deeper body than the white Fulani breed and they share resemblance with the east African Boran and the Sudanese Kenana (Mattioli *et al.,* 2000).

The big question is, does genetic variation in the DGAT1 gene exists in indigenous Nigerian cattle breed? If yes, how similar or different is/are these variation(s) to the previously reported variations in cattle of *Bos taurus* origin in Europe? Previous study on the Bovine DGAT1 gene have focused mainly on its functional characterisation in European cattle of *Bos taurus* origin, but variations in the nucleotide sequence of this gene in indigenous Nigerian cattle breeds such as the white Fulani, Sokoto Gudali breeds have remained unstudied, Hence the need for the study which aims to identify the variation in the DGAT1 gene across the Nigerian White Fulani (Bunaji) and Sokoto Gudali cattle breeds.

## MATERIAL AND METHODS

### Ethical Considerations

All procedures involving animals were considered and careful handling according to the approved ethical guideline by the Institutional Animal Care and Use Committee (IACUC). The consent of local cattle owners was also sought-after prior to sample collection.

### Cattle breeds investigated

A total of 154 cattle were investigated in this study (94 White Fulani and 60 Sokoto Gudali). These samples were collected across different farms in Gombe and Kaduna states of Nigeria to ensure genetic diversity within and across the breeds.

### Blood Sample Collection

Based on the conventional method of piercing the ear or the tail of the animal, samples of whole blood were collected from each cow onto FTA$^{TM}$ cards (Whatman$^{TM}$, Middlesex, UK). The samples were allowed to dry, and DNA was extracted and purified based on the two-step methodology as described by Zhou *et al.* (2006).

### Amplification via Polymerase Chain Reaction (PCR)

Using the following forward and reverse primers (5'- CCACTGGGCTGCCACTTG-3' and 5'- GAAGCAAGCGGACAGTGAG-3' respectively) adapted from the work of Li *et al.* (2019), the exon 8 of bovine DGAT1 gene was amplified. The PCR reaction was performed in 15-µL reactions containing the genomic DNA on a 1.2-mm diameter disc of FTA$^{TM}$ card, 0.25 µM for each primer, 150 µM for each dNTP (Eppendorf, Hamburg, Germany), 3.0 mM $Mg^{2+}$, 0.5 U of *Taq* DNA polymerase (Qiagen, Hilden, Germany), and 1 × the reaction buffer supplied with the enzyme.

The PCR amplification was done in a Bio-Rad S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA). The thermal profiles included an initial denaturation at 94 $^{o}$C for 2 minutes, followed by 35 cycles of 94 $^{o}$C for 30 seconds, annealing for 30 seconds at 60 $^{o}$C, extension at 72 $^{o}$C for 30 seconds and a final extension step at 72 $^{o}$C for 5 minutes.

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### Agarose gel electrophoresis

The PCR products were visualized on a 1.5% agarose gel to check the integrity of the PCR reaction.

### Restriction enzyme digestion

The enzyme MspA1 was used to digest the samples at the specific restriction sites prior to being visualised using a 1.5% agarose gel electrophoresis.

### Genotyping and sequencing

Taking into account the PCR-SSCP patterns detected, those cattle samples that were homozygous with uncommon banding patterns were sequenced directly. For the heterozygotes, the unique band(s) was excised from the wet gel, incubated in water at 69 ºC for 1 hour and subsequently sequenced based on the method described by Gong *et al.* (2011).

### Bioinformatics analysis

The generated sequences were analysed using multiple sequence alignment, and other analyses undertaken using DNAMAN (Version 5.2.10, Lynnon BioSoft, Vaudreuil, Canada). Sequence data was aligned and compared to the reference sequence of the DQA1 gene. Nucleotide variations, including SNPs, were identified and annotated. Statistical analysis was carried out to figure out the frequency and distribution of these variations within the White Fulani and Sokoto Gudali Cattle breeds.

## RESULTS

### Sequence variation

The amplified region of exon 8 was visualized on a 1.5% agarose gel as depicted in figure 1 (A). Although no band was observed in the Sokoto Gudali sample (Figure 1 B), a 450bp fragment was detected in the White Fulani samples investigated.

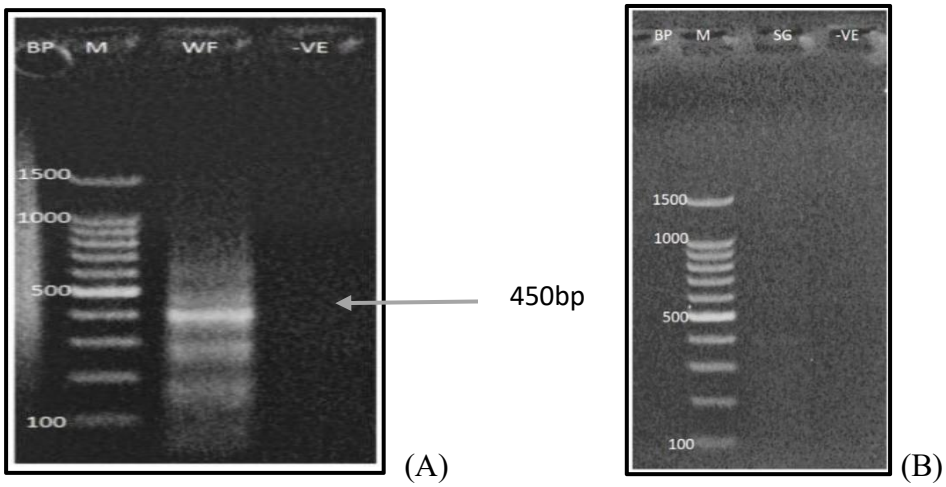


**Figure 1: (A)** Agarose gel electrophoresis results showing a 450bp band observed in White Fulani (WF) cattle, and **(B)** no band was observed in the Sokoto Gudali (SG) cattle breed investigated.

In figure 2, the two restricted fragments were observed, following the restriction of the samples at specific sites using the enzyme MspA1.

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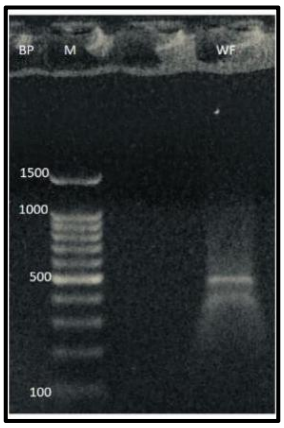


**Figure 2:** Shows the two fragments observed following the Restricted Fragment analysis results of the DGAT1 gene in the White Fulani (WF) cattle breed.

In the analysed samples, the genotypes of DGAT1 responsible for producing *AA*, *AB*, and *BB* at p.K232A were identified (Figure 3), with varying frequencies of 12.9%, 47.3% and 39.8% respectively. The variant with the highest frequency was A (59.9%), followed by B with 40.1%. The *p*-value (0.724) of chi-square in the Hardy Weinberg Equilibrium suggests that the population was at equilibrium.

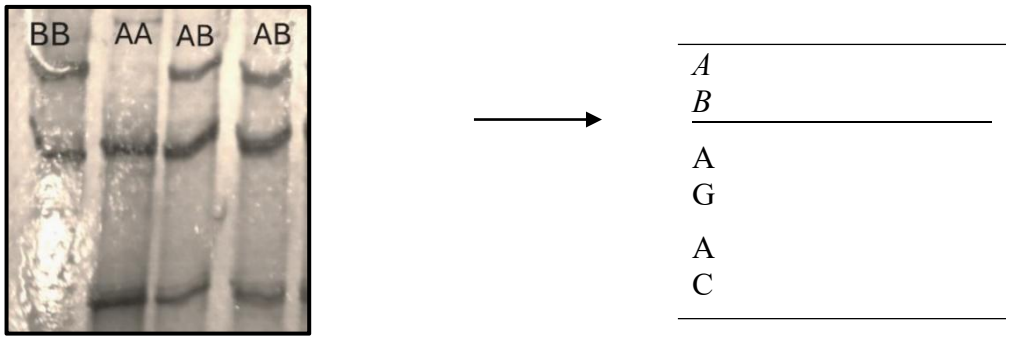


**Figure 3:** Single Strand Conformation analysis result showing the genotypes (*AA, BB & AB*) identified in the region of DGAT1 identified in White Fulani and Sokoto Gudali cattle breeds investigate**.**

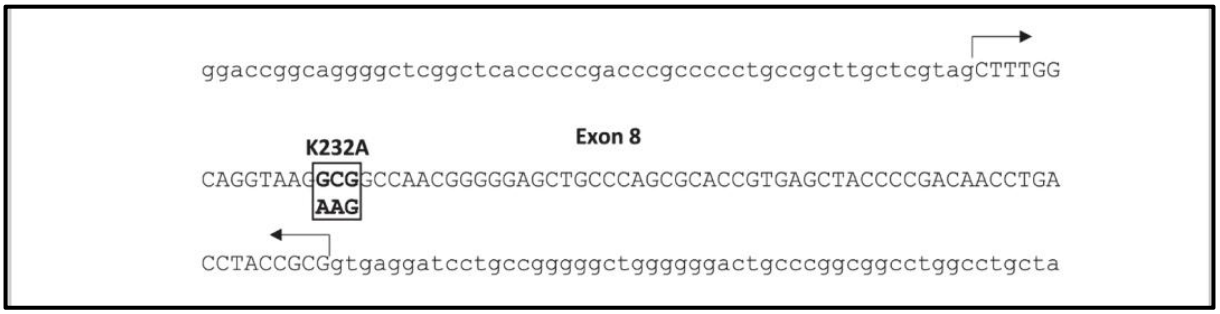


**Figure 4**. The position of the primers for diacylglycerol acyl-CoA acyltransferase 1 gene (DGAT1). The exons are depicted in upper case while the introns are depicted in lower case, with exon/intron boundaries marked with arrows. The regions where the primers bind are underlined, and the p.K232A codon is indicated.

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## DISCUSSION

In recent times, the DGAT1 gene has generated immense interest, this is likely due to its potential use as a gene marker to improve selection of important traits in cattle.

In this investigation, only one genetic variation found in *DGAT1,* as a result of the substitution in exon 8, which is a missense variant (BTA14:611019AA>GC) that underpins p.K232A, and this has been previously reported (Farnir *et al*., 2002; Grisart *et al*., 2002; Winter *et al*., 2002). This variation is an A/A to G/C dinucleotide substitution in the 8$^{th}$ exon, which is responsible for changing a Lysine (K) into an Alanine (A) amino acid at position 232 K232A (Farnir *et al*., 2002; Grisart *et al*., 2002; Winter *et al*., 2002). This change between a Lysine residue that is positively charged with an Alanine residue that is a neutral hydrophobic in the *DGAT1* gene has been found to have a major effect on the content of fat and other traits in milk (Farnir *et al*., 2002; Rahmatalla *et al*., 2008; Sanders *et al*., 2006; Thaller *et al*., 2003; Winter *et al*., 2002). Studies have shown that the lysine variant at *DGAT1* increases fat and protein contents, as well as fat yield, whereas the variant of the *DGAT1* Alanine increases milk and protein yields (Farnir *et al*., 2002; Thaller *et al*., 2003; Winter *et al*., 2002).

Studies have shown that the p.K232A identified here influences the milk fat levels and milk yield in different breeds of dairy cattle. For instance, some studies have shown that the Holstein-Friesian and Jersey cows that possess variant K tend to produce more milk fat (Grisart *et al.*, 2002; Mao *et al.*, 2012; Signorelli *et al.*, 2009; Strzalkowska *et al.*, 2005; Tabaran *et al.*, 2015). In this context, since we were able to identify this variant in the indigenous White Fulani cattle breed, there is a chance that this variant could also be increasing milk fat as well. Other reported variants previously observed in the European cattle breeds in the *DGAT1* were not found here. This may be because of the small sample of cows investigated here. This would suggest that if more samples were investigated across different breeds and locations in Nigeria, it is likely that the variations identified in other studies of *DGAT1* might be found, along with novel ones too.

Additionally, it could also be because of the difference in breed. While the studies conducted in the past were more interested on cattle of *Bos taurus* origin from Europe, our study looked at the indigenous Nigerian cattle breeds in Nigeria, which are of *Bos indicus* origin. Although these two species might have come from a common ancestor, other factors such as the pressure on selection for a particular trait, genetic drift and differences in management practices might have been responsible for their differences. Another reason could be our choice of the gene region investigated. The exons are known to be relatively conserved compared to the introns. Since our investigation targeted the exon 8 region, it is possible that more variations would have been found if the study were to focus on the introns. Hence, it is plausible that these could be the reasons why we could not observe most of the previously reported variants in this study.

## CONCLUSION

In this investigation, the p.K232A variation was identified in the White Fulani cattle breed investigated. This variant has been previously reported, and it's found to affect the milk fat levels and milk yield in different breeds of dairy cattle of European origin. It is therefore likely that this variant might also be responsible for increasing milk in the indigenous White Fulani cattle breed. However, this could not be verified since we were unable to carry out association studies due to lack of phenotypic data in the indigenous cattle breed investigated. Therefore, further studies involving association model need to be carried out in order to ascertain the potential effect of the

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p.K232A variation in the Nigerian cattle breed investigated. Also, while previous reports identified other variations in this gene across several breeds, our inability to identify other variations reported in the previous studies could be attributed to differences in breed, gene region investigated as well as genetic drift and selection pressure for a particular traits.

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