



CARBONYL ANHYDRASE POLYMORPHISM IN EXOTIC AND NIGERIA INDIGENOUS ECOTYPE CHICKEN

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ABSTRACT

The indigenous chicken ecotypes in Nigeria, are known for their hardy nature and ability to survive harsh conditions within the tropics as compared to their exotic counterparts, hence a study on Carbonic Anhydrase polymorphism was carried out to investigate the genetic diversity between the domestic chicken ecotypes (Fulani, Yoruba, Abuja) and the exotic breeds (arbor acre) in Nigeria. A total of 200 birds were used for the study and the procedure for Carbonic Anhydrase typing was carried out using Cellulose acetate Electrophoresis. The result revealed that two co-dominant Carbonic Anhydrase Alleles (CA F, and CA S,) were found in these chicken ecotypes. From the electrophoretic band patterns, three Genotypes (CA FF, CA SF and CA SS) were observed. The frequency of F and S genes were (0.7250, and 0.2750, 0.7000 and 0.3000, 0.6735 and 0.3265, 0.7221 and 0.2779) respectively for Fulani, Yoruba, Abuja and Exotic breeds of chickens, while the Genotype frequency for Carbonic Anhydrase FF (CA FF) are (0.26, 0.14, 0.115, and 0.145) respectively for Fulani, Yoruba, Abuja and Exotic breeds of chicken. The findings of the study suggest little variation in diversity between the local ecotypes and the exotic chicken population. However, based on the observed genetic diversity between both populations studied and the results provide insights and prospective information on the prospects for the improvement of the indigenous chicken population studied. The obtained outcomes can be used as a benchmark for enhancing genetic diversity studies in both indigenous and exotic chickens using biochemical markers.

Keywords: Allele, Carbonyl Anhydrase, Genotype, Frequency, Heterozygosity, Polymorphism, Opportunity

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INTRODUCTION

The expanding population in Nigeria has created a rising need for animal and poultry goods that surpass the current supply, particularly emphasizing the crucial importance of chickens among avian species [1]. Poultry products stand out as a cost-effective and easily accessible source of animal protein for the large population. In Nigeria, chickens, with a population of 166 million birds, are the most widespread among all poultry varieties [2]. Therefore, chickens hold considerable socio-cultural and economic significance in many African communities. The genetic variations found in native livestock species within developing nations are valuable qualities that contribute to the animals' production, adaptation, and ability to withstand endemic diseases. DNA-based technologies have become the preferred methods for genetically characterizing livestock [3]. However, their utilization in developing nations is constrained by complexity, facility availability, and high expenses. Despite this, alternative biotechnological methods have introduced molecular techniques, like regular electrophoresis for identifying variations in proteins and enzymes, alongside serological and immune genetic procedures, enabling the assessment of

genetic diversity [4]. The appearance of multiple forms of protein in a population, known as protein polymorphism, plays a crucial role in genetic variation. This diversity is significant as it prevents the rarest observable characteristics, which has a frequency of more than 0.1 percent, from being maintained solely through repeated mutation [5]. Protein polymorphism has practical applications in gene locations, disease identification, breeding selection, and DNA analysis [6]. Two approaches, namely protein electrophoresis and immunoproteomics, are commonly used to study genetic diversity within a population's gene pool. Electrophoretic mobility across gels is the underlying principle behind these methods, allowing for the identification of allelic group differences accountable for protein amino acid variation [7].

Proteins or allelic enzyme variations remain extremely valuable in emerging countries as a result of their lower complexities, cost-effectiveness, ease of data interpretation, and the rate of genetic information they provide [8]. A significant advantage of biochemical characterization is its independence from environmental factors, stability across developmental stages, and a straightforward mode of inheritance [9]. Despite efforts to study the genetic

variation among livestock using advanced methods like microsatellite markers and other high-tech procedures in the developing world, blood protein markers of livestock are extensively employed. These markers serve purposes such as studying genetic structure and phylogenetics among animal breeds [9], and characterizing and estimating genetic diversity within and across breeds and species [10].

Although there is no direct evidence linking carbonic anhydrase to chicken weight, studies have shown that it does impact the transport and utilization of hemoglobin in chickens. Hemoglobin, in turn, affects the growth rate of chickens. Therefore, the presence of carbonic anhydrase could indirectly influence the growth rate of chickens [11].

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the University of Ilorin Biotechnology Laboratory. A total of 200 birds comprising of 50 Abuja ecotype (G1), 50 Yoruba ecotype (G2), 50 Fulani ecotype (G3) and 50 Exotic breeds (Arbor acre) (G4) were sampled for blood collection and was used in this study.

Blood collection and preparation

Each sampled animal had 5 milliliters (5 ml) of blood collected through wing vein puncture, using via 2ml hypodermal needle. The blood was collected into test tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) as an anticoagulant, and the samples were appropriately labeled. Subsequently, the samples in EDTA bottles were refrigerated at 8°C for a duration of two hours and then transported to the laboratory in container with cold packs and separate samples in plastic bags according to the procedure of NCBI [20].

Cellulose acetate paper soaking

The cellulose acetate papers were immersed in the identical buffer used for the electrode buffer, which

is part of the continuous buffer system. The papers were left to soak for a duration of 20 minutes [19].

Bands scoring

Bands was assessed visually based on their migration, following the guidelines provided by [12, 13]. Gene frequencies were estimated through direct counting. Blood samples were prepared and placed in the wells of a sample plate. Excess moisture on the cellulose acetate paper was removed by blotting it dry between filter paper sheets.

Sample loading

Great care was taken to prevent any shifting of the cellulose acetate paper during sample loading. To secure the paper in place, a drop of gel buffer was applied to the aligning plate before setting the cellulose acetate sheet on it. The aligning base ensured that all samples were applied uniformly. Samples were applied twice to the same position on the plate using an applicator. The loaded plates were rested on the electrodes in the tank without applying any current, while subsequent plates were loaded. The teeth of the applicator were blotted before each new application [19].

Statistical analysis

PopGen 32.0 software was used to estimate population parameters.

RESULTS AND DISCUSSION

Allele frequency of Carbonic Anhydrase for indigenous and exotic chickens

Table 1 revealed that the Carbonic Anhydrase F (CA F) from the Exotic breed population had the greatest frequency (0.7900), whereas (0.2100) from the Exotic breed had the lowest. The results align with the discoveries made by Johari *et al.* [14], who discovered two alleles F and S in chicken. The Carbonic Anhydrase (CA F) and Carbonic Anhydrase S (CA S) co-dominant alleles were responsible for controlling the two Carbonic Anhydrase genotypes that were found [5, 15].

Table 1: Allele frequency of Carbonic Anhydrase for indigenous and exotic chickens

Population Ecotypes	Allele	Sample size	Allele Frequency
Fulani	F	200	0.7250
	S		0.2750
Yoruba	F	100	0.7000
	S		0.3000
Abuja	F	98	0.6735
	S		0.3265
Exotic	F	100	0.7900
	S		0.2100
Average	F	498	0.7221
	S		0.2779

Genotype frequency of Carbonic Anhydrase for indigenous and exotic chickens

Table 2 shows the genotype frequency which varied varies for all parameters measured (0.26, 0.145,

0.115) for CA FF in Fulani, Yoruba and Abuja Ecotype to 0.145 for CA FF in Exotic breed

Table 2: Genotype frequency of Carbonic Anhydrase for indigenous and exotic chickens

Population Ecotypes	Genotype	Sample size	Genotype Frequency
Fulani	FF	200	0.260
Yoruba	FF	100	0.145
Abuja	FF	98	0.115
Exotic	FF	100	0.145

Wright F-Statistic analysis of Carbonic Anhydrase for indigenous and exotic chickens

Table 3 shows the F-statistics estimates of population structure, and all estimates of FIS, FIT, and FST were

not significant ($P < 0.05$) except for the FST value and the FIT which were significant ($P < 0.05$), showing a slight variation from Hardy Weinberg's Equilibrium and a population indicator of outbreeding [16].

Table 3: Wright F-Statistic analysis of Carbonic Anhydrase for indigenous and exotic chickens

Population	(F _{IS})	F _(ST)	F _(FIT)	Nm
C.A	0.0706	0.0093	0.0792	11.4460
Average	0.0706	0.0093	0.0792	11.4460

Effective sample size (N), expected and observed heterozygosity (HE and HO) at Carbonic Anhydrase locus for indigenous and exotic chickens

Table 4 revealed high heterozygosity, where the detected HO, for all chicken kinds in all populations

(0.72), indicates that genetic heterogeneity in chicken populations is growing at the Carbonic Anhydrase locus. This could be explained by the composition of the chicken population being studied [17].

Table 4: Effective sample size (N), expected and observed heterozygosity (HE and HO) at Carbonic Anhydrase locus for exotic and indigenous chickens

Population	N	H _E	H _O
Fulani	200	0.4008	0.4100
Yoruba	100	0.4242	0.2400
Abuja	98	0.4444	0.4082
Exotic	100	0.3352	0.5800

Genetic distance for all breed of both exotic and indigenous chickens

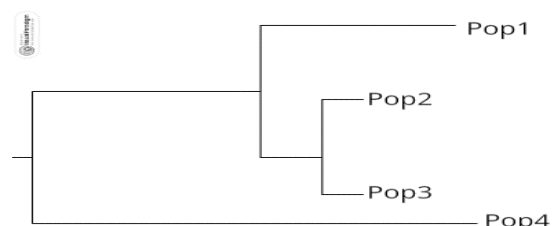
The genetic difference between the populations investigated was minor, but within the range of 0.001 to 0.046 range indicated by Nei [18] different breeds. The difference between Abuja chicken and Exotic

breeds chicken was the greatest, while the distance between Yoruba ecotype and Fulani was the least. In terms of identification, Fulani and Yoruba ecotypes are 99.92% closer, whereas Abuja and Exotic are 96.9% closer.

Table 5: Genetic distance for all breed of birds from all location identity (up) and distance (down)

Population ID	1	2	3	4
1	****	0.9816	0.9859	0.9432
2	0.0185	****	0.9911	0.9810
3	0.0142	0.0089	****	0.9699
4	0.0584	0.0192	0.0305	****

The dendrogram presented in Figure 1. of the four chicken kinds demonstrated that the Yoruba and Fulani chickens are more alike based on their genetic properties than the broiler, all of the chicken types congregated until they reached a spot where the Fulani and Yoruba Ecotype clustered. According to Nei [18], the genetic distance between all breeds of birds from all locations is the same.

**Figure 1:** The Dendrogram of the four chicken ecotypes

Between	and	Length
3	2	0.98354
2	pop1	0.81923
2	1	0.37320
1	pop2	0.44603
1	pop3	0.44603
3	pop4	1.80277

CONCLUSION

The data obtained from this experiment has offered insights into the extent of genetic variation and the occurrence of polymorphisms within Carbonic Anhydrase among the indigenous ecotype and the exotic breed. The results obtained shows that FF groups distribution pattern in Abuja Ecotype conforms with the general pattern across Nigeria. The Allele frequency shows F and S in chickens, where Carbonic Anhydrase F (CA F) from the Exotic breed population had the greatest frequency whereas Carbonic Anhydrase S (CA S) from the exotic breed had the lowest frequency. The genotype frequency from the results varies from the populations for C. The genetic variation observed between the indigenous and exotic chicken populations offers crucial details, which could be employed to improvement or preservation of the indigenous ecotypes in Nigeria. Hence, details of this study will

serve as a guide in identifying definite ideas in the prospects of improvement and conservational strategies for the indigenous ecotypes.

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