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Research Article

Phenotypic Differentiation of Purebred and Crossbred Indigenous Chicken Genotypes Using Multivariate Analysis

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Abstract

Background and Objectives: Indigenous chickens serve as a good source of animal protein, particularly in developing countries. However, these birds have low reproduction and slow growth rate, which limits their commercial use. The growth performance of indigenous chickens can be improved through crossbreeding. This study utilized multivariate analysis to phenotypically characterize purebred and crossbred indigenous chicken genotypes. **Materials and Methods:** A total of 607 chickens were generated through artificial insemination. The parent stock involved indigenous Normal Feather, Naked Neck and Frizzle Feather chickens, along with a broiler breeder, Anak Titan. Weekly body weights (BW) and linear body measurement data were collected from each bird from 1-day-old to 20 weeks of age. Data were then subjected to multivariate analysis. **Results:** Two principal components (PCs), PC1 and PC2 which ranged from 78.00-98.20% and from 0.71-10.90% of total variance, respectively, were generated. Discriminant analysis demonstrated a low level of morphometric differentiation among the chicken genotypes with six highly discriminating variables. **Conclusion:** Multivariate analysis allowed for differentiation of purebred and crossbred chicken genotypes based on morphometric traits. This result demonstrated the utility of multivariate analysis in making crossbreeding and selection decisions of indigenous chickens' improvement.

Key words: Crossbreeding, frizzle feather chicken, growth performance, indigenous chickens, multivariate analysis, naked neck chicken, normal feather chicken

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

INTRODUCTION

Chickens are economically significant poultry species due to their high protein content, short generation interval and global availability^{1,2}. Despite these beneficial traits, protein insufficiency persists as a major issue in developing countries^{3,4}. The low productive and reproductive potential coupled with low egg yield and high mortality contribute to their underutilization of indigenous chickens⁵⁻⁸. However, despite these limitations, indigenous chickens are excellent foragers, possess the ability to tolerate extreme environmental conditions and possess a natural immunity against disease⁹. Thus, efforts to improve growth and reproductive potential of these indigenous species are vital⁹ to ensuring the long term productivity of these species.

Quantitative and qualitative morphological traits are important tools in the characterization and improvement of indigenous chicken genetic diversity³. Indigenous chickens have been characterized using phenotypic traits¹⁰. Multivariate analysis is an effective statistical tool to phenotypically differentiate and characterize indigenous birds⁸. This analysis provides an assessment of variation within each population, which in turn provides small-scale farmers with adequate tools for effective breeding and selection of improved species for commercial use in developing countries⁷. In developing countries phenotypic or morphometric data are currently utilized relative to genomic data due to lack of logistics, finances, capacity and infrastructure¹¹. Therefore, the current study was aimed at phenotypic characterization of purebred and crossbred indigenous Nigerian chicken genotypes using multivariate analytical approach with the goal of providing farmers with information for the selection of superior chicken populations.

MATERIALS AND METHODS

Experimental birds: A broiler breeder, Anak Titan and three purebred indigenous chickens-Naked Neck, Frizzle Feather and Normal Feather were used as the parent stock to produce purebred and crossbred progenies. The parent stock comprised of 15 sires and 80 dams. The chickens were maintained at the Poultry Breeding Unit, Federal University of Agriculture, Abeokuta. These chickens were the seventh generation produced from pure breeding and selection. The foundation stock were from scavenging chickens in the Western part of Nigeria. The progenies were generated through the use of artificial insemination. Both straight and reciprocal crosses from the parent stock were generated. The chickens were reared in deep litter and wing-tagged at 1 day of age for identification purposes. Commercially formulated feed and fresh water were provided *ad libitum*.

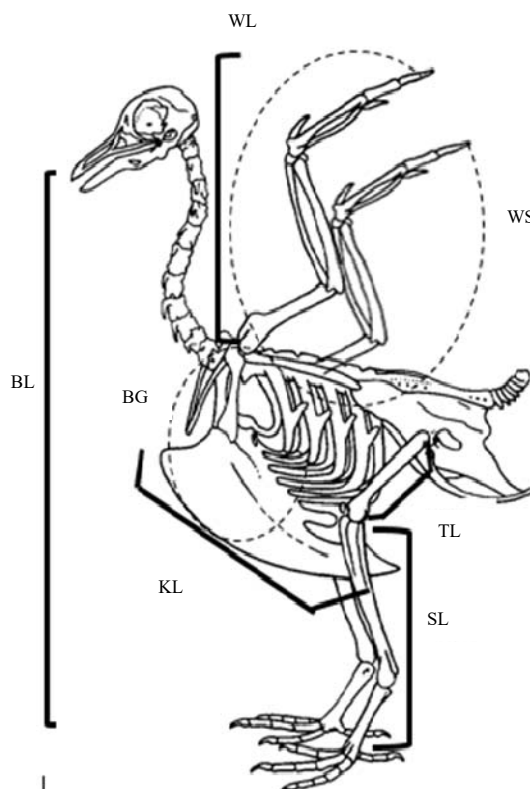


Fig. 1: Body dimensions of chicken (Modified from Wikipedia)

BL: Body length was measured from the tip of the chick's beak to its longest toe, WL: Wing length was measured from the tip of the phalanges to the coracoids-humerus joint, WS: Wing span was measured from the tip of the right wing to the tip of the left wing across the back of the chick, SL: Shank length was measured from the end of the hock joint to the beginning of the tarsometatarsus, TL: Thigh length was measured from the hock joint to the pelvis joint, BG: Breast girth was measure as the circumference of the breast, KL: Keel length was measured from the anterior to the posterior end of the keel

Data collection: Body weight (BW) and linear body measurements (Fig. 1) were collected weekly from individual birds at 1-day-old to 20 weeks of age. BW was determined using a standard scale. Linear body dimensions recorded include, body length (BL); wing length (WL); wing span (WS); shank length (SL); thigh length (TL); breast girth (BG) and keel length (KL).

Statistical analyses: All statistical analyses were performed using the Statistical Package for Social Science (IBM SPSS statistics 24). Multivariate analysis was carried out using principal component (PC) and discriminant analysis. The Kaiser-Meyer-Olkin test, commonly referred to as the KMO test, was used to determine sampling adequacy. Bartlett's test of sphericity was utilized to test the original correlation null

hypothesis in the PC and factor analyses. The validity of the PC was determined by generating communalities, while the number of factors extracted was determined by the cumulative proportion. The reliability of the discriminant functions was tested with the split-sample validation procedure.

RESULTS

Principal component analysis: Purebred and crossbred chicken genotypes had high KMO values which were highly

significant in Bartlett's test for sphericity. High communalities were observed for all chicken genotypes (Table 1). PC1 accounted for the majority of variation, from 78.64-96.03%, while PC2 accounted for 1.13-10.90% of the total variation in the chicken genotypes (Table 2a-c). Variations in the pattern of body dimension loadings are presented in each PC. PC1 of the Anak Titan was classified by WS, WL, TL and SL, while PC2 was classified by BL, BG and KL. In purebred Nigerian indigenous chicken genotypes, all traits were characterized by PC1 with the exception of KL that was characterized by PC2. There was no correlation between the PCs.

Table 1: KMO and Bartlett's test of sphericity

Kaiser-Meyer-Olkin measure of sampling adequacy		0.960
Bartlett's test of sphericity	Approximate chi-square	76997
	Degrees of freedom	28
	Level of significance	0.000*

*p<0.001

Table 2a: Principal components used for the characterization of purebred chickens

Genotypes	Traits	P 1	P 2	Communality
Normal Feathered ^m × Normal Feathered ^f	Wing length	0.952	0.243	0.999
	Wing span	0.950	0.240	0.863
	Shank length	0.937	0.233	0.933
	Thigh length	0.901	0.225	0.959
	Body length	0.880	0.198	0.966
	Breast girth	0.840	0.167	0.734
	Keel length	0.230	0.973	0.814
	Eigen values	5.505	0.763	
	Percentage of total variance	78.640	10.904	
	Naked Neck ^m × Naked Neck ^f	Wing length	0.928	0.317
Wing span		0.927	0.316	0.960
Shank length		0.920	0.327	0.944
Thigh length		0.914	0.328	0.898
Body length		0.896	0.308	0.998
Breast girth		0.817	0.220	0.954
Keel length		0.313	0.949	0.715
Eigen values		5.834	0.598	
Percentage of total variance		93.341	8.536	
Frizzle Feathered ^m × Frizzle Feathered ^f		Percentage of total variance	96.408	1.267
	Wing length	0.941	0.293	0.968
	Wing span	0.935	0.307	0.963
	Shank length	0.933	0.305	0.929
	Thigh length	0.929	0.324	0.969
	Body length	0.918	0.299	0.971
	Breast girth	0.917	0.299	1.000
	Keel length	0.311	0.950	0.932
	Eigen values	6.646	0.179	
	Percentage of total variance	94.944	2.558	
Anak Titan ^m × Anak Titan ^f	Wing length	0.800	0.567	0.983
	Wing span	0.765	0.615	0.986
	Shank length	0.746	0.646	0.961
	Thigh length	0.731	0.643	0.974
	Body length	0.577	0.806	0.984
	Breast girth	0.639	0.760	0.948
	Keel length	0.683	0.760	0.964
	Eigen values	6.722	0.079	
	Percentage of total variance	96.034	1.132	

m: Sire (Male), f: Dam (Female)

Table 2b: Principal components used for the characterization of straight crossbred chickens

Genotypes	Traits	P 1	P 2	Communality
Normal Feathered ^m × Anak Titan ^f	Wing length	0.790	0.605	0.990
	Wing span	0.785	0.612	0.991
	Shank length	0.754	0.650	0.982
	Thigh length	0.715	0.686	0.986
	Body length	0.712	0.697	0.990
	Breast girth	0.705	0.699	0.992
	Keel length	0.600	0.796	0.993
	Eigen values	6.874	0.050	
	Percentage of total variance	98.201	0.712	
	Naked Neck ^m × Anak Titan ^f	Wing length	0.817	0.563
Wing span		0.798	0.591	0.987
Shank length		0.739	0.662	0.981
Thigh length		0.711	0.680	0.971
Body length		0.710	0.683	0.985
Breast girth		0.557	0.819	0.968
Keel length		0.661	0.725	0.961
Eigen values		6.749	0.089	
Percentage of total variance		96.408	1.267	
Frizzle Feathered ^m × Anak Titan ^f		Wing length	0.890	0.426
	Wing span	0.889	0.435	0.980
	Shank length	0.875	0.432	0.952
	Thigh length	0.874	0.473	0.999
	Body length	0.857	0.484	0.987
	Breast girth	0.842	0.494	0.969
	Keel length	0.454	0.785	0.952
	Eigen values	6.510	0.303	
	Percentage of total variance	93.005	4.331	

m: Sire (Male), f: Dam (Female)

Table 2c: Principal components used for the characterization of reciprocal crossbred chickens

Genotypes	Traits	P1	P2	Communality
Anak Titan ^m × Normal Feathered ^f	Wing length	0.927	0.347	0.978
	Wing span	0.925	0.348	0.977
	Shank length	0.924	0.343	0.943
	Thigh length	0.924	0.354	0.969
	Body length	0.919	0.354	0.980
	Breast girth	0.909	0.342	0.972
	Keel length	0.353	0.936	1.000
	Eigen values	6.298	0.520	
	Percentage of total variance	89.970	7.435	
	Anak Titan ^m × Naked Neck ^f	Wing length	0.923	0.340
Wing span		0.922	0.338	0.999
Shank length		0.920	0.330	0.868
Thigh length		0.919	0.353	0.965
Body length		0.914	0.363	0.970
Breast girth		0.887	0.284	0.967
Keel length		0.341	0.940	0.956
Eigen values		6.149	0.545	
Percentage of total variance		87.844	7.779	
Anak Titan ^m × Frizzle Feathered ^f		Wing length	0.846	0.491
	Wing span	0.843	0.503	0.970
	Shank length	0.842	0.515	0.956
	Thigh length	0.837	0.524	0.975
	Body length	0.834	0.540	0.987
	Breast girth	0.811	0.558	0.975
	Keel length	0.521	0.853	0.963
	Eigen values	6.646	0.179	
	Percentage of total variance	94.944	2.558	

m: Sire (Male), f: Dam (Female)

Table 3: The separation of purebred and crossbred chickens based on their morphological characteristics using stepwise discriminant analysis

Traits	Wilk lambda	P level	Tolerance	F to remove
Body weight	0.878	0.001	0.203	182.55
Shank length	0.764	0.001	0.093	46.20
Keel length	0.732	0.001	0.272	7.54
Breast girth	0.733	0.001	0.287	9.60
Body length	0.730	0.001	0.262	5.33
Thigh length	0.731	0.001	0.078	6.56
Wing span	0.729	0.001	0.219	3.84

Table 4: The pairwise distances of the purebred and crossbred chicken genotypes

Genotypes	NF ^m ×NF ^f	NF ^m ×AT ^f	NN ^m ×NN ^f	NN ^m ×AT ^f	FF ^m ×FF ^f	FF ^m ×AT ^f	AT ^m ×NF ^f	AT ^m ×NN ^f	AT ^m ×FF ^f	AT ^m ×AT ^f
NF ^m ×NF ^f	-	22.10***	5.38***	9.02***	16.67***	20.83***	34.95***	27.40***	14.53***	271.26***
NF ^m ×AT ^f		-	23.57***	9.22***	6.35***	18.20***	25.50***	21.52***	5.46***	42.16***
NN ^m ×NN ^f			-	8.17***	19.04***	15.00***	23.70***	17.06***	13.30***	243.19***
NN ^m ×AT ^f				-	6.38***	7.39***	5.98***	3.90***	1.47	95.57***
FF ^m ×FF ^f					-	17.60***	25.74***	22.17***	6.12***	116.43***
FF ^m ×AT ^f						-	3.71**	5.36***	6.91***	106.78***
AT ^m ×NF ^f							-	3.71**	6.92***	177.05***
AT ^m ×NN ^f								-	5.02***	163.81***
AT ^m ×FF ^f									-	56.46***
AT ^m ×AT ^f										-

*p<0.05, **p<0.01, ***p<0.001, m: Sire (Male), f: Dam (Female), NF^m×NF^f: Normal Feather^m×Normal Feather^f, NN^m×NN^f: Naked Neck^m×Naked Neck^f, FF^m×FF^f: Frizzle Feather^m×Frizzle Feather^f, AT^m×AT^f: Anak Titan^m× Anak Titan^f, NF^m×AT^f: Normal Feather^m×Anak Titan^f, NN^m×AT^f: Naked Neck^m×Anak Titan^f, FF^m×AT^f: Frizzle Feather^m×Anak Titan^f, AT^m×NF^f: Anak Titan^m×Normal Feather^f, AT^m×NN^f: Anak Titan^m×Naked Neck^f, AT^m×FF^f: Anak Titan^m×Frizzle Feather^f

Table 5: The percentage of the purebred and crossbred chickens classified into the genetic groups post cross-validation

Genotype	NF ^m ×NF ^f (%)	NF ^m ×AT ^f (%)	NN ^m ×NN ^f (%)	NN ^m ×AT ^f (%)	FF ^m ×FF ^f (%)	FF ^m ×AT ^f (%)	AT ^m ×NF ^f (%)	AT ^m ×NN ^f (%)	AT ^m ×FF ^f (%)	AT ^m ×AT ^f (%)	Total (%)
NF ^m ×NF ^f	32.0	6.6	8.5	2.5	4.3	23.3	10.0	10.5	0.2	2.0	100
NF ^m ×AT ^f	5.3	22.4	5.3	0.0	5.3	32.9	1.3	7.0	0.4	20.2	100
NN ^m ×NN ^f	29.7	3.9	13.1	1.5	3.8	28.1	7.7	10.0	0.3	1.7	100
NN ^m ×AT ^f	11.5	9.6	9.3	1.7	4.9	27.5	13.0	11.1	1.7	9.6	100
FF ^m ×FF ^f	16.4	15.3	6.4	2.3	10.6	23.3	7.4	8.4	1.2	8.7	100
FF ^m ×AT ^f	15.7	4.5	3.8	0.3	6.3	39.4	18.5	10.1	1.0	0.3	100
AT ^m ×NF ^f	16.7	3.2	5.5	2.0	3.5	34.2	18.9	12.2	0.7	3.0	100
AT ^m ×NN ^f	10.7	5.8	10.1	1.9	4.2	27.7	15.2	18.6	0.9	4.9	100
AT ^m ×FF ^f	8.2	5.8	8.9	1.2	6.2	29.6	12.8	10.5	0.8	16.0	100
AT ^m ×AT ^f	2.0	6.0	2.4	1.7	4.8	17.4	5.5	9.0	4.1	47.2	100

m: Sire (Male), f: Dam (Female), NF^m×NF^f: Normal Feather^m×Normal Feather^f, NN^m×NN^f: Naked Neck^m×Naked Neck^f, FF^m×FF^f: Frizzle Feather^m×Frizzle Feather^f, AT^m×AT^f: Anak Titan^m×Anak Titan^f, NF^m×AT^f: Normal Feather^m×Anak Titan^f, NN^m×AT^f: Naked Neck^m×Anak Titan^f, FF^m×AT^f: Frizzle Feather^m×Anak Titan^f, AT^m×NF^f: Anak Titan^m×Normal Feather^f, AT^m×NN^f: Anak Titan^m×Naked Neck^f, AT^m×FF^f: Anak Titan^m×Frizzle Feather^f

Discriminant analysis: The purebred and crossbred chicken genotypes were separated by the following discriminating variables (with respect to F-values), BW, SL, KL, BG, TL and WS (Table 3). The pairwise distances between purebred and crossbred chickens were significant (p<0.05), with the exception of the Anak Titan^m×Frizzle Feather^f and the Naked Neck^m×Anak Titan^f (Table 4). The longest distance (271.26) was observed between the Anak Titan and Normal Feather purebred chicken genotypes, while the shortest distance (1.47) was observed between the Anak Titan^m×Frizzle Feather^f and the Naked Neck^m×Anak Titan^f crossbred chicken genotypes. Approximately 20.78% of cross-validated groups (Table 5) and 23.5% of original groups were classified correctly. On average, 29.6% of Naked Neck^m×Naked Neck^f chickens

were misclassified as Normal Feather^m×Normal Feather^f. The Anak Titan^m×Anak Titan^f and Anak Titan^m×Anak Titan^f displayed the highest percent (47.2%) of correct classification.

DISCUSSION

This experiment demonstrated the reduction of redundant data by principal component analysis. The analysis grouped the phenotypic traits according to their correlation. Each PC only displayed traits, which account for the majority of variation in the initial variables, thereby, making large datasets more manageable¹². PC analysis showed a high positive value in the KMO test (Table 1). This result indicates a high level of variation among the body measurements.

Bartlett's test revealed that the overall correlation matrix was highly significant since the correlation matrix provided sufficient support for the appropriateness of the PC analysis¹³. The high communality values validated the two factors extracted in the PC analysis (Table 2-c). Therefore, PC1 and PC2 represented the majority of variation within the variables^{14,15}. Egena *et al.*¹⁶ and Yakubu *et al.*¹⁴ also reported high variance in Nigerian indigenous chickens. Ajayi *et al.*¹⁷ and Mendes¹⁸ reported high variance between exotic and cross-bred chicken genotypes. However in this study, the communalities for skeletal dimensions (WL and WS) were higher than the flesh dimension (BG).

PCs 1 and 2 of the purebred Anak Titan differed significantly from the purebred indigenous Nigerian chicken genotypes. This indicates the distinctness of the Anak Titan exotic breed from its indigenous counterpart¹⁶. Similar results were observed for the crossbred chicken genotypes. PC1 was strongly correlated with all the morphometric traits except KL. However, KL was characterized by PC2 in all genotypes, indicating that KL provides a poor explanation for the total variance within BW. Therefore, selection based on KL would not lead to a corresponding response in BW¹⁹. Selection of traits within PC1 also showed a high correlation coefficient and would indirectly improve BW as a correlated response. Selection of body measurements with a high positive PC will indirectly lead to an increase in the other correlating PCs. This indicates the presence of a pleiotropic gene action among the variables, particularly in PC1²⁰.

Discriminant analysis reduced the number of measured traits to six highly distinguishable traits as indicated by the Wilks lambda value (Table 3). Similar traits were extracted in a discriminant analysis carried out by Tyasi *et al.*²¹. The discriminant traits allowed for differentiation between the different genotypes, serving as a guide in decisions making regarding conservation, breeding and selection for genetic improvement¹⁹. BW was the most discriminating factor, as the purebred Anak Titan displayed a significantly heaviest mean weight compared to the purebred and crossbred indigenous chickens. Similar results were obtained by Al-Atiyat²². The highest pairwise distance between the purebred Anak Titan and the Normal Feather (Table 4) suggests that crosses between the chicken genotypes can result in higher heterotic gains than crosses with the Anak Titan and other indigenous Nigerian chickens²³. Ajayi *et al.*¹⁷ reported similar findings.

Classification results obtained in this study indicated that the purebred Anak Titan showed the highest classification percentage compared to other chicken genotypes (Table 5).

This further suggests the distinctiveness of the Anak Titan. A high percent of the purebred Naked Neck was misclassified as Normal Feather. This suggests the effect of introgression of genes. Genetic introgression between distinct genotypes could result in their populations becoming more homogenous, reducing genetic variation. This could potentially result in a lower species survival rate by rendering the population more susceptible to genetic drift, such as bottlenecking events²⁴.

The analysis of morphometric structural traits using multivariate analysis was successful in differentiating these chickens populations. The expression of a phenotype is a result of both genetic and environmental factors²⁵. Therefore, it is recommended that prior to selection for breeding, the genetic structure of the indigenous chickens should be determined using molecular DNA-based methods, such as single-nucleotide polymorphisms or high density molecular markers particularly for association study²⁶.

CONCLUSION

The PC analysis predicted the BW of specific genotypes with orthogonal conformation traits and discriminant analysis indicating differential properties between purebred and crossbred chickens. Selection of morphometric traits, which formed PC1, such as SL and TL, would lead to increase in BW. Thus, a multivariate analysis differentiated the chicken genotypes. This potentially would aid conservation and improvement programs for phenotypic traits of indigenous chickens, particularly in smallholder poultry farming. However, application of genomic tools is crucial for faster genetic improvement in indigenous chickens.

SIGNIFICANCE STATEMENT

This study phenotypically characterizes and differentiates between purebred and crossbred indigenous chicken genotypes using multivariate analysis. In smallholder poultry production, findings of this study could help in making breeding decisions especially in developing countries with limited resources.

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