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Research Article
Haemoglobin Polymorphism Characteristics Evaluation Along with Morphological Traits in Duroc Pigs in Imo State, Nigeria

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Abstract

Background and Objective: The component of the oxygen storage and regulation system is Haemoglobin in all living organisms including pigs. This study was carried out to characterize the genetic pool of the indigenous Nigerian Duroc pigs using Hb polymorphism as well as the association of the Hb variants with body weight (BW) and some biochemical traits. **Materials and Methods:** Blood samples were collected from a total of ninety-six mature pigs of both sexes were randomly sampled in Imo State, Nigeria The cellulosic acetic electrophoresis and specific staining procedures were used to indicate the band patterns of the Hb of which two co-dominant alleles causing the occurrence of two genotypes AA and AB were found among the pig. **Results:** The results obtained showed that the frequencies of A and B alleles were 0.85 and 0.15 and the corresponding genotype frequencies of AA and AB alleles in the pig population were 0.70 and 0.30 respectively. Therefore, the observed and expected genotype number was not significant at $p>0.05$, thus supporting the Hardy-Weinberg population. The expected heterozygosity (He) was 0.25 which is the measure of gene diversity while the Shannon diversity index (H) recorded 0.44 indicating non-assortment mating. Hb variants did not have significant effects at $p>0.05$ on the body weight and other measured parameters of the pigs. **Conclusion:** The male pigs were significantly high in all the parameters measured except for breast circumference where there was no variation between male and female Duroc pigs. However, further study should be on the use of neutral markers (Microsatellites) and Single Nucleotide Polymorphism (SNPs) to enhance national conservation and structured production of Duroc pig in Nigeria.

Key words: Haemoglobin, polymorphism, morphometric traits, pigs, Nigeria

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pig production represents an important segment of the food animal industry throughout the world[1]. Today's modern pork is much higher than it was in the past and is widely accepted by consumers. Its consumption increased with an increase in the need for more animal protein for a human segment of the population. The recent high cost of livestock has limited the capacity of an average Nigerian to consume adequate quantity and quality of animal protein. Proteins of animal origin have no alternatives in the growth, development and replacement and repair of body tissues and protoplasm. In Nigeria, commercial pig production has been maintained mainly through the use of imported exotic breed and their crosses[2,3]. Consequently, the availability of breeds differing in their reproductive characteristics is an important source of variations that can allow more rapid adjustments to change economic conditions in the country. The need to develop and conserve pig genetic resources at regional and national levels has been reported[4]. Genetic characteristics through the use of molecular markers are providing new awareness for decision-making choices for conservation at national management of animal genetic resources (AnGR)[5,6]. Protein polymorphisms were the first molecular markers used in livestock[7]. The importance of biochemical polymorphism in the improvement of pigs has been stressed. Some of the polymorphic alleles may be correlated with economic traits due to linkage in pleiotropic or general heterozygosity and can help establish the development of pigs, their original relationships and speciation[8].

One of the important blood proteins is haemoglobin, an "evergreen" red protein[9] which can be used to access adaptability as well as the health status of West African dwarf goats[10]. Adult pigs possess haemoglobin components. The erythrocytes of adult pigs can contain a mixture of functionally distinct haemoglobin isoforms with different biochemical properties and potentially different oxygen-binding properties[11]. Haemoglobin types have been associated with productive traits[12]. Though, there exists published work on ducks and chickens (birds), available kinds of literature show that there is a dearth of information on published works on Duroc pigs in Nigeria. Therefore, the present study aimed at evaluating the genetic structure from the level of the determinant locus of haemoglobin in indigenous Duroc pigs of Imo state, Southeastern Nigeria and to establish the characteristics of the haemoglobin variants with biometric traits.

MATERIALS AND METHODS

Study area: The study was carried out from August to November, 2021. The study area is in Obinze Owerri-West Local Government Area (LGA) of Imo State with headquarters at Umuguma. It is located in Owerri Agricultural Zone, in the rain forest zone about 120 km North of the Atlantic coast and lies on latitudes 4°14'N and 6°15'N, longitude 6°51'E and 8°09'E, respectively.

Sample collection: Blood samples were randomly collected from a total of ninety-six indigenous Duroc pigs of male and female sexes (32 males and 64 females) in some parts of the Imo State, Southeastern Nigeria. The state falls within the tropical rainforest of southeastern Nigeria with average annual rainfall distribution of 2,250-2,800 mm. The state lies between latitudes 05°19" and 05°40"N and longitude 07°45"E. The monthly mean temperature in the state range between 26 and 32°C. The pigs were reared through the extensive management system originated from different productions within the study. Body weight and other linear body measurements (cm) like body length breast circumference, neck length, thigh length, thigh circumference and head length/circumference of each animal were also measured. The anatomical reference point and procedure were as described by the previous study[10].

The 5 mL of blood were collected from each pig by use of a syringe. The blood was drawn into vials bottles containing EDTA (Ethylenediaminetetraacetic acid) anticoagulant. The red cells were separated, washed in saline solution and lysed with distilled water. Haemoglobin was typed using a cellulose acetic electrophoresis[13] with a slight modification[10]. The identification of the haemoglobin types in pigs was achieved following the migration speed of the light spot on the electrophoretic substratum, detected from the start line towards the cathode zone. The direct gene counting method was used to score Hb bands based on the separation of the Hb variants. Haemoglobin typing was carried out at the Federal Medical Centre in Owerri IMO the State of Nigeria. Genotypic and gene frequency of Hb alleles was estimated.

Statistical analysis: Data on the Hb alleles and genotype frequencies were subjected to chi-square analysis to test for goodness of fit for observed and expected frequencies under Hardy-Weinberg Equilibrium (HWE) using the POPGENE statistical software[14]. Observed and expected heterozygosity was estimated as the expected proportion of the

heterozygotes under Hardy-Weinberg equilibrium. The software generated the Shannon diversity index (H). Data obtained were also analyzed using the Generalized Linear Model (GLM) of statistical software to test the fixed effects of the Hb genotype and sex as well as their interactions on body weight, body length, breast circumference, neck length, neck circumference, thigh-length/circumference, head length/circumference. Means were separated using the least significant difference (LSD) at a 95% confidence interval. The following linear model was used[7]:

$$Y_{ijk} = \mu + G_i + S_j (GS)_{ij} + e_{ijk}$$

Where:

Y_{ijk} = Individual observation
μ = Overall mean
G_i = Fixed effect of the ith genotype (I = AA, AB)
S_k = Fixed effect of the ith sex (K = Male, female)
e_{ijk} = Random error associated with each record (normally independently and identically distributed with zero mean and constant variance)

RESULTS

Haemoglobin frequencies: The Hb polymorphism in the duroc pigs was revealed by identification in the electrophoretic field of the two migration zones, the fast Hb named HbAA type and the Hb with intermediate migration labelled HbAB type. The slow Hb designated HbBB type was not found in the pig population. The frequencies of the A and B alleles were 0.85 and 0.15, respectively. The corresponding genotype frequencies for AA and AB in the pig population were 0.70 and 0.30 in Table 1.

Hardy-Weinberg equilibrium: The result of the observed and expected number of the genotypes of Hb and chi-square test of Hardy-Weinberg equilibrium in Duroc pigs is presented in Table 2. The chi-square revealed that the gene genotype frequencies of the pigs were in Hardy-Weinberg equilibrium for both male and female Duroc pigs (chi-square = 1.74 and p = 0.05).

Expected heterozygosity and Shannon diversity index: The chi-square values for observed and expected heterozygosity for Duroc pigs were 0.25 and 0.21, respectively, while the Shannon index diversity index was 0.44 in Table 3.

Haemoglobin types and morphological measurements

Body weight: The result of the bodyweight and linear body measurements of the duroc pigs based on Hb variants is displayed in Table 4. Results obtained showed that the male pig (94.63) was significantly higher in body weight than the female pig (64.35) of either Hb genotype of 84.08 (HbAA) or 68.70 (HbAB).

Body length: The results of body length of male and female pigs showed the same trend as in body weight of which the male pigs were significantly higher ($p<0.05$) than the female pigs.

Breast circumference: The breast circumference of male and female pigs (67.22:67.82 cm) statistically shows no significant difference at $p<0.05$.

Neck length and neck circumference: The neck length and neck circumference of male pigs gave the best performance

Table 1: Distribution of Hb genotype and gene frequencies in the Duroc pigs

		Genotype frequency			Gene frequency	
Sex	No examined	AA	AB	BB	A	B
Male	32	24(0.75)	8(0.25)	0(0.00)	0.87	0.13
Female	64	43(0.67)	21(0.33)	0(0.00)	0.83	0.17
Total	96	67(0.70)	29(0.30)	0(0.00)	0.85	0.15

Table 2: The observed and expected number of the genotypes of Hb and chi-square test of Hardy-Weinberg equilibrium in Duroc pigs

	Genotypes						
	Male			Female			
Parameters	AA	AB	BB	AA	AB	BB	X^2
Observed number	24	8	0	43	21	0	
Expected number	24.19	7.78	0.19	44.16	19.52	1.16	
Chi-square		0.21^{ns}			1.94^{ns}		1.74^{ns}

Ns: Not significant at p>0.05 probability level for male and female and the combined sexes, respectively

Table 3: Estimates of heterozygosity and Shannon diversity index of Duroc pigs

Parameters	Observed Heterozygosity	Expected Heterozygosity	Shannon Index
Male	0.17	0.16	0.27
Female	0.34	0.28	0.56
X^2	0.25	0.21	0.44

Table 4: Bodyweight and linear body measurement of Duroc pigs based on Hb variants

	Haemoglobin Genotypes		Sex	
Parameters	HbAA	HhAB	Male	Female
Body weight	84.08±3.21	68.70±2.32	94.63±3.32[a]	64.35±2.14[b]
Body length	97.44±4.13	98.85±4.16	101.30±4.41[a]	89.47±4.23[b]
Breast circumference	61.14±2.24	59.63±2.11	67.22±2.24[a]	67.82±2.29[a]
Neck length	20.56±1.07	20.34±1.04	23.14±1.21[a]	18.36±1.02[b]
Neck circumference	11.42±1.02	11.03±1.00	14.56±1.07[a]	13.28±1.06[b]
Thigh length	17.16±1.06	15.78±1.02	17.29±1.08[a]	14.33±1.02[b]
Thigh circumference	16.84±1.04	16.78±1.03	16.59±1.08[a]	15.98±1.07[b]
Head length	11.31±1.01	11.12±1.01	12.16±1.02[a]	11.08±1.01[b]
Head circumference	11.96±1.05	10.66±1.00	12.08±1.07[a]	10.37±1.01[b]

Mean along the row with different superscript of letter differ significantly at $p<0.05$ level

of the female pigs. The neck length (23.14 cm) and the neck circumference (14.56 cm) of the male pigs were higher than the female pigs 18.36 and 13.28 cm, respectively.

Thigh-length and thigh circumference: The results of the thigh length and thigh circumference of male pigs were significantly high in length and circumference than the female pigs at $p<0.05$ level.

Head length and head circumference: This shows that the male pigs had 12.16:12.08 cm of head length and head circumference while the female pigs had 11.08:10.37 cm of head length and head circumference.

DISCUSSION

The results from this study have established that the haemoglobin polymorphisms in Duroc pigs in the electrophoretic field were of two migration zones of which the HbAA was the fast haemoglobin and HbAB type was the intermediate migration haemoglobin. This study evaluated the genetic structure from the level of the determinant locus of haemoglobin in indigenous Duroc pigs in Imo State, Nigeria. Hb is one of the most important extensively studied proteins in the aspect of structure, function and relationships and comparative studies of Hb from non-human animals have made an important contribution to this knowledge base[15,16]. This present study was in agreement with the work of Peters *et al.*[17], who observed three genotypes of Hb AA and BB which were controlled by two autosomal alleles A and B on India native chickens and that reported frequency of 0.96 (HbA) and 0.04 (HbB), respectively for white leghorn chickens[18].

The absence of HbBB genotype in the present study could be the analytical method used, rearing and selection pattern and the geographical area reared. The higher male-female ratio in this study was from the management of the piggery farmers who intend to keep more females for production or breeding and offer for sales males not required for rearing. Chi-square analysis showed no significance in the observed and the expected frequencies of Hb types in the Duroc pigs. This indicates that the gene and genotype frequencies of the duroc pig population in the Hardy-Weinberg proportion were unaffected as a result of random mating, genetic drift and possibly mutation. Schenkel *et al.*[10] reported that the gene and genotype frequencies of West African dwarf goats were in Hardy-Weinberg equilibrium in his preliminary investigation of Hb polymorphism and associated with morphometric traits. The low He and H obtained in this study might be due to sampling size and samples being made in a few areas and this can be a further source of investigation. The higher body weight, body length, neck length, neck circumference, thigh circumference, head length and head circumference observed in males could be attributed to sexual dimorphism and agrees with the previous studies[1,16-18] on preliminary observations on effects of Hb genotype and African dwarf and Red Sokoto goats. Pigs with either HbAA or HbAB appear similar in body weight and other body measurements and this could support future rearing and conservation decisions. The use of polymorphic genes as genetic molecular markers is a

promising fact once these genes are proven to be associated with traits of interest in animals.

CONCLUSION

The results obtained in this study indicated the preponderance of HbAA in the pig population which could support decisions on conservation for the improved adaptability. The pigs with either HbAA or HbAB stand a chance of being selected for the improvement of body weight and other measurements of Duroc pigs such as body weight, body length, breast circumference, neck length, neck circumference, thigh length, thigh circumference, head length and head circumference. This has the potential of being applied in the conservation of pig population in crossbreeding programs in other to create genetic stocks with improved adaptability and productivity of indigenous pigs in the tropics.

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SIGNIFICANCE STATEMENT

The knowledge of haemoglobin polymorphism characterization is important in the growth and reproduction performance of pigs for designing breeding programmes for pig production. Increasing genetic potential for pig production requires selection for increased live body weight. This study will help researchers to carry out further studies on the use of neutral markers (Microsatellites) and Single Nucleotide Polymorphism (SNPs) to enhance the national conservation and structured production of Duroc pigs in Nigeria.

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