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GENETIC DIVERSITY AMONG FULANI AND YORUBA ECOTYPE OF NIGERIA INDIGENOUS CHICKEN IN THE DERIVED SAVANNAH ZONE USING MICROSATELLITE MARKERS

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ABSTRACT

Genetic diversity of two different populations of Nigeria Native chickens of was examined by means of microsatellite markers. The populations studied are Fulani Ecotype Chickens (FEC) and Yoruba Ecotype Chickens (YEC). Five di-nucleotide microsatellite markers were investigated to study the genetic diversity and population structure of the two ecotypes. All microsatellite examined were polymorphic across the two populations. A total of 62 alleles were detected across the five loci examined with 32 alleles found in Fulani Ecotype Chicken (FEC) and 30 allele found in Yoruba Ecotype Chickens (YEC). The number of alleles ranged from 4-8 in FEC and 4-10 in YEC. The mean number of alleles (MNA) across all the microsatellite was 6.2 $\pm$ 0.696 across the population. While the mean number per population are 6.4 $\pm$0.784 in FEC and 6.0 $\pm$ 1.265 in FEC and YEC respectively. Highest number of allele was found in loci LEI0094 and MCW0216 in FEC and locus LEI0094 in YEC. Private alleles were found in loci LEI0094, ADL0268, MCW0216 and MCW0248 in both populations. The observed heterozygosity (Ho) was between 0.277 to 0.809 in FEC and 0.383 to 0.660 in YEC while the expected heterozygosity (He) was between 0.392 to 0.670 in FEC and 0.290 to 0.727 in YEC. Fis, Fit and Fst of the two populations ranged between -0.051 to 0.0105, -0.043 to 0.110 and 0.003 to 0.008 for respectively across the loci. The percentage of variation among population was 8% while among individual and within individual was 91% and 1% respectively. Nei's Genetic distance between the two populations (0.015), based on (UPGMA) the two populations are classified as being genetically close to each other. Genetic diversity across the two populations is high. There is low level of genetic differentiation between the two populations which showed absence of clear sub structuring of Nigeria indigenous Chickens from derived savannah zone.

Keywords: Diversity, Indigenous Chickens, Microsatellites, Population

Introduction

Indigenous chickens are widely popular in majority of the households in Nigeria. They constitute 80% of the poultry type raise in the rural areas of Nigeria. Indigenous Chickens provides family members with readily source of protein in terms of egg and meat (Horst 1989). They generally thrive on free range for survival with provision of grains, kitchen waste and farm residue as supplemental diet. They are known to possess inherent advantages over their exotic breed counterpart, this include resistance to different diseases, adaptation to prevailing environmental condition, ability to hatch on their own and self-subsisting. Kitalyi (1998) illustrated poultry production in Africa as being on free range indigenous birds owned by most rural families with production of over 70% of poultry products and 20% of Animal protein intake.

Despite these enormous advantages they remained largely unattended to in terms of conservation and improvement for better contribution to national economy. Characterization of genetic structure and variation of local chicken populations is an important step towards identifying their uniqueness as valuable resources (Muchadeyi *et al* 2007). Characterization of animal genetic resources therefore covers all activities associated with identification, quantitative and qualitative description, and documentation of breed population to which they are or not adapted (FAO 2000). Notter (1999), noted local farms animals are important reservoir of genetic diversity as it is essential to meet their current production needs in various environment and to make possible fast adaptation to changing breeding objectives. Characterization is imperative to safeguard the loss of genetic diversity of these farm animal which has been reported threatened in last decades . Around 22% of the world's livestock breeds are classified as being at risk of extinction due to loss of genetic diversity disgorge in population size by crossbreeding with exotic breeds (FAO 2012). The analyses of breeds' genetic structure is therefore considered imperative and supply the basis for effective conservation programs.

Molecular characterization investigates polymorphis m in selected protein molecules and DNA markers in order to evaluate genetic variation at population level as result, molecular genetic study is undertaken chiefly to explore diversity within and between animal populations and to establish genetic relationships among such populations. Microsatellite markers also known as simple sequence repeats (SSRs), Short Tandem Repeats (STRs) or Simple Sequence Length Polymorphism are tandem repeat of sequence units averagely 5bp in length (Brufort and Wayne, 1993). The repeat are generally di-, tri-, tetra or pentanuleotides with the commonest being dinucleotides repeat and are abundant in genomes of all higher organism including livestock. Micro satellites have identified as one of the useful markers in assessing genetic variability, diversity and relationships because of many advantages such as being abundant and ubiquitous throughout the genome showing higher level of polymorphism and co-dominant inheritance (Tautz, 1989). Polymorphism of microsatellites markers takes the form of dissimilarity in the number of repeats at any particular locus and is normally revealed as fragment length variation in the products of PCR amplification of genomic DNA using primers flanking the selected repeat**.** Zare et al (2002) reported due to their multialleic nature, high level of polymorphism, ease of detection of PCR, reproducibility and co-dominant inheritance, microsatellites have been widely accepted as the most useful marker for population study in livestock.

Two major ecotype Indigenous chickens are found in derived savannah zone of Nigeria and they are Yoruba and Fulani Ecotype chickens (Ige et al 2010). They are well adapted to the environment with social, economic and cultural value. There are few or no study conducted at molecular level to explore their relationship and diversity. The loss of these ecotypes would eliminate unique genetic traits that could be used for future improvement programmes and commercial exploitation, therefore their preservation should be treated as the highest priority. This study therefore investigate the use of microsatellite markers to examine the genetic variability,diversity and relationships of the ecotypes using selected marker primer obtained from database sequence of FAO.

Materials and Method

Sample Collection

Blood samples were collected from 100 indigenous chickens comprising of 50 each of Yoruba ecotype and Fulani ecotype chicken. Yoruba and Fulani ecotype chicken are most important types of Nigeria local chicken population. They are kept at Ladoke Akintola University Teaching and Research Farm whose phenotypic records have been well documented. Between 2 and 5mls of blood samples were collected from individual chickens through wing vein into tubes containing EDTA.

DNA EXTRACTION AND QUANTIFICATION

Genomic DNA was extracted from the blood samples following the procedure of Iranpur and Esmailizadeh (2010) with little modification. The procedure involved various steps of haemolysis, ethanol precipitation and final re-suspension in 50ul TE. The purity of genomic DNA was assessed by observing the ratio of A260/A280 and A260/A230 which were calculated from the spectrums of ultraviolet-visible spectroscope absorbance measurements delivered by NanoDrop,ND-1000spectrophotometer. The quality of DNA were equally checked in 0.8 % Agarose Gel electrophoresis (0.8 g of Agarose in 100ml of TAE buffer) . Resultant gel were checked under UVP, GelDoc-It 310 imaging system to observe the genomic band. The final DNA was stored at 4^{o}c for further use.

Selection Microsatellite Markers

Five microsatellite markers recommended by a joint International Society of Animal Genetics–

FAO working group for biodiversity study of chicken (http://dad.fao.org/). The selection was based on the degree of polymorphism and genome coverage. The characteristics of the marker used including the chromosome location, expected range in base pairs and annealing temperature are shown in table 1.

Table 1: Primer Information for the selected microsatellite markers

Name	DYE	Chrom o-some	Primer sequence	Annealing temp (°C)	Genebank accession Number	Allele range (bp)
ADL0268	5^1FAM	1	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	60	G01688	102-116
ADL0278	5^1HEX	8	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAAACAGTGTG	60	G01698	114-126
MCW0248	5^1HEX	1	GTTGTTCAAAAGAAGATGCATG TTGCATTAACTGGGCACTTTC	60	G32016	205-225
LEI0094	5^1HEX	4	GATCTCACCAGTATGAGCTGC TCTCACACTGTAACACAGTGC	60	X83246	247-287
MCW0216	5^1HEX	13	GGGTTTTACAGGATGGGACG AGTTTCACTCCCAGGGCTCG	60	AF030586	139-149

Polymerase Chain Reaction (PCR) Amplification and Optimization

Primers of the selected markers were synthesized with forward Primers fluorescently (HEX; Green and FAM; Blue) labelled. The primers were standardized and amplification of specific product from Genomic DNA was observed. PCR reaction conditions for single locus amplification were defined as follows:

- 2X PCR Master Mix : 5ul
- 10uM Forward Primer : 0.2ul
- 10uM Reverse Primer : 0.2ul
- 10ng Genomic DNA : 1.5ul
- distilled water : 3.1

2X PCR MasterMix contained Buffer, PCR Polymerase, dNTPs, gel loading dyes and a density reagent. The total reaction volume equals 10ul.

The PCR setting for the Thermer Cycler were as follows:

Initial Denaturation	Denaturation	Annealing	Extension	Final extension
94 ^{O}C	94 ^{O}C	58^{O}C	72 ^{O}C	72^{O}C
5min.	1min.	30S	2min.	7min
	← 35 cycles →			

Multiplexing

PCR multiplex was carried out in two phases. The first phase comprised of markers ADL0278 and LEI0094 with PCR reaction volume of 10ul containing; 5ul of 2X PCR master mix, 0.4ul of 10pmol/ul LEI0094 (Forward and Reverse), 0.6ul of 10pmol/ul ADL0278 (Forward and Reverse), 1.1ul of 10pmol/ul genomic DNA and 1.9 ul of distilled water. Second stage comprised of 5ul of 2X PCR master mix, 0.37ul of 10pmol/ul of both MCW0248 and MCW0216 (Forward and Reverse), 0.16ul of 10pmol/ul of ADL 268 (Forward and Reverse), 1.1ul of 10pmol/ul of genomic DNA and 2.1ul of distilled water.

The cycle condition was the same as above. 2% agarose gel was prepared. 2ul of the PCR products were loaded on the gel and later viewed in UV light to view the amplification at various annealing temperatures.

GeneScan and genotyping

The genotyping was based on master mix containing 9ul of Hi – Di formamide and 0.3ul of Liz – 500 (Size Standard) and 1ul of PCR Product were mixed together in a total volume of 11.3ul, denatured at 95^{o}C for 5min. And the microsatellite genotyping was performed using a genetic analyser 3130xl (Applied Biosystems, USA) and the genotyping result were obtained using Gene mapper **(ver. 3.0, Applied Biosystems, USA.)**

Statistical Analysis

Descriptive statistics was calculated to describe genetic diversity (Nei 1987) with the computation of mean number of alleles for each locus (MNA), allele frequencies and heterozygosity (expected and observed) and standard deviation using the software GenAIEx (Peakall & Smouse, 2001). Estimation of F_{is} and F_{st} statistics and analysis of molecular variance (Amova), was implemented by the same program. Polymorphic Information Content (PIC; Botstein et al; 1980) was carried using CERVUS 2.0 (Marshall et al; 1988), GenAIEx (Peakall & Smouse, 2001), to indicate variation within and between the chicken ecotypes. For clarification of the population structure of the two populations, Dendrogram was constructed by Sequential Agglomerative Hierarical Nested cluster anlysis (SAHN) based on Unweighted Pair-Group Method with an Arithmetic Average (UPGMA) accomplished by NTSYSpc software.

RESULTS

DNA QUANTITY, QUALITY AND MULTIPLEXING

The quantity of DNA extracted ranged from 65ng/ul – 50000ng/ul in FEC and 100ng/ul – 40000ng/ul in YEC. The quality as checked on 0.8 % Agarose Gel electrophoresis (0.8 g of Agarose in 100ml of TAE buffer) indicated high molecular weight DNA. Gel for multiplex of PCR products are shown in figures 1 and 2. Example of electrophoregram generated by genotyping is as presented in figure 3.

Microsatellite Marker Polymorphism

Summary of variations for each of the microsatellite markers with respect to the populations studied are presented in table 1. A total of 62 alleles were detected across the five loci examined with 32 alleles found in Fulani Ecotype Chicken (FEC) and 30 allele found in Yoruba Ecotype Chickens (YEC). All the microsatellite markers were polymorphic across the samples. The number of alleles ranged from 4-8 in FEC and 4-10 in YEC. The mean number of alleles (MNA) across all the microsatellite was 6.2 $\pm$ 0.696 across the population. While the mean number per population are 6.4 $\pm$0.784 in FEC and 6.0 $\pm$ 1.265 in FEC and YEC respectively. Highest number of allele was found in loci LEI0094 and MCW0216 in FEC and locus LEI0094 in YEC. Private alleles were observed in loci LEI0094, ADL0268, MCW0216 and MCW0248 in both populations.

The observed heterozygosity (Ho) were between 0.277 to 0.809 in FEC and 0.383 to 0.660 in YEC while the expected heterozygosity (He) were between 0.392 to 0.670 in FEC and 0.290 to 0.727 in YEC. Values of He and Ho followed the same pattern across the loci in both populations as lowest value was recorded for MCW 248 and highest value for LEI0094. The result indicated high genetic diversity in the two populations across the loci as mean He was relatively higher the mean Ho. Polymorphic Information content (PIC) value ranged om 0.376 to 0.628 in FEC and 0.268 to 0.706 in YEC with mean value of 0.5414 and 0.5266 in FEC and YEC respectively. PIC value for locus LEI0094 was highest in both populations. It consequently point out that highly informative loci were investigated in this study.

Population Diversity

Summary of F-Statistics (Wright, 1965) Fis, Fit and Fst of the two populations in relation to locus were presented in Table 2. The value raged between -0.051 to 0.0105, -0.043 to 0.110 and 0.003 to 0.008 for Fis, Fit and Fst across the loci. Positive Fis value observed in ADL278, ADL268, MCW216 and MCW 248 indicated a degree of homozygosity between the two populations, while negative value for Fis at LEI0094 indicate high heterozygosity. Total inbreeding (Fit) was relatively low across the loci for the two population, so also hetrozygote deficiency (Fit).

Summary of Chi-Square test for Hardy-Weinberg Equilibrium Test (HWE) was presented in Table 3. Significant deviation was observed from ($P<0.05$) HWE at loci ADL278, LEI0094 and MCW 216 in FEC and at loci ADL 278, ADL 268, MCW 216 and MCW 248 in YEC. While FEC was in HWE ($P<0.01$) at loci ADL 268 and MCW 248. YEC was in HWE ($P<0.01$) at only LEI0094. In addition Analysis of Molecular Variance (AMOVA) was carried out to show variation within population and between populations. The percentage of variation among population was 8% while among individual and within individual were 91% and 1% respectively. This revealed that the two populations are closely related demonstrating non-significant geographical

Table 1:Sample Size, No. Alleles, No. Effective Alleles, Information Index, Observed Heterozygosity, Expected and Unbiased Expected Heterozygosity,and Fixation Index

Pop	Locus	N	NA	NE	PIC	I	HO	He	UHE	F
P1	**ADL278**	47	4.000	2.719	0.557	1.122	0.702	0.632	0.639	-0.111
	LEI0094	47	8.000	3.026	0.628	1.434	0.809	0.670	0.677	-0.208
	ADL268	47	6.000	2.579	0.559	1.201	0.511	0.612	0.619	0.166
	MCW216	47	8.000	2.845	0.588	1.251	0.660	0.648	0.655	-0.017
	MCW248	47	6.000	1.644	0.376	0.834	0.277	0.392	0.396	0.294
P2	**ADL278**	47	4.000	3.105	0.616	1.220	0.596	0.678	0.685	0.121
	LEI0094	47	10.000	3.666	0.706	1.732	0.660	0.727	0.735	0.093
	ADL268	43	4.000	2.245	0.503	1.003	0.535	0.555	0.561	0.036
	MCW216	43	8.000	2.524	0.540	1.209	0.581	0.604	0.611	0.037
	MCW248	42	4.000	1.408	0.268	0.577	0.333	0.290	0.293	-0.150

NOTE: P1 = Fulani Ecotype Chicken (FEC), P2 = Yoruba Ecotype Chicken (YEC)

Mean and SE over loci for each populations

Pop		N	NA	Ne	PIC	I	Ho	He	uHE	F
P1	mean	47.000	6.400	2.563	0.5414	1.168	0.591	0.591	0.597	0.025
	SE	0.000	0.748	0.241	0.061	0.098	0.092	0.051	0.051	0.091
P2	mean	44.400	6.000	2.590	0.5266	1.148	0.541	0.571	0.577	0.027
	SE	1.077	1.265	0.384	0.059	0.187	0.056	0.076	0.077	0.047

Grand mean and SE over Loci and Pops

TOTAL	N	NA	Ne	PIC	I	Ho	HE	UHE	F	
Mean	45.700	6.200	2.576	0.5414	1.158	0.566	0.581	0.587	0.0.26	
SE	0.667	0.696	0.214	0.062	0.100	0.051	0.043	0.044	0.048	

NOTE: P1 = Fulani Ecotype Chicken (FEC), P2 = Yoruba Ecotype Chicken (YEC)

population structuring in Nigeria chickens in derived savannah zone.

Phylogenetic Relationship and Genetic Distance

Fig 2 illustrated dendogram generated by sequential Agglomerative Hierarchical Nested Cluster Analysis based on Unweighted Pair Group Method with Arithmatic Average (UPGMA). It is indicated that the two populations should be classified as being genetically close to each other. They were not distinctly clustered as diverse groups according to their origin supporting the reliability of the study, the genetic distance as obtained using Nei's (1978) of pairwise population matrices between the two populations was low (0.015) as compared to pairwise population matrix of Nei Genetic Identity which was very large (0.985), all pointing that the two populations are close to each other at loci investigated in this study.

Table 2: F-Statistics and Estimates of Nm over All Pops for each Locus

POPS	Locus	Ht	Mean He	Mean Ho	Fis	Fit	Fst	Nm
	ADL278	0.658	0.655	0.649	0.009	0.013	0.004	64.311
	LEI0094	0.704	0.698	0.734	-0.051	-0.043	0.008	32.141
	ADL268	0.585	0.583	0.523	0.104	0.106	0.003	96.564
	MCW216	0.631	0.626	0.620	0.009	0.017	0.008	31.993
	MCW248	0.343	0.341	0.305	0.105	0.110	0.005	47.449
				Mean	0.035	0.041	0.005	54.492

Table 3: Summary of Chi-Square Tests for Hardy-Weinberg Equilibrium

Pop	Locus	DF	ChiSq	Prob	Signif
P1	ADL278	6	3.425	0.754	ns
P1	LEI0094	28	32.746	0.245	ns
P1	ADL268	15	66.876	0.000	***
P1	MCW216	28	16.741	0.954	ns
P1	MCW248	15	72.037	0.000	***
P2	ADL278	6	6.440	0.376	ns
P2	LEI0094	45	71.918	0.007	**
P2	ADL268	6	4.827	0.566	ns
P2	MCW216	28	18.454	0.914	ns
P2	MCW248	6	1.680	0.947	ns

Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001
Note P1 = Fulani Ecotype Chicken, P2 = Yoruba Ecotype Chicken

Table 4: Analysis of Molecular Variance based on microsatellite DNA variation

Source	Df	SS	MS	Est. Var.	%
Among Pops	1	51198.354	51198.354	432.830	8%
Within pops	93	937814.972	10084.032	5009.542	91%
Within Indiv	95	6170.000	64.947	64.947	1%
Total	189	995183.326		5507.320	100%

Discussion

The mean number of alleles per population per loci and observed and expected heterozygosity (Ho and He) are the most common parameters for assessing diversity. All microsatellite markers investigated in this study based on the recommendation of FAO/ISAG were polymorphic, in the same vein, they were found useful as molecular markers in the two populations (Yoruba and Fulani Ecotype Chicken). This implied that both population had high genetic diversity, In addition, private alleles were also

detected which is in line with previous studies . A total number of 62 alleles were detected (FEC:32, YEC:30), [3] detected 280 alleles, [15] found 75 alleles while [16] detected 255 alleles . The variation in total number of allele may be attributed to unequal number of Loci investigated in their respective study and Population size and sampling number. [17] obtained total number of alleles close to what was found in this study. Number of alleles found at respective loci was comparable to values got by other researchers . [18], [19] and [15] all reported range of values similar to what was obtained in this study, however [16] and [3] reported higher value of Number of alleles. Generally, differences in number of allele per loci may be attributed to the degree of genetic diversity and rate of inbreeding in studied populations of each study. Private allele were observed in all the loci except ADL278, earlier , Petit et al 1998 submitted that occurrence of private allele are source of genetic diversity. Value of Ho and He followed the same trend across the loci in both populations, this is in consonance with work of [20]. Range of Ho (FEC: 0.277 to 0.809; YEC: 0.383 to 0.66) and He (FEC: 0.392 to 0.670; YEC: 0.290 to 0.727) is in line with submission of [21] that for marker to be useful for measuring genetic variation , they should have an average heterozygosity ranging from 0.3 to 0.8 in the population, this proved that the two population are heterozygous and that the selected markers were suitable for diversity study. Arising from this, the two population possess a level of genetic diversity that can give room for purposive selection for conservation and improvement of traits of economic importance and probably to develop a strain of chicken adapted to Nigeria environment. [22] had previously used estimated diversity of hetrozygosity and PIC values of microsatellite markers in determining the animal breed selection. [15], [3] and [19] all reported similar value of He and Ho. which suggest admixture of population since they all worked on African Chickens, though it is still subjected to further confirmation of research work, mtDNA study may prove further. Nevertheless, Ho and He value got in this study were lower than value got by [23] who reported 0.709 to 0.882 for He and 0.466 to 0.852 for Ho. Variation of expected heterozygosity may be adduce to differences in location, sample size, population structure and sources of microsatellite markers [15], in addition number of loci investigated could also be attributed to the variation.

[15] opined that PIC is an ideal key to measure the polymorphism of allele fragments. Mean PIC value got in this study FEC: 0.5415 and YEC:0.5266 confirmed a highly informative loci in both populations and therefore agree with submission of [13] that PIC > 0.50 indicates a high revealing and informative locus, they further postulate that 0.50> PIC > 0.25 shows a reasonably informative locus. Hence, mean PIC values in this study agrees with values got by other researchers. [15] reported PIC values of 0.599 and 0.426 respectively in Turkish native chicken. [18], [20] and [19] in Egyptian Local Chickens. Though somewhat low, value of PIC obtained at locus MCW 248 in both populations (FEC: 0.376, YEC: 0.268) yet to some extent confirm informative locus in line with submission of [13].

The simplest parameters for assessing diversity among breeds are the genetic differentiation or fixation index, several estimators have been calculated for Fst, Fit, and Fis values between pairs of populations [24] to test the null hypothesis of a lack of genetic differentiation between population and therefore the partitioning of genetic diversity [25]. The mean value of Fst, Fit, and Fis got in this study for the two populations are 0.035, 0.041 and 0.005 respectively. Fis (inbreeding Coefficient) which indicates the degree of departure from random mating. [19] submitted that when Fis is less than 0.05 the breeds are not in danger, between 0.05 -0.15 they are potentially endangered; between 0.05 – 0.25 they are minimally endangered; between 0.25 – 0.40, they are endangered and more than 0.40 they are critically endangered. Values of Fis obtained in this study at all loci investigated showed that random mating existed in the population and indicative of high level of genetic variation and hetrozygosity. Nonetheless, excessive hetrozygosity was observed at Locus LEI0094 as indicated by the negative value of Fis. It can therefore be inferred that the two populations have not been placed under any form of selection whether conscious or otherwise for any traits of economic importance hence the need for conservation before their valuable genes are lost. [6] cautioned on the need to safeguard the genetic diversity of local farm animals which has been reported threatened. However, [19] reported a deficit of heterozygous and that non random mating existed in their population which suggest that the loci were under morphological and productive selection. Corresponding low value of Fst obtained at all loci in this study indicated that there is low level of genetic differentiation between the two populations which showed absence of clear substructuring of Nigeria indigenous Chickens along ecological types. This observation agree with submission of [3] in Zimbabwe indigenous chickens. It however contradicted with earlier works of [26], [27] who previously differentiated the two population based on protein polymorphism and morphological traits. In

the same way, values obtained for Fit is congruent to other index of fixation parameters.

The Chi - Square test was used to measure Hardy Weinberg Equilibruim (HWE), the result is consistent with the conclusion of fixation index parameters, He and Ho at loci ADL 268 and MCW 248 in FEC and only in LEI0094 in YEC. Some of the loci that deviated from HWE may be linked with genes that were lost through genetic drift and the deviation may also be as a result of sample size as earlier observed by [20] in their work. Our submission is also in concomitant with [28]. The dendogram tree showed that both YEC and FEC are genetically similar and therefore could be proposed that both ecotypes belong to the same population.

Hierarchical analysis of molecular variance (AMOVA) was carried out to appraise the distribution of diversity within and among the two population considered. Inter population differences was significantly lesser than intra population differences. This observation is in line with the value of distance and UPGMA constructed, this thus evidently indicate that they have similar genetic base.

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Conclusion

This study is considered as novel being first of its kind in the derived savannah zone of Nigeria on Indigenous chickens. All microsatellite markers investigated in this study were polymorphic and thus useful in genetic characterization of the indigenous chickens**.** It consequently implied that high degree of genetic variation still exist among the population. Further study should consider more loci for robust characterization and also be extended to SNP.

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