Journal of Experimental Agriculture International

Volume 46, Issue 5, Page 419-427, 2024; Article no.JEAI.114177
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

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

L. O Igbatigbi [a], O. H Osaiyuwu [b*], R. B Fatai [b], O. M. Coker [c] and O. E. Fijabi [d]

[a] Department of Zoology, Faculty of Sciences, Federal University Lokoja, Kogi State, Nigeria.
[b] Department of Animal Science, Faculty of Agriculture, University of Ibadan, Oyo State, Nigeria.
[c] Department of Wildlife and Ecotourism Management, University of Ibadan, Ibadan, Nigeria.
[d] Biological Sciences, University of Alabama, Tuscaloosa, US State, USA.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2024/v46i52393

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/114177

Original Research Article

Received: 14/01/2024
Accepted: 18/03/2024
Published: 30/03/2024

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 chickens in Nigeria. Blood samples were collected from seventy-eight (78) Nigerian indigenous chickens comprising of 41 Yoruba ecotype chickens and 37 Fulani ecotype chickens. Polymerase Chain Reaction-Restriction fragment length polymorphism (PCR-RFLP) method was used and the MboII restriction enzyme cut site 71 of the ghrelin and genetic structure were determined. Population structure was analyzed using allele and genotype frequencies, heterozygosity 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

**Corresponding author: E-mail: cosamede@yahoo.com;*

(0.34) and T (0.66) respectively while C (0.45) and T (0.55) 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 ecotype, 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 Fulani ecotype (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 may be at Hardy-Weinberg equilibrium, while Fulani ecotype deviates for the ghrelin locus. In summary, our results may open opportunities for genetic improvement in Nigerian indigenous chicken due to the polymorphic nature of the ghrelin gene.

Keywords: Genetic diversity; Fulani; ghrelin gene; Yoruba.

1. INTRODUCTION

Natural resources are one of the greatest endowments sustaining the tropical and subtropical economies globally. Indigenous animal genetic resources play a pivotal role in maintaining the socio-economic climate of developing countries. Poultry farming is one of the most attractive husbandries in rural economies due to its ease of rearing, low production cost, provision of quality protein and source of manure [1]. Diversity of genetic resources 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 are 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 characterized based on biochemical indices [4], plumage (normal or frizzled), body structure (naked neck and dwarf types), colour (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 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 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 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 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 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], which thus 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 ecotype chickens raised under the same conditions using PCR-RFLP (Polymerase chain reaction-restriction fragment length polymorphism).

2. MATERIALS 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 kilometers to the north of the city of Ibadan (7˚26'N and 3˚54'E) in Oyo State, 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 to FTA (Flinders Technology Associates) classical cards by dropping it 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 (ACUTIG Genetic Services) 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 the sample disc from the FTA (Flinders Technology Associates) cards containing the blood samples by using the Harris micro-punch card and then placing it into the Eppendorf tubes (five discs per tube), 150 ml of Tris-SDS solution and 200 ml of distilled water were added to each of the Eppendorf tubes and agitated for 10minutes. Then the distilled water was removed and another one added, agitated for another 10 minutes, and then heated at 95˚C for 10 minutes. 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. After 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 Ghrelin) gene using reference sequence Genbank Accession No. AY303688 and the Primer3 web program (Primer 3_www.cgi v0.2) were synthesized by Inqaba Biotec (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 primer, 0.25 µl reverse primer and 0.5 µl DNA, making a total of 6.25 µl. 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 x 5mins, followed by 34 cycles of denaturation at 94˚C ×40s, an annealing temperature of 57ºC and 72˚C for 1min and a final extension step of 72˚C for 10mins. The amplification of Ghrelin gene ran for 2 hours and 18minutes. Amplification of the cGHRN (chicken ghrelin) gene was confirmed by running the PCR (polymerase chain reaction) products on 2% agarose gel and visualizing under UV rays. Bands were seen and pictures taken.

2.5 Primers Used for Selective Amplification of Ghrelin Gene

Forward primer 5’-3’
CATTTCTAAGCTTTTGCCAGTT
Reverse primer 5’-3’
CACTGTTATTGTCATCTTCTC

2.6 Restriction Digestion

The PCR products were digested with *MboII* enzyme, using 1 µl buffer, 0.5 µl of the amplicon (PCR product) from each tube, 7.5 µl of H2O and 0.5µl of *MboII* 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 masking tape, and the comb(s) contained the number of teeth that corresponded 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 were loaded.

2.6.2 Staining and loading of electrophoresis samples

Before loading, the PCR products were first mixed with the gel loading dye. The dye SYBR® GREEN (0.2 µl) was added to stain 4 µl of the DNA 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 sample making it possible for the DNA to drop evenly into the well and also 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, 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. RESULTS AND DISCUSSION

3.1 Results

Our study examined the polymorphism of the Ghrelin gene in Nigerian ecotype chickens that were raised in an intensive system. After DNA extraction from blood samples, amplication and digestion with the *MboII* restriction enzyme cleaved a 369bp fragement 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, and 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 the 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 the 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 Ghrelin locus in Nigerian ecotype chicken. The heterozygosity indices for the ghrelin locus in Nigerian chickens (Table 2) showed that the observed homozygosity for the Yoruba, the Fulani and the overall is 0.51, 0.54 and 0.53 while the expected homozygosity 0.54, 0.50, 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 and the average heterozygosity for the entire population was 0.47. The measure of inbreeding coefficient (FIS) for this population was negative for the Yoruba ecotype (-0.0847) while it was positive for the Fulani (0.00702) and overall (0.0040). The summary of genetic variation of the ghrelin gene showed (Table 3) that two 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).

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 has been characterized, showing different

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**			
	T	0.66	CT	0.49			
			TT	0.41	0.64	0.64	2
Fulani	**C**	**0.45**	**CC**	**0.22**			
	T	0.55	CT	0.46			
			TT	0.32	0.61	0.61	2
Overall	**C**	**0.39**	**CC**	**0.15**			
	T	0.61	CT	0.47			
			TT	0.37	0.93	0.93	2

*$*X^2$ and $*G^2$ = 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*

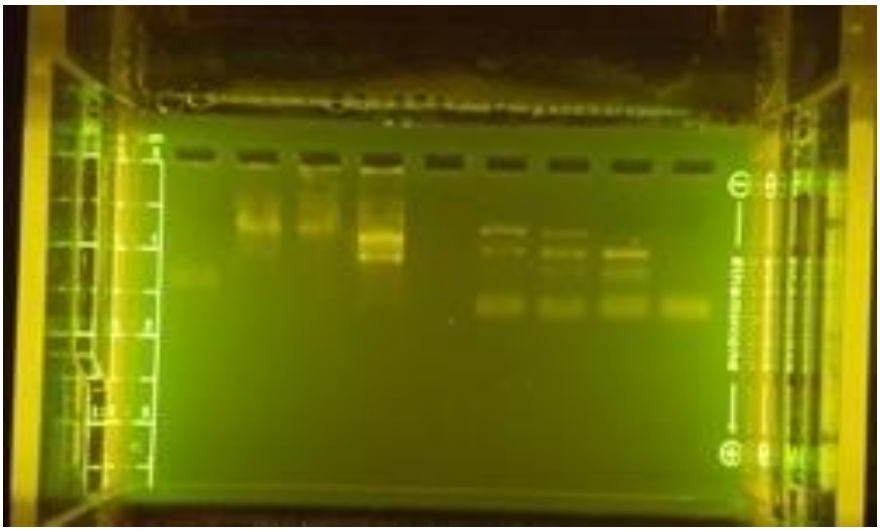

Fig. 1. Electrophoresis pattern of site 71 of the Ghrelin gene in Nigeria indigenous chickens obtained by polymerase chain reaction (PCR)

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

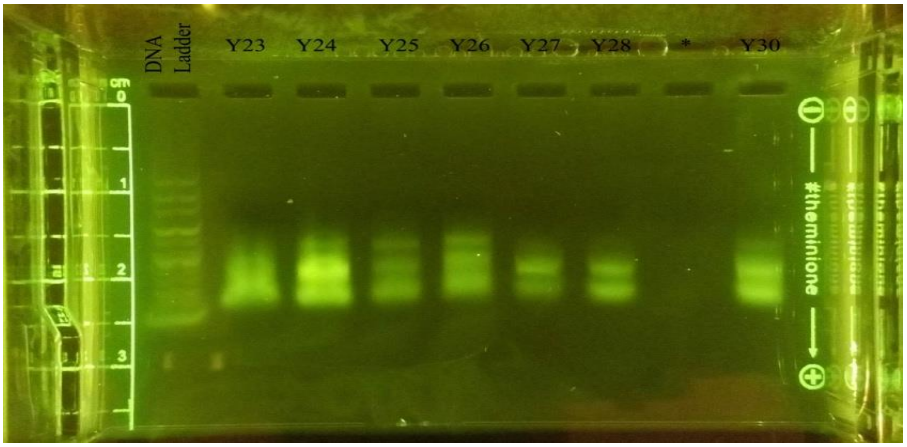

Fig. 2. Electrophoresis product of site 71 of the ghrelin gene in Nigerian indigenous chickens obtained by digesting with *mboii*

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

variations linked growth related traits [21-24]. The present study was geared towards characterizing a major gene encoding an essential modulator of feed intake, growth factor release and fat deposition in chicken [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 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 animal. Thus, our study reveals that alleles C and T are essential for the conservation of Nigerian ecotype chicken. 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 corresponding 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 generation and environmental climes. The differences 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 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 can be derived from these metrics [28]. When $Exp_{het} > Obs_{het,}$ inbreeding is at work in the population with FIS >0 [27]. 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 numbers of alleles for the ecotypes is 2, however, Ne and I 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. Fijabi et al. [14] found similar results in Nigerian turkey 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 in Nigerian indigenous Chickens due to the polymorphic nature of the ghrelin gene.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Guéye EF. The role of networks in information dissemination to family poultry farmers. Worlds Poultry Science Journal. 2009;65(1):115-124. Available:https://doi.org/10.1017/S0043933909000099
2. Drucker AG. Why value ANGR in economic terms? International Livestock Research Institute, P. O. Box 5689, Addis Ababa, Ethiopia; 2023. Available:https://cgspace.cgiar.org/bitstream/handle/10568/3596/casestudy-Drucker-I.pdf
3. Fayeye TR, Adeshiyan AB, Olugbami AA. Egg traits, hatchability and early growth performance of the Fulani-ecotype chicken. Livestock Research for Rural Development. 2005:17(8). Available:http://www.lrrd.org/lrrd17/8/faye17094.htm
4. Ige AO, Salako AE,Ojedapo LO, Adedeji TA. Biochemical characterization of indigenous Fulaniand Yorubaecotypes chickenof Nigeria. African Journal of Biotechnology. 2013:12(50):7002-7008. Available: https://10.5897/AJB2013.12816
5. Ajayi FO, Agaviezor BO, Wihioka SN. Haemoglobin genotypes in the Nigerian indigenous chicken in the Niger Delta region of Nigeria. International Journal of

Advance Biological Research. 2013;3(1):ISSN2250–3579.

6. Momoh OM, Nwosu CC, Adeyinka IA. Egg production of two Nigerian local chicken ecotypes under improved management. ASSET: An International Journal of Agricultural Sciences, Science, Environment and Technology (Series A). 2008:8(2):151-158.
7. Adetayo AS, Babafunso SE. Comparison of the performance of Nigerian indigenous chickens from three agro-ecological zones. Livestock Research for Rural Development. 2001;13(2). Available:http://www.lrrd.org/lrrd13/2/aded132.htm
8. Sola-Ojo FE, Ayorinde KL. Evaluation of Reproductive Performance and Egg quality Traits in Progenies of Dominant Black Strain Crossed with Fulani Ecotype Chicken. Journal of Agricultural Science. 2011;3(1):258-265. Available:https://doi.org/10.5539/jas.v3n1p258
9. Osaiyuwu OH, Salako AE, Adurogbangban O. Body dimensions of Fulani and Yoruba ecotype chickens under intensive systems of management. Journal of Agriculture, Forestry and the Social Sciences. 2009;7(2):195-201. Available:https://doi.org/10.4314/joafss.v7i2.64358
10. Lasagna E, Ceccobelli S, Cardinali I, Perini F, Bhadra U, Thangaraj K et al. Mitochondrial diversity of Yoruba and Fulani chickens: A biodiversity reservoir in Nigeria. Poultry Science, 2020;99(6):2852-2860. Available:https://doi.org/10.1016/j.psj.2019.12.066
11. Moffitt T E, Caspi A, Rutter M. Strategy for Investigating Interactions between Measured Genes and Measured Environments. Archives of General Psychiatry. 2005;62(5):473-81. Available:https://doi.org/10.1001/archpsyc.62.5.473
12. Shoyombo A, Arije D, Ayeni O, Abdulmojeed Y, Adebambo O, Popoola M et al Myostatin gene sequence in helmeted guinea fowl breeds; 2022. Available:https://ssrn.com/abstract=4027429 or https://doi.org/10.2139/ssrn.4027429
13. Fijabi OE, Osaiyuwu OH, Akinyemi MO, Shobanke IA, Oyewola KA, Nwokorie GI et al. Myostatin gene polymorphism and its association with body weight in Nigerian indigenous Turkey. Thai Journal of Agricultural Science. 2020;53(2):58−66.
14. Pradhan G, Samson SL, and Sun Y. Ghrelin: Much more than a hunger hormone. Current Opinion in Clinical Nutrition and Metabolic Care. 2013;16(6):619–624. Available:https://doi.org/10.1097/MCO.0b013e328365b9be.
15. Hayashida T, Murakami K, Mogi K, Nishihara M, Nakazato M, Mondal MS et al. Ghrelin in domestic animals: Distribution in stomach and its possible role. Domestic Animal Endocrinology 2001;21(1):17–24. Available: https://doi.org/10.1016/S0739-7240(01)00104-7
16. Nie Q, Zeng H, LeiM, Ishag NA, Fang M, Sun B et al. Genomic Organisation of the chicken ghrelin gene and its single nucleotide polymorphisms detected by denaturing high-performance liquid chromatography. British Poultry Science. 2004;45(5):611–618. Available:https://doi.org/10.1080/00071660400006263
17. Kaiya H, Van Der Geyten S, Kojima M, Hosoda H, Kitajima Y, Matsumoto M et al. Chicken ghrelin: Purification, cDNA cloning, and biological activity. Endocrinology. 2002;143(9):3454–3463. Available: https://doi.org/10.1210/en.2002-220255
18. Hall, S. Conservation of livestock breeds. In: Proceedings of the research planning workshop held at ILCA Addis Ababa, Ethopia. 19-21 February 1992: 11-16. Available:https://www.researchgate.net/publication/282852043
19. Falconer DS. Introduction to Quantitative Genetics 4th Ed. University of Edinburgh, London: Robert MacLehose and Company Limited, Glasgow; 1960.
20. Yeh F, Rong-Cai Y, Boyle T, Ye Z, Mao J. POPGENE, version 1.32: The user-friendly software for population genetic analysis. Corpus ID: 64471650; 1999.
21. Bahrami A, Behzadi S, Miraei-Ashtiani SR, Roh SG, Katoh K. Genetic polymorphisms and protein structures in growth hormone, growth hormone receptor, ghrelin, insulin-like growth factor 1 and leptin in Mehraban sheep. Gene. 2013;527(1):397–404. Available:https://doi.org/10.1016/j.gene.2013.05.066
22. Jin S, Chen S, Li H, Lu Y, Xu G, Yang N. Associations of polymorphisms in GHRL,

GHSR, and IGF1R genes with feed efficiency in chickens. Molecular Biology Reports. 2014;41(6):3973–3979.
Available: https://doi.org/10.1007/s11033-014-3265-8

23. Ohagenyi IJ, Ndofor-Foleng HM, Ugwu SOC, Okwelum N. Polymorphism of ghrelin genes among four Nigerian chicken populations as tool for improvement of chickens. Indian Journal of Animal Sciences. 2022;92(3):323–326.
Available:https://doi.org/10.56093/ijans.v92i3.122264
24. Li CC, Li K, Li J, Mo DL, Xu RZF, Chen GH et al. Polymorphism of ghrelin gene in twelve Chinese indigenous chicken breeds and its relationship with chicken growth traits. Asian-Australasian Journal of Animal Science. 2006;19(2):153–159.
Available:https://doi.org/10.5713/ajas.2006.153
25. Steinle NI, Pollin TI, O'Connell JR, Mitchell BD, Shuldiner AR. Variants in the ghrelin gene are associated with metabolic syndrome in the Old Order Amish. Journal of Clinical Endocrinology and Metabolism. 2005;90(12):6672–6677.
Available: https://doi.org/10.1210/jc.2005-0549
26. Tyra M, Ropka-Molik K, Piórkowska K, Oczkowicz M, Szyndler-Nędza M, Malopolska M. Association of Ghrelin Gene Polymorphisms with Fattening Traits and Feed Intake in Pig: A Preliminary Study. Animals. 2019;9(7):410.
Available:https://doi.org/10.3390/ani9070410
27. Schmidt TL, Jasper M, Weeks AR, Hoffmann AA. Unbiased population heterozygosity estimates from genome-wide sequence data. Methods in Ecology and Evolution. 2021;12(10):1888-1898.
Available: https://doi.org/10.1111/2041-210X.13659
28. Ritland K. Estimators for pairwise relatedness and individual inbreeding coefficients. Genetics Research. 1996;67(2):175–185.
Available:https://doi.org/10.1017/S0016672300033620
29. Sherwin WB, Chao A, Jost L, Smouse PE. Information Theory Broadens the Spectrum of Molecular Ecology and Evolution. Trends in Ecology and Evolution. 2017;32(12):948–963.
Available:https://doi.org/10.1016/j.tree.2017.09.012

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/114177