DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISM AND GENETIC DIVERSITY AT *IGF-1*GENE IN NIGERIAN INDIGENOUSNORMALFEATHER AND ARBOR ACRE CHICKEN

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Abstract
Insulin-like growth factor 1 (*IGF-1*) which is a growth hormone plays a major role in stimulating systemic body growth, regulating cells growth and development. The *IGF-1* gene sequences were analysed and the polymorphism among the chicken strain was studied. Higher polymorphism was observed in normal feather chicken compared to Arbor Acre. The result showed that the highest number of haplotype (4) and haplotype diversity (0.638) were estimated in normal feather chicken. This shows there is abundance of genetic diversity in this strain of chicken compared to Arbor Acre. The highest average number of polymorphism sites (61), nucleotide diversity (0.017443) and singleton site (60) was also estimated in normal feather chicken. This suggests that this strain of chicken has been subjected to lots of natural selection.

Keyword:*Insulin growth factor-1* gene, Diversity, Polymorphism, Haplotype

Introduction

Nigeria is endowed with many poultry species. These species have lived and produced for several years in the Nigerian environment. Their productivity is poor, owing to stressful environmental factors, problems of diseases, poor housing, and inadequate feeds and feeding. The factor responsible for low productivity of the local poultry resources is the neglect of the local chickens by animal research scientists in preference for exotic breeds (Ndofor-Folen*get al.,* 2010). These local chickens constitute 80% of the 120 million poultry type raised in the rural areas in Nigeria (Ajayi, 2010). They are self-reliant, hardy, and known to possess qualities such as the ability to hatch on their own, brood and scavenge for their food. Insulin-like growth factor 1 (*IGF-1*) is produced primarily by the liver (Kemp, 2007). Its production is stimulated by growth hormone and can be retarded by under-nutrition, growth insensitivity or lack of growth hormone receptors (Miura *et al*., 1992). *IGF-1* gene in vertebrate plays a key role in various physiological and metabolic processes. *IGF-1* gene serves as a mediator in the prediction of growth and meat quality taints in animal genetic improvement schemes.Hormones such as the growth hormone, *IGF*-1, 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-1*) (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). Growth rate stimulation by *IGF* is known in many species of animals. Studies have found that there is no direct dependence between the levels of growth hormone (GH) and the growth rate in chickens, and therefore it might be useful to study insulin-like growth factors (Beccavin*et al*., 2001), as mediators of the functions of the growth hormone (Lei *et al*., 2005). The objectives of the study are as follows;

- To detect single nucleotide polymorphism of *IGF-1* gene in Nigerian normal feathered and Arbor Acre chicken.
- To detect genetic diversity of *IGF-1* gene in Nigerian normal feathered and Arbor Acre chicken.

MATERIALS AND METHODS

The research was carried out at the Poultry Breeding Unit of The Teaching and Research Centre of the Federal University of Agriculture, Abeokuta. The laboratory experiment was carried out at the Biotechnology laboratory of the department of Animal Breeding and Genetics of the Federal University of Agriculture, Abeokuta.The experimental birds that was used for this project consists of Nigerian normal feathered and Arbor Acre chicken. These birds were reared for a period of 8 to 10weeks with adequate care which involve the regular supply of

feed and water, heat, vaccination, protection from predator, and good litre management etc. Blood was collected from the selected chicken per each genotype using a syringe from the right jugular vein, after which the blood collected was transferred into EDTA bottle (Ethylene Dia-mine Tetra-acetic Acid) to avoid coagulation by forming a complex with the oxygen in the environment which can make it useless for the further analysis it was meant for.

DNA Extraction: DNA was extracted using Qiagen DNA extraction kits following the manufacturer procedure.
DNA Quantification: The purity and concentration of the extracted DNA was carried out using Nano-drop spectrophotometer in the Biotechnology centre Federal University of Agriculture, Abeokuta as follow: 2ml of DNA was place in the Nano-drop machine with the help of micro-pipete after which it was measured by the Nano-drop machine and the concentration was noted while DNA samples of low concentration were discarded before analysis.

DNA Amplification: The polymerase chain reaction (PCR) was carried out using the primer and the amplification procedure was done according to Nagaraga*et al.*, (2000). The primer sequence is follow: Forward 5'- GAC TAT ACA GAA AGA ACC CAC-3',Reverse 5'- TAT CAC TCA AGT GGC TCA AGT-3'. Agarose gel electrophoresis was also carried out to know whether the exact region is been amplified.

Sequencing and Sequence Analysis: The amplified DNA sample was sent for sequencing to Starvida, oeiras, Portugal.The sequence nucleotide was then edited using BioEdit sequence alignment editor and the resulting edited sequence served as FASTA format on notepad for further analysis.

Sequence Alignment: The DNA sequence alignment was done using CLUSTALX (www.clustalx) with MEGA 7.0 software and trimmed so as to get a more accurate sequence alignment with the same length.

Genetic Diversity: This was carried out with the use of the DNASP (5.0) software to analyses and determine the number of haplotype '(h), haplotype diversity (hd), nucleotide diversity (N.D), number of polymorphic site (N.P.S), singleton variable site (S.V.S) and parsimony informative site (P.I.S) which were estimated and a table was created to show in the result.

RESULTS

Single nucleotide polymorphism

In *IGF-1* of normal feather chicken, five mutations (SNP) at five positions in 7502 bases were identified. The mutations observed were homozygous 75A>G Glu25Glu (synonymous mutation), 297T>C Phe99Phe (synonymous mutation), and 315G>A Lys105Lys (synonymous mutation).

Table 1. Single nucleotide polymorphism found in normal chicken and their position.

Position	SNP	Amino acid variation
75	A>G	Glu25Glu
297	T>C	Phe99Phe
315	G>A	Lys105Lys
386	T>C	Val129Ala
501	T>A	Thr167Lys

In *IGF-1* of Arbor Acre chicken, 11 mutated bases at four positions were analysed. The mutation observed has two synonymous mutations 159C>T Cys53Cys, 439T>C Leu147Leu and two non-synonymous mutation 249G>T Stp83Tyr, 415T>C Cys139Arg.

Table 2. Single nucleotide polymorphism found in Arbor Acre and their position.

Position	SNP	Amino acid variation
159	C>T	Cys53Cys
249	G>T	Stp83Tyr
415	T>C	Cys139Arg
439	T>C	Leu147Leu

Genetic diversity

The estimated genetic parameters obtained are presented in table 3 below. For the genetic diversity between Arbor Acre and Nigerian normal feather chickens, the highest number of haplotype (4) was estimated in Nigerian normal feather chicken while Arbor Acre has (3) number of haplotypes. The highest haplotype diversity (0.638) was estimated in Nigerian normal feather chicken while (0.318) was estimated in Arbor Acre chicken. Normal feathered chicken had the highest average number of nucleotide diversity, number of variable polymorphic site, total number of mutations, singleton variable site and number of polymorphic segregating sites (0.01744, 61, 61, 60 and 61). The highest number of invariable monomorphic site (488) and parsimony informative site (4) was estimated in Arbor Acre.

Table 3: Estimated genetic diversity in the two strains of Nigerian normal feathered and Arbor Acre chicken

Strain	N.S	H	H.D	N.D	I.M.S	V.P.S	T.M	S.V.S	P.I.S	N.P.S
Normal feathered chicken	15	4	0.638	0.01744	425	61	61	60	1	61
Arbor Acre:	18	3	0.318	0.00198	488	4	4	0	4	4

H= Number of Haplotypes.
H.D= Haplotype (gene) diversity.
N.D= Nucleotide diversity.
I.M.S= Invariable (monomorphic) sites.
V.P.S= Variable (polymorphic) sites.
T.M= Total number of mutations.
S.V.S= Singleton variable site.
P.I.S= Parsimony informative sites
N.P.S= Number of polymorphic (segregating) sites. NS= Number of sequences.

Discussion

Insulin-like growth factor-1 (*IGF-1*) is a protein, which has similar molecular structure to insulin and plays a major role in proliferation, differentiation and metabolism of myogenic cell lines in animals including chicken (Duclos, 2005). The *IGF-1* gene is involved in growth of various tissues like muscle, cartilage and bones (Tirapegui, 1999). Amills*et al*. (2003) discovered a polymorphism in the promoter of the *IGF1* gene in the Black Penedesenca chicken strain, which was associated with average daily gain and feed efficiency. Abbasi and Kazemi (2011) also reported a polymorphism in the 621 bp flanking promoter and 5untranslated regions in the fowl *IGF-1* gene and identified two alleles.

From the result, each strain of the chicken where the SNPs occurs changes the amino acid at a specific location. Highest frequency of SNPs occurred in Nigerian normal feather chicken (5) compared to Arbor Acre chicken (4). Some of the SNPs identified in both strain of chicken were synonymous mutation. This indicates that the region is highly conserved. The Arbor Acre had lower diversity than the normal feather chicken; this may be as a result of differences in their effective population. It has been reported that SNP frequency and nucleotide diversity are affected by several factors, including selection, mutation rates, mating system, effective population size, and demography, gene flow between populations, introgression from hybridisation and historical effects on these factors (Frankham *et al*., (2002).

High nucleotide diversity has been reported in the avian genome (Primmer *et al.,* 2002). The low level that was observed during this study might be connected to the length of the sequence used. Haplotype diversity was higher in normal feathered chicken than the Arbor Acre. This indicated that the diversity of the normal feathered chicken was rich which is as a result of higher nucleotide diversity in the normal feathered chicken. High diversity should enhance the adaptation of these species as it could provide the evolutionary potential to adapt to the rapidly changing environmental condition of the tropical climatic conditions. The highest number of haplotypes (4) was observed in strains of normal feather chicken while Arbor Acre has (3). This suggests that genetic variation will be higher in normal feather when compared to Arbor Acre. Normal feather chicken had the highest haplotype diversity (0.638) compared to Arbor Acre that had (0.318), this suggests the abundance of genetic diversity in normal feather chicken (Sethi, 2003). The highest average number of polymorphic sites (61), nucleotides diversity (0.01744) and singleton site (60) were estimated in normal feather chicken. This suggests that this strain of chicken has been subjected to a lot of natural selection which is in line with (Borghese and Moioli, 1999; Sethi, 2003).

Conclusion

- Most of the SNPs observed were synonymous mutation which showed no alteration in the amino acid.
- The lowest haplotype diversity (0.318) observed in Arbor Acre compared to normal feather chicken (0.638) indicated that the *IGF-1* was highly conserved in Arbor Acre.
- The analyses revealed abundance of polymorphism at the single nucleotide level which prioritise *IGF-1* gene as a valuable candidate gene for association studies with economically important traits in livestock.

Recommendation

More analytical studies should be carried out to check for more *IGF-1* genes by evaluating polymorphism and traits of growth in other breeds and line of chickens and conducting further function studies for defining the effect of SNP in the *IGF-1* gene at a molecular level for improvement of the indigenous chicken.More sequences of *IGF-1* genes of other species need to be studied to give a broader view of evolutionary relationship.

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