



Polymorphism of ghrelin genes among four Nigerian chicken populations as tool for improvement of chickens

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ABSTRACT

Research on polymorphism of ghrelin (GHRL), an acylated peptide that stimulates the release of growth hormone from the pituitary, has shown that it is associated with some essential growth genes in chicken, hence veritable in genomic selection. There is porous information on the polymorphism of GHRL genes among Nigerian chickens. The objective of this study was to determine polymorphism of ghrelin gene among four Nigerian chicken populations. Blood sample (0.5 ml) was collected from the wing vein of 102 birds for DNA extraction. Tested PCR products were sequenced following Macrogen INC. The SNPs were determined using the sequence alignment program, CLUSTAL W, implemented in MEGA software. The sequence results showed 25 SNPs. The Nigerian chickens varied in polymorphic sites from Ogun chickens (348 and 535) to Nsukka chickens (558 and 696) at the GHRL 1 and GHRL2 loci respectively. Polymorphic sites and diversity were higher among the Nsukka chicken than other populations of the Nigerian chickens. SNPs common to one geographic varied in another. The result revealed that genomic selection based on ghrelin SNPs may yield higher predictive accuracy, while Nsukka chickens could be useful for the creation of more superior lines for the global poultry industry. Since past studies have revealed that ghrelin gene stimulates the pituitary and hypothalamus for releasing growth factor (GF) hormone and have strong effect on many organs, we suggest an association study of ghrelin gene and growth traits.

Keywords: Genetic progress, Ghrelin, Nigerian chicken, SNPs

Ghrelin, a quantitative trait loci (QTL), is a natural peptide for growth hormone secretagogue receptor (GHS-R) identified in chicken (Nie *et al.* 2005), but was discovered in human and rat stomachs two decades ago. Ghrelin has a role in the central regulation of feeding. The chicken ghrelin has 24 amino acids (GSSFLSPTYKNIQQQKDRKP TARLH) and has 54% sequence identity with human ghrelin (Kaiya *et al.* 2003). The chicken ghrelin like the rodent ghrelin comprises of 5 exons, 4 introns and 2706 base pairs (bp), which differs from human and mouse (5199 and 4513 bp) counterparts respectively (Nie *et al.* 2005). Ghrelin peptide is associated with obesity, diabetes, hypertension and liver diseases (Agnieszka and Aldona 2018). A mutation in exon 5 (A2355Q) of chicken ghrelin changed glutamine to arginine (Gln113Arg) (Nei *et al.* 2005).

SNPs that occur in one geographical or ethnic group may be scarce in another (Varela 2010). SNPs selection has been implicated for fitter and fast-growing breeds of chicken (Qifan *et al.* 2017). One SNP (T229G) was found

in exon 2 of the Ocx-32 gene of Abuja chicken (Ohagenyi *et al.* 2021).

The Nigerian Heavy Local Chicken Ecotype (NHLCE) weighs slightly lower than the commercial layers chicken (Ohagenyi *et al.* 2021). The Nigerian local chickens are superior to the purebred chicken in heat tolerance, disease resistance and age at first egg (Huang *et al.* 2018). They have been evaluated for variance components, heritability, repeatability, genetic correlations of growth, colour and reproductive traits (Ohagenyi *et al.* 2021) and fairly selected (Agbo *et al.* 2018).

FAO (2021) has reiterated that sustainable agriculture and natural resources management in Nigeria (the 7th most populous country in the world) will provide food security for the predicted world population (7.5 billion) by 2050 and Africa, where every third person is malnourished and 257 million hungry. To meet this future challenge, Nigeria needs to emulate the US poultry which yielded \$35.5 billion in 2020 (USDA 2021). The objective of this study is to determine the polymorphism of ghrelin gene among four Nigerian chicken populations as tool for improving the local chickens.

MATERIALS AND METHODS

The experimental procedures complied with the

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provisions of the University of Nigeria, Nsukka Ethical Committee on the Use of Animals for Biometric Research (2019). Total 102 Nigerian indigenous chickens (25 Abuja chickens, 27 Ogun chickens, 25 Nsukka chickens and 25 Heavy ecotype) were sampled from four Nigerian indigenous chicken populations. Three chicken populations were collected from the local chicken markets in 3 zones of Nigeria namely, Abuja chickens (ABJ), Ogun chickens (OG) and Nsukka chickens (NSK) and the fourth population, the Heavy ecotype (HE), was obtained from University of Nigeria, Nsukka. The HE has been fairly bred through a controlled selection for six generations at the University of Nigeria, Nsukka Animal Science Experimental Farm.

Individuals from 4 chicken populations (ABJ, NSK, OG and HE) were used for SNPs studies. Blood sample (0.5 ml) was collected from the wing vein of each bird in EDTA bottles and genomic DNA was extracted from EDTA-anti coagulant blood.

Primer design: Based on chicken ghrelin cDNA, two pairs of primers with reference sequence Genbank Accession number, AY303688 were designed and synthesized to amplify the fragment (751 and 703 bp) of chicken ghrelin gene. The primer sequence were as follows:

GHRL1 Forward 5'-CATTTCTAAGCTTTTGCC-AGTT-3', Reverse 5'-CACTGTTATTGTCATCTTCTC-3'

GHRL2 Forward 5'-ATAAAGTGAATGCAAGAATAGT-3', Reverse 5'-TGTGTGGTGGGAGTTACTAC-3'.

The primers were synthesized by Integrated DNA Technologies (USA) and PCR reagents synthesized by Norgen Biotek Corporation (Canada). Sample (10 µl) was used for PCR reaction.

PCR reaction and sequencing of PCR products: PCR tubes each containing 10 µl of sample containing 1 µl DNA (template), 0.1 µl of each primer, 3 µl water and 5 µl of 2× master mix (Taq DNA polymerase, DNTPs mixture, magnesium chloride, potassium chloride and PCR enhancer/stabilizer) was placed in a ABI 2700 (Applied Biosystems, Foster city, A) thermal cycler and ran under PCR temperature conditions of 94°C initial denaturation for 2 min, followed by 94°C denaturation for 30 sec, 40 cycles of 58°C annealing temperature for 30 sec, 72°C extension for 2 min and final extension at 72°C for 5 min.

DNA sequencing: DNA sequence was generated using 30 µl of each PCR product (MacroGen DNA sequence protocol 2014). The MegAlign program of Lasergene Version 6.0 (DNASTar) software was used for multiple sequence alignment. The SNPs were determined using the sequence alignment program, CLUSTAL W, implemented in MEGA software (Kumar *et al.* 2018).

RESULTS AND DISCUSSION

PCR and SNPs at locus of ghrelin gene: The sequences of the amplified DNA bands of the Nigerian chicken populations (Table 1) showed that there were 25 SNPs present at the ghrelin locus of the chickens of Nigeria, however these were entirely different from the SNPs

Table 1. SNPs at ghrelin gene locus of 4 populations of Nigerian chickens

No	SNP position	Nucleotide	Codon	Gene locus	Populations found
1	38	G→C	None	GHRL 1	Nsukka
2	51	T→G	None	GHRL 2	Nsukka
3	82	T→C	None	GHRL 2	Nsukka
4	98	A→G	None	GHRL 1	Nsukka
5	150	G→C	None	GHRL 1	Nsukka
6	259	A→C	None	GHRL 1	Nsukka
7	265	A→T	None	GHRL 2	Abuja
8	381	G→A	None	GHRL 2	Abuja
9	383	C→T	None	GHRL 1	Abuja
10	415	C→G	None	GHRL 2	Ogun
11	511	T→C	None	GHRL 1	Nsukka
12	515	A→T	None	GHRL 1	HE
13	629	C→T	None	GHRL 2	Nsukka
14	646	G→T	None	GHRL 1	Nsukka
15	682	T→C	None	GHRL 1	HE
16	745	A→G	None	GHRL 2	Nsukka
17	752	A→C	None	GHRL 1	Abuja
18	780	A→C	None	GHRL 2	Nsukka
19	799	G→C	None	GHRL 1	Abuja
20	905	T→A	None	GHRL 2	Ogun
21	958	C→A	None	GHRL 2	Nsukka
22	970	C→T	None	GHRL 2	Abuja
23	1024	C→T	None	GHRL 2	Abuja
24	1036	A→G	None	GHRL 2	HE
25	1075	A→C	None	GHRL 2	HE

HE, Heavy ecotype

reported earlier for Tswana and Chinese chickens (Nei *et al.* 2005, Kgwatalala *et al.* 2012). All the SNPs were found in the intron.

This study revealed many novel SNPs at the GHRL loci of the Nigerian chicken populations. New variants found in the study conform to earlier report that there are about 15 million SNPs in chickens (Huang *et al.* 2018). These 25 SNPs at the ghrelin locus might be unique (Kgwatalala *et al.* 2012) to Nigerian indigenous chickens. Several SNPs were anticipated at the ghrelin locus of the Nigerian chicken, since there are about 15 million SNPs in chickens (Wong *et al.* 2004). These mutations could be related to chick development, since mutations in human ghrelin gene are related to child development, while some mutations in non-coding regions could play important roles in chicken performance. Similarly, SNPs found at the ghrelin locus shows a higher prevalence of nucleotide change of type A→G, C or T compared to earlier reports (Nei *et al.* 2005, Kgwatalala *et al.* 2012) on Tswana and Chinese chickens, which differed (C→T) nucleotide change.

Nucleotide and haplotype diversity: Haplotype and nucleotide diversity of four Nigerian chicken populations (Abuja, Nsukka, Heavy ecotype and Ogun) is given in Table 2.

At the ghrelin locus, high nucleotide diversity was recorded at cGHRL1 and cGHRL2 loci of four populations (Nsukka, Ogun, Abuja and Heavy ecotype) of indigenous

Table 2. GHRL diversity of four populations of Nigerian chickens

Population	GHRL 1 haplo-type diversity	Nucleotide diversity	Sequence conservation	GHRL 2 haplo-type diversity	Nucleo-tide diversity	Sequence conservation
Nsukka	1	0.6298	0.034	1	0.5685	0.075
Ogun	1	0.4081	0.435	1	0.4941	0.366
Abuja	1	0.5285	0.472	1	0.5343	0.466
Heavy ecotype	1	0.5600	0.440	1	0.5381	0.462

chickens of Nigeria. Nucleotide diversity ranged from 0.4081 in Ogun chickens to 0.6298 in Nsukka, and 0.4941 in Ogun chickens to 0.5685 in Nsukka at cGHRL1 and cGHRL2 loci respectively. The study showed that conserved sequence ranged from 0.034 in Nsukka chickens to 0.472 in Abuja chickens, and 0.075 in Nsukka chickens to 0.466 in Abuja chickens at cGHRL1 and cGHRL2 loci respectively. Diversity of nucleotides observed in the current study attest to the fact that indigenous chickens of Nigeria have not been artificially selected in traits of economic importance and hence exhibit greater variability in performance (phenotype) and the underlying genotypes. The study further showed that Nsukka chickens have more variants than other populations, however Abuja chickens have more conserved nucleotide sequence than the Nsukka, Ogun and HE chickens at GHRL1 and GHRL2 loci. The presence of nucleotide diversity at the ghrelin locus revealed that SNPs common to one geographic or ethnic group may vary in another (Varela 2010). Varying SNPs at different geographical zones reveal the operation of natural selection, which favour the best fit mutant. SNPS frequently occur where selection is fixating genes with most favorable genetic adaptation (Qifan *et al.* 2017), hence some mutations could result to stronger and fitter strains of chicken.

Polymorphic sites and singleton variable sites: Polymorphic and singleton variable sites of four Nigerian indigenous chicken populations is given in Table 3.

The Nigerian chickens varied in polymorphic sites from Ogun chickens (348 and 535) to Nsukka chickens (558 and 696) at the GHRL 1 and GHRL2 loci respectively. Among the Nigerian chickens, the singleton variable sites ranged from 226 and 304 in the Nsukka chickens to 418 and 538 in the Abuja chickens at the GHRL1 and GHRL2 loci respectively.

Variations in the nucleotide diversity, haplotypes

Table 3. Polymorphic sites of chicken ghrelin in four populations of Nigerian indigenous chickens

Population	GHRL 1		GHRL 2	
	Polymorphic site	Singleton variable site	Polymorphic site	Singleton variable site
Nsukka	558	226	696	304
Ogun	348	348	535	535
Abuja	418	418	538	538
Heavy ecotype	390	390	537	537

diversity and sequence conservation are attributes of a random bred population. The four Nigerian chicken populations are large random bred populations, dominated by natural selection in which agencies that tend to change genetic properties operates mildly. Consequently, there are SNP variations at ghrelin loci of Nigerian chicken populations (Khobondo *et al.* 2014). This is because the magnitude of natural selection found in local population where selection, migration and mutation do not exist is always in small quantity from one generation to the next. However, some studies (Kumar *et al.* 2020) reported that artificial selection, which erodes variation and is a stronger systematic process is a tool used by breeders to change gene frequency and genotypic frequency of any population.

Ghrelin SNPs as useful tool for genetic improvement of chickens: Information on SNPs at ghrelin gene locus could be very useful for genetic improvement of several organs affecting growth, reproductive and fitness traits of chickens principally because of their frequent occurrence throughout the genome (Huang *et al.* 2004). One mutation in the exon 5 of chicken ghrelin led to the change of amino acid, which resulted in a different ghrelin precursor instead of mature peptide. Consequently, some SNPs could directly or indirectly affect growth and reproductive traits needed to enhance genetic improvement of chickens, since traits are directly or indirectly controlled by ghrelin stimulant growth hormone (Paredes-Sánchez *et al.* 2015). Past studies have revealed that ghrelin gene stimulates the pituitary and hypothalamus for releasing growth factor hormone (GF) and have strong effect on function and metabolism of stomach, gut and heart (Agnieszka and Aldona 2018). This indicates that numerous traits comprising growth, reproductive and fitness could be associated with ghrelin gene and perhaps its mutations. Furthermore, the role of ghrelin in the central regulation of feeding makes ghrelin highly essential as candidate's genes for traits affected by genotype-environment correlation as well as genotype-environment interaction (Kumar *et al.* 2020). In genotype-environment correlation, breeders are faced with the option of feeding breeder birds or cattle according to their egg yields or milk yield. However, when genotype-environment interaction exists, a superior genotype in environment A may be inferior in environment B.

In conclusion, high genetic variation was found among four Nigerian chicken populations. Most SNPS in Nigerian chicken ghrelin could be used in quantitative trait loci mapping for growths traits in chickens. Again, breeding

decision using information on ghrelin gene SNPs of the Nsukka chicken population could serve as better foundation flock for development of many lines of chicken for both the rural and the global poultry industry. Further study to detect more ghrelin SNPs and probably their association with growth, reproductive and fitness trait for more predictive accuracy is pertinent.

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