The International Journal of Engineering and Science (IJES)
|| Volume || 10 || Issue || 1 || Series I || Pages || PP 01-03 || 2021 ||
ISSN (e): 2319-1813 ISSN (p): 20-24-1805

Polymorphism of Ovocalyxin-32 Gene among Six Nigerian Chicken Populations

*Ohagenyi I. J[1], Oleforuh-Okoleh V. U[2], Ikeh N. E[1], Egom M. A[1],
Nnajiofor N. W[1], Udeh F. U[1], Ogbu C. C[3].
[1] Department of Animal Science, University of Nigeria Nsukka.
[2] Department of Animal Science, River State University Nkpolu, Oroworukwo.
[3] Faculty of Veterinary sciences, Michael Okpara University of Agriculture, Umudike.

----------ABSTRACT----------
Ovocalyxin-32 (OCX-32) is a matrix protein found within the outer layers of the eggshell. Numerous reports in the literature have identified association between variants in the gene encoding this protein OCX-32 and various eggshell quality traits. Thus, OCX-32 is a candidate gene for eggshell traits in commercial poultry populations. Sequencing of exon 1 and 2 of the OCX-32 gene in six Nigerian chicken populations revealed 2 SNPs. Two polymorphic sites (-162T and T229G) were identified in broiler (a commercial line) and Abuja populations respectively. However, SNPs T229G present in exon 2 caused an amino acid change indicating that the polymorphic sites identified in the ABJ population probably represent a small fraction of a more common polymorphic site found in the chicken populations. Commercial broiler had unique variants indicating that more polymorphic site could possibly be found in further study. High heterozygosity within a local Nigerian chicken and commercial broiler chicken indicates selection pressure for certain variants both in the natural environment and during the breeding program. The study also revealed absence of variants (low heterozygosity) within four Nigerian chicken populations and the presence of Hardy- Weinberg equilibrium. This indicates that the three Nigerian native chicken populations (Nsukka, Ogun and Abuja) had undergone little selection and random mating. However, broiler, layer and heavy ecotype could be limited in SNPs due to small sample size. The T229G polymorphism detected in Abuja chicken could be useful in genomic selection and expedite improvement of meat production and egg production traits in Nigerian native chickens.
KEYWORDS: *OCX 32, SNPs, Diversity, Chicken, Populations*

Date of Submission: 16-12-2020 Date of Acceptance: 02-01-2021

I. INTRODUCTION

Egg quality remains an important requirement for today's market to guarantee the integrity of the egg and to reduce the numbers of eggs lost on the way to the consumer. Approximately 6 to 8% of the total egg production is not usable or marketable due to the poor quality of shells (Harms *et al.*, 1996). Major financial losses during routine handling and transport from producer to retail outlets have been recorded (Hunton, 1995). The shell is the most obvious structure related to the safety and integrity as well as the appearance of the egg and has been the focus of most attention for genetic selection. Indeed it is observed that when comparing across traditional and commercial breeds, eggshell traits have been maintained in comparison to other traits suggesting the breeding goals have been successful (Hocking *et al.*, 2003). Therefore, eggshell stability traits play a major role because only eggs with an intact shell are considered salable. Thus, if egg quality, and specifically, eggshell stability, is guaranteed, the layer industry could increase the number of salable eggs produced by each hen housed. Selection for egg quality has therefore been an important component of the breeding strategy of companies marketing egg laying-type hens.

The eggshell is a highly ordered structure resulting from the deposition of calcium carbonate and an organic matrix from the acellular uterine fluid (Nys *et al.*, 2004). Ovocalyxin-32 (*OCX-32*), a 32-kDa protein, is present at high levels in the uterine fluid during the terminal phase of eggshell formation and is localized predominantly in the outer eggshell (Gautron *et al.*, 2001). The timing of *OCX-32* secretion into the chicken uterine fluid suggests that it may play a role in the process of mineral deposition and in the completion of the eggshell (Hincke *et al.*, 2003). SNP in the intron region of the *OCX-32* gene was associated with the thicknesses of the mammillary layer (Dunn *et al.*, 2008). Low egg production strains of Taiwanese country chickens expressed more transcripts of the *OCX-32* gene compared with high strains at egg-laying stages and suggested that the *OCX-32* gene is a potential molecular marker associated with different rates of egg production (Yang *et al.*, 2007). These results indicate that the *OCX-32* gene might have a direct effect on egg quality traits. The

objective of the present study was as to identify a polymorphism of the chicken *OCX-32* gene in different indigenous Nigerian chicken populations.

II. MATERIALS AND METHODS

Collection of blood samples and DNA extraction

Blood samples were collected from the wing vein of a total of 180 Nigerian chickens (30each) consisting of the Abuja, Nsukka, Ogun, Heavy ecotype, Layers and Broilers type chicken. The blood samples were stored at -20°C until DNA extraction. Total genomic DNA was extracted using Quick-gDNA Mini Prep kit of Norgen Biotek Corporation following the manufacturer's protocol.

Primers and PCR amplification

Two pairs of primers were used for selective amplification of cOCX-32 gene based on the chicken *OCX-32* mRNA information (accession no. NM_204534) and genome sequences (accession no. AADN02021077) on chromosome 9 in GenBank. The primers were synthesized by Integrated DNA Technology (USA). The primer sequences were:

F'- 5'GGCAGGACCCGAGCGAGGAGTT-3'; R'-5'GGCTAAGGCGTGAGGACCGAAACC-3'

F'-5'GCCCACTGGTCAGAAAAGAA-3'; R'-5'CCTGCAGAGGAAAAGAGCTG-3'

Selective amplification of different regions of OCX-32 gene was performed using thermocycler GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) and PCR reagents synthesized by Norgen Biotek corporation Canada. PCRs were performed in a programmable thermocycler with the following protocol: 94°C for 5 min; followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min; with a final extension step of 72°C for 10 min. Amplification of the different segments of cOCX-32 gene was confirmed by running the PCR products on 1% agarose gel and visualizing under UV rays. Thirty microliters of each PCR product was purified and sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit by standard protocol.

Sequence analysis

Chromatographs generated from sequencing were processed using Cluster W and sequence trimming was carried out on BioEdit. Both forward and reverse primer sequences were then aligned using the ClustalW multiple sequence alignment program (http://www.ebi.ac.uk/clusatlw/) to determine the presence of genetic polymorphisms. Sequences were blasted against database on NCBI and reference sequences acquired (NC_006096.5 and NP_989865.1).

III. RESULT AND DISCUSSION

DNA polymorphism

The intron/exon location (Loc), the affected cDNA and position of SNP in OCX 32 locus of the Nigerian chicken are shown in Table 1. Variants were numbered according to their order of occurrence in the gene sequence. Following the May, 2006 chicken genome build (WUGSC 2.1/gal Gal 3) at UCSC (http://genome.UCSC.edu) the position of individual variant was determined.

Table 1: The affected cDNA and position of SNP in OCX 32 locus of the Nigerian chicken

Name	Loc	Position	JF	NSK	OG	ABJ	HE	LA	BRL
OCX 32-1	I_1	22596162	-	-	-	-	-	-	T
OCX 32-2	E_2	22596229	T	T	T	G	T	T	T

Note; JF (Jungle Fowl), NSK (Nsukka), OG (Ogun), ABJ (Abuja), HE (Heavy Ecotype), LA (Layers) and BRL (Broiler)

The Populations of Nigeria Chicken differed slightly in terms of number of variants found within and between populations. Only two polymorphic sites were found among the populations used in the study. The two polymorphic sites (-162T and T229G) were identified in BRL (a commercial line) and ABJ populations respectively, however only SNPs T229G present in exon 2 caused an amino acid change indicating that the polymorphic sites identified in the ABJ population probably represent a small fraction of the more common polymorphic site found in the chicken populations. Commercial broiler had unique variants indicating that more polymorphic site could possibly be found in further study.

SNP Detection

SNPs and their locations in OCX 32 gene sequence among population of Nigerian native and commercial chicken are shown in table 2. One novel SNP (-162T) was found in this study. The novel SNPs (-162T) discovered in this study differed from those reported earlier, while the other SNPs (T229G) had been reported earlier (Takahashi *et al.,* 2010). SNP T229G found in ABJ brought about change in amino acid (leu83Arg), while SNP – 162T also found in commercial broiler did not cause change in Amino acid. SNP T229G had been reported earlier to be in linkage disequilibrium (LD) with five other variants present in exon 2 (Fulton *et al.,* 2012). Those six variants always appeared together. Hence the SNP T229G can be used alone to identify the genotype of the entire cluster. For the size of 305 bp analysed in this study, there is one SNP in an average of 152 bp sequence of chicken OCX 32.

Table 2: SNPs and their locations in OCX 32 gene sequence among population of Nigerian native and commercial chicken

Position	Location	N.N.change	Codon and AA change
22596162	Intron 1	g - > T	None
22596229	Exon 2	T > G	CTT>CGT:p.leu83Arg

IV. CONCLUSION AND RECOMMNDATION

The result suggested that very low heterozygosis was observed between populations. In conclusion only three chicken populations (Nsukka, Ogun and Abuja) followed the Hardy Weinberg equilibrium, indicating that the three Nigerian native chicken populations (Nsukka, Ogun and Abuja) had undergone little selection and random mating. However, Broiler, Layer and Heavy ecotype chicken with selection pressure, could be limited in SNPs due to small sample size. The T229G polymorphism detected in Abuja chicken could be associated with meat production and egg production traits in Nigerian native chickens and therefore should be a subject for further enquiry.

REFERENCES

[1]. Dunn, I.C., Joseph, N.T., Bain, M., Edmond, A., Wilson, P.W., Milona, P., Nys,Y., Gautron, J., Schmutz, M., Preisinger, R. and Waddington,D. (2008). Polymorphisms in eggshell organic matrix genes are associated with eggshell quality measurements in pedigree Rhode Island Red hens. *Animal Genet* 40: 110-14.

[2]. Fulton, J.E., Soller, A.R., Lund, A.R., Arango, J. and Lipkin, E. (2012). Variation in the ovocalyxin-32 gene in commercial egg-laying chickens and its relationship with egg production and egg quality traits. *Anim. Genet*. 43:102-113.

[3]. Gautron, J., Hincke, M.T., Panheleux, M., Bain, M., McKee, M.D, Solomon, S.E. and Nys,Y. (2001). Ovocalyxin-32, a novel chicken eggshell matrix protein. Isolation, amino acid sequencing, cloning and immunocytochemical localization. *J Biol Chem* 276: 39243-39252.

[4]. Hall, T. A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/nt. *Nucl. Acids. Symp., Ser. 41*, 95-98

[5]. Harms, R. H., Douglas, C. R. and D. R. Sloan. (1996). Midnight feeding of commercial laying hens can improve eggshell quality. *J.Appl. Poult. Res.* 5:1–5.

[6]. Hincke, M.T., Guest, S., Gautron, J. and Nys,Y. (2003). Gene organization of ovocalyxin-32, an eggshell matrix protein. In: Proceedings of the 10th *European symposium* on the quality of eggs and egg products, vol 3. Saint- Brieuc, France, pp 13-19 (23-26 September 2003).

[7]. Hocking, R.M.G., Bain, M., Channing, C.E., Fleming, R. and Wilson, S. (2003). Genetic variation for egg production, egg quality and bone strength in selected and traditional breeds of laying fowl. *British Poultry Science* 44, 365-73.

[8]. Hunton, P. (1995). Understanding the architecture of the egg shell. *World's Poult. Sci. J.* 51:141–148.

[9]. http://genome.UCSC.edu

[10]. Kumar S, Stecher G, Li M, Knyaz C, and Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35:1547-1549.Publication PDF available at: http://www.megasoftware.net

[11]. Nys,Y., Gautron, J., Garcia-Ruiz, J.M. and Hincke, M.T. (2004). Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. *Compters Rendus Palevol.* 3(6):549-62.

[12]. Takahashi, H., Sasaki, O., Nirasawa, K. and Furukawa, T. (2010). Association between ovocalyxin-32 gene haplotypes and eggshell quality traits in an F2 intercross between two chicken lines divergently selected for eggshell strength. *Animal Genecis* 41, 541-4.

[13]. Yang, K.T., Lin, C.Y., Liou, J.S., Fan, Y.H., Chiou, S.H., Huand, C.W., Wu, C.P., Lin, E.C., Chen, C.F., Lee, Y.P., Lee, W.C., Ding, S.T., Cheng, W.T. and Huang, M.C. (2007). Differentially expressed transcripts in shell glands from low and high egg production strains of chickens using cDNA microarrays. *Anim Reprod Sci* 110: 113-124.

Ohagenyi I. J, et. al. "Polymorphism of Ovocalyxin-32 Gene among Six Nigerian Chicken Populations." *The International Journal of Engineering and Science (IJES)*, 10(01), (2021): pp. 01-03.