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SEQUENCE ANALYSIS OF EXON 1 AND INTRON 1 OF GROWTH HORMONE GENE IN SIX CHICKEN GENOTYPES RAISED IN TROPICAL ENVIRONMENT

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Abstract: Chicken growth hormone (*cGH*) is a polypeptide hormone secreted by the pituitary gland which is responsible for several functions such as tissue growth and reproduction in chickens. This study was conducted to characterize six chicken genotypes using exon 1 and intron 1 regions of *cGH* gene sequences. One hundred and thirty-four (134) chickens comprising Normal feather (19), Naked neck (21), Frizzle feather (8), Arbor Acre (24), FUNAAB Alpha-1 (dihybrid) (31), and FUNAAB Alpha-2 (trihybrid) (31) were used for the study. Blood samples were collected from the birds into EDTA bottles for DNA extraction. The exon 1 and intron 1 regions of *cGH* were amplified using published primers. The product of the polymerase chain reaction was subjected to Sanger sequencing. DnaSP5 software was used to determine the diversity indices and MEGA6 software was used to determine the phylogenetic relationships among the six chicken genotypes and other chicken sequences. Fifteen (15) SNPs were identified in intron 1 and none in exon 1 of the *cGH* gene in all the six genotypes, and nine (9) of the SNPs occurred as transitions while others were transversions. The allele frequency ranged from 0.30 to 0.95 while the highest heterozygosity (0.66) was observed in mutation 410A>C in Naked neck genotype and lowest heterozygosity observed in Arbor Acre at SNP 330C>T. Polymorphic Information Content (PIC) was at the maximum in SNP 410A>C in Naked neck genotype with a value of 0.92. The exon 1 phylogeny tree revealed two clades where all the genotypes diverged. Intron 1 revealed two clades where Frizzle feather clustered with FUNAAB Alpha-1, Naked neck and FUNAAB Alpha-2 clustered together at one of the sub-clades in the second clade. Network analysis revealed Normal

feather chicken as the major ancestor of all the genotypes. The study concluded that intron 1 of *cGH* is polymorphic in all the six chicken genotypes investigated, and this can be used as candidate gene for selection in growth-related traits.

Key words: Diversity, FUNAAB Alpha**,** Growth hormone gene, Nigerian indigenous chicken, SNPs

Introduction

Poultry production is an important aspect in agriculture that contributes to the enhancement of the living standard of the people where income is provided, socio-cultural, and religious needs of farmers are met (*Akinola and Essien, 2011*). Chicken, out of all the poultry birds, is mostly consumed in the tropics because of its quality meat, egg, and short generation interval with reasonable feed efficiency (*Oluyemi and Roberts, 2007*). These attributes make it more preferred compared to other animal protein sources (*Peters, 2000*). Attention has been drawn to indigenous chickens in the tropics due to their adaptability and the quality of meat they produced (*Kaya and Yildiz, 2008*). Previously, it had been reported that Nigerian indigenous chickens exhibited slow growth rate and lower egg production compared to the exotic strains. *Oladeji (2016)* revealed that these inadequacies aided quest for better poultry chicken in Nigeria. However, the challenge being faced by breeders and geneticists in importing commercial chickens is enormous and this can be reduced by making use of the existing indigenous chickens to reduce the cost of importing exotic ones *(Chineke, 1998).* The indigenous birds are more adapted to the tropical environment with high disease resistance than their exotic counterpart (*Nwosu, 1987).* This led to the development of improved indigenous chicken, FUNAAB Alpha (Dual-purpose and broiler). The breed was developed for over six generations of selection and inbreeding, growth and productive performance were improved upon through crossbreeding with exotic lines *(Adebambo et al., 2018)*.

Growth rate in chicken is an economic trait and a set of multiple genes control growth performance in broiler production (*Vasilatos et al., 1997).* Growth hormone (*GH*) gene is one of the important genes responsible for various physiological functions in animals especially in growth and reproduction of chicken (*Enayati and Rahimimianji, 2009*). *Tanaka et al. (1992)* reported a nucleotide length of 4,101 base pairs with five exons and four introns. Polymorphisms in chicken growth hormone gene (*cGH*) have been studied generally using sequencing and restriction fragment length polymorphism (RFLP) and this had earlier been reported by different authors. One of these studies earlier reported is the polymorphisms in the intron 1 of chicken growth hormone gene which was positively associated with egg production in chickens according to

Kuhnlein et al. (1997). SNPs identified in intron 1 and exon 1 regions of *cGH* had been reported to be significantly associated with chicken body weight (*Ghelghachi et al., 2013; Kaur et al., 2008*) while some of the alleles identified in the introns had been linked to egg production traits and Marek's disease resistance in chickens (*Ip et al., 2001; Yan et al., 2003*). This present study aimed to determine the genetic diversity in the exon 1 and intron 1 of growth hormone gene of, Nigerian indigenous Normal feather, Naked neck, Frizzle feather, FUNAAB Alpha-1, and FUNAAB Alpha-2 chicken genotypes in comparison with the exotic Arbor Acre commercial strain.

Materials and Methods

Experimental birds and their sources

A total of 134 birds were sourced from FUNAAB Alpha project farm, Federal University of Agriculture, Abeokuta. The chickens consist of the following: 19 Normal feather, 8 Frizzle feather, 21 Naked neck, 24 Arbor Acre, 31 each of FUNAAB Alpha-1 and FUNAAB Alpha-2.

Blood sample collection

Blood samples were collected from 134 birds at their 8th week of age at the Biotechnology Laboratory of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta. This was done by collecting about 0.5ml of blood from brachial vein using a new needle and syringe per bird and stored in an ethylene diamine tetra acetic acid (EDTA) bottle to prevent coagulation.

DNA Extraction and Quantification

DNA was extracted from the whole blood samples using Qiagen DNA extraction kit following the manufacturer's procedure. The DNA was quantified using NanoDrop Spectrophotometer in order to determine the purity and the concentration of the DNA.

Amplification and sequencing of exon 1 and intron 1 of six chicken genotypes

Polymerase Chain Reaction (PCR) was carried out using designed primers Fwd 5'-ATCCCCAGGCAAACATCCTC-3' and Rev 5'-GACTATACAGAAAGAACCCAC-3' primers to amplify 776 bp region covering the exon 1 and intron 1 of the *cGH* gene. During the amplification, 5µL of genomic

DNA was added to a reaction mixture containing 17.8 µl of nuclease-free water, 2.5 µl of 10x PCR buffer, 1.5 mM of 25 mM MgCl2, 1mM of 5 mM dNTP, 1 µM of 10 µM forward and 1 µM of 10 µM reverse primer, and 0.2 U of 10 U/µl surf Hot Taq.

The PCR condition involved an initial denaturation step at 96°C for 15 minutes, 35 cycles of final denaturation at 95°C for 30 seconds, annealing at 60°C for 1 second and extension at 72°C for 90 seconds and a final extension at 72°C for 5 minutes carried out in a thermocycler (Agilent Surecycler 8800). To visualize the PCR product, gels were stained using ethidium bromide, microwaved and ran through a distinct well and a voltage of 100 volts after being loaded with a DNA ladder of 100 base pairs. The amplified fragment was visualised by an ultraviolet (UV) trans-illuminator and photographed. Sequencing of PCR product was carried out using Sanger-based capillary automatic sequencers (ABI prism 3130 system).

Trimming, Cleaning and Multiple Sequence Alignment

The nucleotide sequences were viewed with Bioedit software and trimmed using MEGA 6 software to remove noise in the sequences. The sequence obtained for exon 1 and intron 1 were aligned with *cGH* reference sequence NM_204359 and the alignment was carried out using Clustal W software (*Thompson et al., 1994*) integrated in MEGA 6 software.

Identification and Analysis of SNPs

Codon Code Aligner (Codon Code Corporation, Dedham, MA, USA) and MEGA 6 software were used to identify the SNPs in *cGH* gene in the six genotypes. The allele frequencies, heterozygosity and polymorphic information content were calculated manually.

Genetic Diversity

The genetic diversity indices were determined using DnaSP version 5 software (*Librado and Rozas, 2009).*

Evolutionary Analysis

The nucleotide frequency present in exon 1 and intron 1 of c*GH* gene in six chicken genotypes was determined using MEGA 6 software. MEGA6 software was also used to determine the phylogenetic relationship among exon 1 and intron 1 of *GH* gene in the six chicken genotypes used for this study and other downloaded sequences from the Genbank (Table 1). The phylogenetic tree was inferred using

unweighted pair group method and the reliability of the inferred tree was evaluated using bootstrap analysis of 1000 replications.

Table 1. Retrieved chicken growth hormone (*cGH*) gene sequences from NCBI with their accession numbers

Common name	Accession number	Base pair	Region	Origin
Dokki-4	MG906785	467	Exon	Egypt
Elmandra	MG906787	467	Exon	Egypt
El-salam	MG906782	467	Exon	Egypt
Inchas	MG906789	467	Exon	Egypt
Yellow wai chow	EF472953	776	Intron	China
Indian Cornish-3	JN403373	776	Intron	India
NG	JN403372	776	Intron	India
Rhode Island Red	EF452679	776	Intron	USA

Results and Discussion

PCR amplification of the *cGH* gene surveyed a region of 776 bp in six chicken breeds. Fifteen SNPs were identified at the intron 1 region of *cGH* gene (Table 2) while no SNP was found at the exon 1 region. The first SNPs identified frequently occurred in all the genotypes and SNPs 673T>C and 696G>A occurred frequently in Frizzle feather chickens while SNPs 279G>A was found specifically in Naked neck chicken genotype only. SNPs 330C>T was only peculiar to Arbor Acre and one of the FUNAAB Alpha chicken genotypes (TRH) and SNPs 410C>A was found only in Arbor Acre chicken genotype. The identified SNPs represent the occurrence of genetic variations in the intron 1 of growth hormone gene of the six chicken genotypes. This SNPs identified was higher than the one earlier reported by *Nie et al. (2005). Lyons et al. (2005)* reported more transition mutation more than transversion in their research and this is in line with the result in this research but in contrast with the report of *Nie et al. (2005)* where transversion was more than transition. According to *Ip et al. (2001*), high substitution rate of transition than transversion showed that selection had occurred severally among the chicken genotypes and this generally does not in any way favour transversion mutation.

Table 2. Single nucleotide polymorphisms identified in intron 1 of *GH* gene of Nigerian and improved indigenous chicken genotypes

Polymorphism	Type of mutation
267T>C	Transition
279G>A	Transition
282C>A	Transversion
310A>G	Transition
330C>T	Transition
410A>C	Transversion
410C>A	Transversion
453A>C	Transversion
453C>A	Transversion
454A>C	Transversion
492C>T	Transition
576C>T	Transition
576T>C	Transition
673T>C	Transition
696G>A	Transition

The high heterozygosity observed in some of the loci at the intron 1 in this study in Table 3 could be as a result of large heterozygous alleles present (*Chatterjee et al., 2010*). According to *Guo and Elson (1999),* polymorphic information content measures the informativeness of a genetic marker. *Hildebrand et al. (1992)* reported that a PIC greater than 0.7 is highly informative while bi-allelic markers PIC of 0.375 is at the maximum value and this can be observed in Naked neck with PIC 0.92 in Table 3. The Tajima's D estimated for all the chicken genotypes were not significant. Normal feather and Naked neck had negative values of -0.033 and -0.459 respectively. The negative and non-significant Tajima's D observed at the intron 1 in Normal feather in the first two indigenous chickens showed that the genotype has undergone positive selection with no demographic changes happening to them at the studied loci. This negative Tajima's D compares the average number of pairwise nucleotide differences with the total number of segregating sites (*Alonso and Armour, 2001; Durvasula, 2015*). This negative Tajima's D can be as a result of rare alleles produced by population expansion and purifying selection at the first intron (*Hahn et al., 2002*). The positive Tajima's D observed in other genotypes indicated that each population was undergoing balancing selection at each particular locus where alleles are at the intermediate frequencies (*Larsson et al., 2013; Durvasula, 2015*).

Table 3. Allele Frequencies, Heterozygosity and Polymorphic Information Content (PIC) of SNPs identified in intron 1 of *GH* gene of the six chicken genotypes

Genotype	SNP	MAF	H	PIC
Normal Feather	267T>C	0.89	0.19	0.17
	410A>C	0.78	0.34	0.28
	492T>C	0.89	0.19	0.17
	576T>C	0.78	0.34	0.28
Naked Neck	267T>C	0.88	0.21	0.19
	279G>A	0.88	0.21	0.19
	282C>A	0.82	0.29	0.29
	310A>G	0.88	0.21	0.19
	410A>C	0.71	0.66	0.92
	453A>C	0.82	0.29	0.25
	576T>C	0.88	0.21	0.19
Frizzle Feather	267T>C	0.50	0.50	0.38
	282C>A	0.62	0.47	0.36
	310A>G	0.87	0.23	0.20
	410A>C	0.62	0.47	0.36
	454A>C	0.50	0.50	0.38
	492C>T	0.62	0.47	0.36
	576T>C	0.75	0.38	0.37
	673T>C	0.87	0.23	0.20
	696G>A	0.87	0.23	0.20
Arbor Acre	267T>C	0.67	0.12	0.62
	282C>A	0.95	0.12	0.10
	310A>G	0.56	0.49	0.37
	330C>T	0.95	0.10	0.10
	410A>C	0.78	0.34	0.28
	453C>A	0.56	0.47	0.37
	454A>C	0.89	0.19	0.17
	492C>T	0.83	0.28	0.24
	576C>T	0.72	0.40	0.32
FUNAAB Alpha-1	267T>C	0.89	0.19	0.17
	310A>G	0.67	0.12	0.62
	410A>C	0.39	0.48	0.37
	453C>A	0.39	0.48	0.37
	454C>A	0.94	0.11	0.10
	492C>T	0.50	0.54	0.38
	576C>T	0.78	0.34	0.28

	267T>C	0.81	0.30	0.26
	282C>A	0.92	0.15	0.14
	310A>G	0.73	0.39	0.31
FUNAAB Alpha-2	410A>C	0.31	0.43	0.34
	453C>A	0.69	0.43	0.34
	454A>C	0.31	0.43	0.43
	492C>T	0.38	0.47	0.36
	576C>T	0.30	0.42	0.33

MAF= Major Allele Frequency; H= Heterozygosity; PIC= Polymorphic Information Content; SNP= Single Nucleotide Polymorphism

The diversity of chicken growth hormone gene at the intron 1 in all the genotypes studied is presented in Table 4. The number of polymorphic site is similar in three of the genotypes (Frizzle feather, Arbor Acre, and FUNAAB Alpha-2) studied while FUNAAB Alpha-1 with (7) polymorphic sites was closer to Naked neck genotype. The nucleotide diversity was highest in Frizzle feather and lowest in Normal feather (0.002) and this was lower than the one earlier reported by *Nie et al. (2005).* This was in line with the report of *Ilori et al. (2016)* where they reported that differences in effective population size of indigenous chicken could be responsible for a higher nucleotide diversity and this was recorded in Frizzle feather, an indigenous chicken. *Frankham et al. (2002)* had earlier reported that effective population size is one of the factors that affect SNP frequency and nucleotide diversity. The average number of nucleotide difference lowest value was observed in Normal feather (1.150) while the highest value was observed in Frizzle feather (3.929). According to *Abebe et al. (2015),* mating individuals that are genetically identical can result to a low heterozygosity and this was observed in Arbor Acre having the lowest heterozygosity in all the genotypes used. The genotypes used in this study were sourced and raised on the same farm and this may likely have contributed to the low heterozygosity observed.

The phylogenic tree at the exon 1 (Figure 1) showed that the six chicken genotypes can be separated into two clades with first clade, Dokki-4 chicken distinct itself on the evolutionary scale while other chickens diverged progressively. The second clade consists of the other chickens with Frizzle feather and Naked neck forming a sub-clade. This suggests that there was no difference within the genotypes and that they all shared a common ancestor.

Table 4. Diversity indices of intron 1 *GH* gene in Nigerian indigenous and improved indigenous chickens

Diversity indices	n	Eta	pi	k	Tajima's D
Normal feather	18	4	0.001	1.150	-0.033
Naked Neck	17	8	0.002	2.059	-0.459
Frizzle feather	8	9	0.006	3.929	0.646
Arbor Acre	18	9	0.004	3.033	0.563
FUNAAB Alpha-1	18	7	0.003	2.693	1.097
FUNAAB Alpha-2	26	9	0.005	3.382	1.394

n= Number of sample; Eta= total number of mutation; pi= nucleotide diversity; k= Average number of nucleotide change.

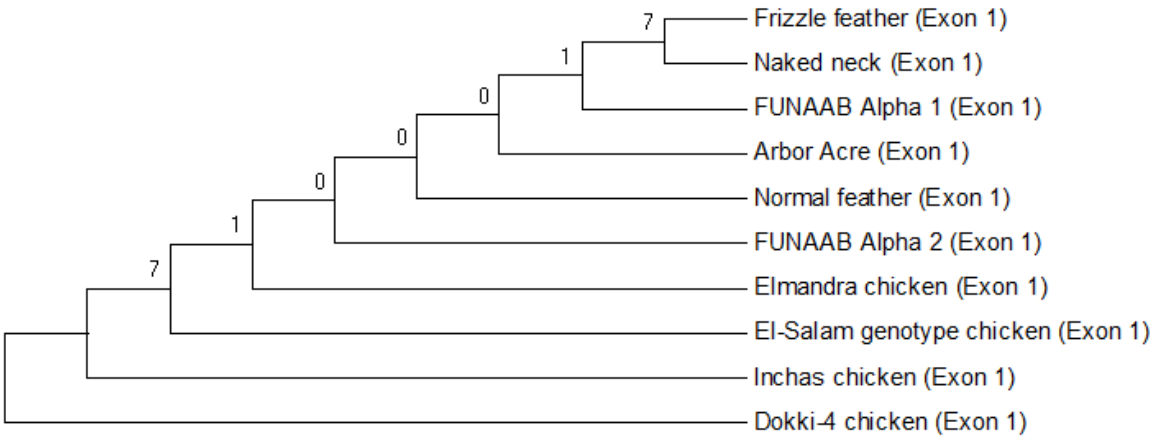

Figure 1. Phylogenetic relationship based on exon 1 of *GH* gene of the six chicken genotypes and other chicken genotypes.

At the intron sequence, commercial strain Rhode Island Red and Normal feather indigenous chicken are closely related since they form a sub-cluster with one another and they clustered with Indian Cornish-3 chicken and Yellow Wai Chow and also sub-cluster with Frizzle feather and FUNAAB Alpha-1 (Figure 2). This may be because the indigenous chicken was improved with an Indian chicken

to develop FUNAAB Alpha chickens and the it also suggested that all Indian chicken breeds are genetically similar at the inron1 of the growth hormone gene. The FUNAAB Alpha-2 closely related with Naked neck indigenous chicken and clustered with sub-cluster of NG and Arbor Acre chickens. This could be because of the development that has taken place in all the genotypes. Close relationship between intron 1 of normal feather and Rhode Island Red implied high comparability and evolution from a most common ancestor.

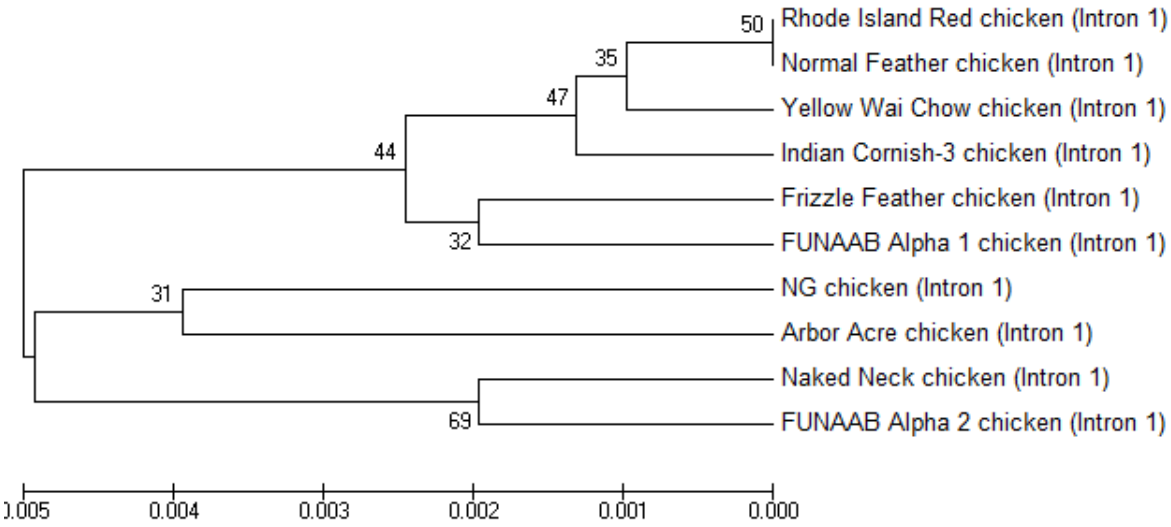

Figure 2. Phylogenetic relationship based on intron 1 of *GH* gene of the six chicken genotypes and other chicken genotypes.

Conclusion

Analysis of exon 1 and intron 1 of growth hormone gene in Normal feather, Naked neck, Frizzle feather, Arbor Acre, FUNAAB Alpha-1 and FUNAAB Alpha-2 chickens showed the existence of polymorphisms at the intron 1 region in all the chicken genotypes other than the exon 1. The Frizzle feather chicken had the highest number of polymorphism with higher nucleotide and haplotype diversity. Number of polymorphic site, haplotype diversity and nucleotide diversity showed the level of allelic variation in the GH gene of chicken which result in the genetic diversity of the GH gene in all the chicken genotype used. The phylogenetic tree showed that small genetic differentiation exists at the exon 1 among the chicken genotype studied while the intron 1 *GH* gene of Arbor Acre was closely related with NG strain from Indian while all the genotypes were

related with the same NG strain except Normal feather. This can also be seen in intron 1 phylogenetic tree where Normal feather sub-clustered with a commercial strain (Rhode Island Red) and clustered with an Indian strain (Yellow wai chow chickens). Associating the alleles with the genotypes of Normal feather, Naked neck, Frizzle feather, FUNAAB Alpha-1, and FUNAAB Alpha-2 is very necessary in order to know if they actually associate with production traits. This will help to channel the selection in any breeding program in order to improve their growth.

Analiza sekvence egzona 1 i introna 1 gena hormona rasta kod šest genotipova pilića uzgajanih u tropskom okruženju

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Rezime

Hormon rasta (*cGH*) je polipeptidni hormon koji luči hipofiza i koji je odgovoran za nekoliko funkcija kao što su rast i reprodukcija tkiva kod pilića. Ovo istraživanje je sprovedeno kako bi se okarakterisalo šest genotipova pilića korišćenjem egzon 1 i intron 1 regiona sekvenci gena *cGH*. Grupa od sto trideset i četiri (134) pileta, koja se sastojala od pilića normalnog perja (19), golovratih pilića (21), pilića kovrdžavog perja (8), Arbor Acre (24), FUNAAB Alpha-1 (dihibrid) (31) i FUNAAB Alpha- 2 (trihibrid) (31), je korišćena za ispitivanje. Uzorci krvi su sakupljeni od ptica u EDTA boce za ekstrakciju DNK. Regioni egzona 1 i introna 1 *cGH* su amplifikovani korišćenjem objavljenih prajmera. Proizvod lančane reakcije polimeraze je podvrgnut Sangerovom sekvenciranju. Softver DnaSP5 je korišćen za određivanje indeksa diverziteta, a softver MEGA6 je korišćen za određivanje filogenetskih odnosa između šest genotipova pilića i drugih sekvenci pilića. Petnaest (15) SNP-ova je identifikovano u intronu 1 i nijedan u egzonu 1 gena *cGH* u svih šest genotipova, a devet (9) SNP-ova se dogodilo kao tranzicije, dok su ostali bili transverzije. Frekvencija alela se kretala od 0,30 do 0,95 dok je najveća heterozigotnost (0,66) primećena kod mutacije 410A>C u genotipu golovratih pilića, a najniža heterozigotnost uočena u Arbor Acre na SNP 330C>T. Polimorfni informacioni sadržaj (PIC) bio je na maksimum u SNP 410A>C u genotipu golovratih pilića sa vrednošću od 0,92. Stablo filogenije egzona 1 otkrilo je dve klade u kojima su se svi genotipovi razišli. Intron 1 je otkrio dve klade gde su pilići kovrdžavog perja grupisani sa FUNAAB Alpha-1, golovratim i FUNAAB Alpha-2 grupisanim u jednoj od podklasa u drugoj kladi. Mrežna analiza je otkrila da su pilići sa normalnim perjem glavni predak svih genotipova. Studija je zaključila da

je intron 1 *cGH* polimorfan u svih šest ispitivanih genotipova pilića i da se može koristiti kao gen kandidat za selekciju u osobinama vezanim za rast.

Ključne reči: diverzitet, FUNAAB Alpha, gen hormona rasta, nigerijski autohtoni pilići, SNP

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Conflict of interest

The authors declare no conflict.

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