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Polymorphism and genetic diversity of chicken growth hormone in selected chicken breeds in Nigeria

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Abstract

The study was carried out to investigate polymorphism and genetic diversity of the chicken growth hormone gene of selected chicken breeds (Fulani ecotype, Noiler, FUNAAB Alpha broiler, Frizzled feathered, Cobb 500) in Nigeria. Genomic DNA was extracted from blood samples collected from 49 birds. Amplification of specific DNA fragments at intron 3 of the cGH gene yielded a product size of 715bp and this was used to analyze for polymorphism using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. The extracted DNA was amplified using PCR and the gene sequenced. The sequenced data was used to evaluate genetic diversity and to carry out phylogenetic analysis. The banding patterns of the gene investigated, revealed the occurrence of three RFLP variants: TT, CT and CC, with genotype frequencies of 0.449, 0.245 and 0.306, respectively. The allele frequencies of the T and C alleles were 0.469 and 0.531, respectively indicating that the C allele was predominant. Chi-square test revealed that the chicken population investigated was in Hardy-Weinberg equilibrium. The mean number of alleles (Na), mean number of effective alleles (Ne), mean Shannon information index (I), and mean observed heterozygosity (Ho) were 2.000±0.000, 1.956±0.014, 0.682±0.004, 0.449±0.067, reapectively. Also, the mean expected heterozygosity (He), mean unbiased expected heterozygosity (uHe), mean fixation index (F), and % polymorphic locus were 0.489±0.004, 0.516±0.004, 0.083±0.138 and 100%, respectively. The gene flow (Nm) for all the population was 13.141. Results of analysis of molecular variance showed that the percentage of molecular variance among and within individuals was 13 and 87%, respectively. Phylogenetic analysis divided the five chickens into two broad groups; Noiler and the frizzled feathered occupied one cluster, while the Fulani ecotype, FUNAAB Alpha and Cobb 500 occupied the other cluster. It was concluded that great genetic diversity exists in the chicken breeds which could be exploited for further improvement.

Keywords: Polymorphism, genetic diversity, chicken, growth hormone, gene

Polymorphisme et diversité génétique de l'hormone de croissance du poulet dans des races de poulet sélectionnées au Nigeria

Résumé

L'étude a été réalisée pour étudier le polymorphisme et la diversité génétique du gène de l'hormone de croissance du poulet de races de poulet sélectionnées (écotype Fulani, Noiler, FUNAAB Alpha poulet de chair, plume grisonnante, Cobb 500) au Nigéria. L'ADN génomique a été extrait d'échantillons de sang prélevés sur 49 oiseaux. L'amplification de fragments d'ADN spécifiques à l'intron 3 du gène HCP a donné une taille de produit de 715 pb et cela a été utilisé pour analyser le polymorphisme à l'aide de la technique Réaction en chaîne par polymérase- Polymorphisme de longueur de fragment de restriction (RCP-PLFR). L'ADN extrait a été amplifié par RCP et le gène séquencé. Les données séquencées ont été utilisées pour évaluer la diversité génétique et effectuer des analyses phylogénétiques.

Les profils de bandes du gène étudié ont révélé l'apparition de trois variantes PLFR : TT, CT et CC, avec des fréquences de génotype de 0,449, 0,245 et 0,306, respectivement. Les fréquences alléliques des allèles T et C étaient de 0,469 et 0,531, indiquant respectivement que l'allèle C'était prédominant. Le test du chi carré a révélé que la population de poulets étudiée était en équilibre Hardy-Weinberg. Le nombre moyen d'allèles (Na), le nombre moyen d'allèles efficaces (Ne), l'indice d'information de Shannon moyen (I) et l'hétérozygotie moyenne observée (Ho) étaient de 2 000 ± 0,000, 1,956 ± 0,014, 0,682 ± 0,004, 0,449 ± 0,067, respectivement. En outre, l'hétérozygotie moyenne attendue (He), l'hétérozygotie moyenne attendue sans biais (uHe), l'indice de fixation moyen (F) et le % de locus polymorphe étaient respectivement de 0,489 ± 0,004, 0,516 ± 0,004, 0,083 ± 0,138 et 100 %. Le flux de gènes (Nm) pour toute la population était de 13,141. Les résultats de l'analyse de la variance moléculaire ont montré que le pourcentage de variance moléculaire parmi et au sein des individus était de 13 et 87 %, respectivement. L'analyse phylogénétique a divisé les cinq poulets en deux grands groupes ; Noiler et le frisé à plumes occupaient un groupe, tandis que l'écotype Fulani, FUNAAB Alpha et Cobb 500 occupaient l'autre groupe. Il a été conclu qu'il existe une grande diversité génétique dans les races de poulets qui pourrait être exploitée pour une amélioration ultérieure.

Mots clés : Polymorphisme, diversité génétique, poulet, hormone de croissance, gène.

Introduction

Growth is a highly complex procedure that is synchronized by an extensive network of neuroendocrine pathways. It is, therefore, challenging to make rapid progress using more conventional methods of genetic selection within breeds (Zhang *et al.,* 2008). However, recent advances in molecular technology have provided new leeways to evaluating genetic variability existing at the DNA level of animals (Kaya and Yildiz, 2008). The candidate gene approach has become a powerful technique for genetic improvement in chicken breeding programs. Applying this approach (the candidate gene approach) will likely result in higher efficiency in detecting desired traits necessary to improve productive performances. The *cGH* gene located on chromosome 27 is one of the most promising candidate genes for improving growth performance and carcass quality traits in chickens (Anh *et al.,* 2015). Growth hormone and the transforming growth factor-β subfamily are the most important hormones that play crucial roles in many physiological functions such as growth and reproduction. Growth hormone affects traits in broiler chickens production (Harvey, 2013). It promotes muscle and bone growth, development and regulation of fat content and its metabolism (Zhang *et al.,* 2008). Apart from affecting various physiological functions, *cGH* is reported to be one of the most important genes affecting skeletal muscle growth in chicken (Vasilatos-Younken *et al.,* 2000). It also plays significant roles in egg production, aging, and reproduction (Kansaku *et al.,* 2008). The chicken Growth Hormone (*cGH*) is a 22-kDa protein containing 191 amino acid residues consisting of 4,101bp, having five exons and four introns (Hrabia *et al.,* 2008; Kansaku *et al.,* 2008). It is a polypeptide, produced and secreted by the pituitary gland. A thorough look at the growth performance of the various chickens reared in Nigeria will show that they do not grow at the same rate. It is possible that differences in the cGH of the chickens manifest in differences in the birds' performances, especially, when comparing the exotic and indigenous ones. The current study was therefore, conceived to investigate polymorphism at intron 3 of the cGH gene and evaluate genetic diversity at

the gene locus focusing on selected chicken breeds in Nigeria.

Materials and methods

Study area

The study was carried out at **Minna, Niger state, Nigeria. Minna is located** in the Southern Guinea Savanna zone on latitude 9° 31 and 9° 42' North and longitude 6° 29 and 6° 41' East with annual rainfall of 1,200-1,300 mm, and temperature of 38-40° C averaging **27.3 ° C**. It lies on an altitude of 1,475 m above sea level, and is characterized by two seasons; wet (April-October), and dry (November-March).

Management of the birds

The study was carried out using five chicken breeds commonly reared in Nigeria (Fulani ecotype, Frizzle feathered, Noiler, FUNNAB Alpha broilers, Cobb 500). The Noiler, FUNNAB Alpha broilers and Cobb 500 chickens were managed intensively, while the Fulani ecotype and Frizzled feathered chickens were managed extensively.

Blood sample collection

Blood samples (5mls) were collected from the brachial vein of the chickens using 5ml syringes (separate needles and syringes were used for individual birds to avoid cross-contamination). The blood samples were collected into Ethylene Di-amine Tetra-acetic Acid (EDTA) bottles. Samples were stored on ice packs for onward transfer to the laboratory where genomic DNA was extracted.

DNA extraction, PCR

This was done at African Biosciences Ltd., Ibadan, Oyo State, Nigeria. The DNA was extracted using a gSYNC™ DNA extraction kit (Geneaid) according to the manufacturer's protocol. The quantity and quality of the extracted DNA was checked by spectrophotometer and agarose gel electrophoresis, respectively. The PCR products (2 samples per breed) were sequenced and then aligned against the chicken genome with the BLAST programme to verify their identity. Prior to sequencing, the DNA was cleaned to ensure that the gene is not contaminated with impurities. This was done using a DNA clean and concentrator kit according to the manufacturer's protocol (ZYMO Research). The primer used for the study was specific to the *cGH* gene and was designed using the National Centre for Biotechnology Information (NCBI) website. The *cGH* forward primer 5'TCAGTACGCAGACCTACCCTC3' and 5'TGCACATCATGTCCCACGTTT3' (reverse) were used for the study. The extracted DNA was diluted with autoclaved distilled water to 30ng per µl. A total of 21µl of the reaction mixture for the primer was mixed in the Eppendorf tube. The reaction volume (21µl) containing the *cGH* primers was amplified using PCR-RFLP method; each of 1.5µl, PCR Master Mix (10µl) contained 2.7µl water and 5.3µl (90ng) diluted DNA. The reaction mixture was subjected to an initial denaturation at 95°C for 4 minutes, followed by 50 cycles at 94°C for 25 seconds. Annealing then followed at 56°C for 45 seconds, an extension at 72°C for 40 seconds, and a final extension was done for 10 minutes at 72°C. The PCR products were digested with the endonuclease *Taq1*. Restriction enzyme was analyzed on 2.5% agarose gel. For genomic DNA, 1.0% agarose gel was used, while for the PCR product, 2.0% agarose gel was used. Electrophoresis was conducted at a constant voltage of 75 volts for 45 minutes at 37°C using 1.0 x TAE buffer. The DNA ladder (100bp) was used for the sizing of the DNA bands. The stained (ethidium bromide) DNA fragments were photographed using a Blue Light (BL) transilluminator (Biologix®) to visualize the bands.

Statistical analysis

Genotypic frequencies of the different PCR-RFLP patterns were estimated from

the combination of various RFLP alleles generated based on the presence or absence of one or more restriction sites. Different genotypes were identified based on the different patterns observed. The allele frequencies were calculated using standard methods. Chi-square (χ^2) test for goodness of fit was used to test for Hardy-Weinberg equilibrium. The measurement of genetic diversity, including Na, Ne, Ho, He, uHe, I, % polymorphic locus, F, and AMOVA, was estimated using GenAlEx 6.2 software (Peakall and Smouse, 2012). Dendrogram based on Nei's unbiased genetic distances, using the Unweighted Pair Group Method with Arithmetic mean (UPGMA), was generated to show the populations' genetic distances using MEGA X software.

Results

The agarose gel electrophoresis result of isolated genomic DNA of *cGH* of selected chicken breeds in Nigerian is presented in Figure 1. The isolated genomic DNA of the selected chicken breeds indicated high quality and appeared as single bands without sheared fragments. The DNA extraction and quality determination with electrophoresis and spectrophotometer was done for all chickens, and the obtained result was acceptable.

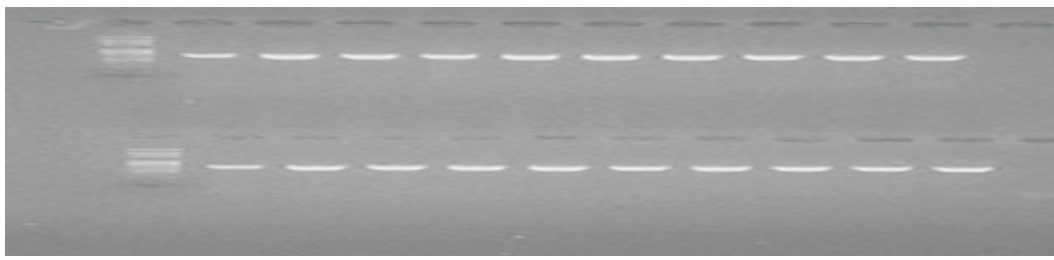

Figure 1: DNA extracted from chicken breeds

The agarose gel electrophoresis results of the PCR amplified *cGH* is presented in Figure 2. Amplification of specific DNA fragments at intron 3 of the *cGH* gene was successfully performed for the total samples using the designed primer pair.

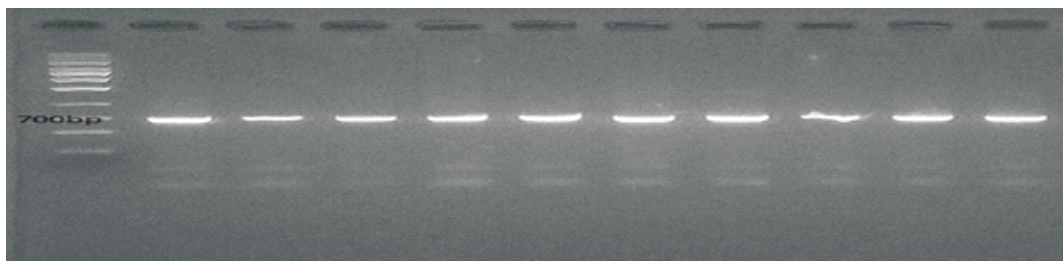

Figure 2: PCR amplified products at intron 3 of *cGH* gene

The agarose gel electrophoresis result of the PCR-RFLP analysis amplified *cGH* is presented in Figure 3. The banding pattern revealed three RFLP variants in the entire chickens sampled, which were assigned as CC, CT, and TT genotypes. The CC, CT and TT genotypes were detected by the presence of two bands, three bands and one band, respectively. The frequencies of the different RFLP fragments at intron 3 of the *cGH* gene are presented in Table 1. Out of the 49 chickens studied, 15 had CC, 22 had CT, and 12 had TT genotype, respectively.

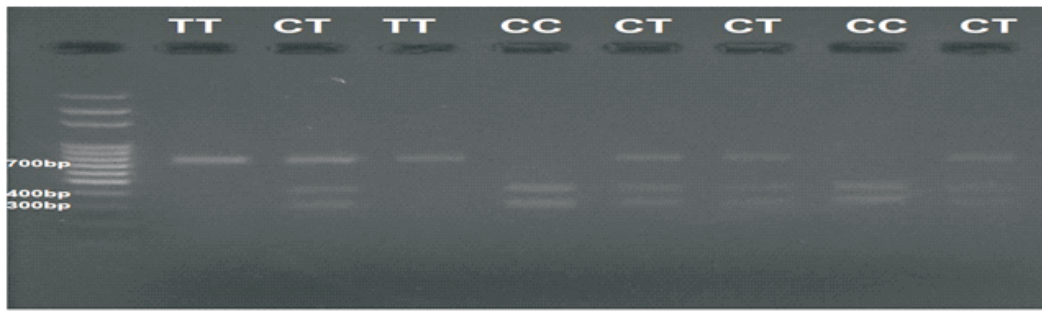

Figure 3: Genotypes amplified products at intron 3 region of *cGH* gene

Table 1: Frequency of RFLP patterns at intron 3 region of *cGH* gene

Pattern	Genotype	Frequency	Percentage
Two bands	CC	15	30.6
Three bands	CT	22	44.9
One band	TT	12	24.5

The genotype and gene frequencies with respect to intron 3 of the *cGH* gene is depicted in Table 2. The genotype frequencies were 0.306 for CC, 0.449 for CT, and 0.245 for TT genotypes, respectively. The calculated Chi-square value (0.312) showed that the studied population was in Hardy-Weinberg equilibrium with respect to intron 3 of the *cGH* gene (Table 3).

Table 2: Genotype and gene frequencies at intron 3 region of *cGH*

Genotype	Number	Frequency
CC	15	0.306
CT	22	0.449
TT	12	0.245
Allele		
C	52	0.531
T	46	0.469

Table 3: Hardy-Weinberg equilibrium at intron 3 of *cGH* gene

Genotype	Observed (O)	Expected (E)	$(O-E)^2/E$	X^2
CC	12	11.028	0.087	0.312ns
CT	22	23.944	0.158	
CT	15	14.028	0.067	

ns = not significant ($p>0.05$)

The genetic differentiation at the *cGH* gene locus of the chicken breeds is presented in Table 4. The FUNAAB Alpha and Cobb 500 had lower Ne (1.923), I (0.673), He (0.480) and uHe (0.505). The lowest Ho (0.200) was observed in the Cobb 500 while the highest F (0.583) was observed in the Cobb 500 broilers chicken. The entire gene locus was found to be polymorphic (100%) and the Nm over all populations for each locus was 13.141.

Table 4: Genetic differentiation at the *cGH* gene locus of five chicken breeds

Population	N	Na	Ne	I	Ho	He	uHe	F	%P	Nm
Noiler	10	2.00	1.980	0.688	0.500	0.495	0.521	-0.010	100	
Fulani ecotype	9	2.00	1.976	0.687	0.444	0.494	0.523	0.100	100	
FA	10	2.00	1.923	0.673	0.600	0.480	0.505	-0.250	100	
Ff	10	2.00	1.980	0.688	0.500	0.495	0.521	-0.010	100	
Cobb 500	10	2.00	1.923	0.673	0.200	0.480	0.505	0.583	100	
Mean	9.80	2.00	1.956	0.656	0.516	0.464	0.477	-0.075	100	13.141
SE	0.20	0.00	0.014	0.031	0.126	0.030	0.031	0.225	0.00	

FA=FUNAAB alpha, Ff=Frizzled feather, SE=standard error, Na=number of alleles, Ne=number of effective alleles, I=Shannon's information index, Ho=observed heterozygosity, He=expected heterozygosity, uHe=unbiased expected heterozygosity, F=fixation index, %P=percentage of polymorphic locus, Nm=gene flow.

Variation within and between populations of chicken breeds estimated using AMOVA (Table 5) revealed that a large proportion (87%) of the observed variance occurred within the breeds and 13% of the variance was contributed due to differences among individuals within populations. Variation among populations was 0%.

Table 5: AMOVA table showing variation within and between chicken populations

Source of variation	Df	SS	MS	Estimate of variance component	% variation
Among population	4	0.464	0.116	0.000	0
Among individual	44	12.944	0.294	0.035	13
Within animal	49	11.000	0.224	0.224	87
Total	97	24.408		0.259	100

A cluster analysis generated based on Nei's standard distance matrix (Figure 1) showed that Noiler and frizzled feathered chickens were in the first cluster, while Fulani ecotype, FUNAAB Alpha and Cobb 500 were in the second cluster.

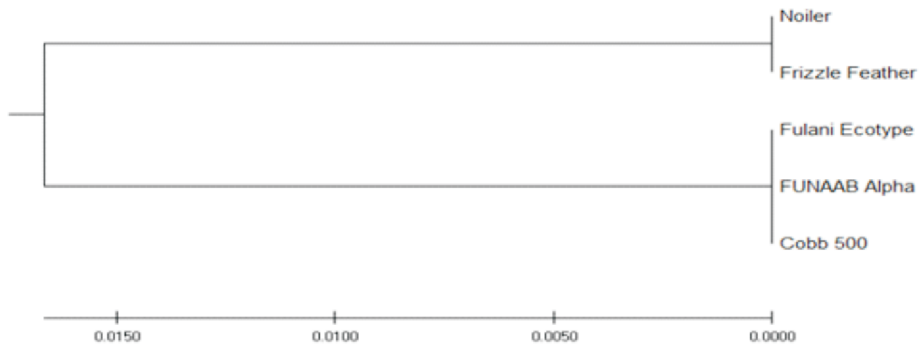

Figure 4: Phylogeny showing genetic relationships between chicken breeds at the *cGH* gene locus

Discussion

The amplified products appeared as clear single bands, and occupied the appropriate positions with approximately 715bp of amplicon size; no variation in sizes between the chicken breeds was observed. The amplified products were consistent with the target fragments and had a reasonable specificity, which could be directly analyzed through the PCR-RFLP technique. These findings at intron 3 of the *cGH* gene are in tandem with Muin and Lumatauw's (2013) report in Indonesian native chickens. However, Rahmadani *et al.* (2014) and Saikhom *et al.* (2017), all reported product sizes of 367bp and 713bp,

respectively. The differences observed in the PCR fragments' bp suggest the possibility of insertion/duplication of the sequence. The occurrence of three genotypes in the present study is in agreement with the observations of Bingxue *et al*. (2003), who also reported three genotypes (AA, AB and BB) in a population of hybrid chickens (broilers x Silky) in China with respect to the chicken growth hormone gene. Also, Tanmankaurd *et al.* (2008) analyzed 776bp amplicon of *cGH* gene digested with *Msp*1 with the results revealing three different genotype patterns (AA, AC and CC) created by a combination of polymorphic *Msp*1 cut sites present in exon-1 and intron-1, respectively. The results, however, disagree with Thakur *et al.* (2009), and Kulibaba (2015) who reported two genotypes AA and AB in Kadaknath chickens and, AB and BB in local chicken breeds of Vietnam. Bingxue *et al*. (2003) reported three distinct genotypes with genotypic frequencies of 0.759, 0.213, and 0.027, respectively in a population of Chinese hybrid chickens. Thakur *et al.* (2009), and Kulibaba (2015), however, reported two genotypes in Kadaknath and local chicken breeds of Vietnam; the frequencies were 0.4151 and 0.5849, and 0.070 and 0.930, respectively. The results of Makhsous *et al.* (2013) revealed six genotypes (AA, AB, AC, BB, BC, CC) while studying the *cGH* gene in the native chickens of Iran. The allele frequencies of the C and T alleles (0.531 and 0.469) indicate that the C allele was predominant. A higher frequency of A allele (0.9028) compared to B allele (0.0972) was reported by Muin and Lumatauw (2013) in Indonesian native chickens. Other authors who also observed preponderance of A allele over B allele include: Bingxue *et al*. (2003), where A allele was 0.8655 and B allele was 0.1335; Thakur *et al.* (2009), where allele A was 0.7075 and B was 0.2925. Makhsous *et al.* (2013), however, reported allele frequencies as follows: 0.599 (A), 0.102 (B) and 0.299 (C). However, Kulibaba (2015) observed a higher frequency of the B allele (0.964) than the A allele (0.036) in local Vietnamese chicken breeds, disagreeing with the outcomes of the current study. This indicated that evolutionary forces like migration, mutation and selection might have acted on the studied chicken breeds at the locus studied. This is in agreement with earlier observations (Khoa *et al.,* 2013: Makhsous *et al*., 2013; Rahmadani *et al.,* 2014). The mean Na over the loci for each population (2.000) is much lower than the 5.10-6.28 and 3.52-6.62 reported by Lyimo *et al.* (2013), and Mtileni *et al.* (2010) in Tanzanian and South African chickens, respectively. The lower Na as observed in the current study when compared to these other chicken populations indicates the presence of a relatively limited sample of the gene pool. The mean expected heterozygosity (0.464) concurs with the 0.351-0.434 reported by Marle-Koster and Nel (2000). Mtileni *et al.* (2010) reported expected heterozygosity of 0.67-0.69 among South African free-range chickens, while Lyimo *et al.* (2013) reported expected heterozygosity values of 0.58-0.67 in Tanzanian chicken populations. The difference observed in heterozygosity values may be due to variation in geography, chicken types, sample sizes, laboratory and sources of microsatellites used. Expected heterozygosity value of <0.5 may be due to inbreeding associated with population constraints and bottlenecks (Fariba, 2008). The negative fixation index (F is an inbreeding index) suggests out-breeding is still occurring in the Noiler, FUNAAB Alpha, and frizzled feathered chickens. The reverse is the case in the Fulani ecotype and Cobb 500. The high values could be due to the high level of selection involved in creating Cobb 500; the close population in which Fulani ecotype

chickens are reared probably, explains the positive nature of the fixation index. A negative fixation index in a population indicates that homozygous deficiencies may have arisen from population subdivisions owing to null alleles, genetic drift and selection against inbreeding (Pemberton *et al.,* 1995). The 100% polymorphic loci observed in the study showed that the markers used were highly informative in showing genetic diversity. Hassen *et al.* (2007) used polymorphic information content to assess how informative markers were and got an average value of 0.71. The main effect of gene flow (Nm), is the homogenization of allele frequencies between populations. The more the flow of a particular gene between populations, the more their similarity (El Hentati *et al.*, 2012). The estimated Nm in the studied chicken's populations (13.141) is high; this is evidence of the widespread use of commercial chicken lines to improve indigenous ones. The impact of such introgressions is rather limited, possibly due to poor adaptation of exotic birds to village conditions/consumers' preference for local chickens. Diffusive gene flow prevents substantial genetic differentiation due to genetic drift if gene flow is greater than unity (Slatkin, 1985 cited by Udeh, 2015). The closeness of the Fulani ecotype chicken to FUNAAB Alpha and Cobb 500 might not be too surprising because it has some exotic bloodline; Ogundipe (1990), and Tiamiyu (1999) opined that, the Fulani ecotype chicken could have been developed from crosses between exotic cockerels (Rhodes Island Red) used in previous improvement programmes, and indigenous hens. Hassen *et al.* (2007) clustered Ethiopian indigenous chickens into two clusters (similar to this result), showing the presence of two major breeds; according to the authors, cluster shows the level of inbreeding, and populations in it could be having coancestry.

Conclusion

In conclusion, the presence of observed genetic variants in the *cGH* gene could be exploited as a candidate gene in marker-assisted selection to improve the studied chicken breeds' performance.

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