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GENETIC DIVERSITY OF BROILER CHICKEN BRANDS RAISED IN ARID AND SEMI-ARID ZONES OF NORTHERN NIGERIA USING MITOCHONDRIAL DNA

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ABSTRACT
A sector of broiler production is growing very fast to meet the high demand of meat in Nigeria. However, high mortality rates among the broilers especially during the hot dry season in arid and semi-arid zones is worrisome. There is a need for molecular genetics study that could aid in management, conservation and sustainable exploitation of this species. To evaluate the genetic diversity of broilers raised in these regions, a total of forty-six broilers were randomly sampled from eight different brands (Agrited, Amo, Chi, Fol-hope, Obasanjo, Olam, Yammfy and Zatech) for mitochondrial DNA analysis. Four haplotypes were detected among all the samples used that belong to the four strains. The sequences of mitochondrial regions revealed high haplotype diversity (0.78600) and low nucleotide diversity (0.00286). Lower genetic diversity observed may increase the chances of rapid disease infection and distribution during any disease outbreak. Introduction of new strains of broilers with high genetic diversity is highly recommended. Future study should be conducted on the performance of these strains during the extremely hot temperature period in arid and semi-arid zones of Nigeria. This is to provide reliable information for the sake of local broiler farmer's benefit who invests largely on this sector. The study will also help the geneticists from these brands to develop a strain that could survive and perform excellently under severe climatic conditions of the rural areas of arid and semi-arid zones of Nigeria.

Keywords: broiler brand, chicken, strains, mitochondrial DNA, Nigeria

INTRODUCTION

Population in Nigeria hits 200 million people with an estimated annual growing rate of 3.5% (Khaleel *et al*., 2020). The need for animal protein source (meat and egg) is increasing and attract an intensive profit in the meat and egg industries (Tamburawa *et al*., 2018; Ashiru *et al*., 2020). The number of farmers who own broiler parent and grand-parent stocks has increased rapidly, resulting in the increase in the production of broiler chicken in Nigeria. These farms produce and sell a variety of broiler strains under various brand names. The common broiler strains circulating in the Nigeria markets include Abor Acre Plus, Anak, Cobb, Ross, Hubbard, Marshall, Neoiler/Kroiler, and Cockerel (Olawumi and Fagbuaro, 2011; Amao *et al*., 2015). Broiler parents are commonly imported from temperate areas around the world (Udeh *et al*., 2011). In addition, different strains of birds have been developed with an intention to obtain maximum meat production (Kebede, 2017).

The assumption is that broiler chickens with better productivity, capable of adapting to the Nigerian environment and disease resistivity should be chosen by the broiler farmers. In native chicken, the existence of genetic diversity among/within the breeds provides a resistance towards disease infections (Ha *et al*., 2017). Therefore, such variations are also expected to exist between these broiler brands because of the parent stock quality, breeding efficiency and differences in the lineage from which the brand may have emerged (Deeb and Lamont, 2002). Commonly, the birds in the grand-parent stock facilities are raised in a climate-controlled confined housing facility characterised with a cool, bio-secured and hygienic environment. This is coupled with the use of latest poultry technology to maximize flock welfare and performance. In contrast, broiler chicks produced and distributed to the local broiler farmers are raised in a natural and hot environment with a poor facility (Sudik *et al*., 2020). Thereby, causing the

risk of diseases outbreak and high mortality rate that may lead to the huge loss during the production phase.

Northern Nigeria is characterised with an extremely hot temperature during the dry season period (March to July). Arid and semi-arid regions receive low rainfall, high temperature and low humidity (Alori *et al*., 2020). Raising broilers at this period is challenging and igniting certain diseases outbreak due to the heat stress. Casualties become extremely higher with several farms been affected. Sometimes, a high mortality rate among broilers is questionable as whether these broiler strains from different brands do acclimatized to the Northern Nigerian habitat. Therefore, two questions were raised in the current study, (1) Does these broilers from different brands belong to the same strain? (2) Are there any genetic differences among the broiler strains from different brands. Mitochondrial DNA (d-loop region) sequences have been widely used to determine the genetic diversity and phylogeographic structure of numerous species populations (Ha *et al*., 2017; Ahmad-Syazni *et al*., 2017; Khaleel *et al*., 2019; Lasagna *et al*., 2020). For enlightening and educating the small and big scales broiler farmers in arid and semi-arid zones of Nigeria about different brands and strains. The current study was aimed to determine the genetic diversity among broiler strains from different brands raised in Northern Nigeria using mitochondrial DNA analysis.

MATERIALS AND METHODS

Sampling

A total of forty-six samples of broiler chicken from eight major brands were collected from September to November 2020 by reputable suppliers (Table 1). The brands selected were the major distributors of broilers raised in the arid and semi-arid zones of Northern Nigeria. A small chicken wing portion from each individual was cut and preserved in 95% ethanol for DNA extraction (Ha *et al*., 2017).

Table 1. List of sample sizes and sample abbreviations for mtDNA analysis according to the various brands

Brand Name	Strain	location	Abbreviation	Sample size
Fol-hope Limited	Cobb 500	Oyo state	FUL	6
Olam Hatchery Limited	Cobb 500	Kaduna State	OLA	5
Zartech Hatchery Limited	Cobb 500	Oyo state	ZAT	7
Amo Farm Sieberer Hatchery	Arbor Acres plus	Oyo state	AMO	5
Chi Farms & Hatchery Limited	Arbor Acres plus	Oyo state	CHI	6
Yammfy farms Nigeria Limited	Arbor Acres plus	Kwara state	YAM	5
Obasanjo Farms Hatchery	Marshall	Ogun state	OBJ	6
Agrited Hatchery Limited	Ross 308	Oyo state	AGR	6

DNA Extraction, Amplification and Sequencing

The total genomic DNA of each sample was isolated using Favorgen DNA extraction Kit (Favorgen Biotech Corp., Ping-Tung 908, Taiwan) by following the manufacturer's protocol. The partial control region of mitochondrial DNA was amplified using a pair of primers, forward primer L16750 5'-AGGACTACGGCTTGAAAAGC-3' (Desjardins and Morais, 1990), and reverse primer H547 5'-ATGTGCCTGACCGAGGAACCAG-3' (Komiyama *et al.*, 2003) by polymerase chain reaction (PCR). The PCR was carried out in a 25 µl reaction volume containing 18.2 µl sterile distilled water, 2.5 µl Taq buffer, 2.0 µl dNTP Mix (2.5mM), 0.5 µl of each primer (10 µM), 0.3 µl of 5 unit/µl Taq polymerase (TaKaRa) and 1 µl template DNA (1-50 ng/µl) on a thermal cycler PCR machine Veriti 96 Well Thermal Cycler (Applied Biosystem, California, USA), under the following thermal cycling conditions. Initial denaturation at 95°C for 5 min, 35 cycles including denaturation at 95°C for 20s, annealing at 61.5 °C for 20s and elongation at 72 °C for 30 s, followed by final extension for 10 min at 72 °C and the PCR product was maintained at 4 °C. Sequencing was succeeded using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) by following the manufacturer's instructions, performed on an ABI Prism 3730xl Genetic Analyser (Applied Biosystems).

Data Analysis

The sequence chromatograms from the automated sequencer were checked with the Chromas version 2.4 (Technelysium Pty. Ltd., Queensland, Australia). The sequences were aligned and edited using GENETYX v9.1.3 multiple sequence alignment program. Nucleotide composition and number of variable sites were assessed using DnaSP v.6 (Rozas *et al*., 2017). All the aligned sequences were used to analyse the genetic variation using ARLEQUIN version 3.5 (Excoffier *et al.*, 2005). Genetic diversity in each brand was measured as haplotypic diversity (Nei, 1987) and nucleotide diversity (Tajima, 1983). A neighbour-joining tree of the haplotypes was constructed under the model of the Kimura 2-parameter using MEGA v.7 (Kumar *et al*., 2016), and evaluated with 1,000 bootstrap replicates.

RESULTS AND DISCUSSIONS

Sequence Variation

A total of forty-six 539 base pair fragment were sequenced and aligned successfully from the samples used. Among the

539 sites, 535 were invariable (monomorphic) sites and 4 were variable (polymorphic) sites. Three variable sites were parsimony informative sites with a single singleton variable site (Table 2). The sequences of the present study showed higher transition frequency (75%), lower transversion frequency (25%) with no insertion or deletion. Lower sequence variation observed have reflected the closeness of maternal lineage between the broiler strains used in the current study. In contrast, a higher sequence variation was observed in the previous studies on different local chicken breeds analysed (Ha *et al*., 2017; Lasagna *et al*., 2020).

Genetic Diversity

Among all the samples, four haplotypes were detected (Table 2). The FUL, OLA and ZAT shared a single haplotype (Hap-1), whereas Hap-2 consist of AMO, CHI and YAM. Hap-3 and Hap-4 were belonging to the OBJ and AGR brands respectively. The overall haplotype diversity and Nucleotide diversity were 0.78600 and 0.00286 respectively. The current study showed high level of haplotype diversity and low nucleotide diversity. Haplotype diversity (also known as gene diversity) represents the probability that two randomly sampled alleles are different, while nucleotide diversity is defined as the average number of nucleotide differences per site in pairwise comparisons among DNA sequences (Nei, 1987).

Table 2. Nucleotide polymorphism observed in partial control region of 46 broiler chicken sequences and their frequencies (N). Dots (•) indicate identity with reference sequence and different base letters denote substitution

	Nucleotide position				
Haplotype	**177**	**222**	**330**	**342**	**N**
Hap-1 (Cobb 500)	A	G	T	A	18
Hap-2 (Arbor Acres plus)	•	A	C	G	16
Hap-3 (Marshall)	•	A	C	•	06
Hap-4 (Ross 308)	T	A	C	•	06

Phylogenetic Relationships

The neighbour-joining (NJ) tree was constructed from the 4 haplotypes of mtDNA control region using the Kimura 2-parameter model (Figure 1). Two genus of *Gallus gallus*; *Gallus gallus gallus*, GenBank accession number AB007720 and *Gallus gallus bankiva*, GenBank accession number AB007718, that were retrieved from the National Centre of Biotechnology Information (NCBI) were included in the tree as out-groups. The tree showed no obvious genealogy among the four haplotypes. The topology of the tree was shallow and there were no significant genealogical clusters of samples corresponding to the strains. Broiler chickens from four strains are genetically closer to *Gallus gallus gallus* and relatively far away from *Gallus gallus bankiva*. Similar result was reported by Ha *et al*. (2017) that local chicken in East Coast of Peninsular Malaysia is closer to the *Gallus gallus gallus*. Hence, Ross 308 strain was closer to the ancestor (*Gallus gallus gallus*) compared to other strain.

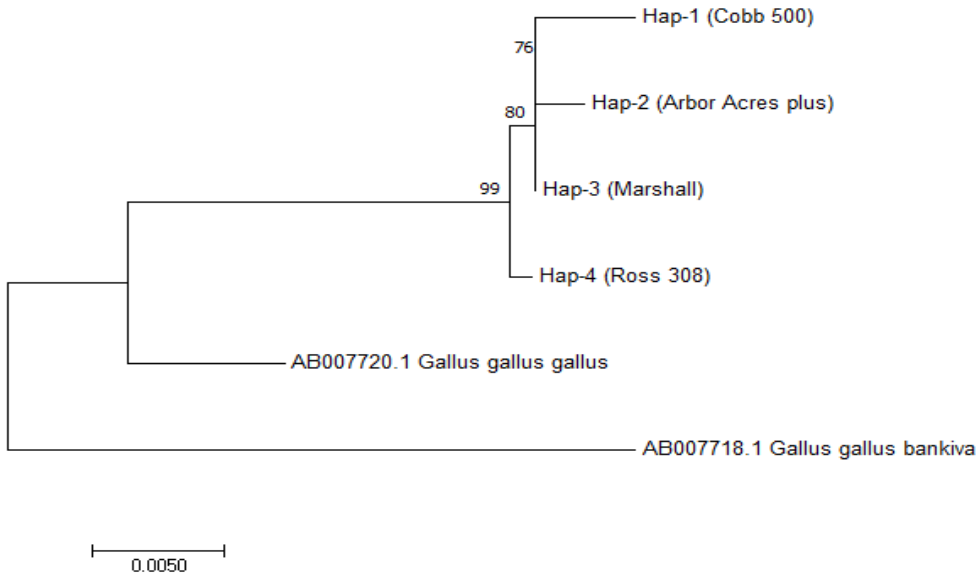

Figure 1. Neighbour-joining tree constructed using MEGA version 7 from 4 haplotypes identified in the eight brands producing and distributing broiler chicken to arid and semi-arid zones of Nigeria

For years, many populations have been chosen for their phenotypes as well as their ability to adapt to the environment and resist prevailing disease (Mpenda *et al*., 2019). This is illustrated by a wide range of chicken breeds and ecotypes seen across the world (Di Lorenzo *et al*., 2015). A broiler is any chicken (*Gallus gallus domesticus*) bred and raised exclusively for meat production. Figure 2 show the clear pictures of Arbor Acres plus, Cobb 500, Marshall, Ross 308 strains available in the Nigerian market. These strains are very difficult to be differentiated at day-old to one week stage. Some companies do not produce the chicks, rather they obtain a day-old chick from verified hatcheries. For instance, Fol-hope day-old birds are sourced from Chi, Amo, Agrited, Grinphield, Olam, Zartech, Sayed, etc.) and packaged in cartons of 50 chicks (afrimash.com). As such, single strain may dominate the production which can lead to the strain homogeneity in the system.

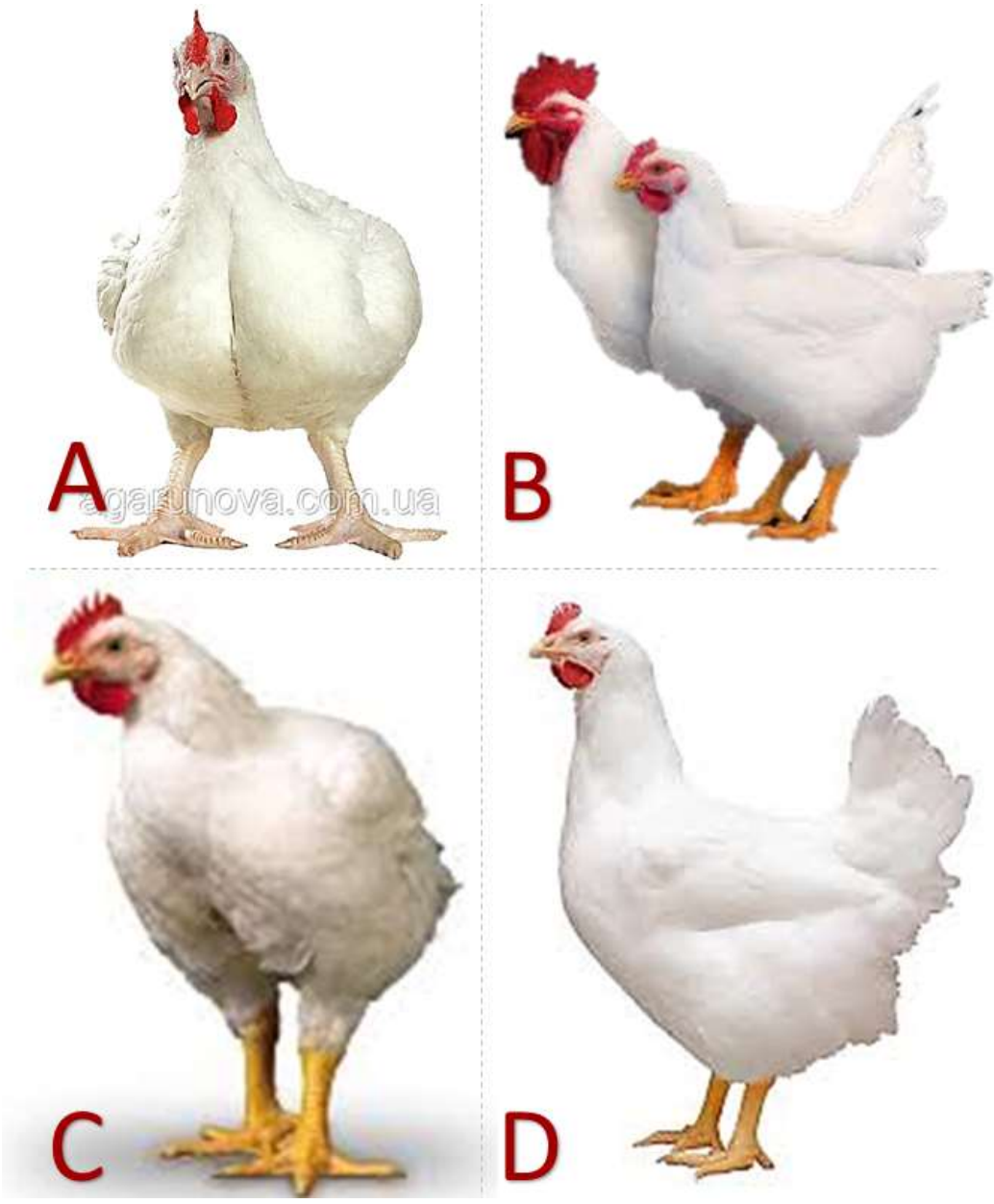

Figure 2. Broiler strains in Nigerian markets. **A:** Ross 308 (agarunova.com.ua) **B:** Marshall (eu.aviangen.com) **C:** Arbor Acres plus (us.all.biz) **D:** Cobb 500 (thebizbookproject.org)

In the preliminary survey conducted by the authors of this study, Olam dominate the production market in the arid and semi-arid zones due to its closer location to the region, followed by Zatech. Both these brands shared similar strain (Cobb 500, Hap-1). In the event of any disease outbreak such as bird flu, the mortality rate will be higher in those farms raising the most popular strain. Most broiler farmers in Nigeria do not understand the breeding principles and concepts of these strains. Because of high demand of broiler especially during festivals, farmers source their broiler chicks from the brands available in the market. Sometimes, colleague who may be from different vegetation (e.g., humid

zone) can also suggest the brand to a farmer in the arid or semi-arid zones. This incompatibility explains the reasons why farmers in the arid and semi-arid zones are suffering from casualties. Nowadays, many broiler farmers exit the business due to the unstable nature of the system.

Vegetation in Nigeria is complex and may vary within a 100 km (Figure 3). It will be challenging to produce a broiler strain that would couple to all vegetations present in the country. Recently, a study was conducted at Jos Plateau on the performance of three broiler strains from different brands (Sudik *et al*., 2020). The authors recommended these strains (Arbor Acres Farm Support, Arbor Acres Grinphield and Marshall Grinphield) to the farmers for expressing similar genetic potential and performance with less than 5% mortality rate. This might be due to the unique vegetation of Jos which is characterised with a lower temperature. In subhumid zone of Nigeria (Makurdi) however, the performance of Marshal and Hubbard strains was reported to be grossly affected by heat stress compared to Arbore acre strain (Gwaza *et al*., 2017). The scenario may vary if similar studies are carried out in arid, semi-arid and humid zones of Nigeria.

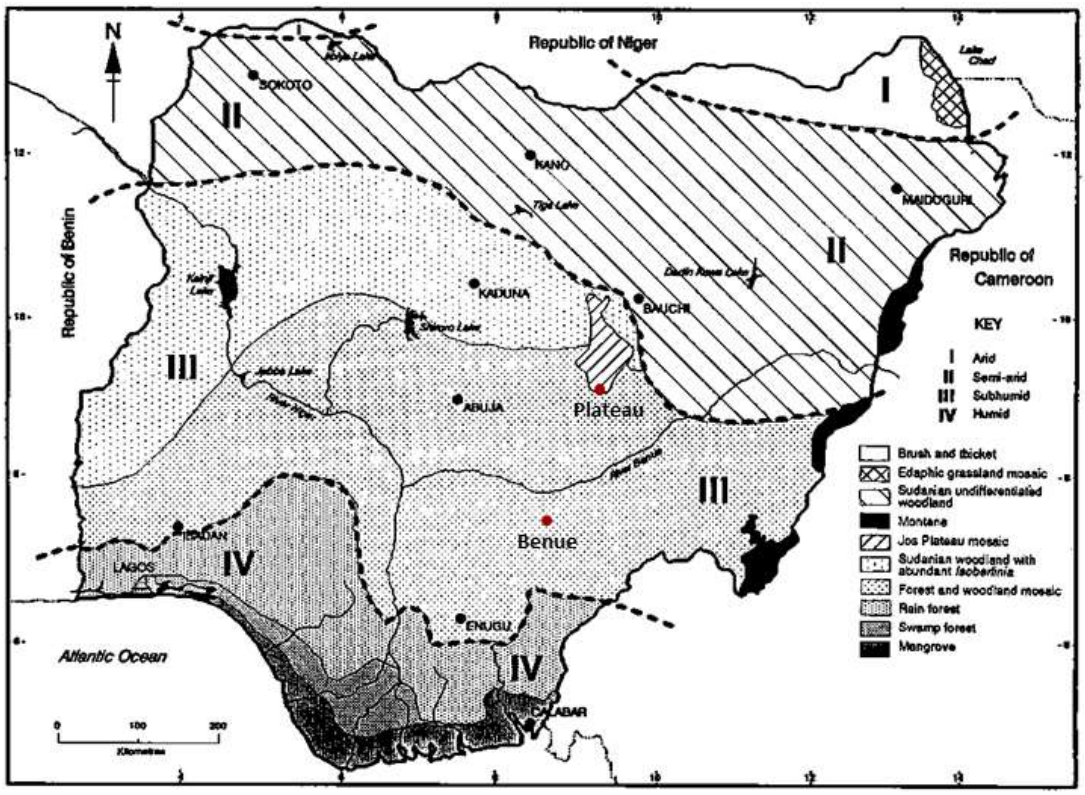

Figure 3. Vegetation map of Nigeria (adreed5nigeria.weebly.com)

CONCLUSIONS

This study represents the first comprehensive overview of the maternal lineages of broiler strain in Nigerian through the analysis of the mtDNA control regions of 46 samples belonging to the eight brands. The sequences of mitochondrial regions revealed 4 haplotypes with high haplotype diversity and low nucleotide diversity. Introduction of new strains of broilers with high genetic diversity is highly recommended. It is also recommended that study should be conducted on the performance of these strains during the extremely hot temperature period in arid and semi-arid zones of Nigeria. This is to provide reliable information for the sake of local broiler farmer's benefit who invests largely on this sector. The study will also help the geneticists from these brands to develop a strain that could survive and perform well under severe climatic conditions of the rural areas of arid and semi-arid zones of Nigeria. Finally, we are suggesting that these brands should clearly state the name of the strain in their cartons for simple verification and choice of the farmers.

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