POLYMORPHISM OF SELECTED CANDIDATE GENES IN GHANAIAN SHEEP BREEDS

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ABSTRACT
Polymorphisms of candidate genes BMP15 and GDF9, GH and CAST that influence fecundity, growth, and carcass traits, respectively, were studied in two Ghanaian sheep breeds to determine whether these breeds were polymorphic or monomorphic at the above gene loci. DNA was extracted from blood samples collected from 63 Sahel and 49 Djallonké sheep and analyzed by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) technique. Restriction enzyme digestion of the BMP15-FecX^{B} locus revealed two genotypes (AA and AB) in both populations with genotype AA recording the highest frequency in both populations. At the BMP15-FecX^{G} locus, genotype Gg had the highest frequency (0.548) in the Djallonké population whereas GG frequency (0.719) was the highest in the Sahel population. Analysis of GDF9-FecG^{H} with DdeI endonuclease also revealed two genotypes, AA and AB in both populations with the genotypic frequency of AA being the highest in both populations (Sahel= 0.933 and Djallonké= 0.98). At the GH gene locus, restriction digestion with HaeIII showed three genotypes (AA, AB, BB), with genotype AB recording the highest frequency in both populations. In addition, PCR-RFLP analysis at the CAST-MspI gene locus showed three genotypes (MM= 0.048, MN= 0.919, NN= 0.032) in the Sahel population whereas only two genotypes (MM= 0.367, MN= 0.633) were observed in the Djallonké population. Results from this study showed that the selected candidate genes were polymorphic in the two studied sheep breeds of Ghana. These results will form the basis for candidate gene association studies in the two sheep breeds.

Keywords: *Djallonké sheep, Sahel sheep, genetic polymorphism, PCR-RFLP*

INTRODUCTION
The enhanced understanding of the molecular basis of phenotypic variation in natural populations remains an important foundation, yet elusive goal of evolutionary and developmental genetics (Erickson *et al.,* 2004; Fitzpatrick *et al*., 2005). The increasing availability of genomic resources and cost-effective molecular profiling techniques have improved the likelihood of identifying genes responsible for these phenotypic variations (Gratten *et al*., 2007). The knowledge of polymorphisms in genes influencing special traits, and the understanding of the biological effects of these variations, will enable genomic data to be applied most effectively and efficiently in the selection of animals and breeding programmes (Nanekarani and Goodarzi, 2014).

Candidate gene polymorphism has become an important subject of study to better understand the phenotypic variations observed in natural populations. He *et al*. (2010) and Barakat *et al.*

(2017) have outlined the candidate genes associated with fecundity (prolificacy) traits and their mutations in Chinese goats and Egyptian sheep breeds respectively. Sheep have been used as a model species for research into the genes involved in mechanisms that control the rate of ovulation (Polley *et al*., 2009). Furthermore, substantial evidence has been reported after vast investigations carried out on the genetic variations which have been linked with ovulation rate in sheep (Bindon *et al*., 1996).

Reproduction is seen as a complex process (Drouilhet *et al*., 2009), and traits like rate of ovulation and litter size are regulated genetically by a set of different genes collectively known as fecundity genes (*Fec genes*) (Davis *et al*., 1982; Piper and Bindon, 1983). Booroola gene (*FecB*) was the first main gene for prolificacy in sheep to be identified preceding the discovery of these fecundity genes (Polley *et al*., 2009). Three genes associated with fecundity traits were discovered in sheep. These include Growth differentiation factor 9 (*GDF9*) also known as *FecG*, Bone morphogenetic protein receptor type 1B (*BMPR1B*) or activin-like kinase 6, known as *FecB* and Bone morphogenetic protein 15 (*BMP15*) called *FecX* (Galloway *et al*., 2000; Souza *et al*., 2001; Hanrahan *et al*., 2004). Mutations in these fecundity genes have been reported to be associated with the alteration in the rate of reproductive processes including litter size, fertility, and rate of ovulation (Polley *et al*., 2009).

The main aim of sheep production is for one or more of the following purposes: wool, milk, and meat (El Fiky *et al*., 2017). However, all the breeds of sheep in Ghana are kept for meat and therefore growth and carcass composition are of commercial importance for mutton production. In breeding programmes for meat purposes, animals with high genetic merits in terms of growth and body measurements are of top priority (Singh *et al.,* 2015). This implies that growth traits are essential to sheep production with consumers having preference for animals with increased weight and low body fat. This has greatly impacted sheep rearing to ensure an increase in the size and weight of the animal. The rapid growth of lambs to reach market weight at a much younger age implies a shorter feeding duration and less risk of death loss**.** The function of the growth hormone (*GH*) gene has been observed in many tissues, including muscle, bone, and adipose tissues (Kumari *et al*., 2014) all of which translate to increased body weight and size.

Meat quality improvement and meat tenderness have been a top priority for the meat industry and primary indicators of consumers' preferences, respectively (Morgan *et al*., 1991). To aid solve the issue of variations in the tenderness of meat, a lot of studies on the inheritance of meat tenderness traits such as the activity of calpastatin, have been documented (Marshall, 1994; Bertrand *et al*., 2001; Riley *et al*., 2003). Asadi and Khederzadeh (2015) identified the calpastatin (*CAST*) gene as an important functional candidate gene for the formation of muscle, degradation, and tenderness of meat after slaughter.

The identification of polymorphism of these genes in Ghanaian sheep breeds specifically the Djallonké (WAD) and the Sahelian sheep (WALL) would be a major step to an understanding of their association with growth and reproductive traits, thereby providing essential information for developing marker-assisted selection (MAS) studies which will promote and enhance selection and breeding of sheep in Ghana.

Moniruzzaman *et al*. (2014) outlined that as most of the traits considered in animal genetic improvement programmes are quantitative traits, selection is based on phenotypes for classical and conventional breeding. Animal breeding in Ghana, with minimal impact of molecular genetics, has heavily relied on these classical and conventional breeding methods without knowledge of which genes influence traits that are being selected for. There is hence the need to improve on the knowledge of the molecular genetic basis of traits and the genetic polymorphisms associated with such traits that are important in sheep breeding in Ghana to ensure effective and efficient breeding programmes.

This study, therefore, sought to identify polymorphism at the $FecX^{G}$ and $FecX^{B}$ loci for the *BMP15* gene, and the *GDF9*-$FecG^{H}$ locus and their frequencies, the genetic variability of the

CAST gene and growth hormone (*GH*) gene in the WAD and WALL.

MATERIALS AND METHODS

Sampling and Extraction of Genomic DNA

A total of 112 unrelated sheep (Djallonké, n = 49 and Sahelian, n = 63) were sampled from three parts of Ghana: the northern (Bawku, Bolgatanga, Pong-Tamale, Walewale), middle belt (Ejura municipal, Atebubu) and the southern part (Amrahia and its environs). Five millilitres of venous jugular blood was collected into vacutainer tubes containing EDTA and stored at -20° C. Genomic DNA extraction was later carried out using the QIAGEN® blood and tissue kit according to the manufacturer's protocol.

PCR Analysis

Polymerase chain reaction (PCR) was done under optimised conditions to amplify the target regions of the genes in this study. Amplified products were subsequently digested with restriction enzymes (Table 1). A total reaction volume of 10 µL for each sample containing 5 µL of AmpliTaq Gold™ 360 Master Mix (Thermo Fisher Scientific, Foster City, CA), 3 µL UltraPure™ DNase/RNase-Free distilled water, 0.5 µL each of forward and reverse primers and 20 ng of DNA was prepared.

RFLP Analysis

For the restriction fragment length polymorphism conditions, 3.8 µL of DNase/RNase–free water and 0.2 µL of restriction enzyme for $FecX^G$, *CAST*, *GH* loci and 3.65 µL of DNase/RNase–free water and 0.35 µL of restriction enzyme for $FecX^B$ and $FecG^H$ were used. The addition of 1 µL of 1x buffer and 5 µL of PCR product led to a total reaction volume of 10 µL for each locus. The mixture was incubated at

Table 1: Primers for selected candidate gene loci in sheep

Gene	Locus	Fragment Size (bp)	Forward and Reverse Primers (5'-3')	R.E.	Reference
BMP15	$FecX^G$	141	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC GATGCAATACTGCCTGCTTG	*HinfI*	Hanrahan *et al*., 2004
	$FecX^B$	153	GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCT TA TTCTTGGGAAACCTGAGCTAGC	*DdeI*	Hanrahan *et al*., 2004
GDF9	$FecG^H$	139	CTTTAGTCAGCTGAAGTGGGACAAC ATGGATGATGTTCTGCACCATGGTGTGAAC-CTGA	*DdeI*	Hanrahan *et al*., 2004
CAST		622	TGGGGCCCAATGACGCCATCGATG GGTGGAGCAGCACTTCTGATCACC	*MspI*	Nanekarani and Goodarzi. 2014
GH		422	GGAGGCAGGAAGGGATGAA CCAAGGGAGGGAGAGACAGA	*HaeIII*	Kuulasma, 2002; Hua *et al*., 2009

R.E. – Restriction Enzyme

Table 2: Optimised PCR conditions

Locus	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	No. of cycles
$FecX^G$	94ºC for 5 mins	94ºC for 30 sec	63ºC for 40 sec	72ºC for 30 sec	72ºC for 10 mins	40
$FecX^B$	95ºC for 5 mins	94ºC for 30 sec	64ºC for 40 sec	72ºC for 30 sec	72ºC for 5 mins	45
$FecG^H$	94ºC for 5 mins	94ºC for 30 sec	62ºC for 40 sec	72ºC for 30 sec	72ºC for 4 mins	45
CAST	95ºC for 5 mins	95ºC for 30 sec	62ºC for 45 sec	72ºC for 1 min	72ºC for 10 mins	40
GH	95ºC for 5 mins	95ºC for 45 sec	60ºC for 45 sec	72ºC for 45 sec	72ºC for 10 mins	33

37ºC for 1 hour for all the loci. The restriction enzymes *HinfI*, *MspI* and *HaeIII* were inactivated at 80ºC for 20 mins while *DdeI* was inactivated at 65ºC for 20 mins. The products of digestion were run on 2% agarose gel stained with Gel-red. Electrophoresis was carried out at 100V for 30 mins and the products were visualised by exposure to ultraviolet light.

Statistical Analysis

The allelic frequencies, genotypic frequencies, observed heterozygosity, expected heterozygosity, and Chi-square test to analyse the deviation from Hardy-Weinberg equilibrium (HWE) were estimated using GenAlEx 6.5 genetic analysis software programme (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

BMP15-FecX^B

Restriction enzyme digestion of the amplified 153 bp PCR products (Plate 1) of all samples in this study with *DdeI* revealed two genotypes: homozygote AA and heterozygote AB. Genotype AA had 122 bp and 30 bp (Plate 1) while genotype AB had two fragments with lengths 153 bp and 122 bp (Plate 2).

Higher allele A frequency was recorded in both populations (0.944 in Sahel and 0.959 in Djallonké), with allele B frequency of 0.056 and 0.041 in Sahel and Djallonké respectively (Table 3).

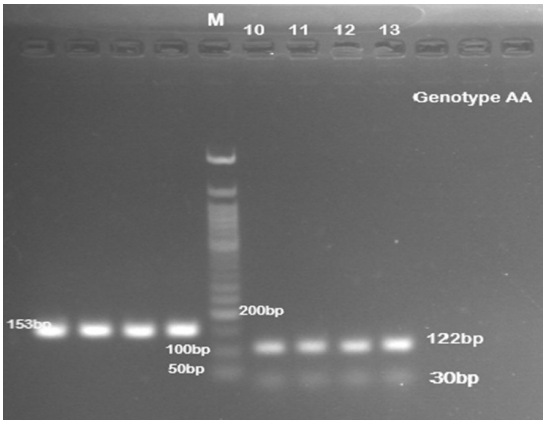

Plate 1: 2% agarose gel electrophoresis image for PCR product of *FecX^B* showing amplification of 153 bp product on the left-hand side of the 50 bp DNA ladder (M). PCR products of *FecX^B* digested with *DdeI*. Lane 10 – 13 shows AA genotype on the right-hand side of the DNA ladder.

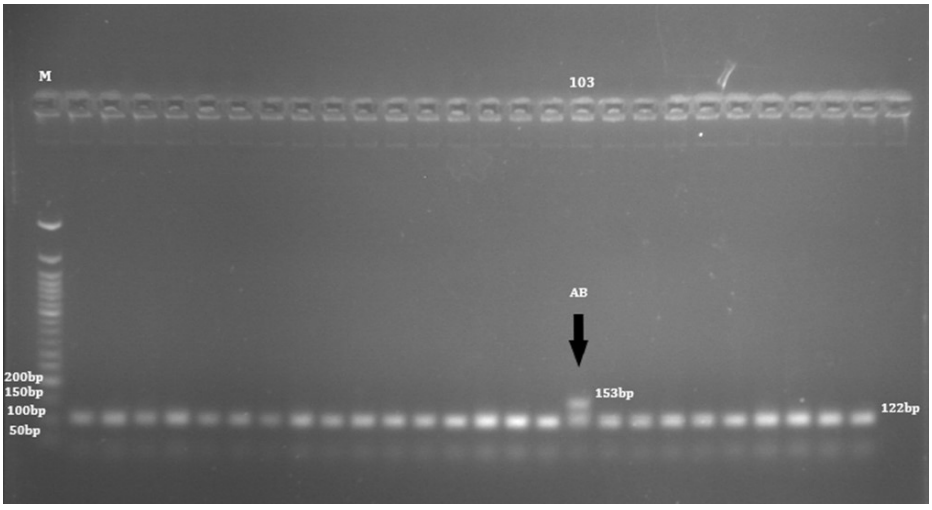

Plate 2: PCR products of *FecX^B* digested with *DdeI*. Lane M is 50 bp DNA ladder. Lane 103 (shown by arrow) shows AB genotype

Table 3: Allelic and Genotypic Frequencies, Observed (H_o) and Expected (H_e) Heterozygosity, Chi-Square Test Values for HWE of the various Loci

Loci	Breed	N	Genotypic Frequency			Allelic Frequency		H_o	H_e	χ^2	p	Significance
FecX^B			**AA**	**AB**	**BB**	**A**	**B**					
	Sahel	62	0.887	0.113	0	0.944	0.056	0.113	0.107	0.222	0.638	ns
	Djallonké	49	0.918	0.082	0	0.959	0.041	0.082	0.078	0.089	0.766	ns
FecX^G			**GG**	**Gg**	**gg**	**G**	**g**					
	Sahel	57	0.719	0.281	0	0.86	0.14	0.281	0.241	1.519	0.218	ns
	Djallonké	42	0.452	0.548	0	0.726	0.274	0.548	0.398	5.971	0.015	**
FecG^H			**AA**	**AB**	**BB**	**A**	**B**					
	Sahel	60	0.933	0.067	0	0.967	0.033	0.067	0.064	0.071	0.789	ns
	Djallonké	49	0.98	0.02	0	0.99	0.01	0.02	0.02	0.005	0.942	ns
CAST			**MM**	**MN**	**NN**	**M**	**N**					
	Sahel	62	0.03	0.92	0.05	0.492	0.508	0.919	0.5	43.663	0	**
	Djallonké	49	0	0.633	0.367	0.316	0.684	0.633	0.433	10.490	0.001	**
GH			**AA**	**AB**	**BB**	**A**	**B**					
	Sahel	63	0.063	0.873	0.063	0.5	0.5	0.873	0.5	35.063	0	**
	Djallonké	49	0.204	0.735	0.061	0.571	0.429	0.735	0.49	12.25	0	**

***: significant ns: non-significant*

Genotypes AA and AB recorded in both populations in this study were consistent with results by Jemmali (2017) who also indicated two genotypes in Barbarine sheep: heterozygote AB (153 bp, 123 bp) and AA homozygote (123 bp, 30 bp). This could be attributed to reports that carriers of the homozygous mutation (BB) are infertile (Galloway *et al*., 2000; Hanrahan *et al*., 2004). According to Jemmali (2017), due to selection for prolificacy in Tunisian national prolific ewes programme with the aim of establishing a prolific population, the frequency of the A allele increased in the Barbarine strain. With heterozygote ewes found to have an elevated rate of ovulation (Davis, 2004) which could influence litter size and thus prolificacy, the selection for prolific ewes could have inadvertently led to genotype AA and AB being recorded. The results of this study, however, were not in agreement with the work by Mohamed *et al.* (2020) who reported monomorphism where only the genotype AA (wild type) (122 bp, 31 bp) was identified in Watish Sudanese desert sheep. Furthermore, DinÇel *et al*. (2018) only reported genotype AA (wild type for *FecX^B* mutation) in Chios (Sakiz) sheep, a breed which according to Avdi & Chemineau (1998) has a prolificacy of 2.04 ± 0.77 in Spring and 2.13 ± 0.85 in Autumn while Ligda *et al.* (2000) and El-Nakhla *et al.* (2002) both reported 1.9. Also, results from Shokrollahi (2015) in Markhoz goats and Barakat *et al.* (2017) in Egyptian sheep breeds (only genotype AA was found) reported monomorphism at the BMP15-FecX^B locus. Sithi Marjitha *et al*. (2015) reported in Nilagiri sheep, a breed that has also been reported by Chaudhari *et al*. (2019) of having prolificacy of 1.14 ± 0.07 for wild type (++) of the *FecB* gene and 1.34 ± 0.07 for B+ carriers. Karsli *et al*. (2012) also found Akkaraman and Awassi sheep breeds were also monomorphic. These breeds have prolificacy of 1.27 (Güngör *et al*., 2019) in the Akkaraman and a prolificacy range of 1.02 – 1.12 (Juma and Alkass 2005) in the Awassi sheep breed. The Sahel breed has a prolificacy between 1.01 – 1.13 (Mbah *et al*., 1988; Wilson 1988) and the Djallonké breed is 1.15 (Hagan *et al*., 2022). It is imperative to further investigate the

effect of these polymorphisms on the prolificacy of the Ghanaian breeds.

Genetic diversity, which is low in most of the populations of foreign breeds indicated above, forms the basis for resilience and persistence in any population. Reductions in population size and lack of gene flow can result in reduced genetic diversity, reproductive fitness, and a limited ability to adapt to changes in the environment thereby increasing the risk of extinction (Furlan *et al*., 2012).

The chi-square values for both populations suggest that they were not significantly different. This suggests that both populations in this study were not deviating significantly from HWE for *BMP15-FecX*^{B} gene locus. High heterozygosity observed in both populations also indicated high genetic variability.

BMP15-FecX^{G}

A 141 bp region was amplified from the *BMP15 -FecX*^{G} locus in both populations. Restriction enzyme digestion with the *HinfI* endonuclease revealed genotypes GG (141 bp) and Gg (141 bp and 111 bp) (Plate 3) with the absence of genotype gg.

Genotype GG frequency was the highest in the Sahel population, however, genotype Gg was the highest in the Djallonké population (Table 3).

The H_o and H_e values (Table 3) for the Sahel population were 0.281 and 0.241 respectively, while the χ^2 value for this population was 1.519 ($p = 0.218$). The Djallonké population, however, had H_o, H_e and χ^2 values of 0.548, 0.398 and 5.971 ($p = 0.015$) respectively.

The absence of genotype gg in both populations in this research could be associated with reports by Hanrahan *et al*. (2004) who indicated that homozygote carriers at the *FecX*^{G} locus are known to be sterile in Belclare and Cambridge sheep. Findings from this study were consistent with studies by Chu *et al.* (2007), Elkorshy *et al*. (2013), Barakat *et al*. (2017) and Asharani *et al*. (2018) who also reported genotypes G+ (141 bp and 111 bp) and genotype ++ (111 bp). This study, however, conflicted with findings by Zhang *et al*. (2011) and Nadri *et al*. (2016) who reported all three genotypes: GG (141 bp), G+ (141 bp and 111 bp) and genotype ++ (111 bp) in Small Tailed Han sheep from Yellow River Valley of China and Mehraban and Lori sheep breeds respectively. Kasiriyan *et al.* (2009) and Singh *et al.* (2019), however, reported that the

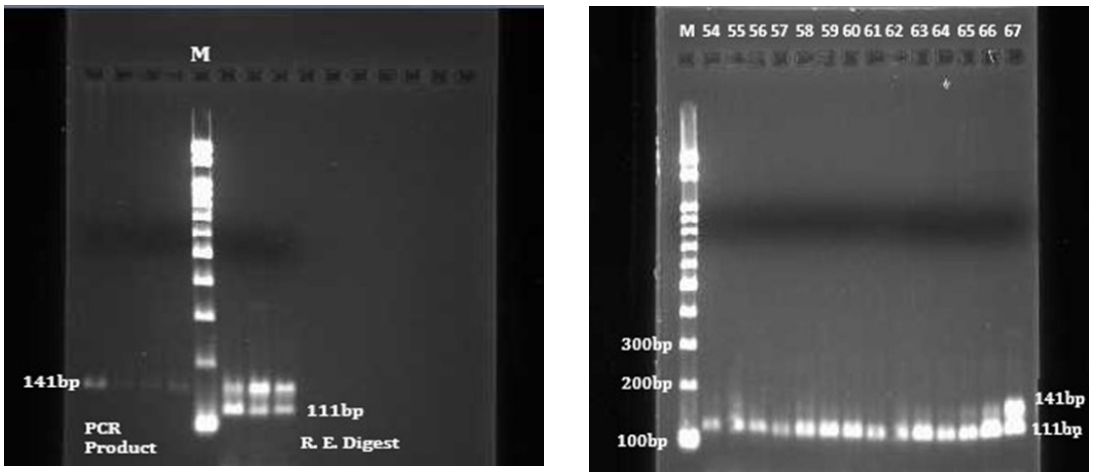

Plate 3 Left: Agarose gel image for PCR product of *FecX*^{G} showing amplification of 141 bp (left of DNA ladder). M: 100 bp DNA ladder. Right: Gel image of restriction digest of the amplified region of Sahel and Djallonké *BMP15-FecX*^{G} locus. Lanes 66 & 67: Gg, Lane 54 – 65: GG genotypes

*Hinf*I PCR-RFLP assay revealed only the GG genotype (141 bp) in Muzzafarnagari sheep and Sangsari sheep breeds, thus finding these breeds to be monomorphic. Polley *et al.* (2010), Sithi Marjitha *et al*. (2015), Bahrami *et al*. (2018), DinÇel *et al.* (2018) and Pineda *et al.* (2018) found only the ++ genotype in the Garole breed, Nilagiri, Hisari, Chios and Colombian Creole breeds, respectively.

The higher allele G frequency recorded in both populations which could be attributed to the absence of the gg genotype was consistent with findings by Chu *et al*. (2007), Zhang *et al*. (2011), Barakat *et al*. (2017) and Asharani *et al.* (2018) who all reported higher allele G frequency. Findings from this study, however, were not in agreement with Elkorshy *et al*. (2013) and Asharani *et al*. (2018) who obtained higher allele g frequency.

The high genotype Gg frequency in the Djallonké population obtained in the study was consistent with results reported by Chu *et al*. (2007), Zhang *et al*. (2011), Elkorshy *et al*. (2013) and Barakat *et al*. (2017). Reports from Hanrahan *et al*. (2004) indicated that Belclare and Cambridge sheep homozygote recessive at the $FecX^G$ and $FecX^B$ loci were found to be sterile, however, heterozygote individuals had higher ovulation rates.

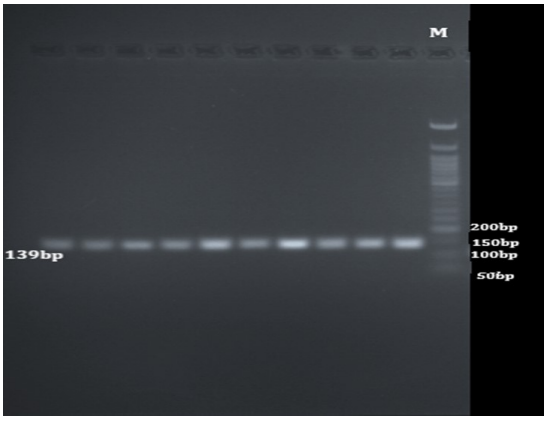

Plate 4: Gel image for PCR product of $FecG^H$ showing amplification of 139 bp. M: 50 bp DNA ladder.

The χ^2 values obtained suggest Sahel population was in Hardy-Weinberg equilibrium whereas the Djallonké population significantly deviated from HWE. There was genetic variability in both populations based on the observed and expected heterozygosity.

Growth Differentiation Factor 9 - *$FecG^H$*

The PCR products of the *GDF9-$FecG^H$* locus with a size of 139 bp (Plate 4) digested with *Dde*I showed two genotypes AA (108 bp and 31 bp) and AB (139 bp, 108 bp and 31 bp) were present in both populations in this study (Plate 5).

The genotype AA frequency was higher in both populations. The Sahel population had AB frequency of 0.067 and AA frequency was 0.933. Genotype AB frequency was 0.02 and AA frequency of 0.98 in the Djallonké population.

The H_o and H_e values shown in Table 3 above for the Sahel population were 0.067 and 0.064 respectively, while the χ^2 value in this population was 0.071 (p = 0.789). The Djallonké population, however, had H_o, H_e and χ^2 values of 0.020, 0.020 and 0.005 (p = 0.942) respectively. The χ^2 values obtained in both populations suggests that both populations did not significantly deviate from HWE.

The genotypes (AA and AB) recorded in this study were also reported by Barakat *et al.* (2017) in Rahmani, Barki and Ossimi breeds in Egypt. However, these findings did not agree with

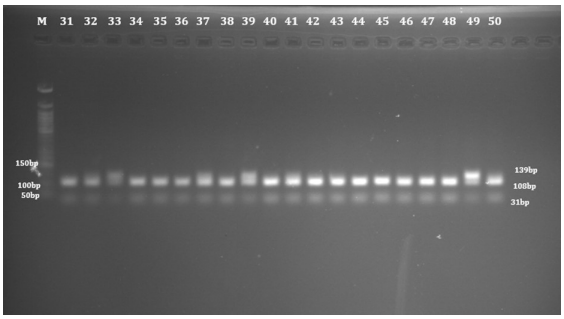

Plate 5: RFLP analysis of the amplified region of Sahel and Djallonké for *GDF9-$FecG^H$* locus digested with *DdeI*. Lane M: 50 bp DNA ladder. Lanes 33, 37, 39 & 48: AB, Lane 31 – 32, 34-36, 38, 40-47 & 50: AA genotypes

those of Mohamed *et al.* (2020) who recorded only one restriction fragment identified as the wild type denoted by ++ (AA in this study) with fragment sizes 108 bp and 31 bp in both single litter and multiple litter Watish Sudanese Desert ewes. The absence of the BB genotype in both populations in this study could be attributed to the fact that this genotype has been reported to be lethal or sterile (Barakat *et al*., 2017).

The higher genotype AA frequencies recorded in this study agreed with studies by Mohamed *et al*. (2020) in Watish Sudanese Desert sheep and Barakat *et al*. (2017) who reported higher genotype AA frequency in Barki, Ossimi and Rahmani sheep breeds. They indicated the genotypic frequency of AB and AA were 0.22 and 0.78 respectively for Barki, 0.46 and 0.54 for Ossimi, 0.36 and 0.64 for Rahmani. The findings in this study were consistent with reports by Barakat *et al*. (2017) and Mohamed *et al*. (2020) who indicated higher allele B frequency. Homozygous individuals of *FecG*^{H} were also reported to be sterile in the Cambridge sheep breed (Hanrahan *et al*., 2004).

The H_o and H_e values for the Sahel population were 0.067 and 0.064 respectively, while the Chi -square (χ^2) value for this population was 0.071 (p= 0.789). The Djallonké population, however, had H_o, H_e and χ^2 values of 0.020, 0.020 and 0.005 (p= 0.942), respectively. This implies that both populations were in HWE for the *GDF9-FecG*^{H} gene locus.

Calpastatin (*CAST*) gene

A 622 bp fragment was amplified from the CAST locus in both populations (Plate 6). Restriction enzyme digestion with the *Msp*I showed alleles M and allele N with NN (336 bp and 286 bp), MN (622 bp, 336 bp and 286 bp), and MM (622 bp) genotypes (Plate 6 and 7). All three genotypes were observed in the Sahel population, however, the Djallonké population had only genotypes MN and MM.

The frequency of allele N was higher than the allele M in the Djallonké population (M = 0.316 & N = 0.684) and the Sahel population (M = 0.492 & N = 0.508) in this study (Table 3).

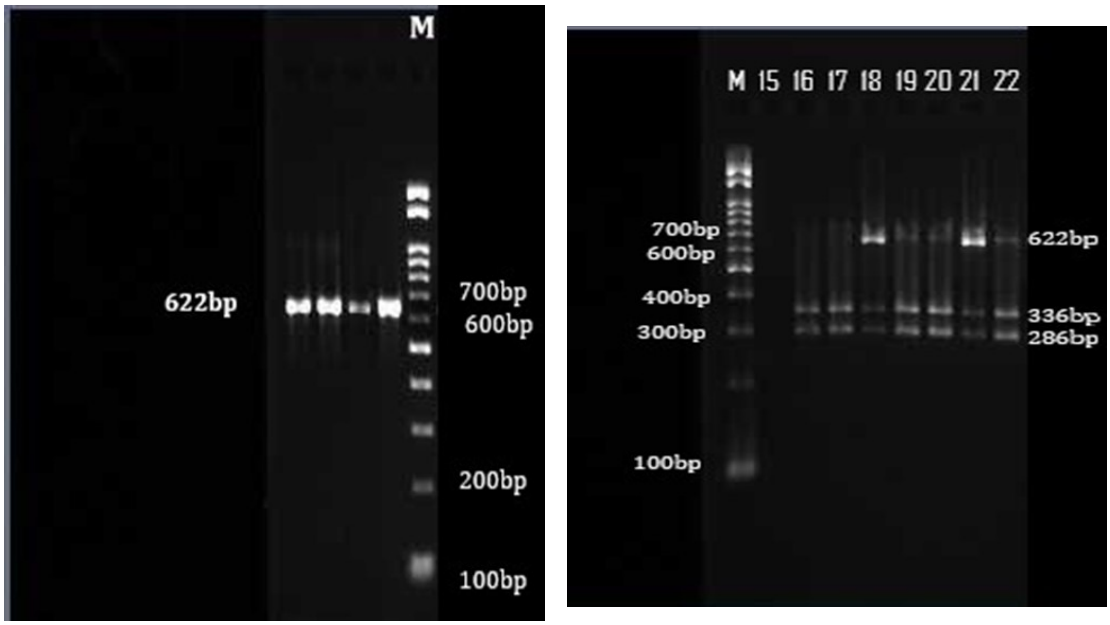

Plate 6 (left): Gel image for PCR product of *CAST* showing amplification of 622 bp. M: 100 bp DNA ladder. Right: Genotyping of CAST gene. Lane 16 and 17: NN genotype Sahels

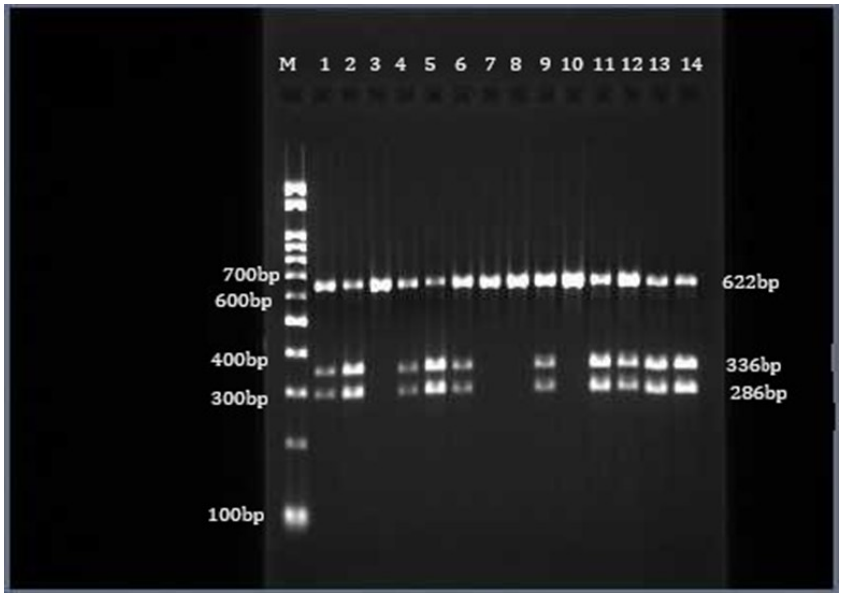

Plate 7: Gel image of *CAST* genotypes. Lane M: 100 bp ladder. Lane 3, 7, 8, 10: NN genotype Djallonké. Lane 1, 2, 4, 5, 6, 9, 11 to 14: MN genotype Sahel.

An observed heterozygosity (H_o) of 0.919 and an expected (H_e) heterozygosity of 0.5 were recorded for the Sahel population with a χ^2 value of 43.663 (p = 0). In the Djallonké population, the H_o, H_e and χ^2 values were 0.633, 0.433 and 10.490 (p = 0.001), respectively.

The observed genotypes (MM, MN, NN) in the Sahel population were consistent with studies by Palmer *et al*. (1998) in 22 unrelated Corriedale rams, Eftekhari Shahroudi *et al*. (2006) in Iranian Karakul sheep, Suleman *et al*. (2012) in Lohi and Kajli breeds and Gorlov *et al*. (2015) in Soviet Merino. Mohammadi *et al*. (2008) in Arabic sheep and Asadi and Khederzadeh (2015) in Lori sheep also reported the genotypes AA, AB, and BB (corresponding with genotypes MM, MN and NN in this study). However, this study is not in agreement with study by Gábor *et al*. (2009) in Slovakian breeds and Gorlov *et al*. (2015) who worked on the Salsk breed recorded only genotype MM (fragments 336 bp and 287 bp) and genotype MN (fragments: 622 bp, 336 bp, 287 bp). The NN genotype was not present in work by Eliasi *et al*. (2005) in the Ghezel x Arkharomerino and Arkharomerino sheep breeds.

The *CAST* gene locus was found to be polymorphic in both the Sahel and Djallonké populations, which agrees with Suleman *et al*. (2012) in the Pakistani sheep breeds Lohi, Kajli and Thalli. This is however not in agreement with Bozhilova-Sakova and Dimitrova (2016) who reported the *CAST* gene locus to be monomorphic in the Bulgarian Karakachan sheep breed (only genotype MM was observed) as did Gábor *et al*. (2009) in purebred Lacaune and East Friesian and Hristova *et al*. (2015) in Bulgarian breed, Local Karnobat where only allele M was detected.

The genotype frequency of MN was found to be the highest in both populations indicating high genetic variability at the *CAST* locus within the populations and this was consistent with findings by Asadi and Khederzadeh (2015) who also found the genotype AB (corresponding with MN in the current study) frequency to be the highest in Lori sheep. The results obtained in this study, however, were not in agreement with that reported in a study by Eftekhari Shahroudi *et al*. (2006) who reported 0.61, 0.36 and 0.03 for genotypes MM, MN and NN, respectively. Another study by Mohammadi *et al*. (2008) also found

AA genotype frequency to be the highest with genotypes AA, AB, and BB (corresponding with MM, MN and NN in this study) frequencies being 0.703, 0.283 and 0.009, respectively. Findings from this study were also not consistent with work by Szkudlarek-Kowalczyk *et al.* (2011) who found the MM genotype to have the highest frequency (0.56) in the Polish Merino sheep with MN and NN having 0.40 and 0.04 respectively.

Studies have been carried out by several researchers to find out the association of these identified genotypes with phenotypic traits such as skin with back fat thickness value, average daily gain (ADG), back fat thickness value, live weight. Findings by Saeed-ul-Hassan *et al.* (2012) in Balkhi and Kajli sheep indicated that individuals carrying the NN genotype of *CAST* gene showed lower live weight averages, however, individuals carrying the MN genotype showed significantly higher average daily gain than carriers of the other two genotypes from birth to 8 months of age in the Balkhi breed. Genotype MN carriers in the Kajli population showed higher ADG than the two other genotypes from birth to 4 months of age. Yilmaz *et al*. (2014) in Kivircik sheep also reported that individuals carrying genotype NN of *CAST* gene showed lower ADG, lower back fat thickness (BT) and lower skin with back fat thickness (S+BT) values. Results from other studies conducted also showed that individuals with genotypes MM and MN recorded higher performances in terms of live weight, ADG, and weaning weight than genotype NN animals (Sutikno *et al*., 2011; Chung and Davis, 2012). These findings provide an idea of the possible association that might exist between the genotypes identified in the Djallonké and Sahel populations and performance traits.

Both populations deviated from the HWE at the *CAST* gene locus. The findings in this study did not agree with work by Avanus (2015) in the Karakul, Red Karaman, Karayaka, and Imroz breeds which were in HWE. However, the Hemsin and Kivircik populations deviated from HWE at the CAST locus.

Growth Hormone (*GH*)

Three different genotypes in both populations after restriction enzyme digestion of the 422 bp PCR amplicons (Plate 8) were revealed. These genotypes included AA (422 bp), AB (422 bp, 366 bp, 56 bp) and BB (366 bp and 56 bp) (Plate 9).

Allele A and allele B frequencies recorded were the same in the Sahel population (i.e., A = 0.5, B = 0.5) while in the Djallonké population, allele A frequency was higher (0.571) than allele B (0.429) in this study. The *GH* gene locus was found to be polymorphic in the Sahel and Djallonké populations. The genotype frequencies of AA, AB and BB recorded for the *GH* locus were

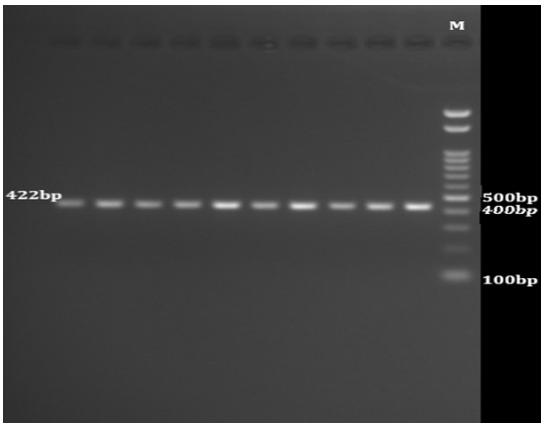

Plate 8: Gel image of *GH* locus showing amplification of 422 bp. M: 100 bp DNA ladder.

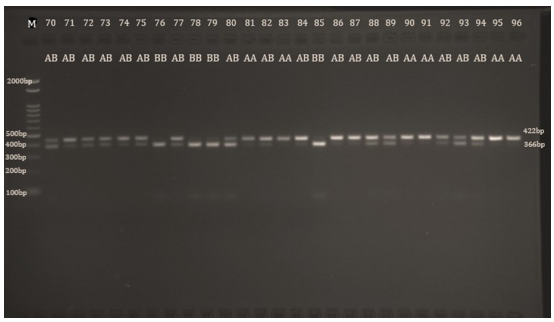

Plate 9: Gel image of *GH* genotypes. Strands with 422 bp for AA genotype (lanes: 81, 83, 90, 91, 95 & 96), 422 bp and 366 bp for AB genotype (lanes: 70-75, 77, 80, 82, 84, 86-89 & 92-94), and 366 bp for BB (lanes: 76, 78, 79 & 85). Lane M: 100 bp ladder

0.063, 0.873 and 0.063 respectively in the Sahel population and 0.204, 0.735 and 0.061 in the Djallonké population (Table 3) thus genotype AB frequency was the highest in both populations.

An observed heterozygosity (H_o) of 0.873 and expected (H_e) heterozygosity of 0.5 were recorded for the Sahel population with χ^2 value of 35.063 ($p = 0$). For the Djallonké population, the H_o, H_e and χ^2 values were 0.735, 0.49 and 12.25 ($p = 0$).

The recorded genotypes in this study were consistent with results from studies by Malewa *et al.* (2014) in East Java and Donggala sheep, Abd Al -Muhsen *et al*. (2018) in Nuimi and Awassi sheep, Mahdi *et al*. (2018) in Awassi, Hamdani, and Karadi sheep breeds in Iraq. The results, however, are not consistent with findings by Othman *et al*. (2015) who showed two genotypes in Egyptian small ruminants which were identified as GG (366 and 56 bp), and AG (422, 366 and 56 bp) (corresponding with BB, and AB, respectively in this study). Kumari *et al.* (2014) reported only genotype AA (366 bp and 56 bp) (corresponding with BB in this study), and genotype AB (422 bp, 366 bp and 56 bp) at *GH-Hae*III (A781G) locus in native Indian sheep breeds.

The equal A and B allelic frequencies in the Sahel population found in this work were not in agreement with work by Mahdi *et al.* (2018), however, the higher allele B frequency in the Djallonké population agreed with their findings in all three breeds which are Hamdani (A= 0.45, B=0.55), Awassi (A= 0.15, B= 0.85) and Karadi (A= 0.20, B= 0.80). A high allele A frequency was, however, reported by Jia *et al.* (2014) in Tibetan sheep (A= 0.8532, B= 0.1468), Small Tail Han (A= 0.6607, B= 0.3393) and 1.00 in both German Merino and Polled Dorset sheep.

Genotype AB frequency of 0.735 recorded in the Djallonké population for instance could be because 78.3% (36) of the animals sampled were from the Ejura Sheep Breeding Station where selection of breeding animals is based on individuals with high growth trait parameters (e.g., birth weight, weaning weight, etc.). This may have inadvertently led to a selection bias favouring the AB genotype, however, further studies on the association of these identified genotypes with growth traits is required.

Malewa *et al.* (2014) indicated in their study that animals with genotype AA had significantly higher weaning weight compared to genotype BB while animals with genotype AB recorded no significant differences in weaning weight in the breeds in their study. Analysis by Hua *et al*. (2009) of *GH gene* polymorphism also indicated that genotype AA had a significant decrease in girth of birth chest and weaning weight compared to genotype AB carriers. Al-Muhsen *et al*. (2018) and Malewa (2014), however, reported that genotype AA individuals had significantly higher weaning weights than genotype BB. Awassi breeds with genotype aa (corresponding with genotype BB in this study) were found to have higher birth weight and weaning weight values, however, genotype Aa (AB in this study) recorded lower birth weight and weaning weight values (Al-Salihi *et al*., 2017; Abd Al-Muhsen *et al*., 2018).

The high observed heterozygosity value recorded in both populations suggests a high level of genetic variability within the two populations. The χ^2 values in both populations also suggest that the populations deviated significantly from HWE.

CONCLUSION

Three genotypes were revealed at the *CAST gene* for the Sahel population but only two genotypes in the Djallonké population. At the *GH* gene locus, three genotypes were obtained in both populations with genotype AB recording the highest frequency. Two genotypes were revealed at the $FecX^B$ gene, $FecX^G$ gene and $FecG^H$ gene loci. The populations under this study were found to have deviated from HWE at the $FecX^B$ gene locus, $FecG^H$ locus and the Sahel population at the $FecX^G$ locus.

All the selected candidate genes in this study were found to be polymorphic in both the Sahel and Djallonké populations sampled across the country, indicating a substantial level of genetic variability in both populations at the loci analyzed. The results of this work will necessitate further studies to ascertain the association of these polymorphisms with traits of economic importance.

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