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Research Paper

Polymorphism In Pou Class 1 Homeobox 1 (Pou1f1) Gene In Nigerian Indigenous And Improved Chickens Raised In South-South Nigeria

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Abstract
This study was carried out to investigate the polymorphism in POU Class 1 Homeobox 1 (POU1F1) gene in Nigerian indigenous and improved chickens raised in the South-south region of Nigeria. Genomic DNA was extracted from the blood of Frizzled feathered, Normal feathered, Naked neck and Noiler chickens which was used to amplify the POU1F1 gene and later sequenced. Sequences were aligned and analyzed using Mega 6.0 and DNA Sp. The results revealed variation in polymorphism site across the four strains of chickens studied. Noiler breed had the highest numerical value for number of pairwise comparison (136) and least was observed in Naked neck (3) and highest number of segregating site (26) and least was observed in Naked neck (0). Normal feather recorded the highest numerical value of indel site analyzed for insertion-deletion polymorphism (91) though Noiler chickens recorded the highest number of indel site (605). Highest estimates of average evolutionary divergence over sequence pairs of chicken breeds was observed in Noiler (0.666) and least in naked neck (0.01). Genetic distance in POU1F1gene across the chicken breeds studied revealed that the highest genetic distance was between frizzle and Noiler (0.519) and the least genetic distance existed with normal feather and naked neck (0.073). The result obtained from this study revealed a clearer understanding of the genetic diversity in POU1F1 gene across the four strains of Nigerian local and improved chickens. The information obtained from this study canbe harnessed for better policies for conservation and breeding programs.

Keywords: *Diversity, PIT1 gene, Strain, Nigeria, Chicken*

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I. Introduction

Present animal and veterinary sciences are geared towards the molecular approach of studying biological organisms. Till date, growth and physiological mechanisms in animals are much more understood at the molecular level. Recently, success has been recorded in the identification of major genes affecting different traits in animals, by the use of candidate gene approaches which are widely employed (Octura *et al*., 2014). *POU1F1* is a tissue specific transcription factor that regulates gene expression, particularly in somatotrophs, lactotrophs, and thyrotrophs, which are responsible for growth related hormone secretion (Bona *et al*., 2004). Major challenges of animal breeding and genetics in sustainable production in Agriculture and environment in Nigeria has been explained by different researches on different candidate genes and how they affect the performance of the Nigerian indigenous chicken have been carried out (Adebambo *et al*., 2009; Ajayi and Agaviezor (2012). Ajayi *et al*., 2013; Amusan *et al*., 2013; Agaviezor *et al*., 2018). Several researches have been done both at the phenotypic and molecular levels for improvement of the Nigerian indigenous chicken. Adebambo *et al*. (2009) worked on the Mitochondrial DNA D-Loop analysis of South Western Nigerian Chicken. The use of *POU1F1* gene in genetic studies has been researched by some authors (Qinghua *et al*., 2008; Jin *et al*., 2018). Other improvement research on the Nigerian local chicken has been examined by

Agaviezor *et al*. (2018) in their work on Single nucleotide polymorphism in growth hormone gene and its association with growth performance in chicken. This study was therefore carried out for us to the polymorphism in *POU1F1* gene in Nigerian indigenous and improved chickens raised in the South-South region of Nigeria and the information generated from this study will be used in the improvement of the growth traits of the Nigerian local chicken breeds.

II. Materials and Methods

This research was carried out at the Poultry unit of the Research and Teaching Farm of the Faculty of Agriculture, University of Port Harcourt, Rivers State. Sixty birds comprising of four strains of birds, 15 Frizzle feathered (male and female), 15 Naked-neck (male and female), 15 Noiler (male and female) and 15 Normal feathered (male and female) chickens were randomly selected from a flock of about 100 chickens. Three (3) mls of blood was collected from each bird from each bird from the wing vein in a 5ml bottle gently mixed with the EDTA to prevent coagulation. The bottles were kept in an ice pack and transferred to the laboratory where they were preserved at -4^{o}C until DNA extraction. DNA was extracted from the blood of the chicken using Quick-DNA Miniprep Plus kit by Zymo Research, following the manufacturer's instruction. Polymerase Chain Reaction was done using the DNA and *POU1F1* primer. 2.5ul of 10xPCR buffer, 1.0ul of 25MmMgCl2, 1.0ul of 5pMol forward primer (GGACCCTCTCTAACAGCTCTC) and1.0 ul of 5pMol. Reverse primer (GGGAAGAATACA CAGGGAAAGG). Also added was 1.0 ul DMSO, 2.0 ul of 2.5 Mm DNTPs, 0.1ul Taq 5u/ul, 3.0 ul of 10ng/ul DNA and 13.4ul water. A touch down PCR condition that involves initial denaturation at 94°C for 5 minutes, 9 Cycles of denaturation at 94°C for 15 seconds, annealing temperature at 62°C at 20 seconds and extension at 72°C for 30 seconds was carried out. This was followed by 35 cycles of denaturation at 94°C for 15 seconds, annealing temperature at 58°C for 20 seconds and extension at 72°C for 30 seconds and a final extension at 72°C for 7 minutes. PCR products of *POU1F1*gene of the chickens were sent for sequencing at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. DNA sequences were aligned using Mega 6.0. Also, sequence variation parameters and genetic similarities and diversities were determined using Mega 6.0 and DNA sp Version 5.

III. Results

Table 1 shows the variation in polymorphic site in *POU1F1* gene across chicken strains studied. Analysis pair wise comparison showed that the highest total number of sites observed in Naked neck (547) followed by Normal feathered (241), Frizzle feathered (209) and the least total number of sites were recorded by Noiler chickens. The highest number of pair wise comparison was observed in Noiler (136), followed by Frizzle feather (91), Normal feathered next (55) and the least was observed in Naked neck (3). However, Naked neck has highest average number of sites analyzed (564), followed by Normal feathered (493), Frizzle feathered next (456) and least average number of sites analyzed was observed in Noiler (385). The highest average number of differences was observed in Noiler (171), followed by Frizzle (147), Normal feather next (116) and least number of differences were recorded by Naked neck. For nucleotide diversity, the highest value was recorded by Noiler (0.48) followed by Frizzle feathered (0.34), Normal feather next (0.26) and least value were observed in Naked neck (0.11). Column by column analysis at individual site revealed that the highest number of site analysed was recorded by Noiler breed (641) followed by Frizzle feather (634), Normal feathered (627) and the least number of site analysed was observed in Naked neck breeds (598). The highest number of polymorphic sites were observed in Noiler (617) followed by Frizzle feathered (546), Normal feathered next (434) and the least value recorded by Naked neck (96). The highest average number of differences was observed in Noiler breed (293) followed by Frizzle feathered (216), Normal feathered next (158) and least value by Naked neck (71).For nucleotide diversity, the highest value was recorded by Noiler (0.44) followed by Frizzle feathered (0.39), Normal feathered (0.25) and least value by Naked neck (0.12). For theta-w per sequence, highest value was observed in Noiler (207) followed by Frizzle feathered (182), Normal feathered next (157) and least value recorded by Naked neck (68). For Theta -w per site, the highest vale was recorded by Noiler breed (0.32) followed by Frizzle feathered (0.29), Normal feathered next (0.25) and least value observed in Naked neck (0.11). Combined variation in polymorphic sites I *POU1F1* gene across chicken breeds studied, revealed that the number of sequence used was 45, number site 641 and total number of site was 26, the number of pair wise comparison was 990, average number of site analyzed was 444.58, average number of differences 148.05, nucleotide diversity pi 0.36. For combine column by column analysis, the number of site analyzed was 641, number of polymorphic site S 636, average number of differences was 230, nucleotide diversity pi was 0.35, theta- w per sequence was 157 and theta w per site was 0.24.

Table 1 Variation in polymorphic site in *POU1F1*gene across chicken breeds studied

Parameters	Frizzle feathered	Naked neck	Noiler	Normal feathered
Number of sequence	14	3	17	11
Number of site	641	641	641	641
Total number of site	209	547	36	241
Number of pair wise comparison	91	3	136	55
Average number of sites analyzed	456.12	564.00	385.33	493.07
Average number of difference	147.04	63.00	171.89	116.51
Nucleotide diversity, Pi	0.33712	0.11254	0.48024	0.25580
Analysis at individual site (column by column)				
Number of site analyzed	634.0	598	641	627
Number of polymorphic sites S	526	96	617	423
Average number of difference	216.50	71.00	293.77	158.00
Nucleotide diversity Pi	0.34149	0.11873	0.45830	0.2520
Theta – W, per sequence	182.38	68.00	207.33	157.72
Theta – W, per site	0.29	0.11	0.32	0.25

Table 2 shows the variation in gene flow and genetic differentiation in *POU1F1*gene in chicken strains studied. Number of segregating site was highest in Noiler (26). This was followed by Frizzle (15), Normal feathered (10) and least Naked neck (0). For number of haplotype, the highest value was observed in Noiler (14), followed by Frizzle feathered (10), Normal feathered (5) and the least value were observed in Naked neck (1). Haplotype diversity varied across the four breeds with Noiler having the highest value (0.956) followed by Frizzle feathered (0.890), Normal feathered (0.618), Naked neck had the least value of 0. For Nucleotide diversity, the highest value was observed in Noiler (0.39) followed by Frizzle feathered (0.17), Normal feathered (0.11). For average number of difference k, the highest value was recorded by Noiler (10) followed by Frizzle feathered (4.3), Normal feathered (2.8) and least value recorded by Naked neck (0). Combined or total data estimate for gene flow and genetic differentiation in *POU1F1* gene among chicken strains studied revealed the following values for number sequence (45), number of segregating site, S (26), number of haplotypes h (26), haplotype diversity Hs (0.826), nucleotide diversity (0.24) and average number of nucleotide difference k (6.20).

Table 2 Gene flow and genetic differentiation in *POU1F1*gene among chicken strains

Parameters	Frizzle feathered	Naked neck	Noiler	Normal feathered
Number of sequence	14	3	17	11
Number of segregating sites S	15	0	26	10
Number of haplotypes, H	10	1	14	5
Haplotype diversity, HD	0.89011	0.01	0.95588	0.618
Average number of difference K	4.34	0.02	10.22	2.81
Nucleotide diversity, Pi	0.16695	0.01	0.39310	0.10839
Nucleotide diversity with JC, PIJC	0.19994	0.01	0.66620	0.12708

Table 3 shows the variation in insertion deletion polymorphism in *POU1F1*gene in the four strains of chickens studied. Total number of indel site analysed showed the highest value in Normal feathered (91) chickens. This was followed by Frizzle feathered (79), Noiler (45) and Naked neck with the least value (17). Number of indel site observed was highest in Noiler (605), followed by Frizzle feathered (432), Normal feathered (393) and least value observed in Naked neck (64). The total number of excluded over lapping indel site had the highest value in Noiler (560) followed Frizzle feathered (353) then Normal feathered (302) and least value observed in Naked neck (47). The highest number of indel and non indel sites was observed in Naked neck (564) followed by Normal feathered (332) Frizzle feathered (288) and Noiler with the least value (81). For total number of indel event analyzed1, the highest value was recorded by Normal feather (56) followers by Frizzle feathered (48), Noiler (17) and Naked neck (16).

For the total number of indel event, Noiler had the highest value (246) followed by Normal feathered (170), then Frizzle feathered (164) and Naked neck with the least value (27). The total number excluded over lapping indel event was highest in Noiler (229) followed by Frizzle feathered (116), Normal feather (114) and least value were recorded in Naked neck chickens (11). For average indel length event, the highest value was observed in Noilers (3.65) followed by Frizzle feathered (1.98), Normal feathered (1.77) and Naked neck with least value (1.06). Average indel length also varied. Noiler had the highest value (3.40) followed by Frizzle feathered (1.66), Normal feathered (1.49) and least value was observed in Naked neck (1.04). Number of indel

haplotypes was biggest in Frizzle feathered (11) followed by Noiler (10), then Normal feathered (8) and least value were recorded by Naked neck (3). For indel haplotype diversity, Naked neck had the highest value (1.0), followed by Frizzle feathered (0.93), Normal feathered (0.89) and least value recorded by Noiler (0.79). Indel diversity was highest in Normal feathered (11.55) followed by Naked neck (10.67), Frizzle feathered (8.51) and least value was observed in Noilers (2.31). For diversity per site, Normal feathered had highest value (0.04) followed by Frizzle feathered and Noiler (0.03) and least by Naked neck (0.02 while considering theta per sequence from 1 theta (1), the highest value was observed in Normal feathered (19.12) followed by Frizzle feathered (15.09), Naked neck (10.67) and least value observed in Noilers (5.03).

Table 3 Insertion Deletion polymorphism

Parameters	Frizzle feathered	Naked neck	Noiler	Normal feathered
Total number of indel site analyzed	79	17	45	91
Total number of indel site	432	64	605	393
Total number of excluded over lapping indel sites	353	47	560	302
Total number of (indel and non indel) site analyzed	288	564	81	332
Total number of indels events analyzed 1	48	16	17	56
Total number of indel events	164	27	246	170
Total number of excluded overlapping indel event	116	11	229	114
Average indel length event	1.938	1.063	3,647	1,768
Average indel length	1.662	1,038	3,400	1,491
Number of indel Haplotypes	11	3	10	8
Indel Haplotypes diversity	0.934	1.000	0.794	0.891
Indel diversity k	8,505	10.667	2,309	11.564
Indel diversity per site pi	0.030	0.019	0.029	0.035
Theta (per sequence from 1, Theta (1)Tajima	15.094	10.667	5.029	19.119

Figure 1 represents the estimates of evolutionary divergence over sequence pairs within chicken strains. Analysis was conducted using the maximum composite likelihood model (Tamura *et al.*, 2004). The analysis involved 45 nucleotides sequences. Codon position included was 1st + 2nd + 3rd + Non-coding. All position containing gas and missing data were eliminated. There were a total of 26 positions in the final data set. Evolutionary analysis was conducted in MEGA 6 (Tamura *et al.*, 2013). The highest estimates of average evolutionary divergence over sequence pairs within chicken strains was observed in Noilers (0.666) and the least in Naked neck (0.01).

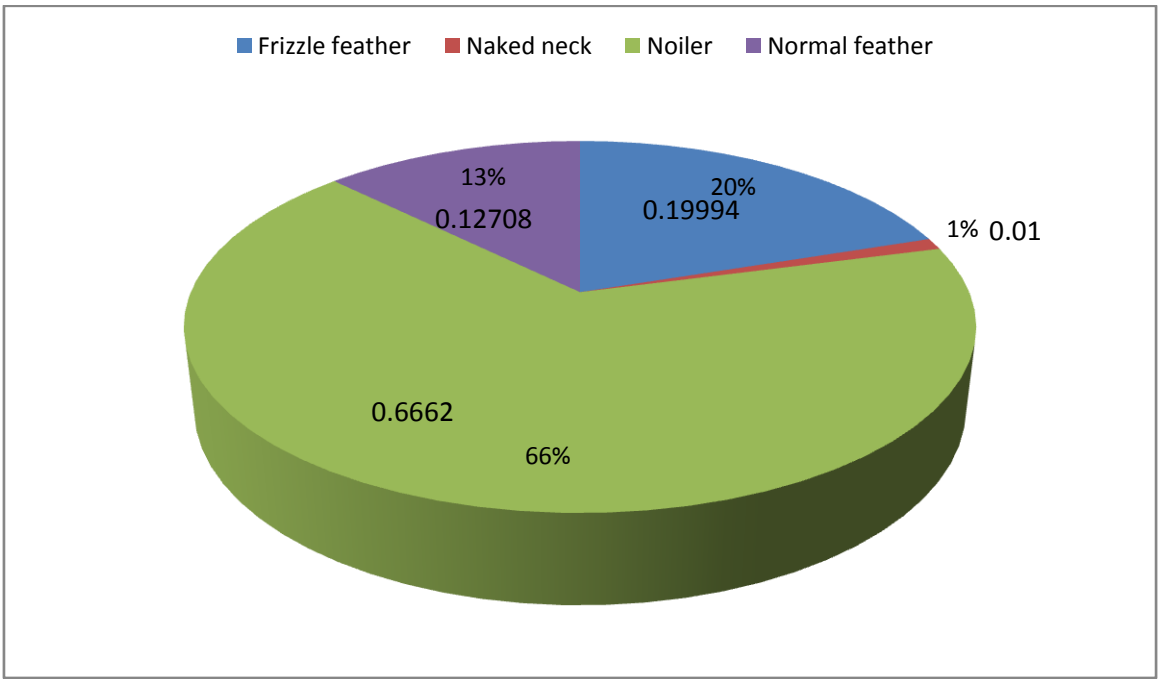

Figure 1: Estimates of Average Evolutionary Divergence over Sequence Pairs within chicken strains

Table 4 revealed the genetic distance in *POU1F1*gene between the four strains of chickens studied. The highest genetic distance is between Frizzle feathered and Noiler (0.519). The least genetic distance is between

Normal feathered and Naked neck (0.073). The higher genetic distance seen in Noilers was expected because Noiler is an improved indigenous strain. Frizzle feathered, Normal feathered and Naked neck are indigenous Nigerian strains of chickens.

Table 4 Genetic distance in *POU1F1* gene across the chicken strains studied

	Normal feathered	Frizzle feathered	Naked Neck	Noiler
Normal feathered	1			
Frizzle feathered	0.179	1		
Naked Neck	0.073	0.128	1	
Noiler	0.474	0.519	0.425	1

Figure 2, present the phylogenetic tree using unweighted pair groups method with arithemetic mean (UPGMA) across individual chicken *POU1F1*gene sequence. The phylogenetic tree across the individual chicken *POU1F1*gene sequenced were clustered and revealed low genetic distance indicating that the four strains were closely related and this could be as a result of continuous inbreeding over the years.

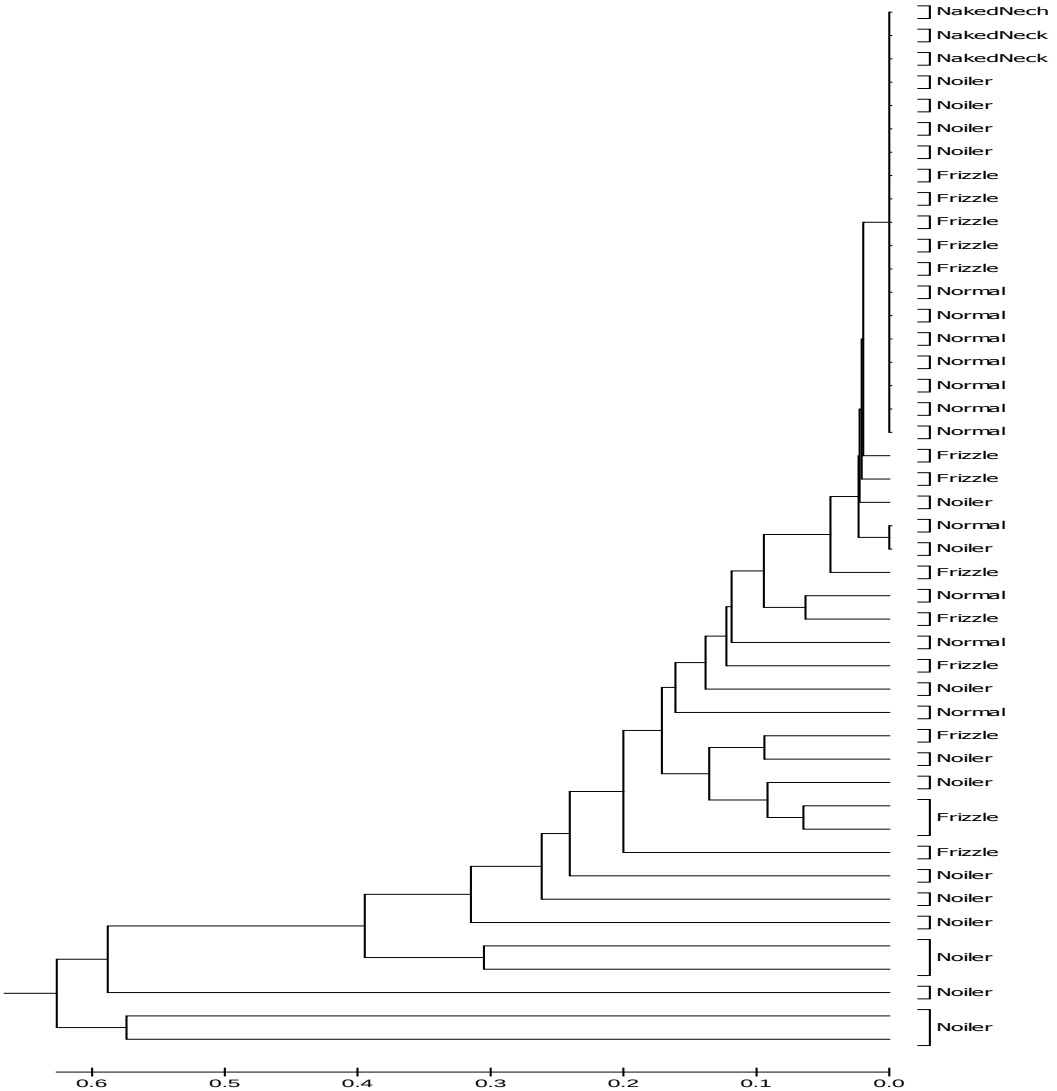

Figure 2: Phylogenetic tree using unweighted pair group method with arithmetic mean (UPGMA) across individual chicken *POU1F1*gene sequences

Figure 3 present the phylogenetic tree using unweighted pairs group method with arithemetic mean (UPGMA) between the four strains of Nigerian local chicken based on their *POU1F1* gene sequences. The four strains of chickens' genetic distance were far from each other, Normal and Noiler were closely related, though both Noiler

and normal feather had closer genetic distance with naked neck than frizzle fathered chickens. Frizzle feather is on its own and had different ancestors.

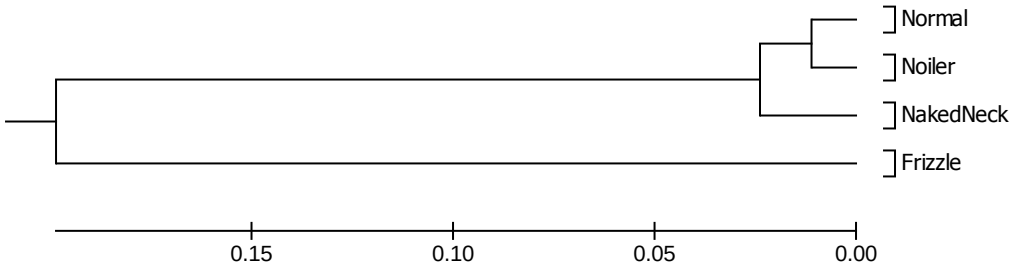

Figure 3: Phylogenetic tree using unweighted pair group method with arithmetic mean (UPGMA) between Nigerian local chicken Strains based on their *POU1F1*gene sequences

IV. Discussion

Candidate gene approach is a very powerful method used to investigate association of gene polymorphisms with economically important traits in farm animals (Rothschild and Sollar, 1997). The use of *POU1F1* gene in genetic studies had been researched by some authors (Qinghua *et al*., 2008: Jin *et al*., 2018: Agaviezor and Chukwuemeka, 2020). Variations in polymorphic sites were observed across the poultry breeds. These variations could be as a result of mutation and adaptation over the years. Variation in DNA polymorphism was observed in average number of difference. This result is supported by Agaviezor and Chukwuemeka (2020) who observed nucleotide diversity that ranged from 0.13 - 0.40 but not supported by Gao *et al.*, (2017) who observed values ranging from 2.720 - 7.571 and nucleotide diversity ranging from 0.00221 - 0.00615. The variation in gene flow and genetic differentiation were observed across poultry breeds. These variations occurred as a result of series of changes undergone by the different poultry breeds over the years. These variations were seen in areas such as number of segregating sites which was within the range of 0 - 26 as reported by Pandey*et al*., (2002) but was not in accordance with the report by Agaviezor and Chukwuemeka, (2020) who recorded number of segregating sites which ranged from 41 - 174. Haplotype diversity reported in this study were approximately the same with the work done by Agaviezor and Chukwuemeka (2020) but not in concordance with the report of Gao *et al* 2017, whose work recorded haplotype diversify which was within the range of 4 - 23. The nucleotide diversity in this study was in accordance with the work done by Agaviezor and Chukwuemeka, (2020) who observed nucleotide diversity value that ranged from 0.13 - 0.40 in their work on genetic diversity of *pituitary Transcriptive Factor 1(PIT 1)* gene in Nigerian local and exotic chickens. Furthermore, nucleotide diversity was observed to be 23 by Qinghua *et al*. (2008) in their work on *PIT 1* gene polymorphism associated with chicken growth traits which was not in accordance with the value observed in this study.

The variation in insertion deletion polymorphism, the value obtained in number of indel haplotype which ranged from 3-11, indel diversity 6-11 and indel diversity per site (0.02 - 0.04) reported by Agaviezor and Chukwuemeka, (2020) on their work on genetic diversity of *pituitary Transcription Factor 1 (PIT 1)* gene of Nigerian local and exotic chickens was in conformity with the outcome of work done in the present study. The genetic distance between poultry breeds as observed by Pandey*et al*. (2002) was within the range of 0.5609-0.8982 but was not in agreement with the values obtained in this study. Genetic distance between chicken breeds in the present study is in agreement with the values recorded by Agaviezor and Chukwuemeka, (2020) which was within the range of 0.264-0.578.

V. Conclusion

Results obtained from the study revealed a clear understanding of genetic diversity in *POU1F1* gene across the four breeds of chicken. The low diversity and high genetic similarities obtained in this study could be attributed to possibility that major changes have not taken place in the genome of these chickens that could be detected by chicken growth hormone gene.

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