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Effect of breed and Diacylglycerol acyltransferase 1 gene polymorphism on milk production traits in Beninese White Fulani and Borgou cows

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Diacylglycerol acyltransferase 1 (*DGAT1*) is a potential candidate gene with a non-conservative substitution of lysine by alanine (K232A) in exon 8 having a major effect on milk production traits in cattle. The aim of this study was to analyze the allele and genotype frequency, and investigate the association of the *DGAT1* K232A polymorphism with milk production traits in indigenous White Fulani and Borgou cattle breeds in Benin. In total, 103 White Fulani and 103 Borgou were genotyped by Polymerase Chain Reaction–Restriction Fragment Length Polymorphisms and validated by Sanger sequencing. The genotypic frequencies of KK, KA and AA in White Fulani and Borgou breeds were 0.83, 0.16, 0.01 and 0.57, 0.39, 0.04 respectively. Frequencies of K and A alleles were 0.91 and 0.09, and 0.77 and 0.23 in White Fulani and Borgou breeds respectively. The White Fulani cows showed higher daily milk yield (P< 0.01), lactose content (P<0.001), protein yield (P< 0.01) and fat yield (P< 0.01) compared to Borgou. The *DGAT1* KK genotype was significantly (P<0.05) associated with higher fat yield in White Fulani. Therefore, the *DGAT1* locus could serve as a genetic marker for selection of fat yield in indigenous White Fulani Cows. Further studies would be needed to investigate the effect of *DGAT1* gene on milk fatty acids variation between the two breeds.

Keywords: *DGAT1*, Milk traits, Borgou, White Fulani, Benin.

INTRODUCTION

Milk and dairy products are an important source of high quality protein, nutrients and vitamins, especially in developing countries (Dugdill *et al*., 2013). In Benin, cow milk represents a cheap and significant source of proteins. Indigenous cattle are the main producers of milk for the rural population. The cattle population of Benin is estimated

to be 2,166,000 head (2013) and consists mainly of indigenous *Bos taurus* cattle breeds namely, Borgou, Somba, and Lagune and *Bos indicus* namely, M'bororo, Gudali and White Fulani and their different crosses (FAO, 2015). The Borgou cattle breed represents more than 50 % of the total cattle population in Benin and is widely distributed throughout the country (Koutinhouin *et al*., 2003). The White Fulani cattle are the second most common breed in Benin and are mainly located in the Northern and Central regions of the country (FAO, 2015). The two breeds are well adapted to the local environmental conditions and have not undergone any selection for milk or beef production. Raw milk produced in Benin is consumed at household level and mostly processed into yogurt, curds and traditional cheese known as *Wagashi* for commercialization (Aïssi *et al*., 2009). A rich nutritional cheese produced by women, *Wagashi* is the highest consumed dairy product in Benin (Aïssi *et al*., 2009). In the traditional breeding system, the white Fulani and Borgou cattle produce an average daily milk yield of 1.45 l and 0.99 l, respectively,over eleven months of lactation (Kassa *et al*., 2016). Because of the low productivity of the cows, the herds are not able to guarantee the full coverage of the milk demand of the country (Youssao *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 increase fat and protein contents, as well as fat yield while the Alanine variant (232A) is associated with increased milk and protein yields (Winter *et al*.,2002; Sanders *et al*.,2006; Rahmatalla *et al*., 2015).

In Benin, previous studies have focused on cattle breeding system (Assani *et al*., 2016), bovine pathologies challenging milk production (Farougou *et al*., 2006) and nutritional values of beef (Salifou et al., 2013). However, to date, there has been no study on *DGAT1* K232A polymorphism frequency and its association with milk traits in indigenous White Fulani and Borgou cattle breeds. Moreover, no detailed data on milk composition of these breeds is available. Such data would be useful for gathering knowledge on nutritional value of indigenous cow milk for the dairy industry in Benin and to understand the genetic variation for milk production between breeds.

The aim of this study was to analyze the allele and genotype frequency, and investigate the association of the *DGAT1* K232A polymorphism with milk production traits in indigenous White Fulani and Borgou cattle breeds. The identified significant associations with milk production traits could serve as potential genetic markers in a breed improvement program.

MATERIALS AND METHODS

Sampling sites and animals tested

The indigenous Borgou cattle breed is characterized by medium horns size and a flat face profile. The coat is usually white, gray and sometimes piebald. The white Fulani breed is characterized by a fairly developed hump and an inclined ridge. The head is long and thin. The horns are generally in lyre, strong at the base and directed forward. The dominant coat is White. 103 indigenous Borgou and 103 White Fulani cows were sampled from three government farms (Samiondji, Betecoucou and Okpara) and traditional breeding farms located in the peri-urban areas of Parakou District in Benin (Figure 1). The government farms were selected because they are the centers of conservation of the indigenous cows breeds and practice the breeding system based only on natural grazing. The cows were milked in the morning once a day and they were at various stage of lactation (early to late lactation stage) and had different age. The livestock keepers were requested to give a written consent enabling the animal selected to be sampled. The animals were sampled without any pain or stress. In order to ensure that the sampled animals were unrelated, only two to ten lactating cows were sampled per herd and the genealogy of each animal obtained from the herdsman.

Sampling

A total of 206 blood samples (103 Borgou and 103 White Fulani) were collected from the jugular vein of the cows in a 10 ml EDTA vacutainer (BD Vacutainer Systems, Plymouth, UK) tube. Each tube was gently mixed by inversion, labeled with animal number, breed and herd. The blood samples were transported immediately to the laboratory in a cool box containing ice packs and stored at -20°C until further analysis.

Milk was collected in 50ml falcon tubes from 89 Borgou and 93 White Fulani cows. Each milk sample was immedietly treated with one Bonopol milk preservative tablet (MiaGene Biotechnologie Inc., Canada) and kept at -20°C until furher analysis. Milk yields were recorded for

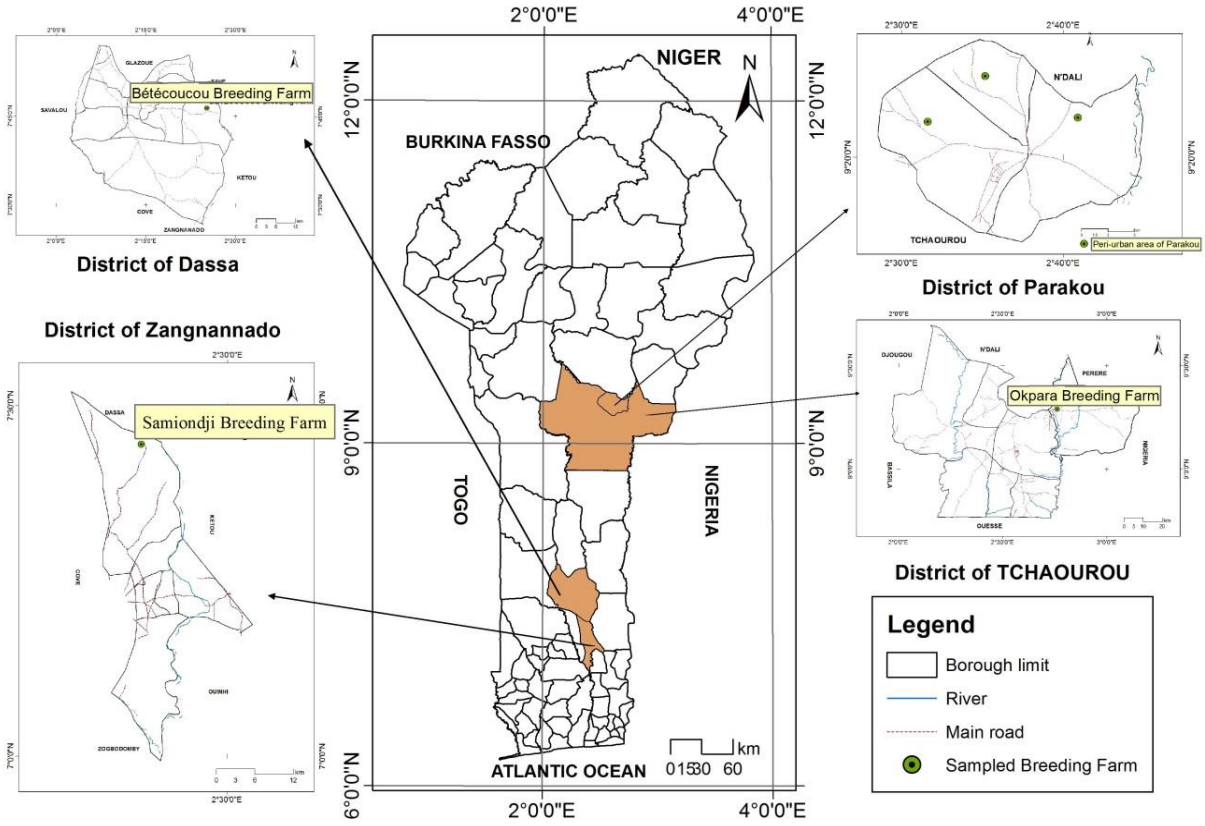

Figure 1: Map of Benin showing the sampling sites

90 days (between May-July 2016) as the total of morning milk production and were collected using a 20 kg weighing scale.

Nutritional analysis

A single day milk sample from each cow was analyzed for total protein, fat, lactose, solid not fat, total solids and casein contents using the infrared method (MilkoScan FT1; Foss Analytical A/S Hillerod, Denmark). The protein and fat yields were determined by multiplying the respective percentages with the milk yield. The collected milk data was used in the association analysis.

DNA extraction

Genomic DNA was isolated from blood samples using the standard method of phenol-chloroform (Sambrook and Russell, 2001) at the Molecular Genetics and Genome Analysis Laboratory at Abomey-Calavi University (Benin). DNA quality and quantity were checked on agarose gel and using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., USA), respectively.

Polymerase Chain Reaction–Restriction Fragment Length Polymorphisms (PCR-RFLP) genotyping

The 411 bp fragment (partial exon-7 to partial exon-9) of bovine *DGAT1* containing the AA/GC (K232A) substitution was amplified in a standard thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The following primers F:5'-GCACCATCCTCTTCCTCAAG-3' and R:5'-GGAAGCGCTTTCGGATG-3' were used (Kaupe *et al*., 2004). The PCR reactions were carried out in a 30µL volume using 45ng of template DNA, 15 µL of PCR Master Mix (Bioneer, Korea) and 4.5 pmol of each primer (3pmol/µL).The PCR conditions included an initial denaturation step at 94°C for 3 min, followed by 35 cycles of 94°C for 45s, 62°C for 60s, 72°C for 60 s, and a final extension step of 72°C for 5 min.

The PCR products were purified using the QIA quick PCR Purification Kit (Qiagen, Germany), electrophoresed in 1.8% agarose gel and visualized with GelDoc-It^2 Imager (Ultra-Violet Products Ltd). Five µLof purified PCR products were mixed with 1µl 10X buffer, 3.8µL dH_2O and 10 U of *EaeI* restriction enzyme (New England Biolabs,

Inc.) and digested overnight at 37°C. The digested fragments were separated at 7V/cm on 1.8% agarose gel in 0.5x TBE buffer. The gel was stained with GelRed™ (Biotium) and visualized under UV light (GelDoc-It^2 Imager). Digestion patterns were visually analysed and recorded.

DNA sequencing

Eighteen (18) PCR fragments representative of the RFLP patterns were sent for Sanger sequencing to validate RFLP results. The 411bp fragment was sequenced at Bioneer (KOREA) using Sanger method (ABI machine, USA). The samples showing a rare mutation in intron 8 after analysis were amplified and sequenced again to confirm the mutation. CLC Main Workbench version 7.8.1 was used for quality control and sequence analysis. The sequence with the rare mutation in intron 8 was compared with *DGAT1* sequences from the *Bos indicus* and *Bos taurus* deposited in the NCBI Gene bank database. The Ensembl genome browser database (http://www.ensembl.org/Bos_taurus/Info/Index) was used to map the position of amino acid change (K232A) as well as the position of the rare mutation in the cow genome (genome assembly UMD3.1).

Statistical Analysis

GENEPOP Program version 1.2 was used to estimate allele frequencies and test for Hardy-Weinberg Equilibrium (HWE) according to Raymond and Rousset (2001). The population inbreeding coefficient (F_{IS}) was calculated using the following formula (Wright, 1951):

$F_{IS=}(H_E-H_0)/H_E$,

Where F_{IS} is the inbreeding coefficient, H_E is the expected heterozygosity (=2pq; where p and q are allelic frequencies of K and A *DGAT1* variants, respectively); Ho is the observed heterozygosity.

The effect of breed and *DGAT1* K232A genotypes on milk components was assessed using the General Linear Model of IBM SPSS version 20 software Package. The linear model used was:

$Y_{ijkl} = \mu + B_i + G_j + (Bi \times Gj)_{ij} + E_{ijk}$

in which Y_{ijk} is the observed trait: milk yield (kg/day) , fat%, protein %, solid not fat %, total solids %, lactose % , casein %, protein yield (g/day) and fat yield (g/day). μ is the population mean, Bi is the fixed effect of Breed, Gj is the fixed effect of *DGAT1* genotype (KK and KA), $(Bi \times Gj)_{ij}$ is the fixed interaction effect between breed and *DGAT1* genotype and E_{ijk} is the random residual associated with each record. The results of breed and *DGAT1* genotype effects are presented as least squares means±Standard Error. Probability less than 0.05 was used to determine the level of significance.

RESULTS

Genotypes and allelic frequencies of *DGAT1* K232A polymorphism

Genotyping of *DGAT1* allelic variation by PCR-RFLP showed a clear distinction of three different genotypes namely KK (Lysine homozygote), KA (Lysine-Alanine heterozygote) and AA (Alanine homozygote). The *EaeI* restriction enzyme digestion revealed uncut single band of 411bp ($DGAT1^{KK}$) and two co-migrating fragments (203bp and 208bp) corresponding to the alanine variant, $DGAT1^{AA}$ (Figure 2). The genotypic frequencies of KK, KA and AA in White Fulani and Borgou breeds were 0.83, 0.16, 0.01 and 0.57, 0.39, 0.04 respectively. Frequencies of K and A alleles were 0.91 and 0.09, and 0.77 and 0.23 in White Fulani and Borgou breeds respectively. The AA genotype was observed in only one individual of White Fulani and in four individuals of Borgou (Table 1). Sequence analysis confirmed the AA/GC dinucleotide substitution in *DGAT1* exon 8 and validated the three restrictions patterns detected by PCR-RFLP (Figure 3). On the basis of the observed *DGAT1* genotype frequencies, the K allele (lysine variant) was present at a higher frequency than the A allele (alanine variant) in the two breeds (0.77 vs. 0.23 and 0.91vs.0.09 for allele K and allele A, respectively) in Borgou and White Fulani cows, respectively. However, White Fulani (*Bos indicus*) cows showed higher frequency of the K allele (0.91) than Borgou (*Bos taurus*) cows (0.77). In this study, *DGAT1* genotypes did not deviate from Hardy-Weinberg equilibrium in the two breeds, $\chi 2$ =0.770 for Borgou and $\chi 2$ =0.069 for White Fulani which are lower than the critical chi-square at one degree of freedom (3.841).

Expected (H_E) and Observed (H_O) heterozygosity and inbreeding coefficient of *DGAT1* gene in this study are presented in Table 1. The observed heterozygosity was higher in Borgou (0.388) than White Fulani (0.155). Furthermore, difference in inbreeding coefficients (F_{IS}) was observed between Borgou and White Fulani. In particular, White Fulani (0.055) had a higher inbreeding coefficient than Borgou (-0.096).

Characterization of a rare Insertion-deletion(Indel) mutation in intron 8 of *DGAT1* gene

Comparison of the 411bp fragments with *DGAT1* reference sequence in GenBank (rsAJ318490) showed a rare Indel mutation in one individual of Borgou breed and two individuals of white Fulani breed. The mutation is characterized by a G nucleotide insertion in intron 8 with genomic coordinates 14:_1802346-1802347 (corresponding

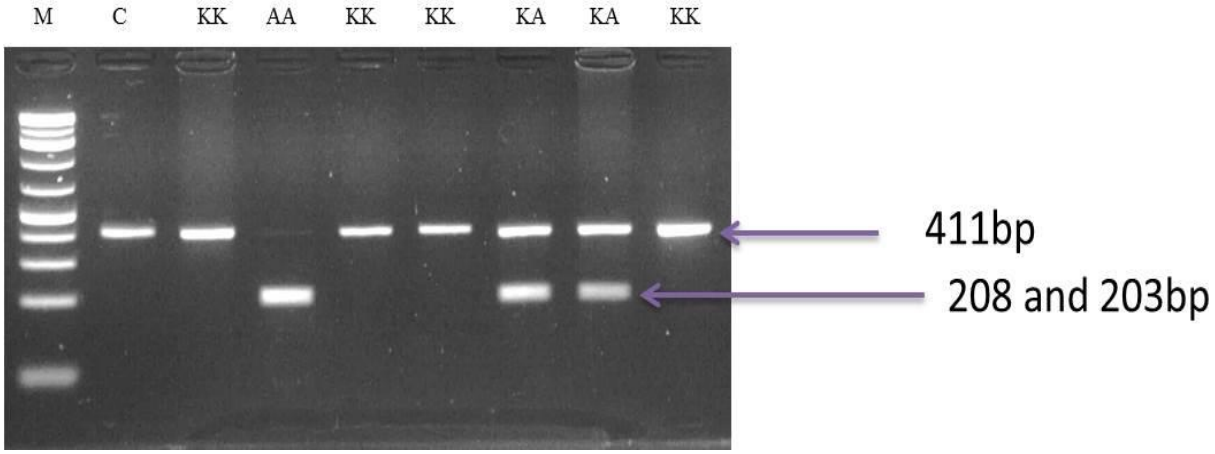

Figure 2: DGAT1 K232A polymorphism by *EaeI*/RFLP showing three different genotypes KK, KA and AA in Borgou and White Fulani Cows. M= 100bp molecular marker. C=Control, uncut PCR product.

Table 1: Genotypes and allelic frequencies, observed (H_O), expected (H_E) heterozygosity and inbreeding coefficient (F_{IS}) of K232A polymorphism of *DGAT1* gene in Borgou and White Fulani cows

	GenotypesFrequency			AllelicFrequency		Heterozygosity			
Breed	KK	KA	AA	K	A	H_O	H_E	Chi-square	F_{IS}
Borgou(103)	0.57(59)	0.39(40)	0.04(4)	0.77	0.23	0.388	0.354	0.794	-0.096
White Fulani (103)	0.83(86)	0.16(16)	0.01(1)	0.91	0.09	0.155	0.164	0.084	0.055

Numbers in brackets correspond to sample size

to the position 271 in the sequence reported in Figure 3) in the *DGAT1* gene. The Indel mutation was associated with lysine variant (KK genotype) in both breeds.The sequences were deposited in GenBank of NCBI under accession numbers MF445054 and MF445055 for White Fulani, and MF445056 for Borgou.

Effect of Breed on milk production traits

Least squares means of milk production traits of Borgou and White Fulani cattle breeds are reported in Table 2. There was a significant difference between breed type for milk yield (P< 0.01), lactose content (P<0.001), protein yield (P< 0.01) and fat yield (P< 0.01). Thus, daily milk yield was higher in white Fulani than Borgou breed (1.07 vs 0.85 Kg/day, P< 0.01). Moreover, White Fulani milk had the highest lactose content (4.74%) compared to Borgou milk (4.51%) (P<0.001). Similarly, White Fulani presented highest daily protein yield (39.76 vs 31.25 g/day, P< 0.01) and highest daily fat yield (50.54 vs 39.16 g/day, P< 0.01). However, no significant difference were observed for fat, solid not fat, total solids and casein contents between the two breeds (P>0.05).

Effect of *DGAT1* K232A polymorphism on milk production traits

The effect of *DGAT1* K232A polymorphism on milk yield and milk composition was revealed by comparing the KK and KA genotypes, because only five animals with the AA genotype were found (four in Borgou and one in White Fulani). The results indicated that the KK genotype was significantly (P<0.05) associated with higher fat yield (49.99 g/day) compared to KA genotypes (39.71 g/day) in White Fulani (Table 3). However, no significant difference was observed for the milk yield, fat (%), protein (%), solid not fat (%), total solids (%), lactose (%), casein (%) and protein yield between the KK and KA genotype (P>0.05) in both breeds. Although, no significant difference was observed for fat and protein content, it's important to note that the cows homozygous for the lysine variant (KK) had higher fat content (4.89 vs 4.75 %) and higher protein content (3.82 vs 3.74 %) compared to the cows with the KA genotype in White Fulani breed. The same trend was observed in Borgou breed for fat content (4.57vs 4.35 % for KK and KA genotypes, respectively), Table 3.

Figure 3: Sequence alignment of the KK genotype, AA genotypes, KA genotype and the Indel mutation (D43, D155 and D184) sequenced samples. Identical nucleotides are replaced by a dot (**.**) and the substitutions are indicated by letter. The simultaneous presence of both nucleotides (heterozygous genotype) is indicated with RM (IUPAC nucleotide code). R for A or G, M for A or C and Y for C or T nucleotides.

Table 2 Effect of Breed on milk production traits in indigenous White Fulani (WF) and Borgou (BO) cattle (Least square means±SEM)

Trait	Breed				P-value
	BO (N=89)	SEM†	WF (N=93)	SEM†	
Milk Yield (Kg/day)	0.85	0.05	1.07	0.06	0.002**
Fat (%)	4.78	0.21	4.85	0.24	0.81
Protein (%)	3.79	0.08	3.77	0.1	0.854
Solid not fat (%)	8.84	0.1	9.13	0.11	0.051
Total solids (%)	13.94	0.28	14.26	0.32	0.437
Lactose (%)	4.51	0.03	4.74	0.04	<0.001***
Casein (%)	2.72	0.05	2.73	0.06	0.949
Proteinyield (g/day)	31.25	1.86	39.76	2.12	0.001**
Fat yield (g/day)	39.16	2.91	50.54	3.33	0.007**

†SEM=Standard Errors of the means; Numbers in brackets correspond to sample size; ** P< 0.01; *** P<0.001

Table 3: Effect of *DGAT1* K232A polymorphism on milk production traits in indigenous White Fulani andBorgou cattle (Least square means±SEM)

Trait	White Fulani					Borgou				
	KK (N=81)	SEM†	KA (N=12)	SEM†	P-value	KK (N=53)	SEM†	KA (N=36)	SEM†	P-value
Milk Yield (Kg/day)	1.03	0.04	0.89	0.07	0.108	0.9	0.1	0.85	0.12	0.645
Fat (%)	4.89	0.17	4.75	0.29	0.683	4.57	0.52	4.35	0.61	0.698
Protein (%)	3.82	0.07	3.74	0.12	0.563	3.62	0.19	3.68	0.22	0.783
Solid not fat (%)	9.06	0.08	8.92	0.14	0.403	8.6	0.23	8.63	0.27	0.895
Total solid (%)	14.24	0.23	13.96	0.39	0.549	13.48	0.68	13.29	0.79	0.801
Lactose (%)	4.64	0.03	4.61	0.05	0.612	4.46	0.08	4.42	0.1	0.723
Casein (%)	2.75	0.04	2.69	0.07	0.507	2.61	0.12	2.65	0.14	0.773
Proteinyield (g/day)	38.42	1.52	32.59	2.58	0.059	32.41	3.69	30.68	4.31	0.667
Fat yield (g/day)	49.99	2.38	39.71	4.05	0.034*	42.52	6.35	36.46	7.41	0.382

†SEM=Standard Errors of the means; Numbers in brackets correspond to sample size, * P<0.05

DISCUSSION

Genotypes and allelic frequencies of *DGAT1* gene

Polymorphisms of DGAT1 K232A have been studied in several breeds from different origins (Kaupe *etal*., 2004; Winter *et al*.,2002; Sanders *et al*.,2006). Genotyping of *DGAT1* polymorphism in different *Bos taurus* and *Bos indicus* from Europe, Africa, Asia, North America and South America showed allelic frequencies ranging from zero to one (Kaupe *et al*., 2004). In this study, the lysine allele $DGAT1^{K}$ was the most frequent in both breeds ranging from 0.77 (Borgou) to 0.91 (White Fulani). Previous studies on *Bos Taurus* and *Bos indicus* identified the *DGAT1* K allele as the wild type and inferred that the A allele substitution would have occurred after the separation of *Bos taurus* and *Bos indicus* lineages (Kaupe *et al*., 2004). The high frequency of the K allele observed in indigenous Borgou and White Fulani cows in this study is in agreement with previous studies where the $DGAT1^{K}$ frequencies were 0.95 and 0.79 in Sudanese indigenous Kenana and Butana dairy cows, respectively (Rahmatalla *et al*., 2015).

The allelic frequencies of K and A observed in Beninese White Fulani (0.91 and 0.09, respectively) are similar to those reported in African White Fulani (0.92 and 0.08, respectively) (Kaupe *et al.,*2004). Borgou cows showed K and A allelic frequencies of 0.77 and 0.23, respectively, similarly to those reported in Iranian Holstein (0.79 and 0.21, respectively) (Mashhadi *et al.,* 2012). No allele fixation was observed in this study. Nevertheless, Winter *et al*. (2002) found fixation of *DGAT1* K in three different Indian Zebus (Fleckvieh, Anatolian Black, and Sahival) while Kaupe *et al*. (2004) found fixation of *DGAT1*A allele in five *Bos taurus* breeds (Belgian Blue_beef, Gelbvieh, Hereford, Pinzgaurer and Slavonian Syrmian).

Observed heterozygosity at *DGAT1* locus of 0.388 and 0.155 for Borgou and White Fulani, respectively, were in the previously reported range of 0.0 to 0.61 (Lacorte *et al*., 2006; Kaupe *et al*., 2004). The highest observed heterozygosity in Borgou Cows (0.388 vs 0.155) indicates that Borgou cattle are more genetically variable at the *DGAT1* K232A locus than White Fulani. Moreover, *DGAT1* genotypes did not deviate from Hardy-Weinberg equilibrium confirming the absence of selection in Borgou and White Fulani breeds. However, different F_{IS} coefficients (inbreeding coefficient) were observed in the two breeds. The F_{IS} coefficients are the classical Wright's *F*-statistic, which estimates the variation within populations. Specifically, it measures the reduction in heterozygosity in an individual caused by nonrandom mating within its subpopulation (Wright, 1951). The F_{IS} coefficient value was positive in white Fulani (0.055) and negative in Borgou (-0.096). This suggests a heterozygosity deficiency in White Fulani cattle population as result of uncontrolled mating leading to reduction of diversity.

The 411bp *DGAT1* fragment sequence analysis revealed the presence of a rare Indel mutation. Our results showed that this rare polymorphism seems to be in complete linkage disequilibrium with the *DGAT1* K allele. The nucleotide BLAST analysis with *DGAT1* sequences in GenBank identified very few sequences

with this rare mutation. However, none of the identified sequences belonged to African indigenous *Bos taurus* or *Bos indicus.*They belong to the Indian Murrah breed of water buffalo (GenBank DQ435292.1)and *Bos indicus* x *Bos taurus* crossbreed (GenBank: KX965998.1). Although the Indel occurred in an intron and not in a coding region of the *DGAT1* gene, it is important to note that introns play an important role in transcription and mRNA splicing (Zhang *et al*., 2010). Therefore, point mutation in introns can activate novel promoters or introduce or eliminate enhancer activity in a gene. However, our study could not confirm at this stage the effect of the insertion mutation on *DGAT1* gene expression and/or regulation, suggesting the need of further research to investigate the effect of the mutation on *DGAT1* gene transcription and mRNA splicing.

Effect of Breed on milk production traits

In this study, the White Fulani breed presented a higher daily milk yield (1.07 kg/day) compared to the Borgou breed (0.85 Kg/day), ($P< 0.01$). These results are similar to the average daily milk yield of 1.45l and 0.99l reported in White Fulani and Borgou cows respectively in Peri-urban area of Parakou, Benin (Kassa *et al*., 2016). However, the daily milk yield of the White Fulani in the present study (1.07 kg/day) is higher than the value reported in the White Fulani (0.16-0.46 kg/day) in Nigeria (Ndubueze *et al*., 2006). Regarding the milk composition, significant differences were observed for lactose, protein yield and fat yield between the two breeds. Several studies reported the effect of breed on milk composition in Nigerian cattle, sheep and goat (Malau-Aduli and Anlade, 2002; Ochepo *et al.,* 2015) and South African indigenous cows breeds (Myburgh *et al*., 2012). Borgou and White Fulani cows presented higher fat content (4.78 and 4.85%, respectively) than the values of 2.01%, 2.68% and 3.79% reported by Myburgh *et al*. (2012) in South African indigenous Tuli, Boran and Afrikaner cattle breeds, respectively. However, in our study, White Fulani and Borgou cows produced milk with lower lactose compared to South African indigenous Boran, Nguni, Tuli and Afrikaner with reported lactose content ranging from 5.16% in Boran to 6.74% in Tuli (Myburgh *et al*., 2012). Moreover, the fat and protein contents of indigenous Borgou (4.78% and 3.79%, respectively) and White Fulani (4.85% and 3.77%, respectively) are in the same order of magnitude and comparable to those reported in Western dairy breeds such as Holstein Friesian (4.01% and 3.47%), Brown Swiss (4.28% and 3.75%) and Jersey (5.59% and 4.07%) (Stocco *et al*., 2016). The white Fulani cows' milk would be preferred by farmers because of the high milk and protein yields favorable for milk transformation into traditional cheese *Wagashi*.

The animals in the present study were raised in their traditional system on natural grazing without feed supplementation. The observed difference between breeds would be therefore due to their genetic background. However, previous studies showed that non-genetic factors such as age, parity number, cow in pregnancy and stage of lactation, affect milk yield and composition in cows (Gurmessa & Melaku, 2012). The animals included in the present study were at different age, parity number, lactation and physiological stages. The difference in milk composition between the two breeds may be also explained by the composition of the forage consumed by the cows. The two breeds are raised in different agro-ecological areas.The White Fulani is mainly found in the Northern part of Benin while the Borgou breed is distributed throughout the country. Moreover, Alkoiret *et al*. (2011) reported protein and fat contents of 4.12% and 5.17% respectively in Borgou cows which are higher than what observed in the current study (3.79% and 4.78%). These high values could be explained by the fact that the researchers in that study supplemented their animals with dried brewer's grain, cassava chips and cotton seed (Alkoiret *et al*., 2011).

Effect of *DGAT1* K232A polymorphism on milk production traits

This study demonstrates a significant association of the *DGAT1* KK genotype with higher fat yield ($P<0.05$) in indigenous White Fulani cows (Table 3). No significant association was found for the fat and protein contents in both White Fulani and Borgou breeds ($P>0.05$). However, the KK genotypes presented high protein (3.82 vs 3.74 %) and fat (4.89 vs 4.75 %) content in White Fulani and higher fat content in Borgou (4.57% vs 4.35%). These results corroborate what Molee *et al*. (2012) and Gautier *et al*. (2007) observed in their study reporting that the cows with the KK (Lysine homozygous) had higher fat yield and higher protein and fat percentages. However, no signicant association of KK genotype with fat yield was found in the Borgou breed, which is similar to a previous study conducted in Sudanese indigenous Kenana and Butana dairy cows (Rahmatalla *et al*., 2015). The least of signicant association found in Sudanese indigenous cows may be due to the low sample size under study, being 40 Butana and 20 Kenana (Rahmatalla *et al*., 2015). Similarly, the least of significance of *DGAT1* effect on all the milk traits in Borgou breed in the current study may be due to the low sample size or the individual variability.

CONCLUSION

This study describes for the first time the *DGAT1* K232A alleles and genotypes frequency distribution in indigenous

White Fulani and Borgou cattle breeds of Benin. The White Fulani breed presented higher milk, protein and fat yields and higher lactose content. The *DGAT1* KK genotype was significantly associated with higher fat yield in White Fulani cows. Therefore, *DGAT1* K232A polymorphism could serve as potential genetic marker for fat yield in White Fulani breed improvement program. Further studies would be needed to investigate the effect of *DGAT1* gene on milk fatty acids composition of Borgou and White Fulani cattle breeds in Benin.

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