MICROSATELLITE ANALYSIS OF WEST-AFRICAN DWARF GOATS IN NIGERIA: A PRELIMINARY INVESTIGATION

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ABSTRACT
This study is aimed at investigating the genetic variability of the West African Dwarf goat of South Western Nigeria. Tissue samples from sixty (60) West African Dwarf goat were genotyped using 25 Microsatellite Markers as proposed by FAO and ISAG. All the Markers were highly polymorphic with allele numbers ranging from 3 (ILSTS005) to 19 (BM6444), and in most cases above 4. High level of genetic variability was obtained as demonstrated by the values of the mean observed and expected heterozygosites (0.584 and 0.679 respectively). The positive F_{is} values obtained at 19 loci indicated some level of inbreeding within the population. The Microsatellite Markers used in this study showed their suitability for analysis of genetic variability in the West African Dwarf goat. However, further research using a larger sample size, together with the three indigenous goat breeds of the country should be considered to ensure possible improvement, conservation of their native germplasm, as well as proper policy formulation.

INTRODUCTION
The indigenous goat populations in Nigeria (West-African Dwarf, Red Sokoto and Sahel goat) have developed over the years certain valuable genetic traits, such as trypanotolerance in some breeds, their ability to survive on low quality forage, climatic stress and high tolerance to infectious diseases and parasites. Goats are important reservoirs of useful genes; hence, genetic improvement of indigenous breeds of goats is essential. According to Groeneveld *et al.* (2010), many breeds of livestock in third world countries may lose their germplasm due to extensive cross-breeding as well as uncontrolled mating in extensive management systems. To obtain better understanding of genetic variation among indigenous goats and formulate conservation policies, better knowledge of the genetic characterization of these breeds should be sought. This would enable breeders and researchers to efficiently strike a balance between genetic improvement and conservation of native germplasm of our indigenous goats. Microsatellites are ideal molecular markers for genetic characterization. They are widely used for the analysis of genetic variability within and between breeds due to their high number, distribution throughout the genome and efficacy of genotyping (Adebambo *et al.*, 2011; Okpeku *et al.*, 2011). Some effort has been directed towards generating baseline information of the genetic variation among Nigerian indigenous goats (Adebambo *et al.*, 2011; Okpeku *et al.*, 2011 and Yakubu *et al.*, 2014). Therefore, this study is aimed at examining the genetic variation within the West-African Dwarf goat in Nigeria using 25 Micro-satellite DNA markers. The results of the study will provide additional preliminary data for further studies aimed at the improvement and conservation of the Nigerian indigenous goat populations.

MATERIALS AND METHODS
Tissue samples were collected from the ears of 60 West African- Dwarf goat using an Allflex ear punch tissue sample collector, and aliquoted into plastic tubes containing the Allflex tissue preservative. DNA was extracted from the tissue cells using commercial Kits (Pure Link™ K1821-0) according to the manufacturer's specifications and protocol. The following 25 Microsatellite Markers were studied: SRCRSP03, ILSTS005, SPS113, CSRD247, MAF209, McM527, SRCRSP5, ILSTS087, SRCRSP9, OarFCB304, ILSTS11, ETH10, MAF065, OarCP34, ILSTS029, INRA023, MAF70, INRA063, BM6444, OarFCB48,INRABERN172, INRABERN185, TCRVB6, SRYM18 and OarFCB20. These Microsatellite Markers were proposed by FAO and ISAG for biodiversity studies in goats. Amplification of markers was by means of Polymerase Chain Reaction (PCR) technique and

PCR products were separated by electrophoresis in an automated sequencer according to Martinez *et al* (2006). The PCR products were multiplexed and genotyped using an Applied Biosystems 3700 sequencer and bands were scored using Peakscanner(R) software (Applied Biosystems Foster City, CA, USA). Allele number, observed and expected heterozygosities, as well as Shanon and Inbreeding index were computed using GenAlEX 6.5 genetic analysis statistical package of Peakall and Smouse (2012). DNA extraction, amplification and sequencing were carried out at the International Livestock Research Institute (ILRI) Nairobi, Kenya.

RESULTS AND DISCUSSION

The number of effective alleles, observed and expected heterozygosity and fixation index for the West African Dwarf goat population are shown in Table 1. The markers used in this population were highly informative with allelic numbers ranging from 3 for ILSTS005 to 19 for BM6444, implying that these markers are suitable for genetic diversity studies in the West African Dwarf goat, as most of them had allele numbers above 4 which is the standard. The Shannon index (I) ranged from 0.236 to 2.429 and in most cases were higher than 1.0, this further reveals high level of polymorphism in the microsatellite markers used. The high polymorphic values for most of the markers are an indication of their application in parentage analysis and biodiversity studies of other goat breeds in Nigeria. The highest value obtained for the effective alleles in this population was 8.571 (SRCSP 9) while the least was 1.116 (ILSTS 005). The observed heterozygosities (Ho) ranged from 0.027 (MAF 65) to 1.000 (SPS 113), while the expected heterozygosity (He) ranged from 0.104 (ILSTS 005) to 0.883 (SRCSP 9). High level of genetic variability was obtained as demonstrated by the values of the mean observed and expected heterozygosites (0.584 and 0.679 respectively).

The inbreeding index (F_{is}) was negative at six (6) loci (ILSTS029, ILSTS087, SPS113, SRYM18, OarFCB34 and MAF209) displaying some level of out-breeding at such loci and was positive (inbred) for the remaining loci, ranging from 0.033 (OarFCB48) to 0.854 (INRABERN172). The negative F_{is} values observed at six (6) loci indicated the presences of more heterozygotes at those loci, while the remaining nineteen (19) loci with positive values suggests an heterozygote deficit, implying an inbred status of the population. The presence of negative F_{is} values at six loci suggests that homozygous deficiencies may have arisen from population sub-division owing to genetic drift, null alleles and selection against heterozygotes or inbreeding. These negative values have also been reported in other studies on goats (Agha *et al.*, 2008; Rout *et al.*, 2008; Dixit *et al.*, 2009, Rout *et al.*, 2012).

CONCLUSION

The genetic variability in the West African Dwarf goat was high as seen in the values obtained for the mean observed and expected heterozygosites (0.584 and 0.679 respectively).The panel of microsatellite markers used in this study showed their suitability for analysis of genetic variability in the West African Dwarf goat. The results will, however, warrant further research using a larger sample size together with the three indigenous goat breeds in the country. This could ensure possible improvement and conservation of the native germplasm.

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Table 1: Allelic Frequencies, Heterozygosity and F-statistics (F_{is}) in West African Dwarf goats

Locus	N	Na	Ne	I	Ho	He	F_{is}
OarFCB20	32	7.000	4.188	1.657	0.594	0.761	0.220
BM6444	60	19.000	8.036	2.429	0.783	0.876	0.105
CSRD247	56	8.000	5.733	1.876	0.732	0.826	0.113
ETH10	60	15.000	4.417	1.948	0.533	0.774	0.311
ILSTS005	55	3.000	1.116	0.236	0.073	0.104	0.300
ILSTS11	43	12.000	3.365	1.705	0.651	0.703	0.073
ILSTS029	60	11.000	2.601	1.380	0.683	0.616	-0.110
ILSTS087	60	14.000	8.072	2.227	0.900	0.876	-0.027
INRA023	41	10.000	5.086	1.837	0.634	0.803	0.211
INRABERNI185	36	5.000	1.468	0.701	0.250	0.319	0.215
INRABERN172	25	9.000	5.580	1.950	0.120	0.821	0.854
MAF65	37	4.000	1.214	0.403	0.027	0.176	0.847
MAF70	59	9.000	4.284	1.711	0.746	0.767	0.027
MCM527	60	5.000	2.374	1.171	0.267	0.579	0.539
OarFCB48	60	11.000	3.411	1.598	0.683	0.707	0.033
OarFCB304	59	13.000	3.920	1.756	0.644	0.745	0.135
SPS113	60	10.000	2.684	1.264	1.000	0.627	-0.594
SRCRSP3	60	5.000	3.216	1.294	0.550	0.689	0.202
SRCSP9	58	10.000	8.571	2.207	0.707	0.883	0.200
SRYM18	60	16.000	4.672	2.021	0.967	0.786	-0.230
TCRVB6	44	9.000	2.076	1.235	0.386	0.518	0.255
OarPC34	48	9.000	5.075	1.841	0.813	0.803	-0.012
SRCSP5	59	12.000	6.574	2.046	0.678	0.848	0.200
MAF209	60	6.000	4.162	1.480	0.767	0.760	-0.009
INRA063	58	6.000	2.530	1.176	0.414	0.605	0.316
Mean					0.584	0.679	

N=sample size, Na= no. of alleles, Ne= no. of effective alleles, I= Shannon index, Ho = observed heterozygosity, He= expected heterozygosity and F_{is} = fixation index/inbreeding coefficient.