CHICKEN GROWTH HORMONE GENE POLYMORPHISM OF SELECTED CHICKEN BREEDS IN NIGERIA

Adigun, U.O., Ojimah, C.O. and Egena, S.S.A.
Department of Animal Production, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria.
Corresponding Author:adigunolagoke@gmail.com

ABSTRACT
The study was carried out to investigate polymorphism at the chicken growth hormone gene of selected chicken breeds (Fulani ecotype, Noiler, FUNAAB Alpha, Frizzled feathered and 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. 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. It was concluded that, the presence of observed genetic variations in the chicken growth hormone gene, could be exploited because of its association with economically important traits.

Keywords: Polymorphism, chicken, growth hormone, gene and RFLP.

INTRODUCTION
Growth hormone affects traits in broiler chickens production such as growth rate, body weight maturation, metabolic rate, egg production, reproduction, appetite control and aging (Harvey, 2013), it also promotes muscle and bone growth and development, as well as regulation of fat content and its metabolism (Zhang *et al.,* 2007). The Chicken Growth Hormone (*cGH*) is a 22-kDa protein, containing 191 amino acid residues and consisting of 4,101bp, having five exons and four introns (Kansaku *et al.,* 2008; Hrabia *et al.,* 2008). A polypeptide, the hormone is produced and secreted by the pituitary gland. Apart from affecting a variety of physiological functions, *cGH* is reported to be one of the most important genes affecting chicken performance traits (Vasilatos-Younken *et al.,* 2000). Its linkage to growth and metabolism, leads to potential correlations between its polymorphisms and economic traits. It is also involved in the processes of sexual differentiation, pubertal maturation and participates actively in gonadal steroidogenesis, gametogenesis and ovulation (Hull and Harvey, 2001). Apart from the above mentioned; it also plays significant roles in a variety of secondary functions such as egg production, aging and reproduction (Kansaku *et al.,* 2008). Accordingly, this study was carried out with the aim of investigating the polymorphism at intron 3 of the *cGH* gene in selected chicken breeds in Nigeria.

MATERIALS AND METHODS
The study was carried out using five chicken breeds commonly reared in Nigerian (Fulani ecotype, Fizzle feathered, Noiler, FUNNAB Alpha broilers, Cobb 500). Blood samples (5mls) were collected from the brachial vein of forty nine 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. This was done at African Biosciences Ltd., Ibadan, Oyo State, Nigeria. DNA was extracted using 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 were cleaned to ensure that the gene is not contaminated with impurities. This was done using DNA clean and concentrator kit according to the manufacturers protocol (ZYMO Research). The primers used for the study was specific to *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 reaction mixture for the primer was mixed in 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 water 2.7µl and 5.3µl (90ng) diluted DNA. The reaction mixture were 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 *Taq*1. 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 volt for 45 minutes at 37°C using 1.0xTAE buffer. The DNA ladder (100 bp) was used for sizing of the DNA bands. The stained (ethidium bromide) DNA fragments were photographed using a Blue Light (BL) transilluminator (Biologix®) to visualize the bands. 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 on the basis of 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.

RESULTS AND DISCUSSION

The agarose gel electrophoresis result of isolated genomic DNA of *cGH* of selection 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. DNA extraction and quality determination with electrophoresis and spectrophotometer was done for all Chickens and the obtained result was acceptable.

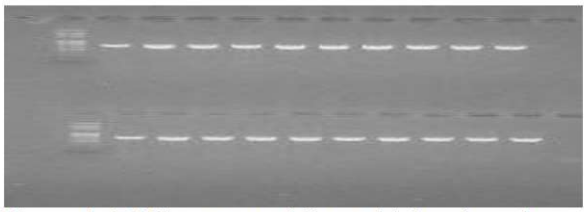


Figure 1: DNA extracted from chicken breeds

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. The amplified products appeared as clear single bands, and occupied the appropriate position with approximately 715bp of amplicon size, with no variation in sizes between the chicken breeds. The amplified products were consistent with the target fragments and had a good specificity, which could be directly analyzed through PCR-RFLP technique. This finding at intron 3 of the *cGH* gene is in tandem with the results of Muin and Lumatauw (2013) in Indonesia native chickens. However, Shahnaz *et al*. (2008), Thakur *et al.* (2009), Khoa *et al.* (2013), Makhsous *et al*. (2013), Rahmadani *et al.* (2014) and Saikhom *et al.* (2017) reported product sizes of 1200bp, 1216bp, 563bp, 1164bp, 367bp and 713bp, respectively. The differences observed in the bp of the PCR fragments suggest the possibility of insertion/duplication of the sequence. The agarose gel electrophoresis result of the PCR-RFLP analysis amplified *cGH* is presented in Figure 3. The banding pattern revealed three RFLP variants which were arbitrarily 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 (30.6%), 22 had CT (44.9%) and 12 had TT (24.5%) genotype, respectively. This occurrence of three genotypes is in agreement Bingxue *et al*. (2003) who reported three genotypes (AA, AB and BB) in a population of hybrid chickens (broilers x Silky) in China. Also, Tanmankaur *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 result however, disagrees with the findings of 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.

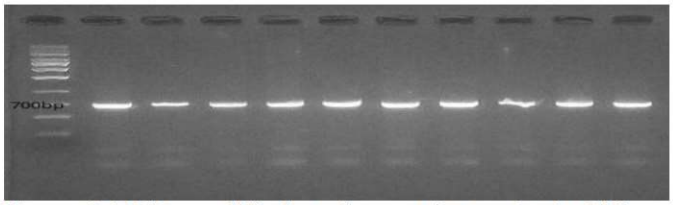


Figure 2: PCR amplified products at intron 3 of *cGH*

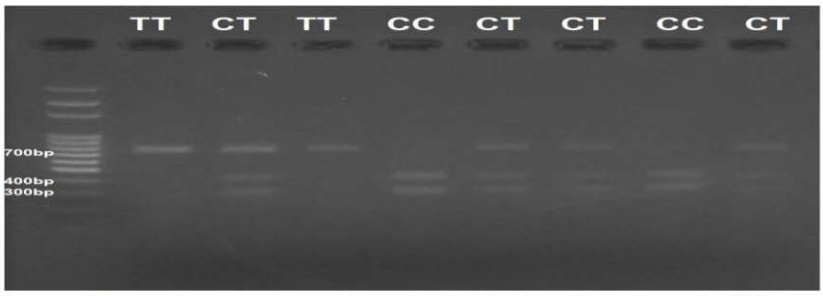


Figure 3: Genotypes amplified products at intron 3 region of *cGH*

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. 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) in the native chickens of Iran.

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

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

The frequencies of C and T alleles (0.531 and 0.469) indicate that the C allele was predominant. 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) reported allele frequencies as follows: 0.599 (A), 0.102 (B) and 0.299 (C), respectively. However, Kulibaba (2015) observed higher frequency of the B allele (0.964) than the A allele (0.036) in local Vietnamese chicken breeds. 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).

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).

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

Genotype	Observed Individuals	Expected individuals	$\frac{(O-E)^2}{E}$	X^2
CC	12	11.028	0.087	0.312
CT	22	23.944	0.158	
CT	15	14.028	0.067	

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 performance of the studied chicken breeds.

REFERENCE

Bingxue, Y., Xuemei, D., Jing, F., Xiaoxiang, H., Changxin, W. and Ning, L. (2003). Single nucleotide polymorphism analysis in chicken growth hormone gene and its associations with growth and carcass traits. *Chinese Science Bulletin* 48(15): 1561-1564.

Harvey, S. (2013). Growth hormone and growth? Genetic Comp. *Endocrinology* 190:3–9.

Hrabia, A., Paczoska-Eliasiewicz, H.E., Berghman, L.R., Harvey, S. and Rząsa, J. (2008). Expression and localization of growth hormone and its receptors in the chicken ovary during sexual maturation. *Cell Tissue Res.,* 332: 317-328.

Hull, K.L. and Harvey, S. (2001). Growth hormone roles in female reproduction. *Journal of Endocrinology.* 168:1-23.

Kansaku, N., Hiyama, G., Sasanami, T. and Zadworny, D. (2008). Prolactin and growth hormone in birds: protein structure, gene structure and genetic variation. *Journal of Poultry Science* 45: 1-6.

Khoa, D.V.A., Khang, N.T.K., Ngu, N.T., Matey, J., Thi, H., Loan, H.T.P. and Thuy, N.T.D. (2013). Single nucleotide polymorphisms in Gh, Ghr, Ghsr and insulin candidate genes in chicken breeds of Vietnam. *Greener Journal of Agricultural Science* 3(10): 716-724.

Kulibaba, R.A. (2015). Polymorphism of growth hormone, growth hormone receptor, prolactin and prolactin receptor genes in connection with egg production in Poltava clay chicken. *Agri. Biol.,* 50(2): 198-207.

Makhsous, S.G., Mirhoseini, S.Z., Zamiri, M.J. and Niazi, A. (2013). Polymorphisms of growth hormone gene in a native chicken population: association with egg production. *Bull. Vet. Inst. Pulawy* 57: 73-77.

Muin, M.A. and Lumatauw, S. (2013). Identification of *Msp*I polymorphism in the fourth intron of chicken growth hormone gene and their associations with growth traits in Indonesia native chickens. *Animal Production* 15(1): 1-7.

Rahmadani, R.P., Sumantri, C. and Darwati, S. (2014). Polymorphisms of growth hormone (GH|MspI) gene in Indonesia local chicken and the crossbred using PCR-RFLP. Sustainable livestock production in the perspective of food security, policy, genetic resources and climate change. *Proceedings of the 16th AAAP Animal Science Congress* Vol. II. 10-14th November. Gadjah Mada University, Yogyakarta, Indonesia.

Saikhom, R., Sahoo, A.K., Taraphder, S., Pan, S., Sarkar, U., Ghosh, P.R., Bhattacharya, D. and Baidya, S. (2017). Polymorphisms of growth hormone gene in Haringhata Black chicken. *Explor. Anim. Med. Res.,* 7(1): 42-47.

Shahnaz, S., Shadma, F., Rank, D.N., Khanna, K. and Joshi, C.G. (2008) Growth hormone gene polymorphism and its correlation with different traits in Bantam and White Leghom chicken. *Indian Journal of Poultry Science* 43(2): 123-127.

Thakur, M.S., Parmar. S.N.S., Chaudhari, M.V. and Bhardwaji, J.K. (2009). Growth hormone gene polymorphism and its association with egg production in Kadaknath chicken. *Livestock Research for Rural Development 21 (8)*

Vasilatos-Younken, R., Zhou, Y., Wang, X., McMurtry, J.P, Rosebrough W., Decuypere, E., Buys, N., Darras, M., Geyten, V. and Tomas, F. (2000). Altered chicken thyroid hormone metabolism with chronic *GH* enhancement *in vivo*: consequences for skeletal muscle growth. *Journal of Endocrinology* 166: 609-620.

Zhang, X.L., Jiang, X., Liu, Y.P., Du, H.R. and Zhu, Q. (2007). Identification of AvaI polymorphisms in the third intron of *GH* gene and their associations with abdominal fat in chickens. *Poultry Science*86: 1079–1083.