International Journal of Science and Technology Research Archive

ISSN: 0799-6632 (Online)

Journal homepage: https://sciresjournals.com/ijstra/

(RESEARCH ARTICLE)

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Identification of SNPs and candidate genes associated with growth traits of Nigerian improved Indigenous chicken genotypes

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International Journal of Science and Technology Research Archive, 2026, 10(01), 086-100

Publication history: Received on 09 January 2026; revised on 25 February 2026; accepted on 28 February 2026

Article DOI: https://doi.org/10.53771/ijstra.2026.10.1.0010

Abstract

For decades, genetic improvement based on measuring growth traits has been successfully applied in the production of meat-type chickens. A genome-wide association study using the chicken 60k SNP panel in 42 Nigerian indigenous chickens (NICs) derived from the crosses between Marshall and pure Nigerian chickens of 500 Chickens' population at 8 weeks of age in T&R farm of FUNAAB, Nigeria to identify Single Nucleotide Polymorphisms on the chromosomes and genes associated with growth trait was carried out. BW was taken for 8 weeks. Blood samples were collected from 42 NICs from purebred and F_2 upgrades to extract DNA, and PCR- RFLP technique was used for DNA amplification in which the Amplicons were sequenced. Molecular analysis using Molecular software packages and bioinformatics approach were used for the sequence analysis.The raw phenotypic data which comprises of the sex and body weight (>2 kg as 1 (case)) and <1 kg as 0 (control)) was analyzed using GenAbel of the R package software to carry out quality checks using some filtering techniques in NICs and their crosses. Selective sweep analysis was applied and ten SNPs were Identified on chromosomes with three candidate genes associated with body weight- (Peroxisome Proliferator-Activated Receptors (PPAR-201) gene, Breakpoint Cluster Region 201 (BCR-201) gene and Glycine Aminodino Transferase Mitochondrial (GATM) gene on Chromosomes 1, 15 and 10, respectively, were associated with body weight of NICs F_2 resource population and (Glycine Aminodino Transferase Mitochondrial (GATM) gene) was a novel gene discovered. These findings made it easier to understand the traits of the good germplasm and their potentials for using the NICs.

Keywords: Body Weight; Candidate Gene; Genome-Wide Association Study; Nigerian Indigenous Chickens; Single Nucleotide Polymorphism.

1. Introduction

Indigenous chicken breeds are developed under the influences of harsh environmental condition with little or no feed supplement given and are artificially selected, and display abundant phenotypic diversities, such as heterogeneous plumage colours (feathers), spurs, comb shape and size which are unique resources for genetic improvements in chickens. They have unique phenotypic traits that distinguish them in terms of morphology, behaviour, plumage colour, and productive abilities. These birds, now considered as indigenous, show greater resistance to various local poultry diseases and parasites compared to exotic and commercially improved chickens. Due to their superior adaptability to local tropical environmental conditions as well as their foraging ability and broodiness, these indigenous birds are often preferred by smallholder farmers for backyard rearing [1; 2].

However, the characteristics of genetic variations in these breeds are still unclear. Theoretically, a thorough understanding of the genetic diversity of the indigenous breeds might support conservation and utilization through suitable breeding programs in the future. In many domestic animals, whole genome re-sequencing technology has already been used to examine the genetic composition of populations [3; 4; 5; 6]. Since the release of the chicken genome

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assembly, many researchers have begun to study economic traits for chickens by combining large-scale genomic and phenotypic information [7]. According to Park *et al*. [8] which used an advanced intercross line (AIL) population from two chicken lineages to demonstrate the presence of quantitative trait loci (QTL) on multiple chromosomes, brought a knowledge into the polygenicity of chicken growth traits. Thereafter, Johansson et al. [9] estimated that the genetic variation of chicken growth traits was the result of more than 100 genome-wide loci. Further researches revealed that genome-wide loci under selection accounted for at least 40% of the phenotypic variation in body weight at 56 days of age in an intercross chicken line, suggesting that bidirectional selection for growth traits acts on many genetic loci, rather than on only a few loci [10; 11]

The use of GWAS in chickens has been to ascertain major loci related to economic traits [12; 13; 14]. Since growth trait of chickens being one of the economic traits are well known for their genetic complexity genome-wide study will through more light to identify underlying genetic mechanisms related to growth traits using large base population. Therefore, in this study, a GWAS was performed to identify potential genomic SNP markers and candidate genes associated with the BW trait in a population of NICs to establish important biological pathways affecting BW of NICs.

2. Materials and Methods

2.1. Experimental site

The research was carried out at the Poultry Breeding Unit of the University Teaching and Research Farm of Federal University of Agriculture, Abeokuta, Alabata Road, Abeokuta. Alabata is at latitude 7°10`N and longitude 3°2`E in Odeda Local Government Area of Ogun State, Nigeria. The area is characterized with a tropical climate with a mean annual rainfall of about 1037mm. The mean monthly ambient temperature ranged from 28- 36°C and 34°C on average. Relative humidity ranged from 60-94%, 82% on the average. The vegetation represents an inter-phase between the tropical rainforest and the derived savannah [15].

2.2. Experimental Management

2.2.1. Parents

Pure indigenous chicken genotypes reared in the breeding unit of the University's Research Farm were used to generate the offspring for this research. The parent stock were basically Normal-feathered (N) of two cocks and ten hens, Frizzled-Feathered (Fz) of one Cock and seven hens and naked neck chicken (NK) of two 2 Cocks and ten hens and Marshall (M) of three Cocks and hens and ten dihybrid cross of ten hens which were cross through artificial insemination method. The combination of these four genotypes at different bloodlines resulted in ten genotypes used in this study: 100% pure breeds, 50:50% M: Ind (di-hybrid) 75:25% M: Ind (F_2 upgrade) fifty chickens per genotype (sample size) making five hundred chickens altogether. The genotypes are presented below:

2.2.2. Mating design

1. M X M = M
2. N X N = N
3. Fz X Fz = Fz
4. Nk X Nk = Nk

50%:50% (M: Ind) bloodline (F_1crosses)

5. M X N = MN (F_1)
6. M X Fz = MFz (F_1)
7. M X Nk =MNk (F_1)

75%:25% (M: Ind) bloodline (F_2 upgrades)

8. M X MN =MMN (F_2)

9. M X MF =MMFz (F_2)

10. M X MNk =MMNk (F_2)

M- Marshall Exotic breed, N-Normal-Feathered, Fz-Frizzle-Feathered,

Nk-Naked Neck chicken genotype, M: Ind = Marshall: indigenous.

2.2.3. Inseminations, egg collection and hatching:

Artificial insemination was carried out on the chickens three times a week. It was done by introducing semen collected from Exotic Marshall cocks (sires) into the reproductive tracts of both exotic and indigenous hens (dams), also, indigenous cock to Indigenous hens following the above mating design to produce the 10 different genotypes of birds studied in this research. The parent stock was housed in the battery cage system singly and fed *ad libitum* with constant water supplied. The pure Marshall genotype served as control. Then the same exotic cocks (Marshal) were crossed to all the genotypes to obtain F_1 birds with 50%: 50% M: Ind bloodline (dihybrid crosses) and F_2 birds with 75%:25% M: Ind bloodline (F_2 upgrades).

2.2.4. Egg collection, incubation and hatching:

Eggs from each mating group were collected daily and labelled according to sire-line. The eggs were then transferred to the University's Teaching and Research farm hatchery unit for incubation and hatching. Assortment of eggs for incubation was done by selecting cracked, discolored or odd- shaped eggs before setting. The eggs were fumigated so as to destroy microbes using fumes from mixture of 35g potassium permanganate in 53cc formalin per cubic meters. Circular air movement in the cabinet and speedy gas removal after 20 minutes fumigation was ascertained. Three day egg collections were set along sire-lines at a temperature of 38-39°C and humidity of 55-66% for the first eighteen days. On the 18th day of incubation, candling was conducted and number of fertile eggs were recorded. The eggs were transferred into the hatcher; the temperature was set to 39-40°C and humidity to 70-75%. The eggs were turned automatically hourly through 90°C in the incubator. On the 21st day chicks were collected and the number of hatched chicks was recorded.

2.3. Data collection

2.3.1. Growth

The chicks body weights (BW) were weighed weekly from day old to 8 weeks of age with the use of a weighing scale with 0.1g limit of sensitivity in the morning before feeding. Other linear body measurements were taken from day-old to 8 weeks using tape rule.

2.3.2. Statistical analyses

Analysis of variance for the body parameters was carried out with [16] software using General Linear Model. Means were separated using Duncans Multiple Range Test at probability of 5% level.

Experimental models

Model 1 :

$Y_{ijk} = \mu + G_i + \varepsilon_{ij}$

Y_{ijk} = Observation of the $_i^{th}$ genotype on the $_j^{th}$ bird

μ = Overall mean

G_i = Effect of the $_i^{th}$ chick genotype ($_i$ = 1, 2 …10)

ε_{ij} = Error due to individual observation

2.4. Genomics

2.4.1. Blood collection

A total number of 200 chickens (offspring), 20 birds per a genotype at equal ratio of male and female were randomly selected from the population of 500 Nigerian indigenous chickens at 8 weeks of age to carry out a genome-wide association study with body weight. Blood samples were collected from 200 chicken's wing veins using needle and syringe and were transferred to Whatman FTA® Classic cards by dropping it in a concentric circular motion within the printed circle area and air dried, then sealed in plastic bag containing silica gel beads. A selection based on representation of sex, genotypes and 8th week body weight at the lower and upper quartiles of the groups were used to select the 42 samples used for genomic studies.

2.4.2. DNA extraction, purification and genotyping

Genomic DNA was extracted from the air-dried blood samples preserved on FTA cards from the selected 42 Nigerian indigenous chickens at 8 weeks, following the FTA extraction method protocol and the quality and concentration of genomic DNA was quantified using Nanodrop® Spectrophotometer and was diluted to 50 ng/µl to fulfil the requirements for the Illumina Infinium SNP genotyping platform. Genotyping using the Illumina 60 K Chicken SNP Bead chip assay was carried out at the Illumina-certified service provider, DNA Land Marks Inc., Canada according to the manufacturer's protocol. Quality control, test of population substructure using clutering algorithm and association analyses was performed in the R package v2.15.0 environment [17],. Combinations of function from the R base, locally developed scripts and GenABEL v1.7.2 package [18] ment core Teaf Developor R[19] was performed, using the check-marker function (attributes: maf=0.05, call= 0.95, p.lev=0ibs.mrk ="ALL", ibs, threshold = 0.95). Prunning was performed on the dataset prior to SNP association tests.

2.5. Statistical analysis on genome-wide association (GWAS) data

2.5.1. Association tests

The fast score test for association between a trait and genetic polymorphism were analyzed with GenABEL in R package using function qtscore to perform tests of allelic association of body weight at 8 weeks and genome-wide SNPs determined from the Illumina chip platform.

2.5.2. Test model formula

qtscore (BWT~ sex,data=data, trait.type = "binomial", times = 1, quiet = FALSE, bcast = 10, clambda = TRUE, propPs = 1, details = TRUE)

Where body weight at week 8 (BWT) is a variable dependent on the covariate sex assuming the trait data set is binomial in nature.

The model BWT~sex (i.e.phenotypic data) used a general linear model (GLM) and later residuals when score test was computed for each of the SNPs in the analysis. Coefficients of regression were then reported for the body weight.

False discovery rate i.e. error involved (test of individual high heterozygosity) was corrected with P-value at 5% significant level [20](Benjamini and Hochbeg, 1995) and overlapping sliding windows of 20 kb was used in selective sweeps using this formula: $Z^{(x+1)} = Z\ (y^x+1)\ /2)$.

x = the window index.

y = the number of SNPs in window index

Z = the position of SNP y.

The mean allele frequency (f) at the minor allele for SNPs within a window was calculated with this formula:

z $T^pf = (T^pf - \mu f)/\delta f$ and $T^Af = (T^Af - \mu f)/\ \delta f$

T^pf = the allele frequencies of samples exhibiting the trait of interest

T^Af = the allele frequencies of samples not exhibiting the trait of interest

μ = mean

δ = standard deviation of mean allele frequencies across the dataset.

Z = $({}_{Z}T^{p}f - T^{A}f)$ final comparative score, indicating the number of deviations from the mean. An arbitrary threshold for significance was 5% using Bonferroni significance threshold.

The genomic region containing the most significant SNPs of a peak was explored by inspecting a 1Mb window around the location of this SNP using GioMart tool and the Ensemble genes 69 database to interrogate 500kb to each side of the marker using the UMD v 3.1 assemblies for the genomic region containing the most significant SNP.

The chicken QTLdb database was also examined to find out if the significant SNP mapped against any described chicken QTL (Quantitative trait loci).

2.5.3. Genome-wide association analysis

A genome-wide association study using the chicken 60K SNP panel in Nigerian chicken population derived from the cross between Marshall and pure Nigerian chickens. The raw phenotypic data which comprises of the sex, genotype and body weight (>2 kg as 1 (case)) and (<1 kg as 0 (control)) was analyzed using GenAbel of the R package software by applying the commands on the custom scripts to carry out quality check in the association test in Nigerian chickens and their crosses. A total of 42 Nigerian indigenous chickens (samples) at 8 weeks of age were used for the SNPs associated test with the body weight of this chicken population. 53313 markers were used for the SNP effects at 5 % Bonferroni genome-wide significance.

3. Results

3.1. Quality checks using genotypic quality control:

To determine the association of SNPs with body weight of Nigerian chickens and their crosses with Marshall at 8 weeks of age genotype and phenotype were connected by converting phenotypic data to genotypic data and analyzed using GWAS method which involved 5 steps at 5 % to 95 % screening rate.

From the result of the quality check control in which body weight of Nigerian indigenous chickens have been subjected to prove if the Nigerian indigenous chickens had deviated Hardy Weinberg Equillibrum (HWE) principle through the Case/control analyses carried out in 42 Nigerian indigenous chicken samples at 8 weeks Appendix 11.

Filtering was done to exclude all false discovery rates such as too high heterozygosity, minor allele frequency, identity by state. These errors were reduced through check marker quality control approach which tends to have shifted the principles of HWE.

Call rate was the first criteria to screen the chicken samples: At high call rate of 95 %, total number of 53313 markers were subjected to each chicken samples in all the 42 chickens at 8 weeks of age involved in this filtering exercise. No chicken was removed at this call rate in the first step for not satisfying the test requirement and none was also removed for deviating HWE test.

Then the test with low 5 % minor allele frequency criteria was carried out and 5374 markers (10.08 %) were removed for not satisfying the requirement no marker was removed from deviating HWE principle. Then low call rate of 95 % was adopted, 2293 markers (4.20 %) were removed for failing the requirement and no marker was removed due to deviating from HWE principle under this test.

Test for heterozygosity (FDR) was carried out and no marker was removed. Mean heterozygosity (FDR) was 0.26±0.09.

The chickens were checked with the criteria of too high identity by state (ibs) of 95 % using 46602 markers with 42 chickens. None was removed for not passing the tested condition. The mean ibs (identity-by -state) was 0.64±0.03. This was based on 46602 (87.41 %) autosomal markers. At the end of it all the 42 Nigerian indigenous chickens passed through all the tests. The second run with the same procedure was repeated but there was no change in the result from the first run.

The graphs represented both the case (BW>2 kg) and control (BW <1 kg) to show the test of HWE using Chi square formula approach. The graph was plotted as observed (x^2) body weight (phenotypic data) against expected (x^2) (HWE constant values) in Figures 1 and 2.

The graph in Figure 1 compared HWE expected data with the growth data (body weight) > 1 kg for pure Nigerian indigenous chicken at 8 weeks of age obtained from this study. The y-axis was the phenotypic data (body weights) known as observed data (x^2) against HWE constant values on the x-axis known as expected (x^2). From the graph it was derived that there was deviation from the standing principle of Hardy Weinberg Equillibrum as both values went at parallel direction with each other. The black line represented the HWE values while the thick large black line was the phenotypic values and the red line indicated the corrected phenotypic data after errors removal.

However, the lines were a little closer to each other, indicating that a slight deviation among the Nigerian purebred indigenous genotypes exhibiting lighter weights used in the control comparison. This little deviation could be due to migration.

Figure 2 for case (body weight) <2 kg was comparison between the Nigerian F_2 upgrades with heavier body weight at 8 weeks of age and the graph was presented in the same pattern of the control. The phenotypic data (body weight) of Nigerian F_2 upgrades at 8 weeks of age was the observed data (x^2) against expected (x^2) which was the HWE values. From the graph it was derived that there was deviation from the standing principle of Hardy Weinberg Equilibrium as both values went at parallel direction with each other. The black line represented the HWE value while the thick large black line was the phenotypic values and the red line indicated after errors were corrected. Similarly, the lines were wide apart from each other, indicating deviation from HWE and this was experienced in the F_2 upgrades of the Nigerian improved indigenous breeds which was affected by the crossbreeding and selection. This confirmed that crossbreeding and selection could be responsible for the variation (genetic mutation).

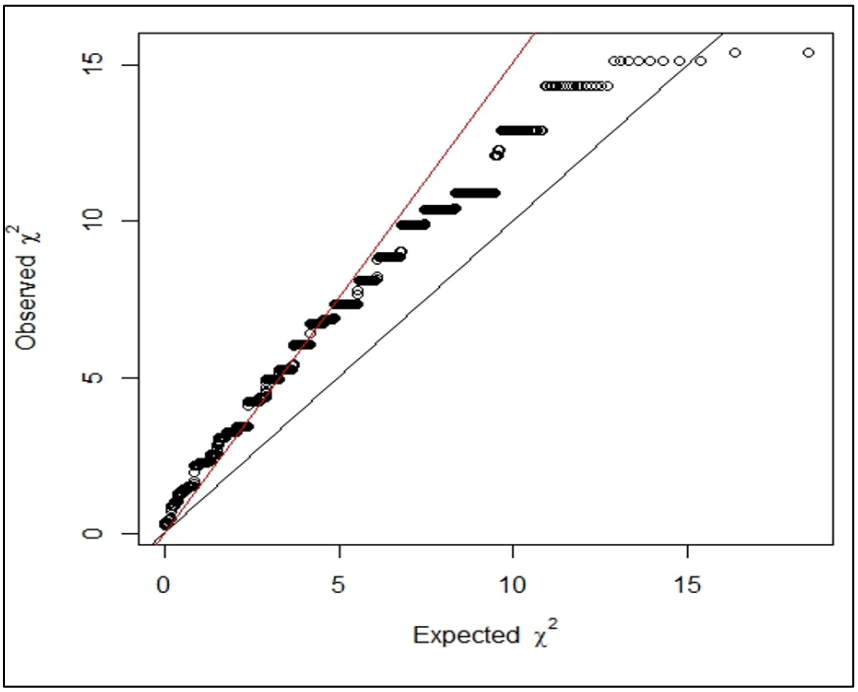

Figure 1 Graph of comparison between Hardy Weinberg Equilibrium principle and body weight of Nigerian indigenous chicken (control i.e >1 kg) at 8 weeks. (Thick black line –the observed body weight data i.e phenotypic data of Nigerian pure indigenous chickens at 8 week; Thin black line – the expected values of Hardy Weinberg Equillibrum; Red line – corrected values)

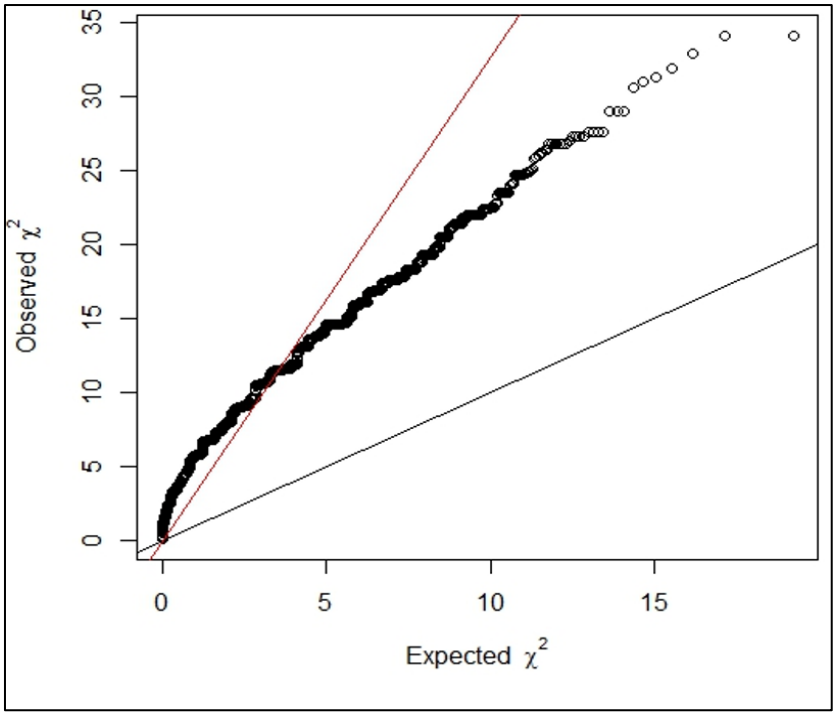

Figure 2 Graph of comparison between Hardy Weinberg Equilibrium principle and body weight of Nigerian F_2 upgrade chicken (case i.e < 2 kg) at 8 weeks. (Thick black line –the observed body weight data i.e phenotypic data of Nigerian F_2 upgrade chickens at 8 week; Thin black line – the expected values of Hardy Weinberg Equillibrum; Red line – corrected values)

3.2. Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses after quality checks.

Table 1 Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses after quality checks

ID	No measured	No poly	Homo	EHomo	Var	F	Callpp	Het
R4	46548	46548	0.89	0.60	0.73	0.99	0.99	0.11
Z4	46378	46378	0.66	0.60	0.53	0.15	0.99	0.34
F29	46492	46492	0.78	0.60	0.75	0.47	0.99	0.21
F14	46430	46430	0.73	0.60	0.72	0.34	0.99	0.26
A31	46525	46525	0.77	0.60	0.72	0.42	0.99	0.23
K19	46496	46496	0.79	0.60	0.76	0.47	0.99	0.21
MK10	46503	46503	0.89	0.60	0.79	0.72	0.99	0.11
C17	46481	46481	0.82	0.60	0.78	0.56	0.99	0.18
A32	46434	46434	0.67	0.60	0.61	0.18	0.99	0.33
A38	46500	46500	0.77	0.60	0.64	0.42	0.99	0.23
A26	46444	46444	0.84	0.60	0.73	0.90	0.99	0.16
A18	46438	46438	0.75	0.60	0.69	0.37	0.99	0.25

ID-identity number, poly- polymorphism, Homo-homozygote, EHomo-expected homozygosity, var- variance, F-inbreeding coefficient, Callpp- call per rate, Het-heterozygote

All the chicken samples involved in this analysis were identified with the markers used for the measurement and computation of number of polymorphism which ranged between 45467 to 46569, the homozygosity ranged between 0.59 and 0.89, expected homozygosity which was 0.60, the variance which occurred ranged between 0.47 and 0.81, the inbreeding coefficient (F) was between 0.02 and 0.99. Generally the inbreeding coefficient was low to high. The low

inbreeding coefficient was associated with the improved Nigerian chickens which are heterozygote while the high inbreeding values were for the pure Nigerian indigenous Chickens because there are more of homozygote since their gene frequencies are not yet altered. Call rate was 0.99 and the allelic heterozygosity ranged between 0.11 and 0.41 as shown on Table 1.

Table 2 Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses after quality checks

ID	Nomeasured	No poly	Homo	E Homo	Var	F	callpp	Het
31	46535	46535	0.87	0.60	0.75	0.67	0.99	0.13
31	46535	46535	0.87	0.60	0.75	0.67	0.99	0.13
L16	46437	46437	0.75	0.60	0.69	0.38	0.99	0.25
O8	46504	46504	0.76	0.60	0.69	0.40	0.99	0.24
A27	46480	46480	0.69	0.60	0.64	0.24	0.99	0.30
L4	46476	46476	0.87	0.60	0.81	0.66	0.99	0.13
L35	46466	46466	0.66	0.60	0.54	0.13	0.99	0.34
A5	46476	46476	0.76	0.60	0.67	0.40	0.99	0.23
C16	46514	46514	0.77	0.60	0.72	0.43	0.99	0.23
O11	46507	46507	0.81	0.60	0.74	0.52	0.99	0.19
F16	46526	46526	0.79	0.60	0.66	0.47	0.99	0.20
H13	46396	46396	0.59	0.60	0.47	0.40	0.99	0.41
C30	46516	46516	0.69	0.60	0.59	0.24	0.99	0.30

ID-identity number, No.Poly- polymorphism, Homo-homozygosity, EHomo-expected homozygosity, var- variance, F-inbreeding coefficient,

Callpp - call per rate, Het-heterozygote.

Table 3 Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses after quality checks

ID	Nomeasurd	No poly	Homo	EHomo	Var	F	Callpp	Het
C29	46486	46486	0.84	0.60	0.72	0.60	0.99	0.16
Z2	46516	46516	0.65	0.60	0.53	0.11	0.99	0.35
L12	46449	46449	0.87	0.60	0.88	0.68	0.99	0.13
R15	46547	46547	0.78	0.60	0.68	0.45	0.99	0.22
R23	46569	46569	0.68	0.60	0.59	0.21	0.99	0.32
R5	46546	46546	0.77	0.60	0.66	0.40	0.99	0.23
E4	46536	46536	0.81	0.60	0.72	0.52	0.99	0.19
F2	45467	45467	0.65	0.60	0.56	0.12	0.99	0.35
E15	46470	46470	0.71	0.60	0.54	0.02	0.99	0.29
O10	46536	46536	0.62	0.60	0.72	0.28	0.99	0.38
E18	46554	46554	0.62	0.60	0.52	0.03	0.99	0.38

ID-identity number,No.poly- Number of polymorphism, Homo-homozygosity, EHomo-expected homozygosity, var- variance, F-inbreeding coefficient Callpp- call per rate, Het-heterozygote.

A graph of Manhattan (Figure 3) was plotted to show the 42 per-id (per identity of each chicken sample) summary of Nigerian chickens samples at 8 weeks of age involved in the association test. However, this graph was plotted P-values

on the y-axis against chromosomes number of the 42 Nigerian chickens sample used on the x-axis in this section of study. The green and blue bubbles indicated the SNPs on the chromosomes. This summarized the general expression of the per-ids of each chicken sample used.

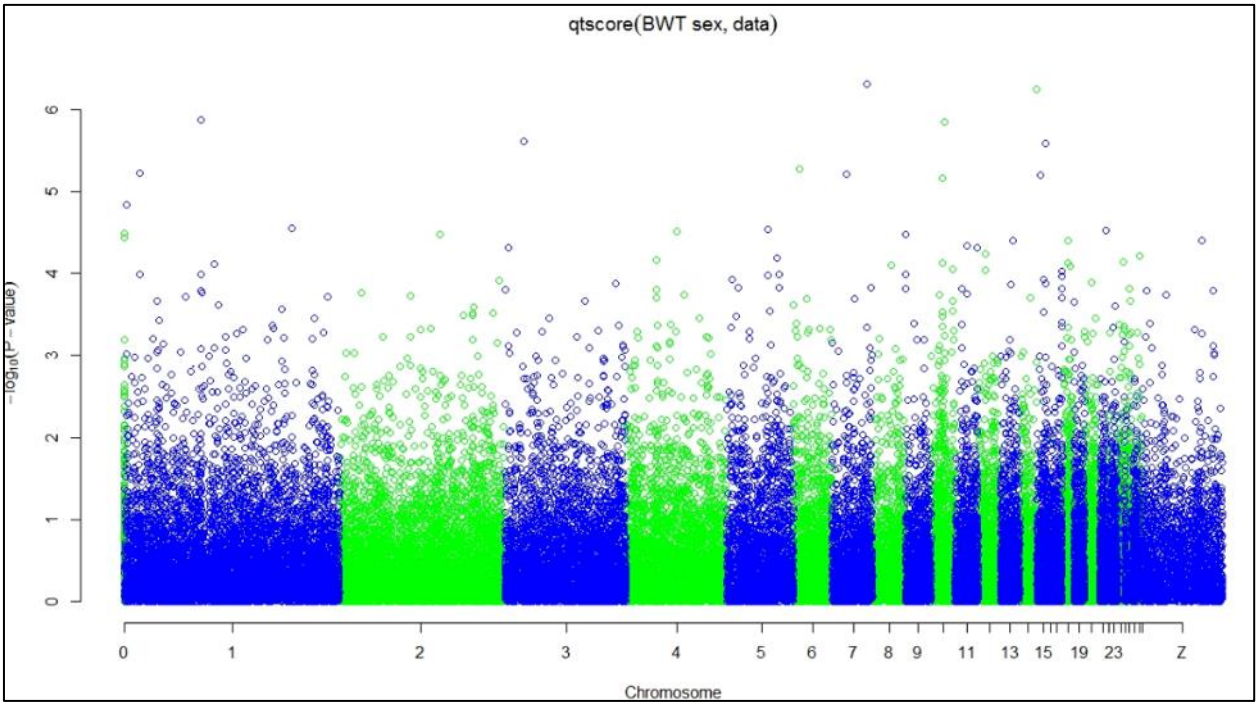

Figure 3 Manhattan plot showing Per-id summary of 42 samples of Nigerian indigenous chicken and their crosses at 8 weeks after quality checks.

3.3. Fast score test association of SNPs of Nigerian indigenous chicken and their crosses on body weights at 8 weeks

A fast score test was conducted with the phenotypic data recorded among the 42 Nigerian chicken samples used for the association test to determine the most significant SNPs associated with body weight trait of Nigerian chickens at 8 weeks of age measured in this study. Its location/position on the chromosome and the gene(s) identified as well as its contribution to the trait. Also, to identify alleles with homozygote or heterozygote and its substitution effects on the body weight trait and the effect of each allele and its interactive effects to this trait. The differences in the means of the effects of alleles (A1 and A2) determined the resultant effect of the individual allele B whether to be selected for improvement in respect to this study. The P-value at 1degree of freedom tested for allelic association while at 2 degree of freedom tested for genotypic association. The principal component (Pc1df) tested for association between SNP and body weight trait and corrected the statistics for possible inflation.

The results in Table 4 indicated that SNP on chromosome 14 was the most significantly associated SNP with body weight of Nigerian broiler chickens at 8 weeks of age, at SNP number GGaluGA105859, located at 15592533 bp downstream direction. The two alleles A_1 and A_2 substituted at T/C, and 41 Nigerian indigenous chickens sample in number were analyzed for. The difference in the means of the two alleles A_1 and A_2 had a negative result of (-11.92±2.23) for the individual allele B indicating that further improvement on this trait will result in poor performances of the chicken since, the individual allele B (A_1) had a negative effect on the body weight. The effect of homozygosity was 28.64 at 1degree of freedom and the p-value of 8.73E-08 but the effect of the heterozygosity was 156 indicating positive contributive effect to the body weight. The multiple combined effect of the heterozygosity was visible though effect of dominant homozygosity could not be analyzed for because of the small sample size. The p-values were 8.73E-08 for 1 and 2 degrees of freedom showing no wide separation in the population. Pc 1df was 5.07E-06, this principal component adjusted for the possible stratification involved in the association test. No candidate gene was identified within this region, might be, there are some genes around the region that interactively affected body weight positively.

3.4. The SNPs and the candidate genes associated with body weight of Nigerian Chickens.

The identified SNP with gene associated with Nigerian chickens body weight at 8 weeks was on chromosome 15 at SNP number GGaluGA109681, located at 8635011bp downstream direction. The two alleles A_1 and A_2 substituted at A/G, and 42 chicken samples in number were analyzed for. The difference in means between the two alleles A_1 and A_2 had a positive result (13.78±2.84) on the A_1 (allele B) indicating that further improvement of this trait would result to a more improved body weight since it has positive effect. The effect of homozygosity was 23.49 at 1 d.f and the p-value of 1.26E-08 but the multiple combined effect of the heterozygosity was 63 indicating positive contributive effect to the body weight at 8 weeks, though the effect of dominant homozygosity could not be visible. The p-value was 2.36E-06 and 3.61E-05 for 1 and 2 d.f showing no wide separation in the population. The gene found at this SNPs region on Chromosome 15 was Breakpoint Cluster Receptor (BCR-201) gene.

The next identified SNP with gene associated with Nigerian chickens body weight at 8 weeks was on chromosome 10 at SNP number Gga_rs14004904, located at 10411414bp downstream direction. The two alleles A1 and A2 substituted at C/T, and 42 chicken samples in number were analyzed for. The difference in means between the two alleles A1 and A2 had a positive result (6.66±1.46) on the A1 (allele B) indicating that further improvement of this trait will result to a more improved body weight since it has positive effect. The effect of homozygosity was 20.29 at 1 degree of freedom and the p-value of 2.75E-05 and 21 for 2 degrees of freedom but the multiple combined effect of the heterozygosity was 8 indicating positive contributive effect to the body weight at 8 weeks, though the effect of dominant homozygosity was invisible due to small sample size. The p-value was 4.79E-06 and 2.75E-05 for 1 and 2 degrees of freedom showing no wide separation in the population. The gene found at this SNPs region was Glycine aminodino transferase mitochondrial (GATM) gene and this was a novel gene discovered.

The third identified SNPs with genes most significantly association with body weight of the Nigerian improved indigenous chicken at 8 weeks of age was on chromosome 1, at SNP number Gga_rs13831454, located at 14027733bp downstream direction. The two alleles A1 and A2 substituted at A/G, and 42 chicken samples in number were analyzed for. The difference in means of the two alleles A1 and A2 had a positive result on the effect of the A1 (allele B) as 6.06±1.33. This showed that allele B had a positive effect on the body weight which supports further improvement of this trait. The effect of homozygosity was 20.72 at 1degree of freedom and the p-value of 5.33E-06. Both the multiple combined effect of the heterozygosity and dominant homozygosity were not visible due to small sample size. The p-values were 2.02E-05 and 0.000105 for 1 and 2 degrees of freedom showing no wide separation in the population.

Although the other most significantly associated SNPs with body weights of the Nigerian improved indigenous chickens at 8 weeks were not mentioned for could be that, there are some genes around which were not found within the protein coding region, but they could the expression effect on body weight by interacting with other genes around it.

Table 4 Fast score test association of SNPs of Nigerian indigenous chicken and their crosses on body weight at 8 weeks.

SNPS no	Chr	Position	Stra	A1	A2	N	EffB	Se effB	1df	P1df	effAB	effBB	2df	p2df	Pc1df
GGaluGA105859	14	15592533	+	T	C	41	-11.92	2.23	28.64	8.73E-08	156	NA	28.64	8.73E-08	5.07E-06
Gga_rs16609686	7	33202174	+	A	G	41	-11. 37	2.68	24.84	6.24E-07	Inf	NA	24.84	6.24E-07	2.15E-05
Gga_rs16729741	3	17838181	+	A	C	40	-12.68	2.58	24.12	9.06E-07	Inf	NA	24.12	9.06E-07	2.83E-05
GGaluGA109681	15	8635011	+	A	G	42	13.78	2.84	23.49	1.26E-06	63	Inf	25.91	2.36E-06	3.61E-05
Gga_rs14005805	10	12252110	+	C	T	41	16.36	3.38	23.36	1.35E-06	52	Inf	25.04	3.66E-06	3.79E-05
Gga_rs1534214	6	6927376	+	G	A	39	-73.46	5.44	22.63	1.96E-06	Inf	Inf	23.31	8.69E-06	5.00E-05
GGaluGA024649	1	71460615	+	G	A	42	9.51	2.02	22.18	2.48E-06	26	Inf	23.15	9.41E-06	5.94E-05
Gga_rs14004904	10	10411414	+	C	T	42	6.66	1.46	20.92	4.79E-06	8	Inf	21	2.75E-05	9.66E-05
Gga_rs13831454	1	14027733	+	A	G	42	6.06	1.33	20.72	5.33E-06	4.6	Inf	21.62	2.02E-05	0.000105
GGaluGA312567	7	13853781	+	A	G	38	9.25	2.04	20.64	5.54E-06	Inf	Inf	23.27	8.87E-06	0.000108

A1 A2-Nuclotide change of Allele 1and 2, position- SNPs position, chrom-chromosome number, stra-strand, effB- effect of the B allele in allelic test, se_effB-Error means of allele B, effAB- effect of heterozygosity in genotypic test, effBB-effect of homozygosity in genotypic test, pc-principal component, P1df: P-value of 1-df-degree of freedom, P2df: P-values of 2-d.f. Pc1df: P-values from the 1-d.f.

The global view P-value of the SNP marker of each trait by GenAbel plot (Fig5) using R-v 2.12(www.project.org) showed that (G*allus gallus*) was identified to have BCR-201 which cut at SNP positional region of 112.34kb on chromosome 15 (GGa4) located at 8.58Mb. This gene significantly associates with body weight of Nigerian improved indigenous chickens at 8 weeks. The protein coding for BCR-201 was shown on the Figure 4.

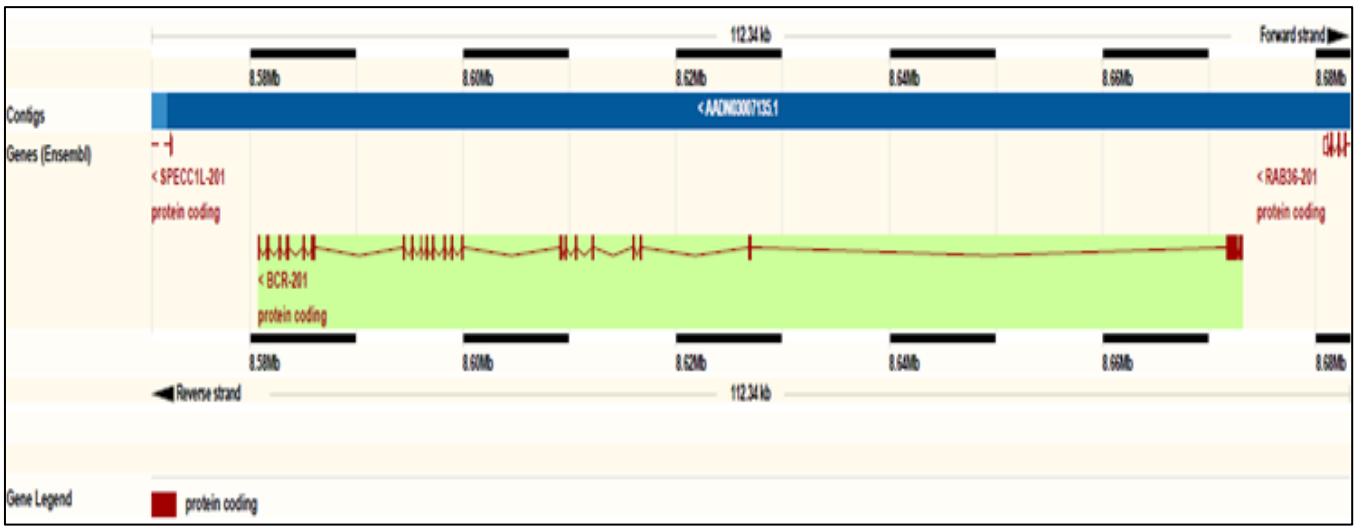

Figure 4 A protein coding region for BCR-201 gene on chromosome 15 of improved Nigerian indigenous chickens

The global view P-value of the SNP marker of each trait by GenAbel plot (fig6) using R-v 2.12(www.project.org) showed that chicken (G*allus gallus*) was identified to have Glycine aminodino transferase, mitochondrial (GATM) gene which cut at SNPs region 33.06Kb on chromosome 10 (GGa4) located at 10.40Mb.This gene associated with body weight of Nigerian improved chicken at 8 weeks. The protein coding for GATM was shown on the Figure 5.

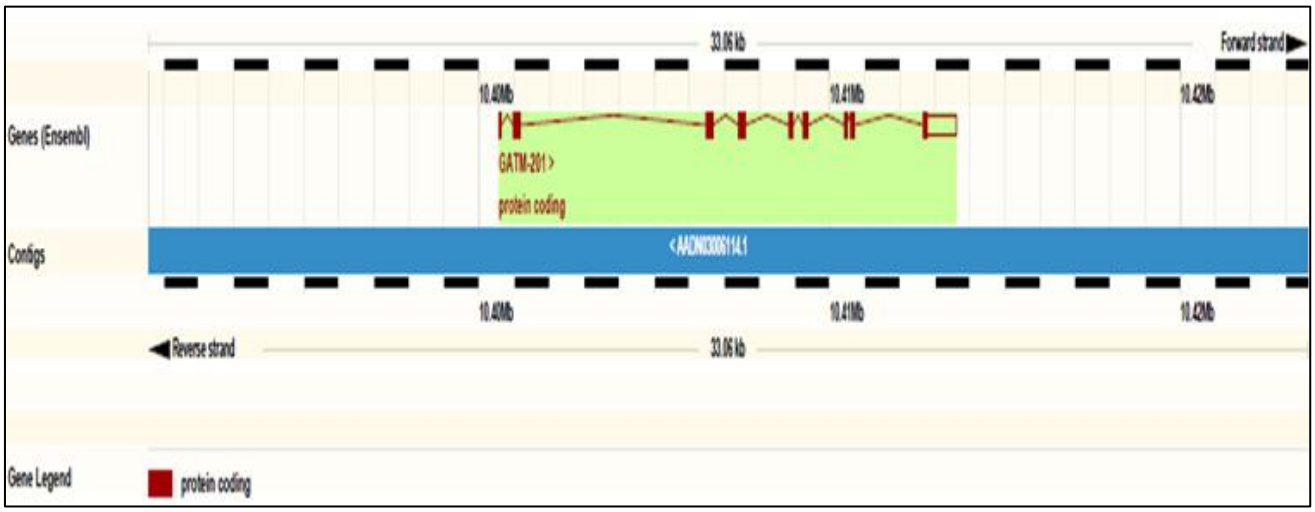

Figure 5 A protein coding region for GATM-201 gene on chromosome10 of improved Nigerian indigenous chicken

The global view P-value of the SNP marker of body weight by GenAbel plot (Fig.7) using R-v 2.15(www.project.org) showed that chicken (*Gallus gallus*) was identified to have proliferator- activated receptors (PPAR-201) gene which cut at SNPs (GGaluGA024649) positional region 51.33Kb at location 71.4Mb on chromosome 1 downstream of Peroxisome proliferator- activated receptors (PPAR-201) gene.

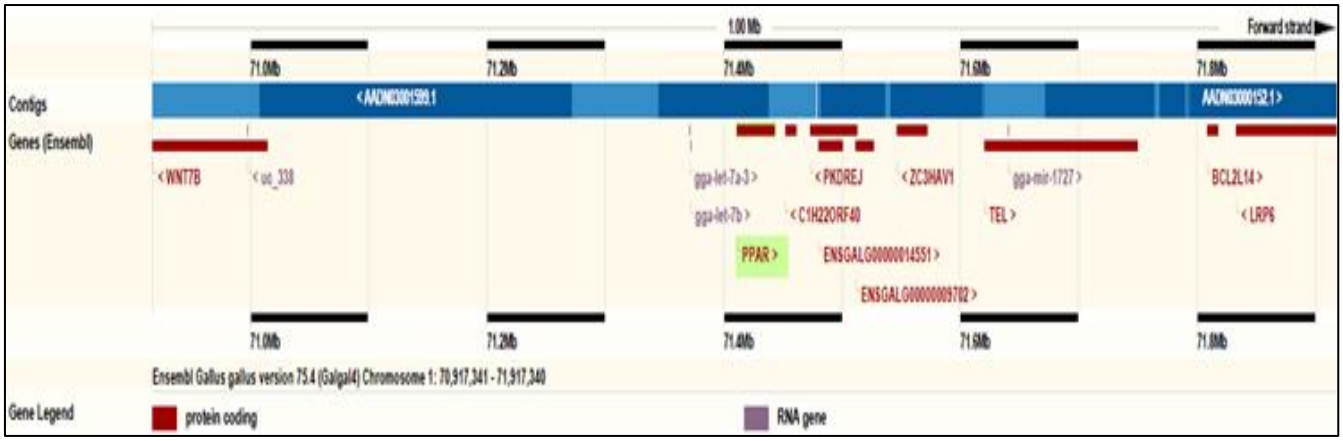

Figure 6 A protein coding region for PPAR gene on chromosome 1 of improved Nigerian indigenous chicken

4. Discussion

A genome-wide association study using the chicken 60K SNP panel in Nigerian improved indigenous chicken population derived from the cross between Marshall exotic and the pure Nigerian indigenous (Normal feathered, Frizzle feathered and Naked Neck) at 8 weeks was used in identify SNPs on the chromosomes and candidate genes associated with body weight of these chickens. Based on the results obtained in this study the purebred Nigerian indigenous had lighter weights and could not reached the 5% Bonferroni genome-wide significance level while F_2 upgrade (Frizzle-Feathered and Naked Neck) were known for heavier weights which reached the 5% Bonferroni genome-wide significance level. This could be due to the Marshall genetic contribution in the Nigerian chicken F_2 upgrades in which Marshall has been under intense selection for body weight and growth rate.

Linkage analyses provided evidence for highly ($p<0.05$) significant SNP associated with body weight of Nigerian improved indigenous genotype. Some positional candidate genes were identified associated with this trait of interest at different SNPs positions on three different chromosomes of the chickens. The candidate gene identified in this study associated with body weight of Nigerian chicken broilers were Peroxisome Proliferator-Activated Receptors (PPAR-201) gene, Breakpoint Cluster Region 201 (BCR-201) gene and Glycine Aminodino Transferase Mitochondrial (GATM) gene in Chromosomes 1, 15 and 10, respectively.

Peroxisome Proliferator-Activated Receptors (PPAR-201) gene was found on chromosome 1 (GGaluGA024649) at SNPs positional region 51.33Kb located at 71.4Mb. Peroxisome Proliferator-Activated Receptors (PPAR-201) genes are expression genes and functions in the regulation of cellular differentiation in the chickens and brain and muscular development in these chickens as postulated by [21]. It also regulates fatty acid transportation and metabolism (carbohydrate, lipid and proteins) in chicken [22]. The allele B of the Proliferator-Activated Receptors genes has a strong association with the body weights of the Nigerian F_2 upgrades (Frizzle-Feathered upgrade) at 8 weeks of age as observed from the present study. The GGA1 had SNP significant ($p<0.05$) effects on body weight at 8 weeks of age with genome-wide significance level.

The Nigerian chickens broilers' body weights have association with the SNPs on chromosome 1 which was consistent with report by Gu *et al*. [23] who observed that chickens on chromosome 1 associated with chickens body weights using 60K SNP panel in F_2 chicken population derived from the cross between Silky Fowl and White Plymouth Rock at 7 to 12 weeks. Likewise [24] who confirmed that chromosome 1 of chickens has association with growth trait. SNP on chromosome 1 was found associated with body weight of Nigerian improved indigenous chickens which was in congruence with the findings of Tatsuda and Funjinaka [25] that QTL controlling chicken's body weight at 7-13 weeks of age in F_2 chickens population was found on chromosome 1.

Another candidate gene identified associated with SNP on chromosome 15 of the Nigerian improved indigenous chickens body weight at 8 weeks of age was Breakpoint Cluster Region-201 (BCR-201) gene. BCR-201 gene cut at SNP positional region of 112.34kb on chromosome 15 located at 8.58Mb as identified in this study. BCR-201 gene does not have direct association on the body weight of the chickens but interact with the genes around and expressed its effects on those genes to affect the body weight positively. According to the function defined by Liu *et al*. [26; 27] that BCR-201 is a fusion protein that has Serine and threonine kinase activity and is a GTPase-activating protein for RAC1 also known as Ras-related C3 botulinum toxin substrate 1. RAC1 is a protein coding gene which is encoded by a GTPase which belongs to

the Rac superfamily of the family Rho family of GTPase i.e GTP-binding proteins. Members of this superfamily appear to regulate a diverse array of cellular events, including the control of cell growth, cytoskeletal reorganization, and the activation of protein kinases [26; 27]. Nassar and Brockmann [24] observed that SNP on chromosome 15 significantly associated with growth traits in chickens which was in consonance with the observation in this study which reported that Chromosomes 15 associated with body weight of Nigerian improved indigenous chickens at 8 weeks of age.

The next candidate gene identified was on chromosome 10, Glycine Aminodino Transferase Mitochondrial (GATM) gene which was a novel gene discovered associated with body weight in this study. This gene has a strong association with body weight of Nigerian improved indigenous chickens. The Glycine Aminodino Transferase Mitochondrial gene functions in the catalysis of the biosynthesis of guanidine-acetate, the immediate precursor of Creatine. Creatine plays a vital role in energy metabolism in muscle tissues and also in embryonic development of the chickens as defined by Humm *et al*.[28] and also plays important role in the central nervous system development of the chickens as defined by Jang *et al*. [29] and Li *et al*. [22]. Nassar and Brockmann [24] observed that chromosome 10 associate with growth traits in chickens which agrees with the observation in this study that chromosomes 10 is associated with body weight of Nigerian improved indigenous chicken broilers at 8 weeks of age. This findings confirm the conclusion by Gu *et al. [23]* that some genes have positive effects on the body weights of chickens F_2 resource population between 7-12 weeks of age.

SNPs on Chromosomes 3 and 7 were found in this study to have been significantly associated with the body weight of the Nigerian improved indigenous chickens at 8 weeks of age. The observation in this study was in consonance with the results of Ikeobi *et al*. [30] who concluded that SNPs on chromosomes 3 and 7 had a strong association with growth trait (abdominal fat) in 30 Chickens family of F_2 chicken population. However, Tatsuda and Fujinaka [25] confirmed chickens chromosome 7 to be associated with body trait (abdominal fat deposition) F_2 chicken population at 7-13 weeks of age which agrees with the result of this study that SNP on chromosome 7 significantly associated with growth trait of the improved Nigerian indigenous chickens at 8 weeks of age and also corroborate the result by Nassar and Brockmann [24] who observed chromosome 7 to associate with growth trait.

The percentage of inbreeding coefficient was high causing a small deviation from the HWE as observed among the Nigerian chicken upgrades which is a common occurrence in commercial stocks. The genetic variation existing in chicken genotypes studied could be due to crossbreeding and selection for fast growth rate.

5. Conclusion

From the study, the following conclusions can be drawn:

The 53313 SNPS marker used in this research passed the 5% Bonferroni Significance threshold.

In Genome-wide association three candidate genes (PPAR-201, BCR-201 and GATM) on chromosomes 1, 15 and 10 respectively, associated with body weight of Nigerian Chickens broilers. A candidate gene "GTAM" is a novel gene discovered in the cause of this study. In this study the use of the Illumina 60k HD Bead chip was successfully used in Nigerian improved indigenous chicken genotypes to determine SNPS associated with growth in chickens genome. Allele B in F_2 Frizzle and naked neck upgrades chickens population should be selected for body weight improvement, since it has positive effect on body weight in this study. In addition, more genes should be identified if higher density chips like 600K and more are used.

5.1. Recommendations

- Introduction of exotic gene in Nigerian indigenous chicken genotype should be encouraged as this would rapidly improve the performance of Nigerian chickens.
- Poultry breeders should improve the Nigerian indigenous chickens to an F_2 upgrades for the achievement of excellent performance especially the Frizzle-feathered and Naked Neck for meat-type purpose.
- Through the practice of crossbreeding and use of marker assisted selection in the breeding program, the Nigerian chickens can be improved for commercial purposes.
- The sample size should be increased to reduce error while carrying out genome-wide association studies with the Nigerian chicken genotypes in order to generate in-depth information that can aid selection for future use, since, this is a preliminary phase SNPs study.
- Allele B in F_2 Frizzle upgrade chickens should be selected for body weight improvement, since it has positive effect on body weight in this study.
- These genes identified should further be studied for future selection purpose.

- In addition, more genes should be identified if higher density chips like 600K and more are used.

Compliance with ethical standards

Acknowledgments

Author is grateful to students who assisted during sample measurement, blood collection and the Institution that allowed this study to be carried out there and the biotechnology centers that did the analysis.

Disclosure of conflict of interest

Author declares that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in this manuscript.

Statement of ethical approval

This research was conducted in accordance with ethical guidelines governing the use of animals and genetic materials in scientific research as stipulated by the Ethical Committee of Department of Animal Science, Faculty of Agriculture, University of Uyo, which is in line with Animal health care constitution.

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