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Polymorphisms of Mx gene and Immune Response Traits in three Genotypes of Nigerian Local Chicken

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Abstract

The study examined polymorphism in Mx gene and its association with immune response traits in three genotypes of Nigerian Indigenous chickens by analyzing single nucleotide polymorphisms (SNPs) across three genotypes: Normal feathered, Frizzle feathered and naked neck. Blood samples from 120 birds (40 per genotype) were collected for haematology and DNA extraction, followed by PCR amplification and sequencing of target regions from a population of 200 birds. Haematological parameters were: Pack cell volume (PCV), White blood cell (WBC), Red Blood Cell (RBC), Eosinophils (Eos), Monocytes (Mono), Haemoglobin (Hb), Lymphocytes (Lym), Heterophils (Heter), Basophils (Bas). The project lasted for 12 weeks. Mx gene polymorphism was genotyp. Then association between the chicken's immune response traits were assessed at weeks 4, 8 and 12 of age. Sequence were analyzed in DnaSP molecular software was used for the molecular analysis and statistical software SAS package was used for the statistical analysis. The genotyping results showed that Mx gene had three genotypes (AA, AB and BB) meaning that it showed variants which is the Polymorphism. AB genotype had the highest effect on RBC, heterophils and eosinophil at different stages of growth. Polymorphism was seen in both naked neck and normal feather while frizzle feather showed mono-morphism. Naked neck chickens were in Hardy-Weinberg Equilibrium while normal feathered chickens were in the disequilibrium. Polymorphism in Mx gene and its association with immune response traits in Nigeria local chickens has been determined.

Keywords: Association; Genotyping Haematology; Immune Response Traits; Mx Gene; Polymorphisms

1. Introduction

The breeds of Nigerian Indigenous chickens carries the genes of three distinct genotypes namely; the naked neck, Frizzle feathered and Normal feathered, which are the basic genotype found in the Nigerian ecotype chicken, thus tracing their root to the country. This local chickens are still the option for supplying the human population with protein and income. Due to their short generation interval and their products, chickens are preferred by majority of Nigerians, having the potentials of providing proteins and meats that can meet the demand of the citizen globally [1]. Livestock and poultry are also seen as important resources that support the livelihood of farmers, consumers and rural people throughout the World. About 35% of the total household income in Nigeria particularly the Niger Delta region is from poultry and livestock production [2]. Controlling diseases incur recurrent expenditure beside posing hazards to human health and threatening animal welfare. Local chicken are resistant to adverse conditions when compared with their exotic counterpart due to some unique adaptive features developed over a long period of random mating in their natural ecosystem. Nigerian local chicken is still found to be self-reliant and hardy birds with the capacity to withstand harsh weather condition and adapt favorably [3]. Genetic resistance to disease is a powerful tool to control and prevent diseases and thereby improving production and dividend in livestock and poultry [4]. According to the review by Ibe [2] who observed that candidate gene with specific mutation affecting a quantitative trait or having many morphological

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variants have been classified as major genes by many researchers, having quantifiable effect on the production performance of chickens; few of which were observed and described in Nigerian indigenous chicken.

According to Li *et al.* [5] the Mx gene is considered to confer positive antiviral responses to the orthomyxovirus in many organisms. The Mx gene is quite rapid in response to infections and interferon which are released into the extracellular environment in neighboring cells and could be used for selection in breeding Programs to improve local chickens [3]. According to Muhammad *et al.* [6] who reported that Tolaki chicken which is an Indonesian local chicken has the ability for anti-viral responses due to the presence of antiviral Mx (myxovirus resistance) gene. According to Ramasamy *et al.* [7] chicken Mx protein confers resistance against viral infections (avian influenza virus). Mx protein is one of the strong antiviral proteins induced by interferon system. The Mx gene response to infections and interferon rapidly and therefore can be used as a precursor to disease and infection in the Nigerian indigenous chickens. This study is therefore initiated towards assessing the anti-viral activity amongst improved Nigerian local chicken based on Restriction Fragment Length Polymorphism (RFLP) gene polymorphism and its association with immune response traits. This may therefore contribute to the genomic knowledge on the efficacy of production of Nigerian local poultry stock. The information garnered will serve as significant contribution to the Nigerian poultry industry towards genomic improvement and selection. It is necessary to study the Mx gene at genomic level among Nigerian local chicken so as to develop breed of birds that are highly resistance to diseases.

2. Materials and methods

2.1. Experimental site

The study was carried out at the Poultry unit, Teaching and Research Farm of the Department of Animal Science, University of Uyo. Akwa Ibom State. Uyo is found between latitude $4^{0}3'$N and 45^{0} 3′S and longitude $7^{0}3'$ and 45^{0} 35′W. The altitude of the area is 38m above the sea level and a mean rainfall of 200mm. The relative humidity is 79% with average Temperature of 28°C [8]. Genomic analysis was carried out at the Biotechnology Laboratory, Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, while haematological analysis was carried out at the Biochemistry Laboratory University of Uyo, Uyo.

2.2. Experimental birds and management of the birds

A total of two hundred and fifty-day-old chicks of Nigerian indigenous origin which comprises of Normal feathered, frizzle feathered and naked-neck were purchased from Federal University of Agriculture, Abeokuta Poultry's Farm.

Birds were reared under intensive system of management. Chicks from each genetic group were allotted to the prepared brooders and brood for 4 weeks before transferred to rearing pen and terminated aw 12 weeks of age. Birds were individually identified by wing tagging according to genotype by sex prior to transfer. The chicks were vaccinated against new castle disease on arrival and other medications were given routinely preventing disease outbreak. Lighting sources: stove and electricity bulb were provided for heat. Feed and water were given *ad libitum.* The beddings were changed weekly if wet. Starter mash (CP- 23% and ME 3462kcal/kg) was provided *ad libitum* throughout the brooding stage. The standard management practice was strictly adhered to till termination at 12 weeks of the study. At rearing stage of 5 to 6 weeks grower ration (20% CP and 300 kcal/kg ME) from 6 to 12 weeks finisher ration (18% CP and 2850 kcal/kg ME) was fed to the chickens.

2.3. Data collection

2.3.1. Haematology

Data were collected for haematological and genomic analyses. Hematological parameters were Packed cell volume (PCV), White blood cell (WBC), Red blood cell (RBC), Haemoglobin (Hb). Blood sample was collected from the wing vein of 120 randomly selected chickens according to their genotype and sex. 40 per genotype and stored in the EDTA bottle for the haematological analysis. Haematological parameters were collected at different ages (4, 8 and 12 weeks) of the birds in the course of the experiment.

2.3.2. Genomic Data

Blood collection

Chickens were randomly selected and blood samples were collected from the wing vein of 120 (40 per genotype) of chickens with the 2ml syringe and needle, then dropped in the marked circle portion on Flinders Technology Associate

(FTA) card. The FTA cards with the blood drop were air dried. The FTA cards were preserved in a cool dry place inside a nylon until the time they were used.

DNA extraction

Five punches of 1mm discs was punched out from the FTA card using 1mm Harris punch on the cutting mat. The discs were placed in the 1.5ml *Eppendorf tubes* and 200µ1 of FTA purification reagent was added into the tubes. They were allowed to incubate for 5 minutes at room temperature before the wash solution was discarded and the card retained inside the Eppendorf tube. The wash process was repeated three times.

100uL of 1XTAE (Tris-Acetate-EDTA) buffer was added to the disc in the Eppendorf tube and it was allowed to incubate for 5 minutes at room temperature then buffer was discarded. The discs were retained inside the *Eppendorf* tube. The process was done two times. The retained discs inside the tube were dried for 10 minutes inside the incubator at 56^0C. 100uL of Nuclease-free water was added into *Eppendorf* tube and spinned at 3500rpm for 3 minutes inside the centrifuge. The DNA extracted was obtained and stored.

DNA quality check

A 10µl from the extracted DNA was subjected to gel electrophoresis at 100 volts in 0.5% agarose gel in 1XTAE buffer containing 1.0µl stained with ethidium bromide. After the electrophoresis, the gel was taken into gel documentation system to view the resulting bands.

Primers

A pair of published primers for exon 13 of the Mx gene was used in amplification of the segment of the DNA through polymerase chain reaction (PCR). The region amplified was exon 13 which was found to be highly polymorphic, Table1

Table 1 The Primer Sequence used for amplification of Mx gene

Gene	Product size	PCR Primer sequence 5^1-3^1	Ref
Mx	100 bp	F: CCTTCAGCCTGTTTTTCTCCTTTTAGGAA R: CAGAGGAATCTGATTGCTCAGGCGTGTA	Seyama *et al.* [9]

2.3.3. Polymerase Chain Reaction

The extracted DNA was amplified using the Polymerase Chain Reaction (PCR-RFLP) method. The amplification reaction was carried out in a programmable thermocycler (Mastercycler pro by Eppendorf). 25 µl of reaction volume mix was prepared comprising 12.5µl master mix, 1µl each for forward and reversed primers, 2µl of DNA template and 8.5µl of nuclease-free water. The contents were thoroughly mixed. The DNA was used as a template for the gene amplification. The PCR conditions of reaction was used with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 60 seconds at 94°C, annealing temperature for 60 seconds at 60°C, and 72°C for 60 seconds and final extension at 72°C for 5 minutes.

2.3.4. Gel documentation and Electrophoresis

A total of 50ml of 4% agarose in TBE buffer was prepared by stirring on a hot plate until agarose is dissolved. The solution was allowed to cool to about 55^oC. Then, Ethidium bromide was added to the concentrated solution of 0.5µg/ml. The gel tray was prepared by sealing ends with tape and placed comb in gel tray. The gel solution was poured into the tray and allowed to solidify for about 20 minutes at room temperature. The comb was removed and the electrophoresis tray was placed in the electrophoresis chamber. 1XTBE electrophoresis buffer was poured into the electrophoretic chamber till the wells were submerged.

The DNA was properly mixed with the loading dye using the micropipette and then loaded onto the proper wells in the electrophoresis chamber. The loading was done toward the cathode (negative terminal) and from right to left.

Following the loading of the wells, electrophoresis was carried out at 80 volts for about 30 minutes and the tracker dye (ethidium bromide blue) migrated a reasonable distance for proper visualization of the DNA bands since the dye has been recorded to have a lower molecular weight compared to the DNA. The result of the gel electrophoresis was visualized under the ultraviolet trans-illuminator.

2.4. Myxovirus resistant (Mx) gene digestion using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

The Mx gene was digested using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Published restriction enzyme (*Rsa1*) was used which recognizes the cutting site GTN | NAC. The RFLP process was performed by adding 1.0 µl Rsa1 enzyme, 2.0µl of 10X NE Buffer and 7µl of nuclease-free water to 10.0 µl of DNA produced by PCR and continuing with 15 minutes incubation at 37°C. DNA fragments became PCR-RFLP products through electrophoresis using electrophoresis device on 4% agarose gel. The device was running using 0.5XTBE buffer at 100 volts voltage for 15 minutes based on manufacturer's recommendation. Electrophoresis gel visualization was performed on gel documentation device.

2.5. The Myxovirus resistant (Mx) genotype scoring and counts

For Mx genotype scoring, samples with one band (having different band sizes) were scored AA samples with two bands were scored AB showing heterozygous for the gene.

2.6. Statistical analyses

The counting of allele frequency, genotype frequency and Mx gene heterozygote were based on genotyping results, the Hardy-Weinberg balance value, correlation analysis, the Polymorphic Informative Content (PIC) and Principal Component Analysis using [10].

2.6.1. Allele frequency formula

$$x_i = \frac{(2nii + \sum_{j=1} nij)}{2N}$$

x_i = *i*-allele frequency

n_{ii} = Individual number of *ii*-genotype

n_{ij} = Individual number of *ij*-genotype

N = Total sample

2.6.2. Genotype frequency formula

$$x_{ii} = \frac{\sum_{i=1}^{n} ni}{N}$$

x_{ii} = ii-genotype frequency

ni = Individual number of ii-genotype

N = Total sample

2.6.3. Hardy-Weinberg (H-W) equilibrium

$$x^2 = \frac{\Sigma(E - O)^2}{E}$$

x^2 = Chi-square value

O = Genotype observation number

E = Genotype expectation number

2.6.4. Heterozygosity

$$H_o = \sum \frac{N_{1ij}}{N}$$

$$H_e = 1 - \sum P_{1i^2}$$

H_o = Heterozygosity observation

N_{1ij} = No. of heterozygous individuals in the 1st locus

N = No. of individuals observed

H_e = Expectation heterozygosity

P_{1i} = Frequency of i-allele at locus-1

2.6.5. Polymorphic Informative Content (PIC)

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

PIC = Polymorphic Informative Content

p_i = Allele frequency-i

p_j = Allele frequency-j

n = Total allele

The other population genetic diversity indices (F_{IS}, F_{ST}, F_{IT}, AMOVA, Tajima and Fu) were computed using GenAlEx [11].

2.7. Phylogenetic tree construction

Phylogenetic analysis was conducted to ascertain the evolutionary relationship of the three Nigerian indigenous genotype chickens using bootstrap 1000.

2.8. Association study analysis

Preliminary analysis will be carried out which include effects of main factors and their interactions. Interaction effects that are not significant will be removed before further analysis. The main effect model is given below:

$Y_{ijkl} = \mu + S_i + C_j + R_k + SC_{ij} + CR_{jk} + SCR_{ijk} + \epsilon_{ijkl}$
where Y_{ijkl} = Observed trait of interest in Nigerian local chickens
μ = population mean
S_i = fixed effect of i^{th} sex (male, female)
C_j = fixed effect of j^{th} Chicken genotype (Frizzled feather, Naked neck, Normal feathered)
R_k = fixed effect of k^{th} Mx genotype (AA, AB, BB)
SC_{ij} = interaction effect of i^{th} sex of s^{th} genotype
CR_{jk} = interaction effect of $_j^{th}$ genotype of $_k^{th}$ gene
SCR_{ijk} = interaction effect of $_i$ sex of $_j^{th}$ genotype in $_k^{th}$ gene
ϵ_{ijkl} = random residual error.

3. Results

3.1. Effect of Mx gene on Immune Response Traits of Improved Nigerian Indigenous Chickens at different ages

The analysis revealed that there was significant differences in the immune response traits of Nigerian Indigenous Chickens at different ages.

3.1.1. Effect of Mx gene on Immune Response Traits of Nigerian Indigenous Chickens at week 4 of age

The result in Table 2 indicated that the Mx gene genotypes (AA, AB, BB) showed significant ($p<0.05$) difference on some of the haematological parameters at week 4 studied. The result indicated that the Mx gene genotypes (AA, AB, BB) showed significant ($p<0.05$) difference on Pcv, Hb, wbc, Heter, Lym, Eos and Mono while Rbc, and Bas had no significant ($P>0.05$) difference as measured in this study at week 4 of age. Genotype BB of Mx gene had the mean value of 32.813±1.89 m/mm^3 for Pcv significantly ($p<0.05$) higher than 26.501±3.20m/mm^3 of AB genotype but significantly ($p>0.05$) similar with (30.251±0.55m/mm^3) of genotype AA at week 8 studied. Genotype BB of Mx gene had the mean value of 10.809±0.63ml for Hb significantly ($p<0.05$) higher than 9.049±1.07ml of AB genotype but significantly ($p>0.05$) similar to AA genotype (10.049±0.18 ml) of Mx gene at week 8 studied. Genotype BB of Mx gene had the mean value of 14.680±0.92 g/dl for wbc significantly ($p<0.05$) higher than AA genotype (12.994±0.27g/dl) and AB genotype (11.569±1.56g/dl) of Mx gene at week 8 studied. Genotype AB of Mx gene had the mean value of 39.391±4.496 um for Heter significantly ($p<0.05$) higher than 929.891±0.78 um of AA genotype and BB genotype (26.267±2.66 um) Mx gene at week 8. Genotype BB of Mx gene had the mean value of 1.209±0.30 cell/McL for Eos significantly ($p<0.05$) higher than AA genotype (1.041±0.51 cell/McL) and AB genotype (0.791±0.44 k/uL) of Mx gene at week 8. Genotype BB of Mx gene had the mean value of 1.419±0.43ml for Momo significantly ($p<0.05$) higher than 1.201±0.12ml of AA genotype but significantly ($p>0.05$) similar with (0.451±0.72ml) of genotype AB at week 8 studied while Bas and Mono had no significant ($p>0.05$) difference in genotypes AA, BB and AB at week 8 in this study.

Table 2 Effect of Mx gene (Genotype) on Immune Response Traits of Nigerian Indigenous Chickens at week 4 of age

TRAITS	AA	AB	BB
Pcv(mL)	30.251±0.55^{ab}	26.501±3.20^{b}	32.813±1.89^{a}
Hb(mL)	10.049±0.18^{ab}	9.049±1.07^{b}	10.809±0.63^{a}
Rbc(m/mm^3)	2.462±0.04	2.462±0.28	2.667±0.16
Wbc(g/dl)	12.994±0.27^{ab}	11.569±1.56^{b}	14.680±0.92^{a}
Heter(um)	29.891±0.78^{b}	39.391±4.496^{a}	26.267±2.66^{c}
Lym(k/uL)	67.127±0.77^{b}	58.877±4.44^{c}	70.187±2.63^{a}
Eos(cell/McL)	0.791±0.09^{b}	1.041±0.51^{ab}	1.209±0.30^{a}
Bas(um)	0.893±0.10	0.643±0.59	0.820±0.35
Mono(ml)	1.201±0.12^{ab}	0.451±0.72^{b}	1.419±0.43^{a}

PCV= Packed Cell Volume Mono= Monocytes Heter = Heterophils WBC= White Blood Cell Hb= Haemoglobin, Bas= Basophils Eos= Eosinophils Lym= Lymphocytes RBC= Red Blood Cell

3.1.2. Effect of Mx gene on Immune Response Traits of Nigerian Indigenous Chickens at week 8 of age

Genotype BB had the mean value of 38.062±1.93 mL for PCV significantly ($p<0.05$) higher than (34.969±0.56 ml) of AA genotype and (30.719±3.26ml) of genotype AB of the Mx gene at 8 weeks of age. Genotype BB (12.680±0.65 mL) and genotype AA (11.657±0.19mL) and AB genotype (10.232±1.10 ml) for Hb of Mx gene of Nigerian local chickens measured at week 8 of age. Genotype BB (17.959±1.58 g/dl) for Wbc was significantly ($p<0.05$) higher than genotype AB (12.232±2.68g/dl) but significantly ($p>0.05$) similar in mean values with genotype AA(15.232±0.46G/DL) and (11.793±1.44mL) while genotype AB and AA were significantly ($p>0.05$) similar. Genotype AB with (69.367±3.43 k/uL) for Lym was significantly ($p<0.05$) higher than BB(68.626±2.03 k/uL) and AA(67.117±0.60 k/uL) . Genotype AA with (30.560±0.66um) for Heter was significantly ($p<0.05$) higher than AB (25.310±3.80 um) and BB (29.326±2.25um) while genotype AA and genotype BB showed no significant ($p>0.05$) difference. Genotype AB with ((3.156±0.61 cell/McL) for Eos was significantly ($p<0.05$) higher than BB (0.656±0.10cell/McL and AA (0.580±0.36 cell/McL) while genotype AA

and genotype BB showed no significant (p>0.05) difference as measured while genotypes AA and BB were significantly (p>0.05) similar for the RBC and Bas studied in genotypes AA, BB and AB at week 8, Table 3

Table 3 Effect of Mx gene (Genotype) on Immune Response Traits of Nigerian Indigenous Chickens at week 8 of age

Traits	AA	AB	BB
Pcv(mL)	29.561±0.45^{ab}	31.311±2.58^{a}	29.932±1.52^{ab}
Hb(mL)	9.772±0.15^{ab}	10.297±0.88^{a}	10.076±0.52^{a}
Rbc(m/mm^3)	2.445±0.04^{b}	2.545±0.24^{a}	2.535±0.14^{c}
Wbc(g/dl)	14.038±0.42^{a}	11.488±2.44^{b}	11.793± 1.44^{b}
Heter(um)	31.811±0.78^{a}	23.811±4.46^{c}	29.471±2.64^{ab}
Lym(k/uL)	67.017±0.73^{b}	73.517±4.21^{a}	68.760±2.49^{b}
Eos(cell/McL)	0.151±0.05	1.151±0.33	0.287±0.19
Bas(um)	0.374±0.08	0.124±0.49	0.576±0.29
Mono(ml)	0.645±0.10	1.39± 0.61	0.904±0.36

PCV= Packed Cell Volume Mono= Monocytes Heter = Heterophils WBC= White Blood Cell Hb= Haemoglobin, Bas= Basophils Eos= Eosinophils Lym= Lymphocytes RBC= Red Blood Cell

3.1.3. Effect of Mx gene (Genotype) on Immune Response Traits of Nigerian Indigenous Chickens at week 12 of age

Genotype BB had the mean value of (38.062±1.93ml) for PCV significantly (p<0.05) higher than AA (34.969±0.56ml) and AB (30.719±3.26ml) and genotype AA (34.969±0.56ml) was significantly (p<0.05) higher genotype AB (30.719±3.26ml) at week 12 of the study. For Hb, genotype BB with (12.680±0.65ml) was significantly (p<0.05) higher than genotype AA (11.657±0.19ml) and AB(10.232±1.10ml) while genotype AA and genotype AB were significantly (p>0.05) similar in means. Genotype BB (17.959±1.58g/dl) was significantly (p<0.05) higher than genotype AB (12.232±2.68g/dl) but similar to AA (15.232±0.46g/dl) for WBC measured at week 12 of the study. AA genotype (30.560±0.66um) was significantly (p<0.05) higher than AB (25.310±3.80um) but significantly (p<0.05) similar to BB genotype (29.326±2.25um) for Heter as measured in week 12 of age, Table 4

Table 4 Effect of Mx gene (Genotype) on Immune Response Traits of Nigerian Indigenous Chickens at week 12 of age

Traits	AA	AB	BB
PCV(mL)	34.969±0.56^{b}	30.719±3.26^{c}	38.062±1.93^{a}
Hb(mL)	11.657±0.19^{ab}	10.232±1.10^{b}	12.680±0.65^{a}
RBC(m/mm^3)	2.935±0.05	2.585±0.32	3.192±0.19
WBC(g/dl)	15.232±0.46^{ab}	12.232±2.68^{b}	17.959±1.58^{a}
Heter(um)	30.560±0.66^{a}	25.310±3.80^{b}	29.326±2.25^{ab}
Lym(k/uL)	67.117±0.60^{b}	69.367±3.43^{a}	68.626±2.03^{ab}
Eos(cell/McL)	0.656±0.10^{b}	3.156±0.61^{a}	0.580±0.36^{b}
Bas(um)	0.697±0.10^{b}	1.447±0.62^{a}	0.355±0.36^{b}
Mono(ml)	0.982±0.13^{ab}	0.732±0.74^{b}	1.118±0.44^{a}

PCV= Packed Cell Volume Mono= Monocytes Heter = Heterophils WBC= White Blood Cell Hb= Haemoglobin, Bas= Basophils Eos= Eosinophils Lym= Lymphocytes RBC= Red Blood Cell

Genotype AB (3.156±0.61cell/McL) for Eos was significantly (p<0.05) higher than genotype AA (0.656±0.10cell/McL) and BB's (0.580±0.36cell/McL) while genotypAA and genotype BB were significantly (p<0.05) similar in means as measured in week 12 of age. For mono, genotype BB with (1.118±0.44 ml) was significantly (p<0.05) higher than genotype AB (0.732±0.74 ml) but significantly (p>0.05) similar to AA genotype while genotype AA and genotype AB and AA were significantly (p>0.05) similar in means.

3.2. Hardy Weinberg equilibrium of Mx gene in Nigerian indigenous chicken genotypes

The results of the Hardy Weinberg equilibrium of Mx gene for Normal feathered chickens showed a very highly significant ($p<0.001$) difference amongst naked neck and frizzle feathered chicken while naked neck and frizzled feather were not significantly ($p>0.05$) different on the basis of Hardy Weinberg equilibrium in Table 5

Table 5 Hardy Weinberg equilibrium of Mx gene in Nigerian indigenous chicken genotypes

Pop	Locus	DF	Chi Sq	Prob	Signif
Frizzle feather	Mx gene	Monomorphic			
Naked neck	Mx gene	1	0.123	0.725	Ns
Normal feather	Mx gene	1	51.000	0.000	***

Pop = population; df = degree of freedom; ChiSq = Chi Square; Prob = Probability; Signif = significance

3.3. Heterozygosity, FStatistics and Polymorphism of Mx gene in Nigerian indigenous chicken's genotypes

The highest number of different alleles (Na) was seen in naked neck and normal feathered chickens (2.000) respectively while the lowest number of different alleles (Na) was recorded in frizzled feather. Observed heterozygosity (Ho) was seen only on naked neck Nigerian indigenous chickens in Table 6.

Table 6 Heterozygosity, FStatistics and Polymorphism of Mx gene in Nigerian Indigenous chicken's genotypes

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
Fz	Mx gene	28	1.000	1.000	0.000	0.000	0.000	0.000	
Nk	Mx gene	10	2.000	1.220	0.325	0.200	0.180	0.189	0.111
NF	Mx gene	51	2.000	1.215	0.321	0.000	0.177	0.179	1.000
Total	Mean	29.667	1.667	1.145	0.215	0.067	0.119	0.123	0.444
	SE	11.865	0.333	0.072	0.108	0.067	0.059	0.061	0.454

Na = No. of Different Alleles, Ne = No. of Effective Alleles, I = Shannon's Information Index, Ho = Observed Heterozygosity, He = Expected Heterozygosity,uHe = Unbiased Expected Heterozygosity, F = Fixation Index

4. Discussion

Polymorphism in Mx gene using RFLP in Nigerian local chicken were presented in Tables 2 to 4. The result of all the Mx gene (AA, AB, BB) genotypes showed significant ($p<0.05$) difference on some of the haematological parameters (PCV, Hb, Heter, Lym, Eos and Mono), while RBC and Bas had no significant ($p>0.05$) as measured in this study at week 4 of age in Table 2. This may be due to stress reactions and some allergic reactions Oon the chickens. Also, the results of all the Mx gene (AA, AB, BB) genotypes showed significant ($p<0.05$) difference on some of the immune response traits (PCV, Hb, WBC, Heter, Lym and Eos) while Bas and Mono measured had no significant ($p>0.05$) difference in this study at week 8 of age in Table 3. It was also observed that the Mx gene (AA, AB, BB) genotypes showed significant ($p<0.05$) difference on some of the immune response traits (PCV, Hb, WBC, Heter and Eos) while Mono had no significant ($p>0.05$) difference as measured in this study at week 12 of age in Table 4.

Heterozygosity values indicates the diversity of a population. Higher value of heterozygosity indicated were more diverse than the observed population as seen in Table 5. Heterozygosity was determined by calculating the allele frequency of a gene, in this study the calculation was based on the allele frequency of Mx genotype [12]. The heterozygosity in this study often refers to the Hardy-Weinberg Equilibrium. If the value of observed heterozygosity is lower than the expected heterozygosity, it means that inbreeding occurred and vice versa, if expected heterozygosity is lower than observed heterozygosity, it means that the effect of isolated separation of a population is being suspected to occur and still in Hard-Weinberg Equilibrium. In this study, the calculation of the expected heterozygosity and observed heterozygosity showed that the overall of the expected heterozygosity (He) was lower than the observed heterozygosity (Ho). The result showed that the observed heterozygocity (Ho) naked neck chicken was still in Hardy-Weinberg Equilibrium (no inbreeding effect). Li *et al.* [5] onfirmed that local chickens were generally still in Hardy-Weinberg Equilibrium. However, especially for normal feather, the value of observed heterozygosity (Ho) was lower (0.000) than expected heterozygosity (He) (0.177). It was probably due to the small sample size studied. However, Normal feathered

chickens in this study were in the disequilibrium population. This could be due to the systematic selection on closed populations, high selection intensity, genetic drift and non-random mating as stated by [13].

5. Conclusion

Conclusively, of the three genotypes of Nigerian local chickens only Frizzle feathered chickens showed mono-morphism which is the variant while naked neck and Normal feathered chickens exhibited Polymorphisms. AB genotype of Mx gene showed the highest effect on Rbc and Wbc components (heterophils and eosinophil) at different ages of growth studied proving that there have been resistant and adaptive features exhibited by these chickens leading to survival in the harsh environmental conditions of the tropical zone of Nigeria. Hence, there is an association between the Polymorphisms of Mx gene on the immune response trait in Nigerian Local Chickens, as such these chickens should be improved and raised commercially for use by the populace to boost animal protein supply in Nigeria.

Recommendation

It is recommended that the local breeds of Nigerian Chicken should be improved and raised commercially for meat production purpose as it has high association effects on the immune response traits leading to surviving the harsh environmental condition of this zone.

Compliance with ethical standards

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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.

Author declaration

All the authors have declared that this article is original article submitted for publication which has not been published anywhere in the world.

Author's Consent

Authors has given an approval and consent for the article to be submitted for publication.

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