Analysis of genetic diversity of eight improved Nigerian Indigenous Chickens population using *Insulin growth factor-1* GENE

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Abstract

The genetic improvement of Nigerian Indigenous Chickens (NIC) globally can be attained by selection and crossbreeding. The production performance characteristics of improved chickens can be further assessed through the use of molecular markers or candidate gene. The Insulin-like Growth Factor-1 (IGF-1) gene polymorphism is a candidate gene of interest useful in predicting productive performance characteristics in the animal genetic scheme. Therefore, purpose of this study was to assess the genetic variation and establish the relationship amongst eight improved Nigerian indigenous chickens using insulin like growth factor 1 (IGF-1*).* Genomic was extracted from 76 DNA samples from eight improved NIC (SIFE = Straight Improved Fulani ecotype, SIFF = Straight Improved Frizzle feather, SINF = Straight Improved Normal feather, SINN = Straight Improved Naked neck, RIFE = Reciprocal Improved Fulani Ecotype, RIFF = Reciprocal Improved frizzle feather, RINF = Reciprocal Improved Normal feather, RINN = Reciprocal Improved Naked neck), PCR was conducted using the primer as designed by Nagaraja et al., 2000, the PCR amplicons were digested with *Pst1* restriction enzymes and fragment were run on 1% agarose gel. The resulting fragments were viewed under UV light and genotyped. All the loci analyzed in IGF-1 showed a polymorphic pattern and total of 2 alleles were observed and the number of alleles per locus was 2.00 with the average observed and expected heterozygosity values of 0.538 and 0.466 respectively. The relative magnitude of gene differentiation (F_{ST}) was 0.336 and was significant between the genotypes while the negative F_{IT} (-0.153) values obtained for all the loci. Dendogram Based Nei's genetic distance exhibited closeness between RINN and RIFE, SIFE and RINF while the widest distance was between SINN and SINF genotypes. The study revealed the existence of moderate genetic diversity in chicken populations studied and also showed that the IGF-1 used were highly informative and can be used in future studies involving chicken populations.

Key words: Genetic Diversity, heterozygosity, alleles, IGF-1*,* Dendogram, Improved Nigerian indigenous chickens

Introduction

Nigerian indigenous chickens possesses several desired traits including the ease of raising and high resistance along with some shortcomings such as low productivity and highly hybridized breeds (Amao, 2018) and improvement were made on these drawbacks through selection and crossbreeding (Amao, 2017) while enhancing and stabilizing breeding quality are one of the methods used to improve these indicators. Many candidate genes associated with poultry productivity have been identified (Amao et al., 2019; Abdi et al., 2014; Fatemi et al., 2012), as a result of the unprecedented improvements of genetic technology, especially molecular genetics. Few attempt were carried out to genetically evaluate improved local breeds carried normal feather, Fulani ecotype, naked neck and frizzle feather chickens in heterozygous status using candidate gene (Wheto et al., 2016). However, little information was known regarding standardizing and characterizing Nigerian Improved Chickens breeds carrying normal feather, Fulani ecotype, naked neck and frizzle feather chickens in homozygous and heterozygous status using any candidate gene. The study aims in determine genetic diversity of eight improved Nigerian Indigenous Chickens' population using IGF-1 gene.

Materials and Methods

The experiment was carried out at the Animal Breeding and Genetics Unit of Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, and Department of Animal Breeding and Genetics, Biotechnology Laboratory, Federal University of Agriculture, Abeokuta, Ogun State – Nigeria.

Chicken Population, Blood Sampling and DNA Isolation

Blood samples were obtained randomly from 76 chickens from 8 different genotypes through the wings vein using 2 ml syringe and transferred to EDTA bottles to serve as anti-coagulating agent, after which the samples were stored in the laboratory at -20 °C. Genomic DNA was extracted from the blood, using the Qiagen DNA extraction kits following manufacturer protocol. Working dilutions of extracted DNA were prepared for each individual at a concentration of 50 ng/µg. Primers forward (5'-GACTATACAGAAAGAACCAC-3') and reverse (5'-TATCACTCAAGTGGCTCAAGT-3') were used for the polymerase chain reaction (PCR) amplification (Nagaraja et al., 2000). Each cycle was consisted of initial denaturation at 94 °C for 5 minutes, 94 °C for 4 minutes, 45 sec at 60 °C, 60 sec at 72 °C and final extension at 72 °C for 10 minutes. The PCR products were run in an agarose gel electrophoresis to verify the result. The PCR product or amplicon were digested with a restriction enzyme, *Pst1* and digested products were electrophoresed for 1 h at 80 V on a 2.5% agarose gel. Individual PCR-RFLP fragment sizes for each gene were determined by visualizing the banding pattern under ultraviolet light. Genotyping was obtained manually following the scoring procedure described by Wheto et al. (2016).

Statistical Analysis

Allele frequencies, Number of different alleles (Na), (through direct counting), Number of effective alleles (Ne), Shannon's Information index (i), observed heterozygosity (H_o), expected heterozygosity (H_e) and unbiased genetic diversity (UH_e) were calculated using GenAIEx 6.5 (Peakall and Smouse, 2006) per locus, for each population of the chicken and for pooled sample.

Genetic distances D_A (Nei et al., 1993) between the genotypes were assessed based on allele frequencies using GenAIEx 6.5 software. The extent of genetic differentiation among the genotype was determined using F_{ST} statistics. The F_{STAT} v.2.9.3 software was used to estimate the inbreeding coefficient within individual (F_{IS}), inbreeding coefficient within subpopulation (F_{ST}) and total inbreeding coefficient (F_{ST}) statistics (Weir and Cockerham, 1984) and their significance was inferred by methods based on randomization. A dendogram showing the relationship of the genotypes was constructed by neighbor-joining method using *MEGA* version 5

(Tamura et al., 2011). The D_A genetic distance is better suited to obtain correct tree topology than other distances, regardless of a mutation model (Takezaki and Nei, 1996). Individual birds were assigned to their presumed population of origin using individual assignment tests using the Gen AIEx 6.5 program, in order to ascertain genetic admixture. The discriminant function analysis was used to determine percentage assignment of individuals into their own populations.

Results

Table 1 showed the population diversity index of IGF-1 gene polymorphism locus in chickens' populations. The results revealed that the number of alleles (Na) were the same (2) for all chicken genotype population. The effective number of alleles (Ne) varied between 1.658 (SINN birds) and 2.000 (RIFF chickens). The total means effective number of alleles was highest in SIFF (2.000) chickens and lowest in SINN birds (1.658) populations. Shannon index (i) was highest for SIFF (0.693) and least value was recorded for SINN (0.586) birds. The mean observed (H_o) and expected (H_e) heterozygosities were 0.556 and 0.475 in RIFE, 0.571 and 0.490 in RIFF, 0.444 and 0.444 in RINF, 0.400 and 0.480 in RINN, 0.300 and 0.455 in SIFE, 1.000 and 0.500 in SIFF, 0.667 and 0.490 in SINF, 0.364 and 0.397 in SINN birds. The observed heterozygosity varied from 0.364 (SINN) to 0.667 (SINF) while the expected heterozygosity was between 0.397 (SINN) and 0.500 (SIFF). The average observed heterozygosity was higher than the average expected heterozygosity in all the chicken populations except in SINN. The mean diversity is indicated by UH_e which had a range of 0.471 for RINF to 0.716 for the SINN birds with total value of 0.498. Highest value of UH_e was recorded for SINN (0.116) while the lowest value of 0.4271 was obtained in RINF. The fixation index (F) of the population ranges from 0.000 (RINF) to 0.341 (SIFE).

The genotype frequencies and Hardy-Weinberg equilibrium (HWE) of IGF-1 in chickens' populations are presented in Table 2. The AA genotype had more values in SIFE and SINN chickens while AC genotype were more in values in RIFE, RIFF and SIFF. The same value (0.44) were obtained for both genotypes AA and AC in RINF birds while the same trend was observed in RINN chicken with value 0.40 for both AC and CC genotypes. The genotypes AA and CC (homozygote individual) had no values in SIFF birds. The expected genotypic frequencies shows that genotype AC (heterozygote individual) was highest in RIFE (0.48), RIFF (0.49),

Table 1. Population diversity index of IGF-1 gene polymorphism locus in the chickens' populations

Genotype	Na	Ne	I	H_o	H_e	UH_e	F
RIFE	2.00	1.906	0.668	0.556	0.475	0.503	-0.169
RIFF	2.00	1.960	0.683	0.571	0.490	0.527	-0.167
RINF	2.00	1.800	0.637	0.444	0.444	0.471	0.000
RINN	2.00	1.923	0.673	0.400	0.480	0.533	0.167
SIFE	2.00	1.836	0.647	0.300	0.455	0.479	0.341
SIFF	2.00	2.000	0.693	1.000	0.500	0.556	-1.000
SINF	2.00	1.960	0.683	0.667	0.490	0.502	-0.361
SINN	2.00	1.658	0.586	0.364	0.397	0.716	0.083
Total	2.00	1.880	0.659	0.538	0.466	0.498	-0.138

SIFE = Straight Improved Fulani ecotype, SIFF = Straight Improved Frizzle feather, SINF = Straight Improved Normal feather, SINN = Straight Improved Naked neck, RIFE = Reciprocal Improved Fulani Ecotype, RIFF = Reciprocal Improved frizzle feather, RINF = Reciprocal Improved Normal feather, RINN = Reciprocal Improved Naked neck. Na = Number of different alleles, Ne = Number of effective alleles, i = Shainnon's information index, H_o = observed heterozygosity, H_e = expected heterozygosity, UH_e = Unibased expected heterozygosity, F = fixation index

RINN (0.48), SIFE (0.46), SIFF (0.50) and SINF (0.49) while genotype AA was only more in SINN (0.58) birds. No difference was obtained for expected genotype frequencies between genotypes AA (0.44) and AC (0.44) for RINF chickens. However, the chi-square analysis showed a significant ($P < 0.05$) difference in the observed and the expected genotypic frequencies of SIFF chickens while other chicken populations had no significant effects. The non-significant ($P > 0.05$) effects was obtained for RIFE, RIFF, RINF, RINN, SIFE, SINF and SINN chicken populations implies that all these gene and their genotypic frequencies of these populations were in Hardy-Weinberg proportion while only SIFF chicken was not in Hardy-Weinberg proportional ($P < 0.05$). The Hardy-Weinberg Equilibrium ranges from 0.00 to 0.58.

The result of the analyses for individual chicken from each population assigned is shown in Table 3. The proportion of the birds from each genotype correctly assigned to their source population ranged from 63.0 percent for reciprocal improved naked neck (RINN) chickens to 93.50 percent for straight improved naked neck (SINN) birds, with an average of 76.71 percent for the entire chicken populations. Considerable proportion of RINN was assigned to other clusters, such as reciprocal improved Fulani ecotype (RIFE) (13.50 percent) and straight improved frizzled feather (SIFF) (13.50 percent). A similar feature was apparent for the proportion of RIFE (12.50 percent) individual birds assigned to RINN while the largest proportion of 16 percent of straight improved Fulani ecotype birds (SIFE) was assigned to SINN birds. Few proportion of RIFE was found in straight improved normal feather birds (SINF) (2.30) while other few clusters proportion of 2.50 percent was assigned for RIFE and SINN birds from reciprocal improved normal feather chickens (RINF) with similar apparent of assignment of 2.50 percent of SIFF from SINN chickens. All individual chickens from SIFF were correctly assigned to their source of population.

The pairwise population F_{ST} values across the chickens' populations are as shown in Table 4. The result revealed the gene differentiation (F_{ST}) indices between all pairs of the chicken populations were positively high and significant ($P < 0.01$) differences amongst the genotypes. The largest F_{ST} value (0.116) was between SINN and RIFE chickens while the smallest F_{ST} value (0.000) was observed between the RINN and RIFE, SIFE and RINF chickens respectively. Table 5 summarizes the inbreeding coefficient

Table 2. Genotype frequencies and Hardy-Weinberg Equilibrium (HWE) of IGF-1 locus in the chickens' populations

	Frequencies of genotypes							
	Observed			Expected				
Population	AA	AC	CC	AA	AC	CC	HWE P-value	Sig
RIFE	0.11	0.56	0.23	0.15	0.48	0.37	0.03	NS
RIFF	0.14	0.57	0.29	0.18	0.49	0.33	0.03	NS
RINF	0.44	0.44	0.11	0.44	0.44	0.11	0.00	NS
RINN	0.20	0.40	0.40	0.16	0.48	0.36	0.03	NS
SIFE	0.50	0.30	0.20	0.42	0.46	0.12	0.12	NS
SIFF	0.00	1.00	0.00	0.25	0.50	0.25	1.00	*
SINF	0.23	0.67	0.10	0.33	0.49	0.18	0.13	NS
SINN	0.55	0.36	0.09	0.58	0.43	0.08	0.01	NS

**P < 0.05, NS = Not Significant*

*AA = homozygote dormant gene, AC = heterozygote gene, CC = homozygote recessive gene, HWE = Hardy – Weinberg Equilibrium, * = Significant level*

Table 3. Percentages of individual birds from each population correctly and incorrectly assigned to source and other population

Source population	Correctly assigned (%)	Assigned to (%)							
		RIFE	RIFF	RINF	RINN	SIFE	SIFF	SINF	SINN
RIFE	70.00	-	0	0	12.50	8.30	0	0	9.20
RIFF	70.00	11.50	-	0	0	0	0	0	8.50
RINF	88.50	2.50	0	-	0	0	6.50	0	2.50
RINN	63.00	13.50	0	0	-	0	13.50	0	10.0
SIFE	67.00	8.50	0	0	0	-	8.50	0	16.0
SIFF	100	0	0	0	0	0	-	0	0
SINF	85.00	2.30	0	0	0	0	7.80	-	3.90
SINN	93.50	0	0	0	0	4.0	2.50	0	-
Total	79.63	38.30	0	0	12.50	8.30	38.8	0	50.1

SIFE = Straight Improved Fulani ecotype, SIFF = Straight Improved Frizzle feather, SINF = Straight Improved Normal feather, SINN = Straight Improved Naked neck, RIFE = Reciprocal Improved Fulani Ecotype, RIFF = Reciprocal Improved frizzle feather, RINF = Reciprocal Improved Normal feather, RINN = Reciprocal Improved Naked neck

within individuals (F_{IS}), Inbreeding coefficient within subpopulation (F_{ST}) and total inbreeding coefficient (F_{IT}). The F probabilities observed were 0.580, 0.584 and 0.336 for $F_{IS,}$ F_{ST} and F_{IT} respectively while the F per locus indicating negatives values for F_{IS} (-0.153) and F_{IT} (-0.153) while 0.0061 was obtained for $F_{ST.}$

Table 6 indicated the genetic distance between the chickens' population using Nei (1978), The smallest genetic distance was observed between RINN and RIFE (0.000), SIFE and RINF (0.000) these relationship were very low while the largest genetic distance was found between the SINN and SINF (0.041) genotypes. Figure 1 shows the neighour-joining dendrogram of the genetic distance. Two main clusters were identified, the first cluster included SINN, RINF and SIFE genotypes while the second one divided into two sub-clusters. One of them included RIFF, RIFE and RINN genotypes while the second incorporated the SIFF and SINF genotypes. The genotypes were clearly clustered as different groups according to their genetic make-up supporting the reliability of the analysis. RINF tends to cluster together and with those SIFE (0.000), RIFE cluster close to RINN (0.000) while SIFF tends to cluster together with SNF (0.001). The widest distance was between SINN and SINF genotypes.

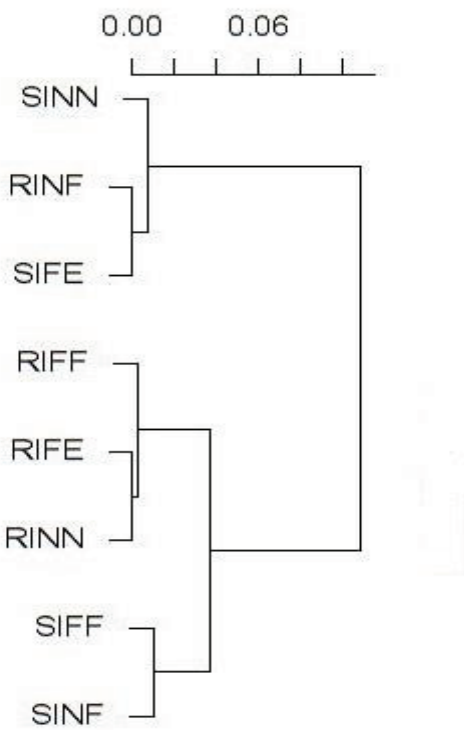

Fig. 1. Dendrogram of genetic distance (D_A) between chicken populations. TERXT

Table 4. Pairwise F_{ST} values across the chickens' populations

Genotype	RIFE	RIFF	RINF	RINN	SIFE	SIFF	SINF	SINN
RIFE								
RIFF	0.002							
RINF	0.077	0.057						
RINN	0.000	0.001	0.071					
SIFE	0.068	0.049	0.000	0.063				
SIFF	0.013	0.005	0.029	0.010	0.023			
SINF	0.033	0.020	0.010	0.029	0.006	0.005		
SINN	0.116	0.091	0.004	0.109	0.007	0.054	0.027	

$^{*}P < 0.01$

SIFE = Straight Improved Fulani ecotype, SIFF = Straight Improved Frizzle feather, SINF = Straight Improved Normal feather, SINN = Straight Improved Naked neck, RIFE = Reciprocal Improved Fulani Ecotype, RIFF = Reciprocal Improved frizzle feather, RINF = Reciprocal Improved Normal feather, RINN = Reciprocal Improved Naked neck

Table 5. F-Statistics

	F_{IS}	F_{IT}	F_{ST}
Probability	0.584	0.584	0.336
Locus	-0.153	-0.153	0.0061

F_{IS} = Inbreeding coefficient within individual
F_{ST} = Inbreeding coefficient within subpopulation
F_{IT} = Total inbreeding coefficient

Table 6. Genetic Distance (D_A) between the chickens' populations

Genotype	RIFE	RIFF	RINF	RINN	SIFE	SIFF	SINF	SINN
RIFE								
RIFF	0.003							
RINF	0.154	0.112						
RINN	0.000	0.002	0.141					
SIFE	0.136	0.097	0.000	0.125				
SIFF	0.024	0.010	0.053	0.020	0.043			
SINF	0.066	0.041	0.016	0.059	0.011	0.010		
SINN	0.224	0.171	0.006	0.209	0.009	0.094	0.041	

SIFE = Straight Improved Fulani ecotype, SIFF = Straight Improved Frizzle feather, SINF = Straight Improved Normal feather, SINN = Straight Improved Naked neck, RIFE = Reciprocal Improved Fulani Ecotype, RIFF = Reciprocal Improved frizzle feather, RINF = Reciprocal Improved Normal feather, RINN = Reciprocal Improved Naked neck.

Discussion

The lowest and same numbers of allelic number were obtained for individual genotype in the present study. The lowest allelic number observed in the study was in close agreement with those reported by Chen et al. (2004) on lowest range for allelic number for Chinese na-

tive chicken population while the same value of allelic number recorded on the study was also agreed with the works of Esmailnejad and Brujeni (2017) for Iranian native chicken. The current result of lowest allelic number contradicted the findings of Keambou et al. (2014) and Fosta et al. (2011). The authors found higher average number of alleles per gene in Cameroon indigenous chicken ecotypes. The number of effective alleles presently was lesser than the values obtained by Keambou et al. (2014) for Cameroon indigenous chicken. The Shannon index value in the present study was lesser than the observation made by Alipanah et al. (2011) for Khazak chicken in Iran. The mean heterozygosity values that ranged from lower to higher may be in accordance with the values reported by Alipanah et al. (2011) for both Khazak and Zabol chickens. Fosta et al. (2011) reported similar range of lower to higher genetic diversity for chickens of 5th agro-ecological zone of Cameroon. The observed heterozygosity (H_o) was more than expected (H_e) values indicating an excess of heterozygotes but the deviation of heterozygosity from expected values may be due to the selection for or against heterozygotes, null alleles, population subdivision owing to the genetic drift.

The non-significant chi-squared effects reported in this study conformed to the findings of Wheto et al. (2016) who reported Hardy-Weinberg proportion among the polymorphism of IGF-1 gene on carcass traits of improved Nigerian indigenous chickens. Abbasi and Kazemi (2013) reported that chi-squared test on the genotype frequencies showed no deviation from Hardy-Weinberg equilibrium in the Mazandaran native fowls population. The obtained results for all the genotypes population showed that frequency of the genotype does not differ from expectation of Hardy-Weinberg equilibrium except for RIFF birds. This was in line with the findings of Jafari et al. (2015) that found differences among genotypes of Isfahan and Mazandaran native fowls to be significant and this indicated that the populations were not in Hardy-Weinberg equilibrium which could due to genetic selection for growth related traits.

The F_{ST} values allow estimation of the number of migrant individuals according to loci in a population per generation. The F_{ST} values obtained in this study were however significant, indicate a certain level of differentiation. The pairwise F_{ST} values obtained among the chicken population was similar to those reported by Eltanany et al. (2010) for Egyptian chicken strains under 29 microsalellite markers investigation. The present findings are also in accordance with the reports of Nigussie et al. (2011) that reported small variation among Ethiopian chicken ecotypes. Meanwhile, the pairwise F_{ST} values across the chicken population are lower than those obtained by Fosta et al. (2011) in the study involving assessment of the genetic diversity of Cameroon indigenous chickens by the use of microsatellites.

The total inbreeding coefficient (F_{IT} value) observed in this study was moderate and it could be linked to the observed mean within heterozygote deficiency (F_{IS} value). Increasing F_{IT} values are an indication of some measure of homozygote excesses or heterozygote deficiency resulting from relatedness of individuals. F_{IS} is an estimate of variation within population that measures the homozygosity or reduction in heterozygosity in an individual that occurs because of non-random mating within population. The F_{IS} values for the loci were negative and it is an indication of no inbreeding. The negative F_{IS} and F_{IT} values observed in this study were consistent with the findings of Folohunso et al. (2018) on genetic diversity between exotic and Nigerian indigenous turkeys at different structural loci. However, the negative values of F_{IS} and F_{IT} contradicted the findings of Keambou et al. (2014), Alipanah et al. (2011) and Clementino et al. (2010). These authors reported positive F_{IS} and F_{IT} values for local indigenous chickens in their different countries and suggested heterozygote excess among the chickens. The homozygote deficiencies might have been linked to pooling together different population in the analysis which are actually subdivided. The estimated F_{ST} values correspond to the amount of genetic viability in the environment and breeding practices. The positive F_{ST} value as reported among exotic and indigenous local turkeys by Folohun-

so et al. (2018) was comparable with this study. Osei-Amposah et al. (2010) found positive F_{ST} values among the genetic diversity of forest and savannah chicken populations in Ghana.

The low genetic distances indicated a close genetic relationship whereas large genetic distance implies a more distant genetic relationship. Within a population genetic distance can be used to measure this divergence between different sub-populations. New standard genetic distance measure assumes that genetic differences arise due to mutations and genetic drift. The current displayed in congruent of genetic distances conformed to the findings of Abou-Emera et al. (2017) and Nigussie et al. (2011) who stated that the multiclustered populations referred to high polymorphism situation. The obtained result on genetic distances currently reflects the fact that these subpopulations are not genetically isolated from each other. Abou-Emera et al. (2017) emphasized that genetic distance measure based on gene frequencies were in good agreement with the genetic diversity of genotype examined, indicating that these approached fit the history of the domesticated chicken well. Meanwhile, the genetic distances that varied from lowest to low observed in the present study was similar to that of Mariandayani et al. (2013) in four Indonesian native and broiler chickens. Fosta et al. (2011) in Cameroon also found similar lowest to low genetic distances values for Cameroonian indigenous chickens. However, the current results were lower than those of five varieties of Egyptian local chickens reported by Abou-Emera et al. (2017) while Ohwojakpor et al. (2012) also found higher genetic distances between three varieties of Nigerian local chickens. These authors' findings contradicted the current low genetic distances obtained.

Conclusion

IGF-1 was recommended to assess molecular genetic structure of the eight improved Nigerian indigenous chickens. The results obtained would serve the appropriate managements on different levels including conservation of such genetic resources, future improvements for these breeds and/or understanding different genome arrangement and knowledge interests.

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