

Original Research Article

Diversity of the Ghrelin Gene in Nigeria's Fulani and Yoruba Ecotype Chickens

ABSTRACT

Ghrelin (GHRL) is a novel 28-amino acid gut-brain peptide linked to a gene associated with the regulation of growth hormones in birds. This study was carried out to investigate the polymorphism of the Ghrelin gene in Fulani and Yoruba ecotypes chickenof in Nigeria. Blood samples were collected from seventy-eight (78) Nigerian indigenous chickens, comprising of 41 Yoruba ecotypes and 37 Fulani ecotypes. Polymerase Chain Reaction-Restriction The Fragment Length length Polymorphism polymorphism (PCR-RFLP) method was used, and the Mboll restriction enzyme cut site 71 of the Ghrelin ghrelin and genetic structure were determined. Population structure was analysedanalyzed using allele and genotype frequencies, heterozygosityheterozygosis, and genetic variation metrics. Two alleles (C and T) and three genotypes (CC, CT, and TT) were observed. In the Yoruba Ecotype, the allele frequencies were C (0.34) and T (0.66) respectively while C (0.45) and T (0.55) was-were observed in the Fulani Ecotype, and the overall population was C (0.39) and T (0.61). The genotype frequencies obtained were, In the Yoruba Ecotypeecotype, CC (0.10), CT (0.48), and TT (0.41) were observed. In the Fulani Ecotype, CC (0.22), CT (0.45), and TT (0.32) were also observed, and in the overall population, CC (0.15), CT (0.47) and TT (0.37) were observed. FIS values for the Yoruba Ecotype (-0.0847) and FE (0.00702) reflects random mating and inbreeding, respectively. The effective number of alleles indicates that the Fulani Ecotype has more effective alleles compared to the Yoruba Ecotype. These results suggest that the Yoruba Ecotype-ecotype may be at Hardy-Weinberg Equilibriumequilibrium, while the Fulani Ecotype-ecotype deviates for-fromthe Ghrelin locus. In sum, our results may open opportunities for genetic improvement of in Nigerian Indigenous-indigenous Chicken-chickens due to the polymorphic nature of Ghrelinghrelin.

Key words: Ghrelinghrelin, Nigerian Ecotypeecotype, Genetic-genetic diversity

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1. INTRODUCTION

Natural resources is-are one of the greatest endowments sustaining the tropical and subtropical economies globally. Indigenous animal genetic resources plays a pivotal role in maintaining the socio-economic climate of developing countries. Poultry farming is one of the most attractive husbandryhusbandries in rural economies due to their-its ease of rearing, low production cost, provision of quality protein, and source of manure [1]. Diversity of genetic resources shave-has been employed as a tool for genetic improvement, thus, diversity of indigenous poultry species presents valuable resources for livestock development [2]. Since

the genetic resources locked up in indigenous poultry ~~is~~ yet to be fully exploited, characterizing the Nigerian ecotype chickens becomes essential.

Chickens make up about 80% of the 120 million poultry found in Nigeria [3]. They have been ~~characterised~~ characterized based on biochemical indices[4], plumage (normal or frizzled), body structure (naked neck and dwarf types), ~~colour~~ color (black, white, brown, and mottled)[5], body size or body weight (heavy and light ecotypes)[6], and location; Yoruba and Fulani ecotypes [7]. The Fulani ecotype chicken is found in the dry savannahs of Nigeria, known for their better meat and egg production[8]. They have been classified for their heavy mature body weight (0.9-2.55 kg) and are known for their growth and reproductive performance potentials[8]. In contrast, the Yoruba ecotype chickens are found in ~~the~~ swamp, rainforests, and derived savannah agro-ecological zones known for their light, mature body weight (0.68-1.5 kg). They have a slower growth rate, a flighty nature, and a fancy ~~colour~~ color pattern of mottling[9, 10]. Cross breeding of superior ecotypes for specific markers may be useful for enhancing the genetic potentials of our local poultry.

Characterizing valuable biological systems is the first step ~~for~~ in their genetic improvement[11]. Several efforts have been made to characterize Nigerian poultry resources using phenotypic traits[3, 4, 9]. This may be misleading because genetic composition does not necessarily translate into phenotype characteristics, and phenotypes are complex interactions between genotype and the environment[11]. It therefore became expedient to uncover the Nigerian poultry local resource potentials ~~from~~ at the genetic or molecular level. Our previous study on turkey and those of others on guinea fowl have started exploring the genetic potentials of local poultry by characterizing genes associated with important economic traits[11,13].

Ghrelin (GHRL), an essential modulator of feed intake, growth factor release, and fat deposition, has captured our attention[14]. This is because the diversity of this gene can help in marker assisted selection for improved growth in Nigerian chicken ecotypes. It functions to increase the secretion of growth hormone and control energy balance[15]. Ghrelin (GHRL) ~~have~~ has been associated with neonatal development in rats, chickens, and coordinates the regulation of growth hormone in birds[16]. The chicken ghrelin (cGHRL) gene is encoded as a 116-amino acid Ghrelin precursor and a 26-amino acid in mature Ghrelin [17], located on chromosome 12, comprising ~~of~~ five exons and four introns[16].

The degree of diversity in any species is a strong determinant of the amount of genetic improvement that can be achieved, and little has been done to enhance the genetic progress and conservation of local chickens at the molecular level in Nigeria. This is because attention has been directed towards commercialization using improved breeds[18], ~~and~~ ~~thus~~ which may lead to the erosion of Nigerian local chicken genetic resources. This present

study was carried out to investigate and identify the polymorphism of [the Ghrelin](#) gene in Fulani and Yoruba ecotypes chickens raised under the same conditions using PCR-RFLP (polymerase chain reaction-[Restriction restriction Fragment fragment](#) length polymorphism).

2.0 MATERIAL AND METHODS

This experiment was carried out at the Poultry Unit of the Teaching and Research Farm of [the](#) Department of Animal Science, University of Ibadan, which is situated 6 [kilometreskilometers](#) to the [North-north](#) of the city of Ibadan (7°26'N and 3°54'E) [in](#) Oyo [stateState](#), South-Western Nigeria, at a mean altitude of 277 meters above sea level.

2.1 Experimental animals

Seventy-eight (78) Nigerian indigenous chickens (NIC) [which](#) comprised of Fulani ecotype (FE) (37) and Yoruba ecotype (YE) (41) were used for this experiment.

2.2 Blood Experimental animals sample collection

Blood samples were collected via the jugular vein of the chickens, using a needle and syringe and were transferred [unto-to](#) FTA (Flinders Technology Associates) classical cards by dropping [#-them](#) on the cards. The samples were allowed to dry at room temperature and then put in sealed nylon envelopes. Extreme temperatures were avoided during storage and while the samples were transported to the [Laboratory-laboratory](#) (ACUTIG Genetic [servicesServices](#)) for analysis.

2.3 DNA extraction

DNA was extracted from air dried blood spotted on the FTA cards using the following procedure (Jena Bioscience); Punching out [of](#)-the sample disc from the FTA (Flinders Technology Associates) cards containing the blood samples by using the Harris micro-punch card and then [placed-placing it](#) into the Eppendorf tubes (five discs per tube), 150 ml of Tris-SDS solution and 200 ml [of](#) distilled water [was-were](#) added to each of the Eppendorf tubes and agitated for 10 minutes. Then the distilled water was removed and another one added, [and](#) agitated for another 10 minutes, and then heated at 95°C for 10 minutes, [-the-The](#) obtained liquid is the DNA. The DNA quality was tested via agarose gel electrophoresis, using agarose at 0.15 g, 1X TBE, i.e. 15 ml, and ethidium bromide, [-and-After](#) [-an-running](#) for 10-20 minutes, bands were seen.

2.4 Polymerase Chain Reaction (PCR) Amplification

The primer used for selective amplification of [the](#) cGHRL (chicken [Ghrelinghrelin](#)) gene using reference sequence Genbank Accession No. AY303688 and [the](#) Primer3 web program (Primer 3_www.cgi v0.2) [was-were](#) synthesized by InqabaBiotec (Hartfield, Pretoria, South Africa). Polymerase chain reactions were performed in a 6.25 µl reaction volume containing 4 µl nuclease free water, 1.25 µl Fast Taq, 0.25 µl forward [primerprimers](#), 0.25 µl reverse

primer₁ and 0.5 µl DNA, making a total of 6.25 µl ~~after~~ After the mixture the tubes were closed well to avoid evaporation of the contents and then vortexed and loaded into the PCR machine to run at an initial denaturation of 94°C x5mins ~~followed~~ followed by 34 cycles of denaturation at 94°C x40s ~~an~~ annealing temperature of 57°C and 72°C for 1min₁ and a final extension ~~step of~~ step of 72°C for 10mins. The amplification of Ghrelin gene ran for 2 hours and 18minutes. Amplification of the cGHRN (chicken Ghrelin~~ghrelin~~) gene was confirmed by running the PCR (polymerase chain reaction) products on 2% agarose gel and visualizing under UV rays ~~bands~~ Bands were seen and pictures taken.

2.5 Primers used for selective amplification of ghrelin gene

Forward primer 5'- 3' CATTCTAAGCTTTTGCCAGTT

Reverse primer 5'- 3'CACTGTTATTGTCATCTTCTC

2.6 Restriction Digestion

The PCR products were digested with *Mbol* enzyme₁ using 1 µl buffer, 0.5 µl of the amplicon (PCR product) from each tube, 7.5 µl of H₂O₁ and 0.5µl of *Mbol*in manufacturer's recommended assay, in a final reaction volume of 9.5 µl. All the components were added and mixed properly before adding the restriction enzyme. The reaction mixture was incubated at 37°C for 10 minutes, and the enzyme was inactivated by heating for 20 minutes at 65°C.

2.6.1 Agarose Gel Preparation and Staining

To make a concentration of 2% agarose, 10 ml of 1 × TBE buffer was added to 0.2 g of agarose powder in a flat-bottomed Erlenmeyer flask. The mixture was swirled briefly and then put in a microwave oven for 40 seconds to dissolve the agarose. To accelerate the dissolution, the slurry was swirled at intervals. After all the agarose had dissolved, the solution was allowed to cool on the bench. Meanwhile, as the solution cooled, the casting tray was prepared for ~~the~~ casting of the gel. The edges of the tray were sealed using a masking tape₁ and the comb(s) ~~contained with~~ the number of teeth that corresponded~~ed~~ to the number of samples slotted in position above the tray. The tray was set horizontally on the leveling stand on top of the bench.

When the gel solution was cold enough (~55°C), the combs were removed to create wells into which the digested products ~~are were~~ loaded.

2.6.2 Staining and loading of electrophoresis samples

Before loading, the PCR products were first mixed with the gel₁ loading dye. The dye SYBR[®] GREEN (0.2 µl) was added to stain 4 µl of the DNA₁ and then loaded into the wells formed. The staining of the gel with SYBR[®] GREEN is important in the detection of DNA under UV light and also because it increases the density of the sample₁ making it possible for the DNA to drop evenly into the well and also ~~enables~~ enabling one to follow the migration front. A

3000 bp (base pairs) ladder was loaded into the first well. 100 V of electric current was applied for the migration of DNA to start, and electrophoresis was allowed to continue for 20 minutes so that the amplicons formed clear bands, and visible bands were seen, and photographs were taken for each set of samples loaded on the gel.

2.7 Statistical analysis

Genotype and allele frequencies were determined by the direct gene counting method [19]. Allele and genotype frequencies of each gene in each strain of chicken were tested for fit to Hardy-Weinberg equilibrium (HWE), as well as genetic diversity parameters, using the POPGENE 1.32 software package [20].

3.0

RESULTS AND DISCUSSION

3.1 Results

Our study examined the polymorphism of the Ghrelin gene in Nigerian ecotype chickens which were raised in an intensive care unit system. After DNA extraction from blood samples, amplification and digestion with the *Mbo*II restriction enzyme cleaved a 369-bp fragment, resulting in Alleles C and T, consequently producing homozygous and heterozygous genotypes (CC, TT, and CT). Table 1 presents the allelic and genotypic frequencies, Table 2 shows the summary of heterozygosity, while Table 3 represents the summary of the genetic variation statistics of the Ghrelin gene in Nigerian indigenous chickens.

Two alleles (C and T) were observed for Yoruba, Fulani, and the overall (table 1) populations with varying frequencies. Frequencies of 0.34 and 0.66 were observed for the Yoruba ecotype, 0.45 and 0.55 for the Fulani ecotype, and 0.39 and 0.61 for overall. Similarly, genotypes CC, CT, and TT were also observed for the Ghrelin locus. We observed frequencies of 0.10, 0.49, and 0.41 in the Yoruba ecotype, while frequencies of 0.22, 0.46 and 0.32 were observed in Fulani ecotype and 0.15, 0.47, and 0.37 for the overall. Hardy-Weinberg test values (X^2 and G^2) for this sampled population revealed the Ghrelin locus is at equilibrium. Alleles T and genotype CT were found to be in highest frequency for the Ghrelin locus in Nigerian ecotype chickens. The heterozygosity indices for the ghrelin locus in Nigerian chickens (table 2) showed that the observed homozygosity for Yoruba, Fulani, and overall is 0.51, 0.54 and 0.53 while the expected homozygosity 0.54, 0.50, and 0.52 respectively. The observed heterozygosity was lower for the Fulani ecotype (0.46) in comparison to the expected heterozygosity (0.50). Nei's genetic distance ranges from 0.45-0.49, with the average heterozygosity for the entire population is 0.47. The measure of inbreeding coefficient (FIS) for this population was negative for the Yoruba ecotype (-0.0847), while it is

was positive for the Fulani (0.00702) and overall (0.0040). The summary of genetic variation of the Ghrelin gene showed (table 3) that 2 alleles (Na) were observed in the sampled population (C and T). However, the number of alleles expected to attain the levels of heterozygosity expected for this sampled population based on HWE (Ne) ranged from 1.82-1.98, with the Yoruba (1.82) having the least while Fulani (1.98) is greater than the overall (1.91). The Shannon index showed that the Fulani ecotype may be more diverse (0.69) than the Yoruba ecotype (0.64).

Table 1: Observed Allele and Genotype Frequencies for Ghrelin Locus in Nigerian Ecotype Chickens.

Ecotype	Allele	Frequency	Genotype	Frequency	X ^{2*}	G ^{2*}	DF*
Yoruba	C	0.34	CC	0.10	0.64	0.64	2
	T	0.66	CT	0.49			
			TT	0.41			
Fulani	C	0.45	CC	0.22	0.61	0.61	2
	T	0.55	CT	0.46			
			TT	0.32			
Overall	C	0.39	CC	0.15	0.93	0.93	2
	T	0.61	CT	0.47			
			TT	0.37			

*X² and *G² = Chi-square and Likelihood ratio test for Hardy-Weinberg equilibrium respectively *DF = degree of freedom for genotype

Table 2: Summary of Heterozygosity for Ghrelin Locus in Nigerian Ecotype Chickens.

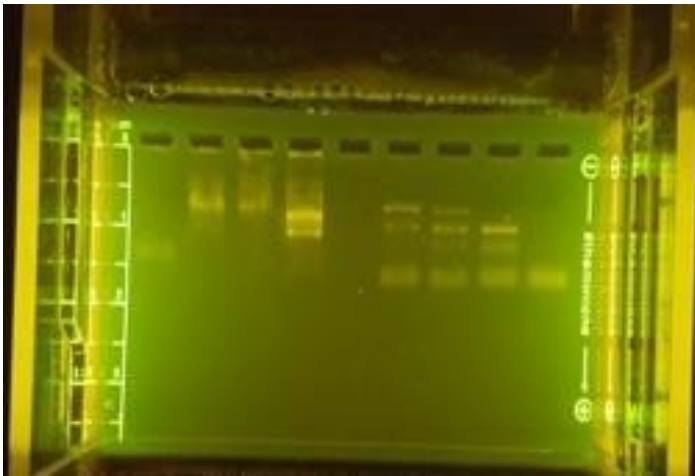
Ecotype	Obs _{hom} *	Obs _{het} *	Exp _{hom} *	Exp _{het} *	Nei's*	Ave _{het} *	FIS*
Yoruba	0.51	0.49	0.54	0.46	0.45	0.47	-0.0847
Fulani	0.54	0.46	0.50	0.50	0.49	0.47	0.00702
Overall	0.53	0.47	0.52	0.48	0.48	0.47	0.0040

*Obs_{hom}=observed homozygosity, Obs_{het}=observed heterozygosity, Exp_{hom}=expected homozygosity, Exp_{het}= expected heterozygosity, Nei's =Nei's expected heterozygosity, Ave_{het}= average heterozygosity, FIS=Wright's Fixation index

Table 3: Summary of Genetic Variation of Ghrelin Locus in Nigerian Ecotype Chickens.

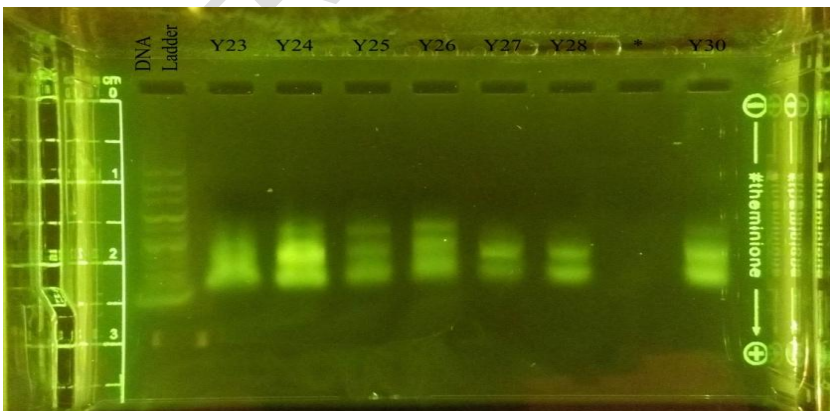
Ecotype	Na*	Ne*	I*
Yoruba	2.00	1.82	0.64
Fulani	2.00	1.98	0.69
Overall	2.00	1.91	0.67

Na* = observed number of alleles, Ne = Effective number of alleles, I = Shannon's information index



Lane 1-8 represents PCR products (amplicons) of Ghrelin gene

Figure 1: Electrophoresis pattern of site 71 of the Ghrelin gene in Nigerian indigenous chickens obtained by Polymerase polymerase chain reaction (PCR).



Band 1:3000bp ladder, band 2 genotype: TT, band 3 genotype: CT, band 4 genotype: CT, band 5 genotype: CT, band 6 genotype: TT, band 7 genotype: TT, band 8 genotype: TT.

Figure 2: electrophoresis product of site 71 of the ghrelin gene in Nigerian indigenous chickens obtained by digesting with *mboii*.

3.2 Discussion

Our study successfully characterized the GHRL (Ghrelin) locus of the Nigerian ecotype chickens for its diversity using the PCR-RFLP method. The diversity of GHRL (Ghrelin) receptors and genes have been characterized, showing different variations linked growth related traits [21-24]. The present study was geared at characterizing a major gene encoding an essential modulator of feed intake, growth factor release, and fat deposition in chickens [25]. This attempt is to understand the polymorphic nature of the GHRL (Ghrelin) gene and how we can assess it for marker assisted selection for improved chicken production in Nigeria. We showed that this locus is polymorphic, with varying frequencies for alleles and genotypes. Allele C is shown to have a lower frequency compared to T in both ecotypes. We suppose that the T allele may have less selective constraints for it to be genetically and environmentally selected for in both ecotypes. Similarly, the CC genotype experienced higher selective constraints, alluding to their lower frequencies across the ecotypes, however, we did not find a correspondingly higher frequency of TT across the ecotypes but observed the heterozygote genotype (CT) to be at a higher frequency. The TT genotype has been linked to lower feed consumption in pigs [26], which may pose a danger for the survival of animals. Thus, our study reveals that alleles C and T are essential for the conservation of Nigerian ecotype chickens. Similarly, allele and genotype frequencies obtained from 12 Chinese chicken breeds for the GHRL (Ghrelin) locus attest to the presence of low frequencies for the C allele with a correspondingly higher T allele [24]. However, they found a correspondingly higher TT genotype frequency for all breeds greater than at CT different GHRL (Ghrelin) sites. The similarity may be attributed to an evolutionary conservation mechanism that is maintained across generations and environmental climes. The differences in between the studies may be due to different markers, primers, and differences in breed which may be confounded by environment-by-genotype interactions. Thus, genotype CT may be more favourable for establishing Nigerian ecotype chickens in different environments.

Heterozygosity is an important metric for assessing the genetic diversity within a population and, as such, a good management metric for threatened taxa [27]. Our data for heterozygosity in the GHRL (Ghrelin) locus (Table 2) reveals that observed heterozygosity (Obs_{het}) is higher than expected heterozygosity (Exp_{het}), implying that random mating is observed for the Yoruba ecotype while the reverse is presented in the Fulani ecotype. Thus, since Exp_{het} and Obs_{het} are dependent on allele frequencies and individual genotypes (which also depends on the genetic variation and inbreeding), respectively,

~~Inbreeding~~ ~~inbreeding~~ can be derived from these metrics[28]. When $Exp_{het} > Obs_{het}$, inbreeding is at work in the population with $FIS > 0$ [27]. ~~The~~ FIS value for the Yoruba is negative and < 1 , and that of the Fulani population is > 1 . In sum, the Yoruba ecotype may be heterozygous due to some randomness of alleles at the locus, while the Fulani ecotype may be homozygous due to inbreeding for the GRHL locus and thus shows departure from HWE.

The allelic diversity reflected by the mean number of alleles, ~~the~~ effective number of alleles, and Shannon's information index is presented in Table 3. ~~The~~ ~~Number~~ numbers of alleles for the ecotypes is 2, however, N_e and I ~~indicates~~ ~~indicate~~ that ~~the~~ T allele may have more impact on the Fulani in comparison to Yoruba ecotype[29]. This shows there is little variation in the GHRL (Ghrelin) locus for the sampled population. Fijabiet *al.* [14] found similar results in Nigerian turkeys using similar methods. Our study provided baseline information to understand the genetic diversity of local chicken breeds in Nigeria and its implications for genetic improvement by assessing the GRHL (Ghrelin) locus. Metrics measured here are sample size-sensitive, and thus larger sample sizes and natural flocks can also be exploited ~~in other~~ to maximize the influence of DNA polymorphisms on GHRL (Ghrelin) activity and function.

4. CONCLUSION

The aim of this study was to determine the genetic diversity between and within Fulani and Yoruba ecotype chickens in Nigeria at the ghrelin gene locus using PCR-RFLP. The results of the study revealed that the sampled populations were polymorphic for ~~the~~ ghrelin gene locus, two alleles (C and T) and three genotypes (CC, CT, and TT) were observed. Conclusively, the results obtained in this research may open opportunities for genetic improvement of ~~in~~ Nigerian ~~Indigenous~~ ~~indigenous~~ ~~Chicken~~ ~~chickens~~ due to the polymorphic nature of ~~Ghrelin~~ ~~ghrelin~~.

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