Nig. J. Anim. Prod. 2020, 47(1):33 - 40
Nigerian Journal of Animal Production

Polymorphism and diversity studies of insulin-like growth factor I gene among indigenous and FUNAAB Alpha chicken breeds in Nigeria

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Abstract

The Insulin-like growth factor gene (IGF-1) is an important candidate gene responsible for growth, body composition, metabolism, skeletal characteristics and fat deposition in chicken. Little or no work has been done on the sequence analysis of IGF1 gene in FUNAAB Alpha chicken breed. Therefore, this study was designed to examine the polymorphisms and genetic diversities of the IGF-1 gene among Nigerian indigenous and improved indigenous (FUNAAB Alpha) chicken breeds. A total of 120 chickens comprising of 20 each from Frizzle feathered, Normal Feathered, Naked neck, FUNAAB-Alpha 1 (DHR), FUNAAB-Alpha 2 (TRH) and Arbor Acre were used for the study. Approximately 2 ml of blood collected from each chicken was used for genomic DNA extraction following the protocol of Qiagen DNA extraction kits. PCR was carried out and the PCR amplicons were outsourced to Stabvida Laboratory, Portugal for sequencing. The results of the analysis indicated a total of 21 polymorphic sites and 12 haplotypes across all the sampled chickens. The number of haplotypes varied from five in DHR and four in Naked neck to three and one in Arbor Acre and Frizzle feathered, respectively. The naked neck was the most superior in terms of nucleotide diversity per site (0.00307), nucleotide difference (1.67974) and the number of variable sites, indicative of its evolutionary adaptability to the prevailing environmental conditions of the tropics and high potential as genetic resources for domestic chicken improvement.

Keywords: IGF-1, SNPs, Genetic diversity, Phylogenetic, Chicken

Introduction

According to Beccavin *et al*. (2001) and Zhou *et al*. (2005), the insulin-like growth factor gene (*IGF*) is a candidate gene that is responsible for growth, body composition, metabolism, skeletal characteristics, growth of adipose tissue and fat deposition in chickens. Insulin-like growth factors belong to the family of polypeptide hormones, they are structural homologues of insulin and also have a similar function (Gouda and Essawy, 2010; Kadlec *et al*., 2011). Hormones such as the growth hormone, IGF, thyroid hormones and insulin, play important and diverse roles in animal growth (Zhou *et al*., 2005). Most of the functions of the growth hormone in chickens are mediated by insulin-like growth factors (IGF) (Lei *et al*., 2005) which stimulate amino acid uptake, glucose metabolism, DNA synthesis (McMurtry, 1998), protein synthesis, and the proliferation of different cell types, and is also involved in the regulation of growth (McMurtry *et al*., 1997). Chicken *IGF1* mRNA is synthesized, as with humans, in many tissues, such as liver, brain, muscle and heart (Kajimoto and Rotwein, 1989). The Nigeria chicken population comprises of the normal feathered, frizzle feathered and naked neck which are the primary indigenous populations that represent important chicken genetic resources of Nigeria. FUNAAB Alpha chickens were developed after intensive crossbreeding and selection over many generations at the Federal University of Agriculture, Abeokuta, Nigeria. This breed of chicken was developed from the Nigerian Indigenous chicken population for meat and egg production. The FUNAAB Alpha 1 is the dual-purpose breed while the FUNAAB Alpha 2 is the meat line. They were developed without losing the adaptive and tolerance traits in the local population (Wheto *et al*., 2017).

Materials and methods

Experimental site

The experiment was carried out at the PEARL Poultry Breeding Unit of Directorate of University Farms, Federal University of Agriculture, Abeokuta, Nigeria. The University is located within latitude 7° 9' 39" N and longitude 3°2'E with an altitude of 76m above sea level and lie in the southwestern part of Nigeria. It has an average temperature of 34°C and a relative humidity of 80% with rainfall of about 1238mm (climate.data.org, 2019). The vegetation in the University represents interphase between the tropical rainforest and the derived savannah.

Experimental birds

The experimental birds comprise of twenty each of the three Nigerian indigenous chicken (Frizzle Feathered, Normal Feather and Naked Neck), twenty (20) FUNAAB-Alpha 1 (DHR), twenty (20) FUNAAB-Alpha 2 (TRH) and twenty (20) Arbor Acre chicken.

Blood collection

Whole blood (2mL) was collected from all the chickens aseptically from the right jugular vein with needle and syringe for each of the bird. The blood was collected into bottle containing Ethylene Di-amine Tetra-acetic Acid (EDTA).

Genomic DNA extraction and quantification

The DNA was extracted from the whole blood using the Qiagen DNA extraction kit following the manufacturer's protocol. The purity and concentration of the extracted DNA were carried out using a nanodrop spectrophotometer and also checked on 1% Agarose gel electrophoresis.

The DNA amplification and sequencing

Polymerase chain reaction (PCR) was carried out using the primers reported by Nagaraga *et al*. (2000). The PCR was carried out in a total volume of 20µl reaction containing 2µl of genomic DNA, 1µl each of the forward and reverse primer (10pmol/µl), 4µl of 5X Firepol PCR premix and 12µl of nuclease-free water. The PCR amplification condition consists of 94°C for 5 minutes then 35 cycles of 94°C for 45 seconds, 60°C for 45 seconds and 72°C for 1 minute, with a final extension step for 10 minutes at 72°C. The PCR amplicons were outsourced to Stabvida, Portugal for sequencing.

Sequence analyses

Sequence alignment, trimming and polymorphism detection was carried out using BioEdit (Hall, 1999) and MEGA 6.0 software (Tamura *et al*., 2004). Haplotype diversity, nucleotide diversity, the number of segregating sites were estimated using DnaSP v5 (www.ub.edu/dnasp). The indices of diversity measured include number of haplotypes, haplotype diversity, nucleotide diversity, number of polymorphic sites, singleton variable site and parsimony-informative site for each population of the chicken. Evolutionary analyses of the *IGF-1* gene were conducted among the chicken genotypes that were determined using the UPGMA method (Sneath and Sokal, 1973) based on the *IGF-I* gene in MEGA7 (Kumar *et al*., 2016). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site.

Results and discussion

The number of the variable sites (polymorphic site), haplotype diversity and nucleotide diversity show the level of allelic variation in the *IGF-I* gene resulting in the genetic diversity of the *chicken population used*. From Table 1, it can be deduced that the Naked neck has the highest number of variable sites (S) with Frizzle Feathered chicken having no variable site from the total of 21 variable sites discovered among the Nigerian indigenous and improved breeds. Also, the total number of haplotype (h) discovered among the Nigerian indigenous and improved breeds is 12 (Table 1) with the highest found within the FUNAAB Alpha 1 (h=5) followed by the Naked neck (h=4), Normal feathered (h=4), FUNAAB Alpha 2 (h=4), Arbor acre (h=3) while the least if found within the Frizzle feathered breed (h=1) but the highest number of Nucleotide diversity per site (Pi) and average number of nucleotide differences (k) was found within Naked neck chicken (Pi = 0.00307, k = 1.67974). Consequently, the Naked neck *IGF-I* gene simply has the highest degree of allelic variation when compared to the other indigenous and improved breed of chicken, used in this study. These results agree with the investigation of polymorphism of the *IGF-1* Promoter and the UTR regions of Nigerian locally adapted chickens by Ilori *et al.* (2016) which reported that the Naked neck chicken had the highest number of polymorphism sites with higher nucleotide and haplotype diversities. By implication, high diversity enhances the adaptation of Naked neck chickens as it could provide the evolutionary potential to adapt to the rapidly changing environmental condition of the tropical climate (Ilori *et al.*, 2016).

From Table 2, a total of 21 SNPs were detected in the population studied which is composed of both transition and transversion type of mutations.

The phylogenetic relationship of the *IGF-1* gene among the Nigerian Indigenous and improved chickens as shown in Figure 1 shows eight clades in which some naked neck is seen to cluster in a single clad while others are mixed in other clads. But the TRH and DHR are seen to appear in all clades since they composed genetically of Nigerian local chickens and exotic chickens.

The phylogenetic tree (Figure 2) showed that the six breeds of the Nigerian indigenous and improved chickens used in the study can be separated into four clades with the first clade consisting of normal feathered and naked neck as distinct breeds on the evolutionary scale while other strains diverged progressively. The second cluster composed of the FUNAAB Alpha 2 and Frizzle feather in the same clade while the FUNAAB Alpha 1 was seen to be in the same clade with the Reference sequence. Only the Arbor Acre exists as a single clade. The phylogenetic analysis shows that the chicken population used shared a recent common ancestor, it also indicated that there exists a small genetic differentiation among the chicken population (Okamoto *et al*., 1999 and Ilori *et al*. 2016)

Table 1: Diversity indices of Nigerian Indigenous and FUNAAB Alpha Chicken genotypes

Breed	**n**	**S**	**Eta**	**G+C**	**h**	**Hd**	**Pi**	**k**	**Tajima's D**
Arbor acre	18	3	3	0.331	3	0.307	0.00146	0.79739	-0.23785$^{\text{N.S.}}$
Frizzle feather	7	0	0	0.332	1	0.000	0.00000	0.0000	-
Naked neck	18	5	5	0.331	4	0.699	0.00307	1.67974	0.49223$^{\text{N.S.}}$
Normal feather	19	3	3	0.331	4	0.614	0.00132	0.72515	-0.42196$^{\text{N.S.}}$
FUNAAB Alpha 1	25	3	3	0.331	4	0.633	0.00182	1.00000	0.64237$^{\text{N.S.}}$
FUNAAB Alpha 2	23	3	3	0.332	5	0.735	0.0019	1.04348	0.72345$^{\text{N.S.}}$
Total	111	21	22	0.331	12	0.658	0.00256	1.40098	-1.92222*

$^{\text{N.S.}}$ $P > 0.10$, * $P < 0.05$ (548.00 sites)

n- Number of samples, S- Number of variable sites, Eta- Total number of mutations, G+C- GC content, h- Number of Haplotypes, Hd- Haplotype (gene) diversity, Pi- Nucleotide diversity (per site), k- Average number of nucleotide differences,

Table 1: Single nucleotide polymorphism of *IGF-I* of Nigerian Indigenous and improved chicken breeds

SNP	Type of mutation
3A>T	Transversion
124A>G	Transition
207T>C	Transition
271T>C	Transition
296T>G	Transversion
346C>T	Transition
442G>A	Transition
462C>T	Transition
486C>T	Transition
529C>T	Transition
532G>A	Transition
533A>C	Transversion
534G>C	Transversion
538C>G	Transversion
540T>G	Transversion
541G>T	Transversion
542A>T	Transversion
543G>A	Transition
544T>C	Transition
545G>A	Transition
546A>C	Transversion

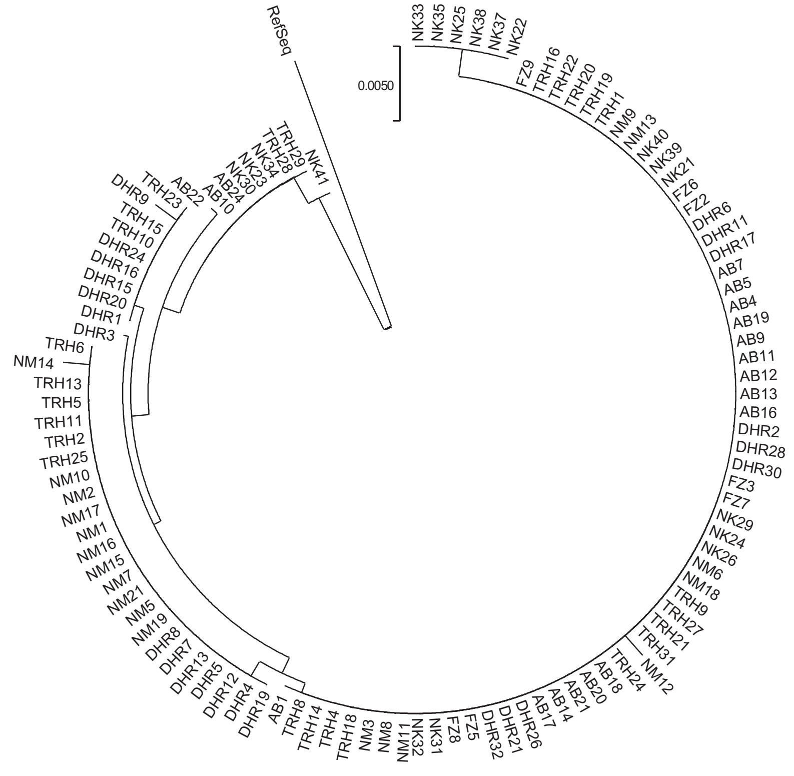

Figure 1: Phylogenetic relationship of *IGF-1* gene among the Nigerian Indigenous and improved Chicken Breeds

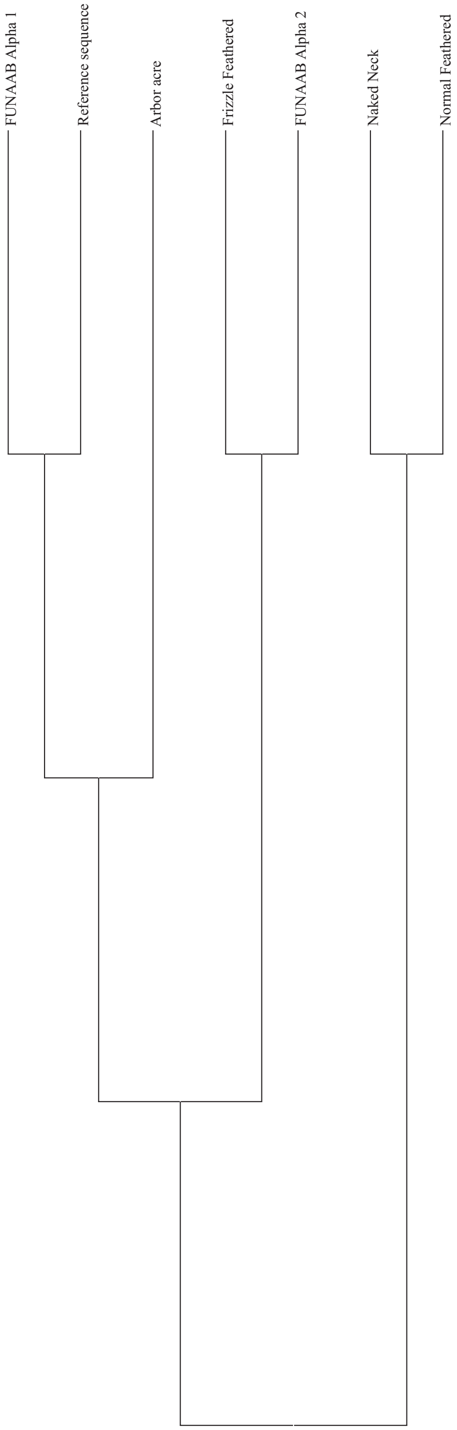

Figure 2. Evolutionary relationship *IGF-1* gene among the Nigerian Indigenous and improved Chicken Breeds

Conclusion

The analysis of the IGF1 gene sequences in FUNAAB Alpha showed that there exist polymorphism in among the chicken population and this polymorphism or variation at the IGF1 gene locus can be used or harnessed for chicken improvement in Nigeria..

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Received: 10th November, 2019
Accepted: 17th February, 2020