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ASSESSMENT OF GENETIC DIVERSITY AND EVOLUTIONARY RELATIONSHIP OF GROWTH DIFFERENTIATION FACTOR 8 GENE OF FOUR BROILER CHICKEN GENOTYPES

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Abstract

This experiment was designed to estimate genetic diversity and analyse evolutionary relationship of exon 1 Growth differentiation factor 8 gene in Arbor Acre, Abor Acre Plus, Cobb and Ross. Two-hundred-day-old chicks of mixed sexes were assigned into five equal-sized cells, each containing forty birds with ten birds per strain. Blood samples were collected via brachial vein of selected birds for molecular analysis. The amplified products were sequenced and genetic diversity was estimated by DNA sequence polymorphism software and phylogeny was analysed by molecular evolutionary genetic analysis software. The analysis revealed polymorphic sites of 7 in Ross and 4 each in Arbor Acre, Abor Acre Plus and Cobb. Ross had 5 haplotypes and other strains had 3 each. Ross exhibited highest haplotype and nucleotide diversity. Positive Tajima's D was revealed in Arbor Acre while negative Tajima's D occurred in Abor Acre Plus, Cobb and Ross. Arbor Acre and Abor Acre Plus were closely related in breeding lineage. The clustering of Cobb and Ross in distinct phylogenetic branches suggested unique ancestral origins. In

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that high genetic diversity is advantageous for breeding programme since it provides a wider genetic pool for selection, resilience against inbreeding depression and better adaptability to environmental changes. However, Ross is the most promising for breeding improvement.

Introduction

Growth differentiation factor 8 (GDF-8) gene also known as *Myostatin,* a member of the Transforming Growth Factor-Beta (TGF-β) superfamily, functions as a negative regulator of skeletal muscle growth by inhibiting muscle cell proliferation and differentiation (Chen *et al.,* 2021). Genetic diversity within the GDF-8 exon 1 region provides valuable insight into variation that shapes muscle development and growth performance in broiler chickens.

Genetic diversity is the basic aspect of phylogenetic study which plays a vital role in the adaptation and improvement of poultry breeds. According to Frankham *et al.* (2017), genetic diversity is important for populations to maintain evolutionary potential, as it enables natural selection to act upon favourable traits that enhance survival and reproduction. Without sufficient genetic variation, populations may face inbreeding depression, reducing their adaptability and fitness over generations. Genetic diversity also engages in the ability of species to adjust to changing environment. As climate change quickens, many species face habitat shifts, new competition and emerging diseases. Jump *et al.* (2009) warned that species with low genetic variation are more likely to experience range contractions and population shrinkage. FAO (2015) documented that over 17% of livestock breeds are at risk of extinction due to the narrowing genetic base caused by intensive selective breeding and commercialization. A genetic erosion in livestock reduces the capacity to respond to new pathogens and environmental shifts, threatening global food security. In broiler chickens, genetic variation directly impacts economic traits such as growth rate, feed conversion efficiency, carcass composition and muscle development (Dekkers and Gibson, 2018).

Phylogenetic analysis is a powerful tool for estimating genetic variation, evolutionary relationships and population structure among different broiler strains. In a phylogenetic study, Muchadeyi *et al.* (2007) employed microsatellite markers to assess genetic variation in both indigenous and commercial chicken populations, including Ross and Cobb lines. Their findings indicated that Ross and Cobb chickens showed lower genetic diversity and formed distinct clusters separate from native breeds, which is associated to intensive selection for production traits. Phylogenetic trees derived from SNPs data showed that Arbor Acre (AA) and Ross

chickens clustered together, consistent with their shared ancestry and breeding goals for broiler efficiency. The study highlighted that commercial broiler, despite brand differences, often originate from a narrow genetic pool (Muir *et al.,* 2008). Islam *et al.* (2019) employed a combination of mitochondrial DNA and microsatellite markers to analyse the genetic structure of Cobb, Ross, AA and Arbor Acre Plus (AAP) chickens. Their results reconfirmed that Cobb and Ross formed a monophyletic group, reflecting their close phylogenetic proximity. Arbor Acre and AAP exhibited slightly greater genetic distances but remained within the broader commercial broiler cluster. The study finalised that while brand distinctions exist, these commercial broiler lines remain genetically similar due to their shared breeding goals and limited founder populations. Phylogenetic data thus serve as a useful tool for tracing breed history and informing cross-breeding decisions. However, limited research has focused on genetic variation and phylogenetic relationships of the growth differentiation factor 8 (GDF-8) gene exon 1 region in broiler strains. Understanding these genetic variations will provide valuable information for enhancing growth.

Materials and Methods

Experimental Site

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Science, Al-Hikmah University, Igbaja Campus, Kwara State, Nigeria located approximately 56kms north-east of Ilorin and north-north-east of Ajase-Ipo, on Latitude 8°23'60"N and Longitude 4°53'60"E at an altitude of 394.97 meters and 1295.83 feet above sea level (Google map). The molecular analysis was carried out at Inqaba Biotech West Africa Limited, Ibadan, Oyo State, Nigeria located on latitude 7°23'47"N and longitude 3°55'0"E (Google, 2023).

Experimental Animals

Data were generated from two-hundred-day-old broiler chicks of mixed sex comprising of fifty chicks per strain namely Arbor Acre, Cobb 500, Ross 308 and Arbor Acre Plus. Birds were raised in a deep litter pen which was divided into five cells containing forty birds per cell. Thereafter, the birds were weighed, leg banded individually and later wing tagged at 4 weeks for easy identification.

Brooding and Rearing Management

Prior to the arrival of the day-old chicks, the brooder pen was washed with Lysol® and fumigated with formaldehyde, lime and potassium permanganate. The dimension of each brooder cell was 8m × 8m. Wood-shaving was spread on the floor of each cell and heat source was placed at the centre of each cell to provide warmth for the chicks. At the arrival of the chicks, they were given multivitamin drugs which served as an anti-stress. The chicks were brooded for two weeks. Adequate heat, ventilation, medication and feeding were provided. The chicks were duly vaccinated and antibiotics was administered as required. The litter was changed at five days intervals to prevent accumulation of ammonia gas. All chicks remained on deep-litter throughout the period of the experiment.

Experimental Feeds and Feeding

The birds had *ad libitum* access to commercial feed and water throughout the experiment. All birds were placed on convectional broiler starter crumble containing 21.0% Crude Protein and 2900kcal/kg Metabolizable Energy for six weeks and finisher pellet containing 17.0% Crude Protein and 3000kcal/kg Metabolizable Energy till the end of the experiment.

Molecular Analysis Procedure

At 12^{th} week of age, 1ml of blood was collected from 40 selected chicken from the flock comprising of 10 birds selected from each of Arbo Acres, Ross, Arbo Acres Plus and Cobb. Blood was collected with needle and syringe via brachial vein into EDTA bottles as described by Ayorinde *et al.* (2001) and Sewalem *et al.* (2002) for molecular analysis.

Extraction of DNA

DNA was extracted from whole blood sample collected with the use of commercially available Quick-DNA™ Miniprep Kit manufactured by Zymo Research Epigenetics Company, USA following the manufacturer's protocol.

DNA Quantification

Extracted gDNA was quantified for purity and concentration using Nanodrop spectrometer. The integrity of the DNA was checked using gel electrophoresis by running Iμl of each gDNA sample on 1% Agarose gel at 120V for 20 minutes.

Primer

The growth differentiation factor 8 exon 1 gene was amplified using the designed forward and reverse primer sequences as used by Tanjung *et al.* (2019).

- Forward: 5′-ATGCAAAAGCTAGCAGTCTATG-3′
- Reverse: 5′-ACTCCGTAGGCATTGTGATAAT-3′

Polymerase Chain Reaction (PCR)

The extracted DNA was amplified using Polymerase Chain Reaction (PCR). The amplification reaction was carried out in PCR tube using programmable thermocycler (Mastercycler pro by Eppendorf). Polymerase Chain Reaction amplification was performed in a volume of 25 µl mixture containing 2 µl of DNA template, 1 µl of each forward and reverse primer and 12 µl of master mix and 9 µl of sterilized distilled water.

Quantification of PCR Products

The concentration of the purified PCR product was determined using UV spectrophotometry or fluorescent quantification.

DNA Electrophoresis

Electrophoresis was carried out on 2 µl sample from PCR amplicon at 100 volts for 45 minutes in 1 % agarose gel in 1 × TAE buffer containing 1.0 µl stain of ethidium bromide. After electrophoresis, the gel was taken into gel documentation machine to view the bands using Ultra-violet illumination. Moreover, for quality check, the PCR products (amplicons) was visualized using Agarose gel electrophoresis for the GDF-8 gene with concentrations of 2.0%.

DNA Sequencing

Polymerase Chain Reaction (PCR) products of the DNA fragment was sequenced unidirectionally with forward primer using the Big Dye version 3.1 sequencing. The sequence data was collected automatically on the ABI PRISM 3100 Genetic Analyser (Applied Bio-systems).

Editing and Analysis of Sequence

The sequence was aligned, trimmed and edited using Bioedit® software. The edited sequence was blasted (BLASTN) (http://www.ncbi.nlm.nih.gov/BLASTN/) against other sequences in the GenBank to ascertain the similarity and identity with other chicken sequences of GDF-8 gene in the database. The edited sequences were further aligned using CLUSTALW for further analyses.

Genetic Diversity in the GDF-8 gene

Nucleotide diversity, number of haplotypes, haplotype diversity, mutation type, average number of pairwise nucleotide differences, Number of polymorphic sites, sequence conservation, theta, singleton variable site and parsimony informative site were estimated from the aligned sequence of GDF-8 gene using DnaSP v6.

Neutrality Test

Tajima's D, Fu's F, Fu and Li's D, Fu and Li's F were applied to test the neutrality of exon 1 of GDF-8 gene in the chicken population sequences according to the procedure of DnaSP v6 (Rozas *et al.,* 2017).

Evolutionary Analysis

A consensus sequence was generated from the chicken population sequences using Bioedit and an evolutionary tree was obtained using Molecular Evolution and Genetic Analysis (MEGA) v11 software showing the relationship between the chicken GDF-8 gene sequences of the chicken population.

Results

The genetic diversities of the Arbor Acre (AA), Arbor Acre Plus (AAP), Cobb and Ross chickens using Growth differentiation factor 8 (GDF-8) gene exon 1 sequence are shown in Table 1. The number of polymorphic sites present in AA, AAP, Cobb and Ross were 4, 4, 4 and 7 respectively. 3 haplotypes were present in each AA, AAP and Cobb while it was 5 in Ross. Arbor Acre and Arbor Acre Plus birds had the same number of Haplotype diversity, followed by Cobb while Ross had the highest. Ross had the highest number of nucleotide diversity and average nucleotide diversity followed by AA while Cobb and AAP had the least. The total mutation that occurred was 20 in which AA had 4, AAP had 4, Cobb had 4 and Ross had 8.

The neutrality test for the four strains is shown in Table 2. Negative Tajima's D occurred in GDF-8 exon 1 of AAP, Cobb and Ross. Negative Fu and Li's D* and Fu & Li's F* values were observed in AAP and Cobb while negative Fu's Fs was observed in only Ross. However, Positive Tajima's D was found in AA, positive Fu and Li's D, Fu and Li's F were observed in AA and Ross while positive Fu's Fs values were observed in AA, AAP and Cobb. Also, Tajima's D, Fu and Li's D, Fu and Li's F and Fu's Fs were observed to have a non-significant ($P>0.05$) deviation.

The phylogenetic relationships of AA, AAP, Cobb and Ross chickens based on GDF-8 gene exon 1 sequence are shown in figure 1. The first clade had four subclades comprising of four genotypes which mean that AA and AAP are closely related while the other two clades revealed relatedness of Cobb and Ross chickens.

Haplotypes and haplotype network of Arbor Acre, Arbor Acre Plus, Cobb and Ross chickens using GDF-8 exon 1 sequence. The haplotype number of the selected individual chicken from each genotype; Arbor Acre (AA), Arbor Acre Plus (AAP), Cobb 500 and Ross 308 chickens using *Myostatin* gene sequence are presented in Table 3. Figure 2 showed the result of median-joining network profile haplotypes observed in Arbor Acre, Abor Acre Plus, Cobb and Ross chicken strains. Each circle represented a haplotype. The circle size is proportional to its total frequency and different coloured circles represented different strain such that Lemon indicated AA, Pink indicated AAP, Green indicated Cobb and Orange indicated Ross. Hap1 represented haplotype 1, Hap2 represented haplotype 2 to Hap10 represented haplotype 10. The results showed the similarity to the neighbour-joining tree. Hap2, Hap4 and Hap5 were mainly distributed in the centre of the median joining network with derivative haplotypes spreading outwards from it.

Table 1: Genetic diversity of Arbor Acre, Arbor Acre Plus, Cobb and Ross chickens using GDF-8 gene exon 1 sequence.

Genotypes	H	Hd	Hdv	HdSd	S	Si	Pa	Pi	Eta	W	K	C
AA	3	0.8	0.02688	0.164	4	2	2	0.00528	4	0.00507	2.000	0.989

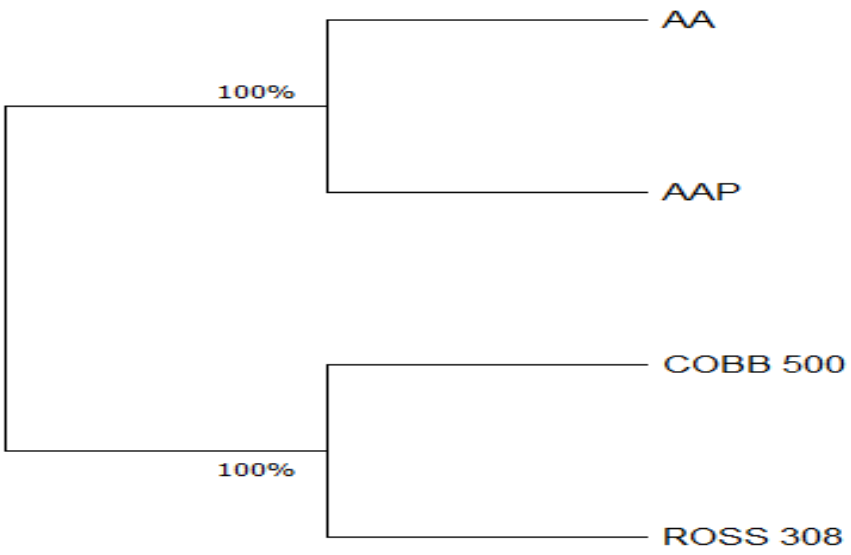
AAP	3	0.8	0.02688	0.164	4	3	1	0.00476	4	0.00508	1.800	0.989
COBB	3	0.7	0.04768	0.218	4	3	1	0.00475	4	0.00507	1.800	0.989
ROSS	5	1	0.016	0.216	7	3	4	0.01	8	0.00884	3.800	0.982

S=Number of polymorphic (segregating) sites, H= Number of Haplotypes, Hd=Haplotype (gene) diversity, Hdv=Variance of Haplotype diversity, HdSd=Standard Deviation of Haplotype Diversity, Pi=Nucleotide diversity, W= Theta, Si=Singleton, Pa=Parsimony, K= Average number of pairwise nucleotide difference, Eta = Total number of mutation and C= Sequence conservation.

Table 2: Neutrality test of Arbor Acre, Arbor Acre Plus, Cobb and Ross chickens using GDF-8 gene exon 1 Sequence.

Genotypes	TAJIMA's D	Fu and Li's D*	Fu and Li's F*	Fu's Fs
AA	0.27345	0.27345	0.27345	0.644
AAP	-0.4102	-0.4102	-0.4102	0.469
COBB	-0.4102	-0.4102	-0.4102	0.469
ROSS	-0.0734	0.28638	0.28638	-1.805

Figure 1: Evolutionary tree of Arbor Acre, Arbor Acre Plus, Cobb and Ross chickens using GDF-8 exon 1 Sequence



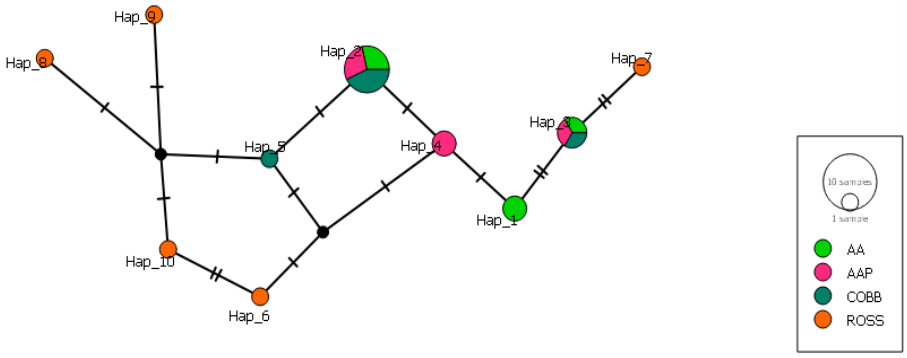
AA: Arbor Acre

AAP: Arbor Acre Plus

Table 3: Haplotypes of Arbor Acre, Arbor Acre Plus, Cobb and Ross chickens using GDF-8 exon 1 sequence.

Chickens	Genotypes	Haplotypes
APB8M	AAP	2
APA7F	AAP	4
APB9F	AAP	2
APC2M	AAP	3
APE10M	AAP	4
AB1M	AA	1
AB2F	AA	2
AE3F	AA	3
AD9F	AA	1
AC3M	AA	2
RD3F	ROSS	9
RC2F	ROSS	7
RB3M	ROSS	8
RC9M	ROSS	6
RE9F	ROSS	10
CA1M	COBB	2
CD9F	COBB	3
CA7M	COBB	2
CB3F	COBB	2
CB9M	COBB	5

Figure 2: Haplotype network of Arbor Acre, Arbor Acre Plus, Cobb and Ross chickens using GDF-8 exon 1 Sequence



Discussion

Genetic diversity is crucial for the adaptability and productivity of poultry populations. In this study, the Growth differentiation factor 8 (GDF-8) gene exon 1 was targeted due to its vital role in muscle growth regulation. The findings revealed the presence of polymorphic sites, indicating genetic variability within and between the four broiler strains. This experiment is consistent with previous studies by Rajput *et al*. (2022) who reported high genetic diversity in *MSTN*. High genetic diversity in Ross birds as recorded in this study agrees with the work of Abdullahi *et al*. (2021). Ross exhibited highest genetic diversity compared to other commercial broiler strains such as Arbor Acres and Cobb which showed moderate to low levels of diversity which may be associated with highly intensive selection for growth traits. This reduction in diversity may reflect selective sweeps, where beneficial alleles are linked to increased muscular hypertrophy have become fixed. This study agrees with the report of Sweeney *et al. (*2021) who documented that Ross had the highest polymorphic sites and nucleotide diversity. Intensive commercial selection decreases diversity and may increase inbreeding risks (Frankham *et al.,* 2017; Gholami *et al.,* 2019). Cobb had undergone rigorous selection as observed by Havenstein *et al*. (2019), and therefore may exhibit signs of a recent selective sweep at the GDF-8 locus.

In the neutrality test, the negative Tajima's D values recorded in the present study suggested that the GDF-8 gene in these broiler strains may be under purifying or directional selection. This is in line with the reports by Chen *et al*. (2020) and Silva *et al*. (2021), who highlighted similar patterns in broiler populations, implying that detrimental alleles might have been erased through selection, especially in commercial breeds. The restraint of harmful polymorphisms ensures the retention of functionally beneficial alleles that promote increased muscle mass and feed efficiency. The experiment supports the work of Chen *et al.* (2020) and Silva *et al. (*2021) also reported negative Tajima's D values in AAP, Cobb and Ross and they suggested purifying selection at the GDF-8 locus, which they attributed to artificial selection for growth traits. Purifying selection likely reflects artificial selection regimes imposed during commercial broiler breeding, which focus on traits like feed conversion ratio (FCR), breast meat yield and early maturity. Arbor Acre showed a positive Tajima's D, possibly indicating balancing selection. The non-significant neutrality tests in Tajima's D, Fu and Li's D* and Fu's Fs revealed weak but relevant selection pressures in the broiler strains (Hudson *et al.,* 2004; Rozas *et al.,* 2017).

Phylogenetic analysis of GDF-8 exon 1 sequences demonstrated evolutionary divergence among the four broiler strains. Arbor Acres and Arbor Acres Plus though closely related in breeding lineage, however, the sequence divergence observed reflects sub-strain specialization, possibly due to regional adaptation and unique selection histories. The clustering of Cobb and Ross with distinct phylogenetic branches suggests unique ancestral origins or independent selection pressures. These results support the hypothesis that although modern broiler strains have been selectively bred for similar performance traits, their genetic pathways may differ significantly. This evolutionary divergence is supported by various studies Zhang and Zhang (2012) and Yin and Zhang (2023) This indicates that breeding strategies employed by parent companies (Aviagen, Cobb-Vantress etc.) result in different genomic footprints, even for functionally similar traits.

Haplotype analysis of GDF-8 exon 1 revealed several distinct haplotypes among the four broiler strains, with variable distribution patterns. The presence of multiple haplotypes suggests that despite selective breeding, a certain degree of genetic variation persists within the GDF-8 gene, especially in Ross. The high haplotype diversity in this study, is an indication of historical recombination events or the retention of ancestral polymorphisms. According to Hudson *et al*. (2004), such diversity ensures a broader genetic base, which is critical for adaptive evolution. In poultry breeding, it allows for selection flexibility when responding to emerging challenges like climate stress or disease resistance. The haplotype network diagram revealed both shared and unique haplotypes. Shared haplotypes across strains point to common ancestry or gene flow due to cross-breeding, while unique haplotypes in specific strains such as Cobb suggests strain-specific evolution or isolated selection histories (Gholami *et al*., 2019). These authors emphasized the importance of preserving rare haplotypes as reservoirs of novel alleles for future breeding.

Conclusion and Recommendation

This study provided valuable insights into the genetic structure, selection patterns and evolutionary relationships among AA, AAP, Cobb and Ross. Ross exhibited higher genetic diversity with the highest number of polymorphic sites and haplotypes than AA, AAP and Cobb. Ross strain also had the highest number of haplotype diversity when compared to other strains in which AA and AAP had the same value of haplotype diversity and Cobb had the lowest value. Neutrality tests revealed negative Tajima's D values in AAP, Cobb and Ross indicating purifying

selection or recent population expansion at the GDF-8 gene locus. However, none of the neutrality test results was statistically significant indicating weak selection. In contrast, AA showed a slightly positive Tajima's D, implying possible balancing selection. The phylogenetic analysis further revealed that AA and AAP are closely related genetically, while Cobb and Ross formed a distinct clade, indicating divergent evolutionary origins. High genetic diversity is advantageous for breeding programme since it provides a wider genetic pool for selection, resilience against inbreeding depression and better adaptability to environmental changes. However, Ross is the most promising for breeding improvement, AA with signs of balancing selection could also be important for maintaining allelic variation that might be lost under strong selection pressure**.** The presence of selection signatures in the genomes of these birds also highlights the evolutionary pressure exerted by intensive breeding, which must be balanced with conservation goals to prevent the erosion of valuable alleles.

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Data Availability Statement

Raw data can be provided upon request.

Authors' Contributions

[1]Adewole R. A**.** is the corresponding author, she contributed to the research work in terms of conceptualization, designing of methodology, data collection, manuscript drafting, manuscript submission and publication process.

[2]Prof. Adedeji T. A. contributed in the supervision of the research work and review of the manuscript.

[3]Prof. Emiola I. A. contributed in the review and editing of the manuscript.

[4,5]Prof. Adebambo A. O. contributed in the data analysis of the research work.

Declarations

Ethical Approval

There was no breach of ethical or regulatory guidelines during the collection of chicken blood for molecular analysis, and the study posed no adverse impact on the community.

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Competing Interest

Authors declare that they have no competing interests.

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