Nigerian J. Anim. Sci. 2020, 22 (1): 74-82

Association of Ovocleidin-116 Polymorphisms with Egg Quality Traits of Nigerian Heavy Local Chicken Ecotype Reared in Derived Savannah

***Ikeh N. E., Ndofor-Foleng H.M., Nnajiofor N.W., Udeh F.U., Egom, M.A., Amaefule B. C., and Udeh V.C**

Department of Animal Science, University of Nigeria, Nsukka

****Corresponding Author:** nnanna.ikeh.pg80080@unn.edu.ng; **Phone Number:** +2347033085209*

***Target Audience**: Geneticists, Breeders, Physiologists, Researchers.*

Abstract

Ovocleidin-116 (OC-116) is a matrix protein observed in the hen uterine fluid during active calcification phase of shell formation. This study was carried out to identify ovocleidin-116 polymorphisms using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method and to determine the association with egg quality traits of Nigerian Heavy Local Chicken Ecotypes. Two hundred and forty laying birds (with three eggs each) were measured for Egg Weight (EW), Eggshell Thickness (EST), Eggshell Weight (ESW) and Egg Shape Index (ESI). The PCR-RFLP products revealed G and C alleles controlling three genotypes, GG, GC and CC with frequencies of 0.6, 0.3 and 0.1 respectively. The genotypes were not distributed according to the Hardy-Weinberg equilibrium (χ^2 = 2.4). Association studies revealed that genotypes had significant ($P<0.05$) relationship with EW, EST, ESI and ESW in the studied population. The GG (53.11±0.49) and GC (53.33±0.69) exhibited higher EW than the CC (45.64±1.21) genotypes while CC (0.33±0.04) had thicker shells than GG (0.31±0.01) and GC (0.31±0.02) genotypes. The ESI and ESW were higher for GG and GC genotypes. These results improve knowledge on the understanding of OC-116 polymorphisms and its potential as a candidate gene for selecting egg quality traits in Nigerian Heavy Local Chicken Ecotype.

***Keywords**: chicken; egg quality traits; ovocleidin-116; polymorphism; Nigerian Heavy Local Chicken Ecotype.*

Description of Problem

Indigenous chickens is a major genetic pool of diversity of the national flocks in Africa (1). Compared to their modern counterparts (commercial strains), indigenous chickens are generally poor producers of eggs and meat. Consequently, they are being overlooked when compared to commercial strains in many developing countries (1). This led to the neglect of the local breed and as such, it poses a threat to the existing genetic diversity of indigenous chickens. Despite their low growth rates and egg production, indigenous chickens are generally better in disease resistance and could maintain a considerable level of performance under poor nutrition and high environmental temperatures compared to commercial strains under village systems (1).

Egg quality trait evaluation and improvement of some Nigerian local chickens have been conducted in some parts of Nigeria (2, 3, 4, 5, and 6). However, most of the genetic progress has been made through phenotypic selection (7). This kind of selection is mainly without the knowledge of the number of genes affecting the trait or the effects of each gene. To overcome the flaws of this pattern of selection,

molecular genetics provides tools to improve the selection procedures.

The rise of genomics as a discipline in the 1980s led to the concept of marker- assisted selection (MAS), in which genetic variants and genes that influence important traits would be identified and used for further genetic improvement (8). The first application of livestock genomics is finding variations that exist in the genome sequences, and characterizing these sequence variations using methods such as Restriction fragment length polymorphisms (RFLPs) and Single Nucleotide Polymorphisms (SNPs). Then, the association between these variants and the observed physical characteristics of animals are established through statistical procedures. The associations between variants and phenotypes are then used to help refine genetic improvement or animal breeding programs. In terms of improving eggshell quality traits through genomics, considerable progress has been made in the identification and characterization of the genes coding for individual eggshell matrix proteins, including those that have been implicated in directing crystal growth during eggshell formation (9).

Table 1: Descriptive statistics of different egg quality traits in the studied population.

Traits	Mean	S.D	C.V	Minimum	Maximum
Egg weight (g)	51.36	5.97	11.62	32.00	67.00
Eggshell thickness (mm)	0.34	0.03	10.75	0.25	0.44
Eggshell weight (g)	5.05	0.71	14.11	3.40	7.20
Shape index (%)	74.26	4.19	5.64	63.59	93.58

S.D; standard deviation, C.V; coefficient of variation

Table 2: Summary of genetic variations in OC-116:c1110.C>G gene markers of the Nigerian heavy local chicken ecotypes.

Marker	A	A_F	G	G_F	Na	Ne	I	Ho	He	χ^2	P
					2.000	1.600	0.562	0.300	0.375	2.40^{ns}	0.121
OC-	**G**	0.75	**GG**	0.60							
116:c1110.C	**C**	0.25	**GC**	0.30							
>G			**CC**	0.10							

A: allele, **A_F:** allelic frequency**; G:** genotype**, G_F:** genotype frequency**, Na**: observed number of alleles, **Ne**: Effective number of alleles, **I**: Shannon's Information index **Ho;** observed heterozygosity**, He;** expected heterozygosity**, χ^2:** Chi Square, **P:** Probability; **ns**: non-significant.

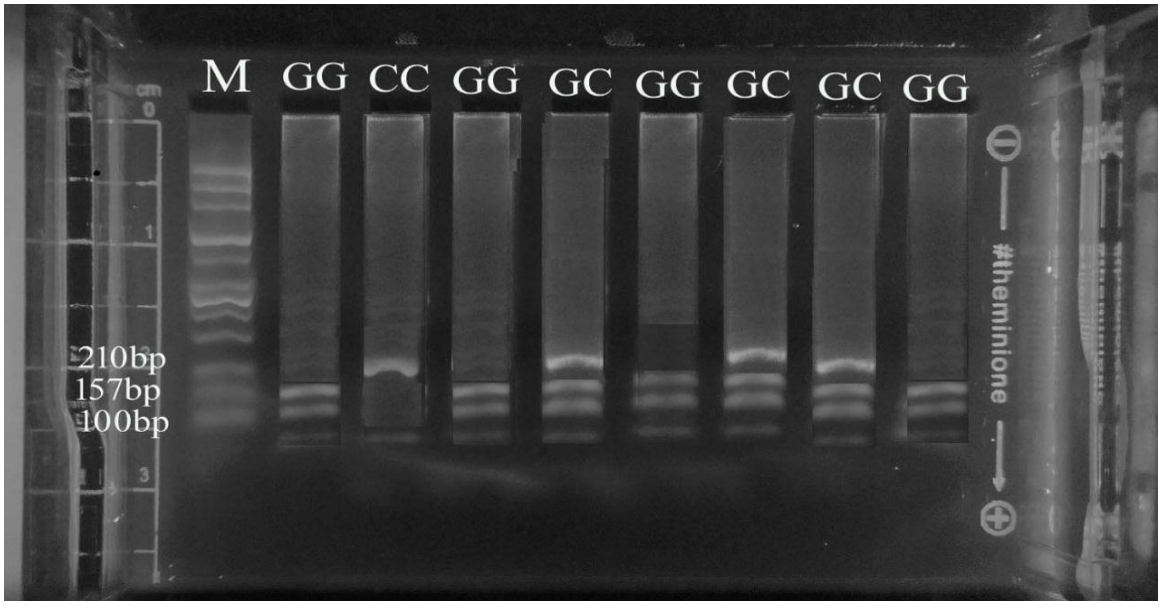
The egg shell is a highly ordered structure resulting from the deposition of calcium carbonate and the organic matrix from the acellular uterine fluid (10). Ovocleidin-116 (OC-116) was the first eggshell matrix protein to be cloned, by expression screening a uterine library using an antibody raised to the abundant 116-kDa protein observed in hen uterine fluid

during the active calcification phase of shell formation (11). OC-116 gene is located in chromosome 4 with four exons (12).

Genomic variations in OC-116 gene has been reported in a number of poultry breeds, including Pureline Rhode Island White layers (12), a Rhode Island Red population (13), and a commercial brown egg-laying line (14). Trait association studies have reported relationships between single nucleotide polymorphisms in OC-116 and numerous egg-related traits including eggshell thickness, elastic modulus, and egg shape (13). Significant association between polymorphisms in exon 4 of OC-116 gene and effective layer thickness, mammillary layer thickness, and average size of mammillary cones (12).

These results suggest the possibility of using molecular markers in OC-116 gene as a tool for improvement of egg production traits in chicken breeding programs. However, little research on evaluation of diversity and in OC-116 gene of Nigerian heavy local chicken ecotype as well as association with egg quality traits has been published. This study, therefore, was designed to evaluate genetic diversity in OC-116 gene of the Nigerian heavy local chicken ecotype and association with egg quality traits.

Figure 1: PCR-RFLP of OC-116:c1110.C>G genotypes on the 2% agarose gel electrophoresis. Deoxyribonucleic acid was digested with *PstI* enzyme.



One fragment (210bp) was present in the CC homozygote, Two fragments (157 and 100bp) were present in the GG homozygote, and three fragments (100, 157 and 210bp) in the GC heterozygote. bp; base pair; M; 3000bp Molecular Marker.

Table 3: Association of different OC-116:c1110.C>G genotypes on egg quality traits of Nigerian heavy local chicken ecotype

Parameters	OC-116:c1110.C>G genotypes			P.Value
	GG	GC	CC	
Egg weight (g)	53.11±0.39[a]	53.33±0.95[a]	45.67±0.85[b]	0.00**
Egg shell Thickness (mm)	0.31±0.01[b]	0.31±0.02[b]	0.33±0.05[a]	0.00**
Egg shell weight (g)	5.00±0.04[a]	5.08±0.09[a]	4.73±0.10[b]	0.04*
Egg shape index	74.62±0.38[a]	74.17±0.39[a]	71.66±0.79[b]	0.00**

[ab]: Means±SEM on the same row with different superscripts are significantly different ($P \leq 0.05$ or $P \leq 0.05$)

Materials and Methods

Animals and Data Collection on Egg Traits

In total, 240 laying birds between 275 to 280 days of age were obtained randomly from a population of Nigerian heavy local chicken ecotype, kept at the Department of Animal science Local chicken research unit, University of Nigeria Nsukka. The birds were housed individually on labeled metal cages and intensively managed. These birds were raised in the same chicken house and fed the same commercial layers mash with crude protein; 16.8%, fat; 3.6%, crude fiber; 4.2%, calcium; 4.2%, available phosphorus; 0.5%, methionine; 0.45%, lysine; 0.85% and metabolizable energy of 2680 kcal/kg. Three eggs each were collected (276 to 287 days of age) and following egg traits were collected.

Egg weight: Eggs were collected from individual birds labelled and weighed immediately using an electronic top loading balance (500g x 0.01g Notebook Series Digital Scale with 5 Digits LCD Display).

Egg shape index was estimated using (15) formula as follows:

Shape index = (egg width/egg length) x 100.

According to this the eggs were classified with respect to shape index (SI), namely as a sharp egg (SI < 72), a normal (standard) egg (SI = 72–76) or a round egg (SI > 76) (15).

Shell thickness: Shell thickness was measured using a micrometer screw gauge.

Shell weight: Shell weight were taken using an electronic top loading balance after allowing the empty egg shells to dry for 48 hours at room temperature after cracking.

DNA Extraction

Blood samples (1ml) were collected from the wing vein of each bird using a syringe and applied to Whatman FTA® Gene Cards directly in the farm, labeled and then stored for laboratory analysis (ACUTIG, Ogun State). A 2-mm diameter Harris Micro Punch and Mat (Whatman); was used to remove 2 disks from each sample and put into a 0.2ml Eppendorf tube. Then 0.15ml of 100mM Tris, 800µL of 1% SDS of FTA purification reagent was added and then rotated up and down for 30 minutes, after which the spent solution was carefully tipped off. Washing was repeated with distilled water and rotated up and down for 10 minutes each after which the spent solution was carefully tipped off. Final wash was done with distilled water for 10 minutes without shaking before tipping off the spent solution. 50µL of distilled water was added and heated at 90^{o}C for 15 minutes and the DNA quality were evaluated by loading genomic DNA on the 2% agarose gel electrophoresis (The MiniOne Systems, San Diego, CA).

Polymerase Chain Reaction (PCR) Amplification

The PCR (The MiniOne PCR Systems, San Diego, CA) was performed in a total volume of 25µL containing: 2µL DNA, 16µL of molecular grade water, 1µL each of the forward and reverse primers (OC-116 F: 5'-AGGGGAGAAGCGGACAGAG-3' and OC-116 R: 3'-CCACCTCTTGCTGGACTCTA-5'), and 5µL of Fast-Taq DNA polymerase (Jena Biosciences, Germany). The PCR conditions were as follows: 96°C for 15 min followed by 35 cycles of 94°C for 60 sec, 55°C for 60 sec, 70°C for 30 sec, and a final extension at 72°C for 6 min. Few samples of the PCR products was randomly selected and evaluated on the 2% agarose gel electrophoresis (The MiniOne Systems, San Diego, CA) to confirm that the procedure was successful before moving on to PCR-RFLP.

Restriction Fragment Length Polymorphism (RFLP) Analysis

The amplified fragments were subsequently digested with *PstI* enzyme for detecting the OC-116:c1110.C>G genotypes. The restriction enzyme digestions were performed using 3µL of PCR product mixed with 5.5µL molecular grade water, 1 µL 10x N.E Buffer with 0.25 µL of the restriction enzyme, which was followed by 37°C for 15min digestion, then 80°C for 20min inactivation. All birds used in this study were genotyped using this method.

Statistical analysis

GenAlEx software (v. 6.503) was used to estimate allele and genotype frequencies as well as estimate genetic diversity within the population. A Chi Square (χ^2) test for goodness-of-fit was performed to verify if genotype frequencies agreed with Hardy-Weinberg expectations.

The effect of OC-116 genotypes on egg traits were assessed using the General Linear Model procedure of SPSS statistical package, where means were found to be statistically different, Duncan multiple comparison test was used for separation of means.

The following model was applied;

$$\mathbf{Y_{ij} = \mu + G_i + E_{ij}}$$

Where;

$\mathbf{Y_{ij}}$ represents the observed values of the traits,

$\boldsymbol{\mu}$ is the population mean,

$\mathbf{G_i}$ is the effect of OC-116 genotypes,

$\mathbf{E_{ij}}$ are the random errors associated with each measured trait.

Results and Discussion

Descriptive Statistics of the Traits Studied.

Descriptive statistics of different egg quality traits in the studied population are presented in Table 1. The egg shell weights showed the highest coefficient of variation while the shape index had the least coefficient of variation, indicating greater variation in the eggshell weights. The average egg weight (51.36g) of the Nigerian local chicken ecotype population was close to the values reported by (16) which was 52.33g for normal and 52.9g for frizzled local chickens, higher than 45.89g and 36.1g obtained by (17) and (18) respectively. The differences in egg weight could be ascribed to the different management systems. The lower egg weights obtained by (17) could be as a result of the fact that the birds were on free range thus spending time and energy in search of food. The birds in the studied population had no competition of feed nutrient thereby producing eggs with heavier egg weights. The current population studied had undergone six generations of selection for egg weight, thereby accumulating selection gains which would be positively correlated to increased egg weights as compared to the values reported by (19) and (4) who obtained average egg weights of 45.06g and 43.77g respectively. It is worthy to note that environmental differences, feed differences as

well as population differences as well as their interactions could contribute to variations in egg weight traits between populations.

Eggshell thicknesses ranged between 0.25-0.44 mm and included thick (≥0.36), medium (0.30-0.36 mm) and thin (≤0.30 mm) shelled eggs according to (20). The mean values of shell thickness found in this study (0.34mm) were lower than findings by (21) who found 0.37mm for normal feathered local chickens. (22) reported higher values of 0.45mm for normal and frizzled feathered chicken while (18) reported 0.32mm which corresponds to the current findings.

The mean egg shape index found in this study (74.26%) was in accordance to the findings by (18) who reported 76.99% as well as the results of (22) who reported similar egg shape index of 75.9% for the local population studied. Values of egg shape index in the current study showed a normal (standard) egg (SI = 72–76) according to (15). Good egg shape index enhances marketing and profitability, in the sense that, high egg index provides the best appearance and low egg index are much likely to be broken during packaging and transportation.

The PCR-RFLP Analysis

The PCR-RFLP analysis using the *PstI* enzyme identified three genotypes for OC-116:c1110.C>G (GG, GC, and CC) as shown in Figure 1. Two fragments of 157 and 100 bp were detected when the genotypes GG was present. The genotype CC was reported by observation of a fragment of 210 bp. The heterozygous exhibited three different amplicon sizes: 210, 157 and 100 bp. The summary of genetic variations in OC-116:c1110.C>G gene markers of the Nigerian heavy local chicken ecotype are presented in Table 2. The GC genotype was found in only 30% of the population and the G allele was most frequent (Table 2). Results of 60% genotypic frequency of GG genotype of OC-116 gene was close to the results reported by (13) on a population of Rhode Island Red chicken (66% for GG). The frequency of the less common allele (C) in this study was 0.25 and was similar to the report of (23). The studied population was not in Hardy-Weinberg Equilibrium (P>0.05) for the polymorphisms as shown by the chi square value of 2.4. This deviation could be as a result of the force of artificial selection that has been applied over time in the studied population thereby concentrating the allele that favors the improved egg traits as reported by (19).

Association of different OC-116:c1110.C>G genotypes on egg quality traits of Nigerian heavy local chicken ecotype.

The results of the association of different oc-116:c1110.c>g genotypes with egg quality traits of Nigerian heavy ecotype local chickens are presented in Table 3. The present study showed that the OC-116:c1110.C>G genotypes had a significant (P<0.01) effect on egg weight, egg width, eggshell thickness, eggshell weights and egg shape index. Egg shell weights were significantly (P<0.05) different among the genotypes. The GC genotypes had the highest egg weights but was significantly (P>0.05) equal to the GG genotypes (53.11±0.39), while the CC genotypes had the lowest egg weights (45.67±0.85). This may probably be due to the inferior expression of the C allele, in the homozygote state and the superior expression of the heterozygote genotype due to heterozygote advantage. According to (23), existence of polymorphisms in OC-116 results in an alteration in the protein sequence thereby influence its expression.

Egg shell of the CC genotypes were significantly (P<0.01) thicker than those of GG and GC genotypes. This may be due the fact that smaller eggs tend to have thicker egg shells than heavier eggs (17). These findings were in accordance with (12) and (13), who found associations between exonic polymorphisms of OC-116 gene and shell thickness.

Egg shell weights of GG and GC genotypes were significantly ($P<0.05$) higher than CC genotypes. This result reflects the positive correlation that exist between egg weights and eggshell weights. Because GG and GC genotypes had higher egg weights, they tend to also have higher eggshell weights.

Egg shape index score of the GG and GC genotypes were significantly ($P<0.01$) higher than that of the CC genotypes. The reason underlying these differences could be attributed to the differences in egg width rather than egg length. The CC genotypes had sharp shape index while the GG and GC had standard shape index according to (15) classification. Standard shape index enhance marketing and profitability in the sense that it provides the best appearance while lower shape index are much likely to be broken during packaging and transportation. This findings are similar to the findings of (13), who found associations between polymorphism in OC-116 gene and shape index in a pedigree Rhode Island Red population.

Conclusions and Applications

The results shows that polymorphisms Ovocleidin-116 gene identified in this present study may be used as;

1. A potential marker to improve egg quality traits in the Nigerian Heavy local chicken ecotype populations.
2. It is also worthy to note that marker assisted selection may also play a role in the selection for a range of traits in full sib males. This is because, in the population structures used in commercial breeding programs, sires have a large contribution to the next generation but no direct measurements can be made of their genetic merit for egg quality traits.
3. To make further progress, there is need to evaluate the relationships between the studied gene polymorphisms and traits of egg quality traits in different Nigerian local chicken populations.
4. Also, for application in breeding practice, validating the SNPs and linkage analyses using more birds and different breeding flocks are needed to definitively demonstrate the functions of these SNPs in egg production traits.

References

1. Bettridge, J M., Psifidi, A., Terfa, Z G., Desta, T T., Lozano-Jaramillo, M., Dessie, T., Kaiser, P., Wigley, P., Hanotte, O., and Christley, R M. (2018). The role of local adaptation in sustainable production of village chickens. *Nature Sustainability*, 1; 574–582
2. Ndofor-Foleng, H.M, Ngongeh, L.A, Uberu, C.P.N, and Nwosu, C.C (2010). Evaluation of the performance of two local chicken and the main cross ecotypes reared in Nsukka, Enugu State. Nigeria. *International Journal of Science and Nature*, 1(2): 179-182.
3. Ndofor-Foleng, H.M., Oleforuh-Okoleh, V., Musongong, G.A., Ohageni, J., and Duru, U. E. (2015). Evaluation of growth and reproductive traits of Nigerian local chicken and exotic chicken. *Indian Journal Animal Research*, 49 (2): 155-160
4. Agu, C. I., Ndofor-Foleng, H. M., and Nwosu, C. C. (2012). Evaluation of economic traits in progenies of Nigerian heavy ecotype chicken as genetic material for development of rural poultry production. *African Journal of Biotechnology*, Vol. 11(39), pp. 9501-9507
5. Oleforuh-okoleh, V., Nwosu, C.C., Adeolu, A.I., Udeh, I, Uberu C.P.N., and Ndofor-Foleng H.M. (2012). Egg production performance in a Nigerian Local Chicken Ecotype subjected to

selection. *Journal of Agricultural Science:* vol 4. No. 6; pp180-186

6. Udeh, F.U., Onodugo, M.O., Udeh, V.C., and Mmadu, P.O. (2018). Response of Two Genetic Groups of Layers Served Ginger Root Powder on the Internal and External Egg Quality Characteristics. *International Journal of Recent Innovations in Academic Research*, 2(7): 235-241.
7. Réhault-Godbert, S.; Guyot, N.; Nys, Y. (**2019).** The Golden Egg: Nutritional Value, Bioactivities, and Emerging Benefits for Human Health. *Nutrients*, *11*, 684.
8. Georges, M., Charlier, C., and Hayes, B. (2018). Harnessing genomic information for livestock improvement. *Nature review Genetics* 20, 135-156
9. Gautron, J., Nys, Y., Schneider, W.J., Mann, K., Garcia-Ruiz J.M. and Hincke, M.T. (2002). Identification and characterization of eggshell matrix proteins. Applications to Eggshell quality. *In proceedings of 11th European Poultry Symposium*, Bremen, Germany.
10. Nys, Y., Gautron, J., Garcia-Ruiz, J.M. and Hincke, M.T. (2004). Avian Eggshell mineralization: biochemical and functional characterization of matrix proteins. *Comptes Rendus Palevo* 3, 549–62.
11. Hincke, M.T., Gautron, J., Tsang, C. P., Mckee, M. D. and Nys, Y. (1999). Molecular Cloning and ultrastructural localisation of the core protein of an eggshell matrix Proteoglycan, ovocleidin-116. *Journal of Biological Chemistry* 274: 32915-32923.
12. Wang, D., Liao, B., Zhang, Q., Liu, J.S., Duan, Z., Hou, Z., and Ning, Z. (2017). Gene Polymorphisms Are Associated with Eggshell Ultrastructure Organization in Hens. *Brazilian Journal of Poultry Science,* 19:1, 129-134
13. Dunn, I.C., Joseph, N.T., Bain, M., Edmond, A., Wilson, P.W., Milona, P., Nys, Y., Gautron, J., Schmutz, M., Preisinger, R., Waddington, D. (2008). Polymorphisms in eggshell organic matrix genes are Associated with eggshell quality measurements in pedigree Rhode Island Red hens. *Animal Genetics 40:110–114*
14. Gautron, J., Hincke, M.T. and Nys, Y. (1997). Precursor matrix proteins in the uterine fluid change with stages of eggshell formation in hens. *Connective Tissue Research*, 36:195–210
15. Duman, M., Şekeroğlu, A., Yıldırım, A., Eleroğlu, H., and Ö. Camcı, O. (2016). Relation between egg shape index and egg quality characteristics. *European Poultry Science*, 80, ISSN 1612-9199.
16. Isidahomen, C. E., Njidda, A. A., and Olatunji, E. A. (2013). Egg Quality Traits of Indigenous and Exotic Chickens as Influenced by Specific Genes. *Biology Agriculture and Healthcare*: 3(1): 53-57
17. Gongolo J, and Tanganyika J (2018) Evaluation of physical egg quality characteristics of free range indigenous Malawian normal feathered and frizzled chicken *International Journal of Avian & Wildlife Bioliogy*;3(1):21–23.
18. Egahi, J.O, Dim, N.I., and Momoh, O.M. (2013). The effect of plumage modifier genes on egg quality indices of the Nigerian local chicken. *Journal of Agriculture and Veterinary Science*, 2(2):04–06.
19. Agbo, M. C., Udeh, F. U., Ndofor Foleng, H. M. Ohagenyi, I. J., Nwosu, C. C. (2018). Genetic Progress of the Production Traits in the Nigerian Heavy Local Chicken Ecotype Obtained by Selection Index in the Derived Savannah Zone of Nigeria. *Conference proceedings of the 23rd Annual Conference of the Animal Science Association of Nigeria*

(ASAN), held at Ilorin, Nigeria. Pp 105-108.
20. Yan, Y.Y., Sun, C.J., Lian, L., Zheng, J.X., Xu, G.Y. and Yang, N. (2014). Effect of uniformity of eggshell thickness on eggshell quality in chickens. *The Journal of Poultry Science*, 51, 338–342.
21. Udoh, U.H., Nwaogwugwu, U.C. and Esiet, E.S. (2012). Egg Laying Characteristics of three (naked neck, normal feathered and frizzled feather) Nigerian Local Chickens. *Journal of Agriculture*, 5(2):84–90.
22. Bobbo, A.G., Baba, S.S., and Yahaya, M.S. (2013). Egg Quality Characteristics of Three Phenotypes of Local Chickens in Adamawa State. *Journal of Agriculture and Veterinary Science*, 4(2):13–21.
23. Bain, M., Dunn, I.C., Edmond, A., Wilson, P.W., Joseph, N., Waddington, D., Solomon. S., De Ketelaere, B., De Baerdemaeker, J., and Preisinger, R. (2003). Marker Assisted Selection To Improve Eggshell Quality: Establishing The Association Between Egg Phenotypic Traits And 'Candidate Genes'. *Proceedings of the Xth European Symposium on the Quality of Eggs and Egg products*, Saint-Brieuc, France Vol.III, 126-1