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Polymorphism of Prolificacy Genes (BMP15, BMPR 1B and GDF9), in the Native Goat (Capra hircus) of Cameroon

Wouobeng P*[1]. Kouam J. S[1]. Meutchieye F[1]. Yacouba M[1]. Achieng G[2]. Tutah J[2]. C. Mutai C[3]. Agaba M[4].

1 The University of Dschang, Faculty of Agronomy and Agricultural Sciences, Department of Animal Productions, Cameroon
2 Interns from the University of Nairobi and Jomo Kenyatta University of Agriculture, Kenya
3 BecA-ILRI Hub, Nairobi, Kenya
4 The Nelson Mandela African Institute of Science and Technology, Arusha, Tanzania

***Corresponding Author**: Patrick Wouobeng. pat3k.wouobeng@gmail.com
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Abstract:
The main objective was to contribute to a better understanding of molecular characteristics of the local goat in order to improve its productivity and specifically to: analyse genetic polymorphism of three prolificacy genes (BMP15, BMPR1B, and GDF9) and test the association of genetic polymorphism and prolificacy of local goats. Tissue samples were collected from 446 animals, and 24 representative female goats were selected to analyse the genetic polymorphism of the prolificacy genes. The selected goats were divided into two groups of 12 females for high prolificacy (more than three kids consecutively in four parity) and 12 females for low prolificacy (less than two kids consecutively in four parity). Chi-square was used to test the association between genetic polymorphism and prolificacy of local goat. The main results showed that BMP15 gene is monomorphic, whereas the two other genes (BMPR1B and GDF9) display polymorphism. For BMPR1B gene, the ten mutations found did not change the corresponding amino acid. Allelic and genotypes frequencies of mutations of this gene varied from one mutation to another and between the two groups of females (high and low prolificacy). Chi-square test of the polymorphism of this gene shows that C34T and A120G mutations of exon 3 are significantly associated ($p < 0.05$) with prolificacy and can be considered as potential genetic markers for improving prolificacy in the native goat. For the GDF9 gene, three mutations were detected in exon 1 with alleles A and G1 of frequency 0.261 and 0.130 for A35G; G2 and C1 of frequency 0.696 and 0.304 for G81C; then G3 and C2 of frequency 0.696 and 0.304 for G255C. The mutations G81C and G255C appeared under BLAST and were missense mutations P27A and A85G respectively while A35G is located in the non-translated 5' region of the gene. Chi-square test between each genotype for any site and the prolificacy was not significant ($P > 0.01$) suggesting that these two characters are not associated. Two mutations were detected in exon 2 at C881T and A1160G sites with C and T and A and G alleles respectively. The two mutations changed the corresponding amino acid from Alanine to Valine at the position 273 in the protein and from Valine to Isoleucine at the position 397 in the protein respectively. Allelic and genotypes frequencies of mutations varied from one mutation to another and between the two groups of females (high and low prolificacy). Chi-square test of the polymorphism shows that, although C881T and A1160G mutations were not significantly associated ($P > 0.05$) with prolificacy, the alleles responsible for the variation of amino acid increased the litter size. Therefore, further studies with increased sample size will help to verify the results.
Keywords: Prolificacy, Litter size, Native goat, Polymorphism, BMP15, BMPR1B, GDF9.

Introduction

Indigenous breeds can adapt and survive in challenging environments. They adapt to a variety of ecological areas and thus represent a valuable genetic resource for livelihood of rural inhabitants (Anderson, 2003). Most food production systems depend heavily on the utilization of locally adapted animal species. However, the common agricultural species kept in most regions include sheep, goats, cattle, horses, pigs, and chicken (FAO, 1994; FAO, 1999).

Goat (*Capra hircus*) is considered the most prolific of all ruminants already domesticated, especially in harsh climatic conditions (Yadav, A., Yadav, B. R., 2008). Goat (*Capra hircus*) is considered the most prolific of all ruminants already domesticated especially in harsh climatic conditions. This is due to their ability to adapt to different environmental conditions and nutritional fluctuations, disease resistance and the ability to survive in low-input systems (Fajemilehin and Salako, 2008; Serrano et al., 2009). Globally, goat farming is one whose number has increased the most over the last twenty years (FAOSTAT, 2010). Africa is a significant goat farming area that holds about a third of the global number (Rege, 1994).

In sub-Saharan Africa, indigenous breeds of goats are more important than cattle for the small-scale farmers since they are easier to acquire and to maintain. Their role often includes the provision of traction, a source of manure, a secure form of investment, a source of savings and insurance (Kunene et al., 2009; Chenyambuga, 2002). Goat meat is also consumed in many regions of the world and in developing countries, goat is even considered as one of the important economic sources of meat (Daniel et al., 1996).

Improving the reproductive efficiency of goat herds in these countries, can increase the efficiency of kid's production (prolificacy) and consequently goat meat (Anous et al., 2009). However, improvement of reproduction by traditional selective breeding methods has proved to be difficult due to the low heritability only about 0.152 and long reproductive cycle (Zhang et al., 2009; Sun et al., 2010; Wang et al., 2012). Molecular breeding through marker-assisted selection is, for this reason, the only effective way to alleviate the inefficiency and long cycle length of traditional breeding. Molecular breeding can shorten the time of new improved variety from 8-10 generations to 2-3 generations (Wang et al., 2012).

Some major genes affecting litter size have been successfully identified in sheep breeds, such as Bone Morphogenetic Protein ReceptorType 1B (BMPRIB) (Fogarty, 2009), Bone Morphogenetic Protein (BMP15) (Chu et al., 2007) and Growth Differentiation Factor (GDF9) (Hanrahan et al., 2004; Vacca et al., 2010), which made a high acceleration of the breeding improvement of litter size. However, there is very little information about these major genes on goats. Therefore, the identification of the genes responsible for prolificacy in goat is also of importance to the goat industry.

The objective was to investigate the polymorphism of exons of the three prolificacy genes and test the association between genes polymorphism and level of prolificacy.

Materials and Methods

Study area

This study was conducted in the Western Highlands and the Bimodal rainfall Forest agroecological zones of Cameroon (Figure 1) situated between 2°6'' and 6°36'' North latitude and 9°18'' and 16°12'' East longitude. This study area included 33 divisions in 5 Regions (Centre, South, East, West, and North-West).

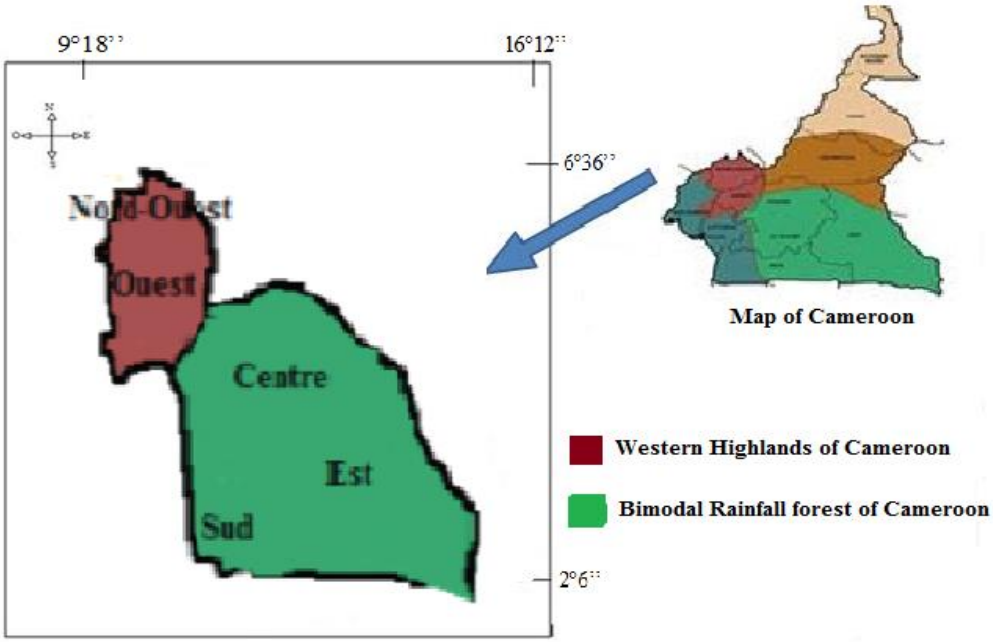


Figure 1: Bimodal rainfall forest and the Western Highlands zone (Source: MINEPIA, (2010); FAO, (2008))

Data collection

Tissue samples of 446 female goats were collected. The tissue samples of individual animals were taken from the ear by tissue puncher tubes. Once collected, the tissues were placed in a 1.5ml Eppendorf tube containing absolute ethanol (80%), and were stored at room temperature and transported to the laboratory with all necessary precautions for analysis.

Molecular experimental procedures reported in this study were carried out on 24 representative animals according to the prolificacy trait and belonging to 24 farms, from the two agroecological zones, with data on litter size in the Current, Previous, Second and First parity. The 24 animals were grouped into two: 12 high prolific (3-6 kids consecutively per parity) and 12 low prolific (1-2 kids consecutively per parity).

DNA extraction

Genomic DNA was extracted by the saturated salt protocol provided by BecA-ILRI hub Laboratory and kept at - 20° C. DNA concentration and purity were evaluated by determination of the spectrophotometric absorbance at wave length λ= 260 and of the 260/280 ratio, respectively on a Nanodrop 1000. Good quality DNA having OD (optical density) ratio between 1.7 and 1.9 was used for further work.

Microsatellite markers, genotyping and PCR conditions

In the present study, eight primer pairs were initially designed at the BecA-ILRI hub laboratory using the primer 3 software (http://frodo.wi.mit.edu/primer3/). Each primer corresponding to one exon of each gene. These Primers were designed from nucleotide sequence GenBank accession number JQ350891.1 (BMP15), AF357007 (BMPR1B) and EF446168.2 (GDF 9). The characteristics of all the primer pairs are described in Table 1.

Table 1: Primer sequences used for PCR.

Gene	Amplified region	Primer name	Primer sequence (5'-3')	Size (pb)
BMP 15	Exon 1	bmp15_exon1_F	CCC ACC TGC TGT TTC TGT TT	20
		bmp15_exon1_R	GCA ATG TGA AGC CTG ACA GA	20
	Exon 2	bmp15_exon2_1_F	AGG GCT GCT TGT CAG TTT GT	20
		bmp15_exon2_1_R	GGG GAG AGC ACT TGG GTT GA	20
GDF9	Exon 1	gdf9_exon1_1_F	CAA GGG CCA ACT CCT TTA TG	20
		gdf9_exon1_1_R	CTA GCC CAC CCA CAC ACC TA	20
	Exon 2	gdf9_exon2_F	CAA GGG CCA ACT CCT TTA TG	20
		gdf9_exon2_R	CTA GCC CAC CCA CAC ACC TA	20
BMPR1B	Exon 2	bmpr1b_exon2_F	GCT AAA TAC TGA CCC ATCA	20
		bmpr1b_exon2_R	TTG GCA CAG AGT ACA AGAGC	20
	Exon 3	bmpr1b_exon3_F	TTG ACT TGA TGG AGT ACC TG	20
		bmpr1b_exon3_R	GTA GCT TCA TTC TGC TTG TC	20
	Exon 4	bmpr1b_exon4_F	GTG CCT GAA AGA GAC TCA G	20
		bmpr1b_exon4_R	GTA GCT TCA TTC TGM TTG TC	20
	Exon 10	bmpr1b_exon10_F	GAT CTG CAC TCT CTG TTK AT	20
		bmpr1b_eon10_R	GCT TGC ACT CAG TCA AAT AC	20

Genomic DNA was amplified in 20µl reaction volume. For the reaction, 1 µl genomic DNA was amplified with 0.8 µl of each primer, 0.3 µl BSA, 0.3 µl Hidi, 16.8 µl water and 1.8 µl of initial premix. The genomic DNA was genotyped using the Sanger platform.

The Polymerase Chain Reaction (PCR) thermal conditions, performed on a PCR machine, consisted of an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at different temperature and different time by the primer (60 °C for 40 seconds and 56° C for 40 seconds), extension at 72°C for 90 seconds, and concluded with a final extension step at 72°C for 7 minutes on Mastercycler. The PCR product (20µl) was digested with Hidi restriction enzyme at 37°C for 2 h, and the resulting products were separated on a 1.5% agarose gel for 45 minutes, ethidium bromide was added to increased luminescence during visualisation and detected by UV transilluminator. The gels consisted of 0.5×Tris-borate-EDTA (TBE) buffer, 8% or 10% or 12% polyacrylamide.

Sequencing and statistical analysis

The identity of DNA fragments from each genotype was confirmed by direct sequencing in both forward and reverse directions. 17µl of each PCR product was purified with the PCR Qiagen Kit and eluted in 30 µl of distilled water. Analysis of nucleotide sequences and deduced amino acid sequences was performed with Bioedit (www.mbio.ncsu.edu/BioEdit/) software, DNASTAR, and Ugene. Comparison among sequences and multiple alignments were accomplished using ClustalW software (http://align.genome.jp/).

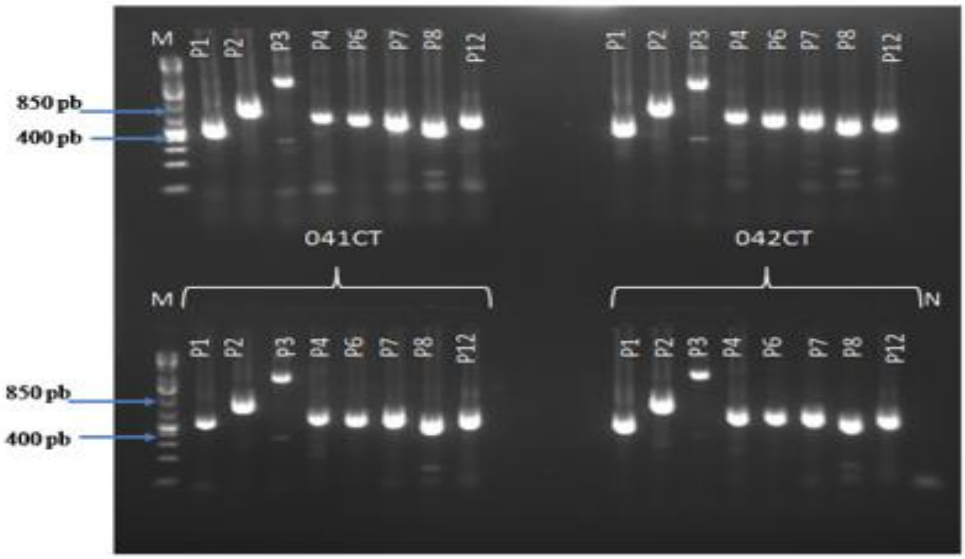
Genotypic polymorphism data were analysed with the GenePop software (http://genepop.curtin.edu.au/) and GeneScreen program (http://dna.leeds.ac.uk/genescreen/) for allele and genotype frequencies. The test of association between gene polymorphism and level of prolificacy was analysed using the chi-square method.

Results

Polymorphism of BMP15 gene

Exons amplification of BMP15.

Figure 2 shows the electrophoresis image of BMP15 exon 1 and 2. Genomic DNA was amplified using a pair of primers that covered the exons sequence of the gene.



Legend: M = Marker, CT = Sample ID, N = Blank

Figure 2: Electrophoresis image of amplified exon 1 and 2 of BMP15 gene using specific primer.

The results showed that desired fragments amplified properly that is consistent with the target ones and had good specificity so that they could be sequenced directly.

Sequencing results and genotype differences of BMP15

Alignment of the sequences of the two exons between them did not reveal any mutation. Also, alignment of the sequences with the reference query protein (GenBank accession number AFH75124.1) in NCBI BLAST (tblastn) did not reveal any specific difference. So, all the tested female samples were monomorphic for the BMP15 gene.

Polymorphism of BMPR1B gene

Exons amplification of BMPR1B >

Figure 3 shows the electrophoresis images of the four exons of BMPR1B gene.

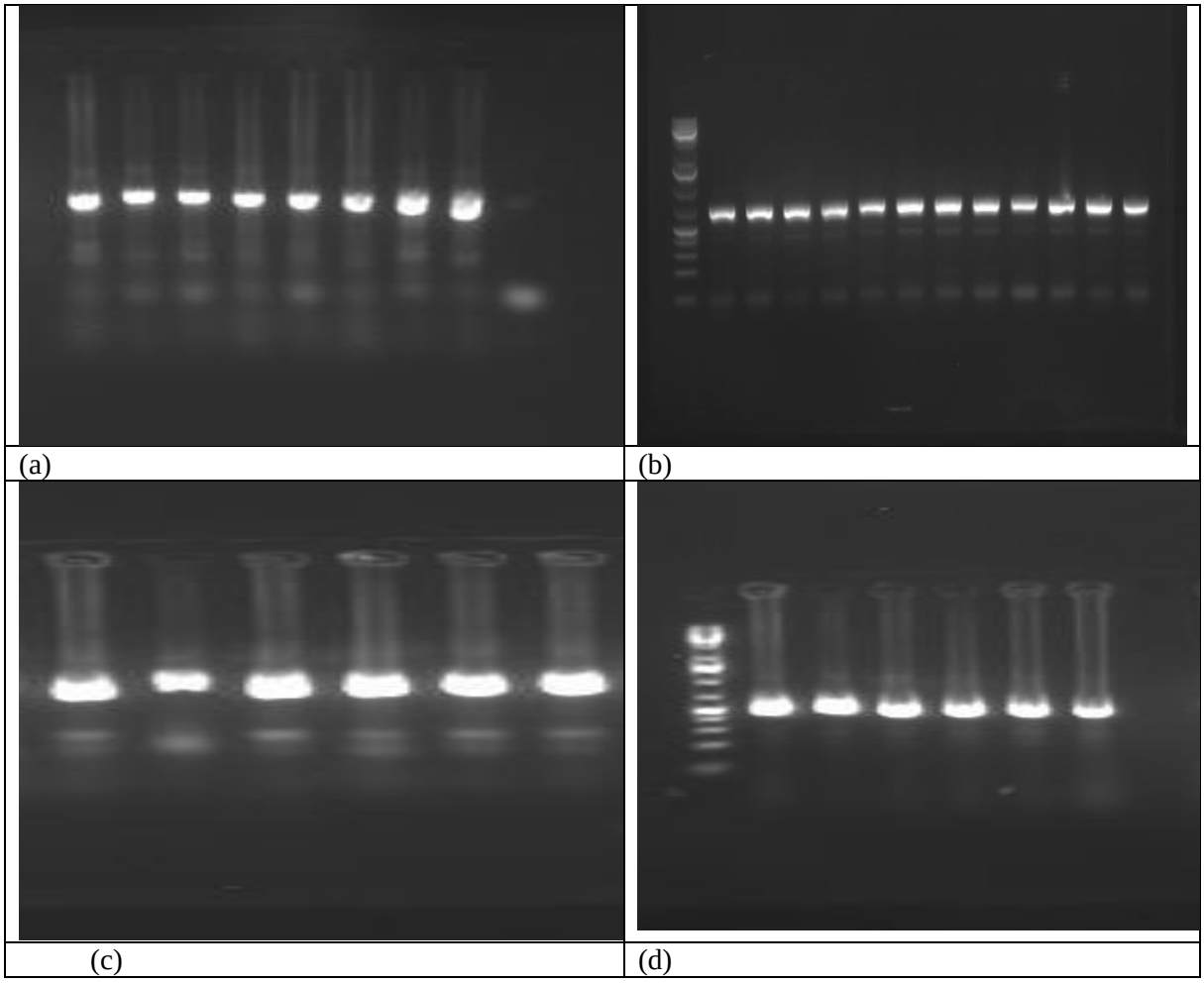


Figure 3: Electrophoresis images of amplified exon 2 (a), exon 3 (b), exon 4 (c) and exon 10 (d) of BMPR1B gene using specific primers.

Figure 3 showed that desired fragments appropriately amplified and had good specificity. Also, this Figure shows multiple bands meaning that the primers of BMPR1B gene had polymorphisms.

Sequencing results and genotype differences of BMPR1B

A total of 10 mutation sites were detected. Table 2 gives the distribution of these mutations according to exons and their impact at the level of the corresponding amino acid.

Table 2: Distribution of mutations by exons of BMPR1B.

Gene	Name of the exon	Number of mutations	Change of amino acid
BMPR1B	2	1	No
	3	4	No
	4	2	No
	10	3	No

Table 2 shows that the highest number of mutation was recorded in exon 3 with 4 mutations, followed by exon 10 with three mutations. The two others, exon 2 and exon 4 had respectively 1 and 2 mutations. Alignment of the sequences with the reference query protein (GenBank accession number AHG55161.1) in NCBI BLAST (tblastn) revealed that none of the mutations induces a change in the corresponding amino acid.

Table 3 gives the distribution of the genotype differences and the position for each mutation of BMPR1B exons.

Table 3: Different genotypes and positions of BMPR1B mutations by exons.

Exon name	Mutations	Position of the base	Different genotypes
2	1	C84T	CC and CT
3	1	C34T	CC, CT and TT
	2	A120G	AA, AG and GG
	3	C473T	CC, CT and TT
	4	C624T	CC, CT and TT
4	1	C381T	CC, CT and TT
	2	C532T	CC, CT and TT
10	1	A44G	AA, AG and GG
	2	G387T	GG, GT and TT
	3	C441T	CC, CT and TT

Table 3 shows that, sequencing of exon 2 revealed a single nucleotide polymorphism (SNP) at the coding base position of 84 (C to T). Exon 3 revealed four single nucleotide polymorphisms (SNP) at the coding base position of 34 (C to T), 120 (A to G), 473 (C to T) and 624 (C to T). Exon 4 revealed two single nucleotide polymorphisms (SNP) at the coding base position of 381 (C to T) and 532 (C to T). Exon 10 revealed three single nucleotide polymorphisms (SNP) at the coding base position of 44 (A to G), 387 (G to T) and 441 (C to T).

Chi-square analysis for BMPR1B genotype polymorphism

Analysis of the genotype polymorphism by chi-square method was done, and the test result is summarised in Table 4. Some genotype frequencies are shown to be variable ($P < 0.05$) among the two groups of females (high and low prolificacy). An average mean was calculated for these variable genotypes in order to see their effect on the litter size.

Ho: There is no association between genotypes and level of prolificacy (low and high).

Ha: Genotypes are associated with the level of prolificacy.

Table 4 shows that only the mutations of exon 3 at position 34 and 120 were significantly associated ($p < 0.05$) between the high and low prolific female goats. The other genotypes were equally distributed among high and low prolific female goats. The mean litter size according to genotypes confirmed that, for the first mutation, the female with genotype CT had 2.50 kids more than those with genotype CC and 1.23 kids more than those with genotype TT. For the second mutation, the female with genotype AG had 0.10 kids more than those with genotype AA and 2.17 kids more than those with genotype GG. These results indicated that mutations of exon 3 of BMPR1B gene could be associated with high prolificacy in Cameroon native goat of the western highland and bimodal rainfall forest zones.

Table 4: Chi-square testing of genotype polymorphism of BMPR1B gene.

Group of doe	Exon	Mutation	Genotypes	Chi-square value	P-value	Association
High prolific	Exon 2	C87T	CC	0,069	0,801	NA
Low prolific			CT			
High prolific	Exon 3	C34T	CC	6,851	0,013	A
Low prolific			CT			
			TT			
High prolific	Exon 3	A120G	AA	6,569	0,049	A
Low prolific			AG			
			GG			
High prolific	Exon 3	C473T	CC	3,733	0,155	NA
Low prolific			CT			
			TT			
High prolific	Exon 3	C623T	CC	0,110	0,740	NA
Low prolific			CT			
			TT			
High prolific	Exon 4	C381T	CC	0,202	0,653	NA
Low prolific			CT			
			TT			
High prolific	Exon 4	C532T	CC	0,002	0,964	NA
Low prolific			CT			
			TT			
High prolific	Exon 10	A44G	AA	3,069	0,216	NA
Low prolific			AG			
			GG			
High prolific	Exon 10	G387T	GG	1,209	0,546	NA
Low prolific			GT			
			TT			
High prolific	Exon 10	C441T	CC	4,579	0,101	NA
Low prolific			CT			
			TT			

A = Association and NA = No Association

Polymorphism of GDF9 gene

Exons amplification of GDF9

Genomic DNA was amplified using a pair of primers that covered exon 1 and exon 2 sequence of GDF9 gene. The electrophoresis image (Figure 4) showed that desired fragments amplified properly that is consistent with the target ones and had good specificity so that they could be sequenced directly.

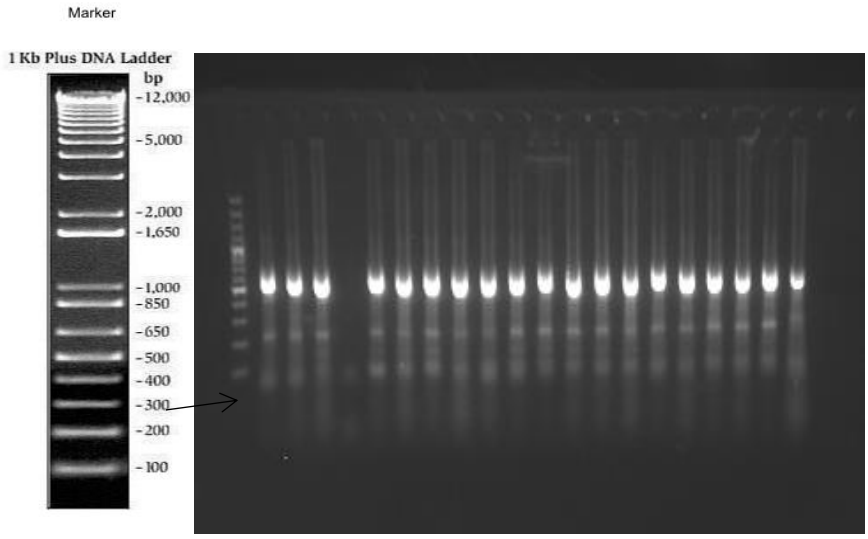


Figure 4: Electrophoresis image of amplified GDF9 gene using a specific primer.

Figure 4 shows the electrophoresis image of GDF9 gene. Genomic DNA was amplified using a pair of primers that covered the exon 2 sequence of GDF9 gene.

Sequencing results and genotype differences of GDF9 gene

A total of five mutations sites were detected. Table 5 gives the distribution of the genotype differences, the position for each mutation and their impact at the level of the amino acid. This table (table 5) shows that sequencing of exon 1 revealed three single nucleotide polymorphism (SNP) at the coding base position of 35 (A to G), 81 (G to C) and 255 (G to C). Sequencing of exon 2 revealed two single nucleotide polymorphism (SNP) at the coding base position of 881 (C to T) and 1160 (G to A).

Table 5: Distribution of mutations of GDF9 gene.

Gene	Name of exon	Number of mutations	Position of the base	Different genotypes	Change of amino acid
GDF9	1	3	A35G	AA, AG and GG	No
			G81C	GG, GC and CC	Yes
			G255C	GG, GC and CC	Yes
	2	2	C881T	CC and CT	Yes
			G1160A	GG, GA and AA	Yes

Table 5 also shows that alignment of the sequences with the reference query protein (GenBank accession number AEW47006.1 and AEM23949.1) in NCBI BLAST (tblastn) revealed that some of these Single Nucleotide Polymorphisms induce a change at the level of the corresponding amino acid.

The first nucleotide change did not cause any replacement at the level of amino acid. The second nucleotide leads to a replacement of Proline with Alanine at position 27 (P27A) of the protein. The third nucleotide caused replacement of Alanine with Glycine at position 85 (A85G) of the protein. The fourth

nucleotide change caused replacement of Alanine with Valine at position 273 (A273V) in the coding residue. The fifth nucleotide caused replacement of Valine with Isoleucine at position 396 (V396I) in the coding residue.

Inspection of exon 2 of GDF9 gene showed two mutation sites. The first is at nucleotide No. 881 (C881T) with a change in amino acid from Alanine to Valine (A273V) and the second is at nucleotide No. 1160 (A1191G) with a change in amino acid from Valine to Isoleucine (V397I). Looking closely at two of the ten SNPs identified so far in sheep GDF9 gene (Table 6), the second mutation (A1160G) in Cameroon native goat with a change from Valine to Isoleucine can be comparable to G6 (A994G or V332I) mutation in sheep identified by Hanrahan et *al.*, (2004).

Table 6: Major mutations of GDF9 gene identified in sheep.

	Base change	**Coding base (bp)**	**Coding residue (amino acid)**	**Mature peptide residue (amino acid)**	**Amino acid change**	**Reference**
G1	G to A	260	87	-	Arg to His	Hanrahan et *al.* (2004)
G2	C to T	471	157	-	Unchanged Val	-
G3	G to A	477	159	-	Unchanged Leu	-
G4	G to A	721	241	-	Glu to Lys	-
G5	A to G	978	326	8	Unchanged Glu	-
G6	G to A	994	332	14	Val to Ile	-
G7	G to A	1111	371	53	Val to Met	-
G8	C to T	1184	395	77	Ser to Phe	-
FecGSI	T to G	1034	345	27	Phe to Cys	Melo et *al.* (2008)
FecTT	A to C	1279	427	109	Ser to Arg	Nicol et *al.* (2009)
A152G	A to G	152	51	-	Asn to Asp	Li et *al.* (2003)
T692C	T to C	692	231	-	Leu to Thr	Gao (2007)

Association between genotype polymorphism and Prolificacy

Analysis of the genotype polymorphism by chi-square method was done, and the result is summarised in Table 7. For the mutations causing a changed at the corresponding amino acid, least-

squares means were calculated for those genotypes in order to see their effect on litter size (Table 8 and Table 9).

Table 7: Chi-square testing of genotype distribution of GDF9 gene.

Group of doe	Exon	Mutation	Genotypes	P-value	Association
High prolific	Exon 1	A35G	AA	0.261	NA
Low prolific			AG		
			GG		
High prolific	Exon 1	G81C	GG	0.522	NA
Low prolific			GC		
			CC		
High prolific	Exon 1	G255C	GG	0.522	NA
Low prolific			GC		
			CC		
High prolific	Exon 2	C881T	CC	0,178	NA
Low prolific			CT		
High prolific	Exon 2	A1160G	AA	0,173	NA
Low prolific			AG		
			GG		

A= Association; NA= No Association

Table 7 shows that none of the genotypes of GDF9 were significantly associated ($p > 0.05$) with the level of prolificacy. A low sample size of females can explain these results. Therefore, for economic reasons, only 24 females were used.

For C881T mutation, animals with CT genotype had a change in their amino acid, and the frequency of this genotype responsible for the amino acid change was higher (0.42) in the group of high prolific animals. The mean litter size by genotypes was 3.4 (SD=1.40) and 2.29 (SD=0.97) for CT and CT genotypes respectively.

Table 8: Least squares means and standard error for litter size of CC and CT genotypes of GDF9 gene exon 2.

Genotype	Observation (does)	Mean of litter size	Std. Deviation
CC	17	2.29	± 0.97
CT	7	3.43	± 1.40

As shown in Table 8, the female with genotype CT had 1.4 kids more than those with genotype CC. The results indicated that allele T was significantly correlated with high prolificacy in Cameroon native goat.

Table 9: Least squares means and standard error for litter size of AA, AG, and GG genotypes of GDF9 gene exon 2.

Genotype	Observation (does)	Mean of litter size	Std. Deviation
AA	3	2.33	± 1.16
AG	10	2.80	± 1.03
GG	11	2.55	± 1.44

For A1160G mutation, animals with AA and AG genotype had a change in their amino acid, and the frequency of this genotype responsible for the amino acid change was higher (0.17 and 0.50 respectively) in the group of high prolific animals. The mean litter size by genotypes was 2.33 (SD=1.1), 2.83 (SD=1.03) and 2.55 (SD=1.44) for AA, AG and GG genotypes respectively. As shown in Table 9, the female with genotype AA and AG had 2.8 kids more than those with genotype GG. The results indicated that allele A was significantly correlated with high prolificacy in Cameroon native goat.

Discussion

In this research, Cameroon native goat BMP15, BMPR1B, and GDF9 gene exons were sequenced for the first time. Several genes are defined to have associations with fecundity in some animals.

Goat breeding is just like sheep; we also need to find out the key mutations in the prolificacy genes and know how these mutations affect reproduction and how to increase the reproductive capability including reproductive seasonality and litter size, which will be a rapid and economical method to improve the goat breeding speed.

Some of the prolificacy genes were studied in goats (Hua, et al., 2008; Ran et *al*., 2009; Arefnezhad et al., 2010; Ren et *al*., 2010 and Hadizadeh et *al*., 2014;) and it is determined that their influence in prolificacy of goats were not as significant as in sheep.

Polymorphism of BMP15, BMPR1B, and GDF9 gene was studied by a simple PCR followed by sequencing. This technic was also used by Ran et *al*. (2009), Ren et *al*. (2010) and Yosefabad et *al.* (2011) to study the polymorphism of these same genes.

Exon 1 and 2 of BMP15 gene are monomorphic for all the tested samples in the study area. This result is comparable to those of Arefnezhad (2007) and Hamid et *al.* (2009) cited by Alakilli et *al.* (2012) respectively on Markhoz goats and Iranian local goats and also comparable to those of Polley et *al.* (2009) on Black Bengal goats. Similar results were also observed by Hua et *al.* (2008) and by Palai et *al*. (2012) who mentioned the absence of FecXI, FecXH, FecXB, et FecXG mutations on Boer, Haimen, Huanghuai, Nubi, Matou, Raighar and Boer-Huanghuai crossed breeds of goats. So, it appears as in many other breeds that, BMP15 gene may not be the factor responsible for the variability of types of birth observed on goats of the western highlands and bimodal rainfall forest zones of Cameroon. However, contradictory results have been observed in other breeds. Polymorphism of BMP15 gene was reported in many studies and having or not a significant effect on the litter size. On the White goat of Guizhou, Lin et *al*. (2007) reported an S99I mutation, only present on Does having three kids per kidding. This mutation was then confirmed by Ran et *al*. (2009) who revealed four other mutations on

this same breed. Chu et *al.* (2007a) cited by Zhu et *al.* (2013) identified two point mutations in BMP15 gene and was significantly associated with litter size of Jining Grey goats. A similar result was reported by Abdel-Rahman et *al*. (2013) on 25 Anglo-Nubian goats. Also, the study of Wang et *al*. (2011) reported four SNPs in exon 2 of BMP15 gene on Funiu white breed and Taihang black breed goats, and which seams affecting the litter size of the Funiu white goat. Recently, Feng et *al.* (2014) suggested that the mutation S32G of the amino acid could be responsible for prolificacy on Matou breed and also on Jining Grey breed. Nevertheless, the contribution of BMP15 gene on prolificacy remain with doubt and still need to be master.

BMPR1B gene polymorphism results showed ten mutations: C87T (exon 2), C34T, A120G, C473Tand C624T (exon 3), C381T and C532T (exon 4), A44G, G387T and C441T (exon 10). None of the ten mutations changed the corresponding amino acid, so all were silent mutations. The study of five Chinese native goat breeds (Jining Grey, Anhui White, Wendeng Dairy, Liaoning Cashmere and Beijing native goats) had no Q249R mutation (Chu et *al*., 2006). The present study identified ten mutations which are not similar to what was obtained by other authors. None of the mentioned mutations above was recorded in the Cameroon native does. The FecB mutation (A746G or Q249R) had no significant influence on litter size in Jining Grey, Boer, Anhui White, Wendeng Dairy, Liaoning Cashmere or Beijing native goats (Chu et *al*., 2006). The results in the present study are not similar to the results mentioned above.

GDF9 gene has two exons. The available literature reports very little on the polymorphism of exon 1 of goats, the essential of studies are on exon 2. Three SNPs A35G, G81C, and G255C were identified during this study. None of the mutations is identical to those observed in the available literature. Wang et *al*. (2011), Hanrahan et *al*. (2004) reported a mutation site on exon 1 respectively on goat and sheep. Yosefabad et *al.* (2011) observed a silence mutation on exon 1 of GDF9 gene of Markhoz goat. These results are therefore contradictory to those of Polley et *al*. (2009) who reported a monomorphism on exon 1 on Black Bengal goats tested with the G1 (R87H) mutation of sheep. None of the mutations in exon 1 has been reported so far to be significantly associated with prolificacy on mammals. Also, according to Zhang et *al*. (2012), the structure, the mechanisms of action and the control of important functions of genes have not been totally clarified.

Four mutations G423A, A959C (Q320P), C881T (A273V) and G1189A (V397I) in exon 2 of the GDF9 gene had been detected extensively in several goat breeds. Mutation G423A was detected in Jining Grey, Liaoning Cashmere and Boer goats (Wu et *al*., 2006; Feng et al., 2011), Wendeng Dairy and Beijing native goats (Wu et *al.*, 2006) and Guizhou White goats (Feng et *al.*, 2010). Mutation A959C was found in Yangtse River Delta White and Huanghuai goats (Zhang et *al*., 2008), Jining Grey, Liaoning Cashmere, and Guizhou White goats (Feng et *al.*, 2011) and Boer goats (Zhang et al., 2008; Feng et al., 2011). Mutation C881T was found in Beetal goats (Hadizadeh et *al.*, 2014); mutation G1189A was identified in Jining Grey, Liaoning Cashmere and Boer goats (Wu et *al*., 2006; Feng et *al*., 2010), Guizhou White goats (Du et *al*., 2008; Feng et *al.*, 2010), Wendeng Dairy and Beijing native goats (Wu et *al*., 2006; Feng et *al.*, 2010). In the present study, two mutations were detected in exon 2 of GDF9. The first mutation which changes the amino acid from Alanine to Valine was the same identify in Beetal prolific goats (Hadizadeh et *al.*, 2014). The second mutation A1189G which change the amino acid from Valine to Isoleucine is comparable to G6 mutation found in sheep (Hanrahan et *al*., 2004) and mutation identified in Jining Grey, Liaoning Cashmere and Boer goats (Wu et *al*., 2006; Feng et *al.*, 2010), Guizhou White goats (Du et *al.*, 2008; Feng et al., 2010), Wendeng Dairy and Beijing native goats (Wu et *al*., 2006; Feng et *al*., 2010). In this study, for the GDF9 exon 2 mutation, the means were 3.43±1.40 (n=7) for the heterozygous CT genotype and 2.29±0.97 (n=17) for the homozygous CC genotype. Does with genotype CT had 1.14 ($P < 0.05$) kids more than those with genotype CC. The result showed that GDF9 gene is either a significant gene that influences the prolificacy in Cameroon native goat in the study area or a molecular genetic marker in close linkage with such a gene. Further extensive sampling and DNA analysis would be required to verify these results. Hanrahan et *al*. (2004) found that the least-squares mean for ovulation rate of Belclare ewes with wild type and G8 mutation heterozygote were 1.92±0.28 (n=11) and 2.67±0.89 (n=1), respectively, and which of Cambridge ewes were 2.27±0.49 (n=10) and 4.28±0.31 (n=28), respectively. Ewes with G8 mutation homozygote were

infertile in Belclare and Cambridge sheep (Hanrahan et *al*. 2004). Hadizadeh et *al.*, (2014) found that goat with genotype CT had 1.43 ($P < 0.05$) kids more than those with genotype CC. The results indicated that allele T was significantly correlated with high prolificacy in Beetal goat.

Conclusion

The current study was designed to evaluate the polymorphism of Prolificacy genes (BMP15, BMPR1B, and GDF9) on Cameroon native goats. BMP15 gene was monomorphic, whereas BMPR1B and GF9 genes displayed polymorphism. Some few polymorphisms found so far in the genomes of many prolific breeds throughout the world were present in the native goat we examined especially for GDF9 exon 2. The allelic and genotypes frequencies of these mutations also varied from one mutation to the other and between the two groups of females (high and low prolificacy). Although C881T and A1160G mutations were not significantly associated with prolificacy, the alleles responsible for the variation of amino acid increased the litter size. In Cameroon native goats, the genetic factors controlling twinning, triplet, and quadruplet are related to the mutations in the Booroola gene and probably in the GDF9 gene. Further studies with the increase in the sample size are required to confirm these results.

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