Assessment of Haemagglutination Titre and Serum Lysozyme Concentration in Nigerian Indigenous Chicken Genotypes

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Abstract

This research was conducted to assess immunocompetence of Nigerian indigenous chicken (NIC) genotypes (normal-feather, frizzled-feather and naked neck) reared in the sub-humid Southwest region. The chickens were inoculated with sheep red blood cells (SRBC). A total of 268 chickens were used to test the primary antibody response to sheep erythrocytes. At 8 weeks, the birds were inoculated with 1ml of 1% suspension SRBC via the jugular vein and antibody responses at 5, 10 and 15 days post inoculation (dpi) were measured. Data collected on the haemagglutination (HA) titre and serum lysozyme concentration (SLC) were analysed using the Linear Model procedure of R version 4.3.0. Titre was expressed as log_2 of the highest dilution showing complete haemagglutination. Results showed that genotype significantly ($P < 0.05$) affected HA titre at 5 and 10 dpi. At 5 dpi, naked neck chickens had 2.11 ± 0.02 which was higher ($P < 0.05$) than 1.99 ± 0.03 obtained for frizzle-feather chickens. At 10 dpi, naked neck and normal-feather had similar values, but were higher ($P < 0.05$) than the estimate for frizzle-feather chickens. There was a significant ($P < 0.05$) interaction effect of genotype and sex for SLC at 5 dpi, in which female frizzled-feather chicken had higher value than female normal-feather chicken. In conclusion, HA titre for Nigerian indigenous chicken could be selected for at 10 dpi. Also, Nigerian indigenous naked neck chickens with higher HA titre and female frizzle-feather chickens with higher SLC, could be selected for the improvement of such traits.

Key words: haemagglutination, immune response, indigenous chicken, serum lysozyme, srbc.
Running title: Nigerian indigenous chicken genotypes

Evaluation Comparative Du Titre D'hémagglutination Et De La Concentration De Lysozyme Sérique Dans Des Génotypes De Poules Indigènes Nigérianes

Résumé

L'amélioration de l'immunocompétence des poules et leur résistance aux pathogènes par sélection génétique devrait réduire les coûts et les pertes liés aux maladies. Cette recherche a été réalisée pour évaluer l'immunocompétence des génotypes de poules indigènes nigérianes (plumes normales, plumes frisées et cou nu) élevées dans la région sub-humide du Sud-Ouest. Les poules ont été inoculées avec des globules rouges de mouton (GRM). Un total de 268 poules (61 à cou nu, 70 à plumes frisées et 137 à plumes normales) a été utilisé pour tester la réponse anticorpale primaire aux érythrocytes ovins. À 8 semaines, les oiseaux ont été inoculés avec 1 mL de suspension de 1 % de SRBC par la veine jugulaire et les réponses anticorporelles ont été mesurées à 5, 10 et 15 jours après l'inoculation (dpi). Les données collectées sur le titre d'hémagglutination (HA) et la concentration de lysozyme sérique (CLS) ont été analysées en utilisant la procédure du modèle linéaire de R version 4.3.0. Le titre a été exprimé comme log2 de la plus forte dilution montrant une hémagglutination complète. Les résultats ont montré que le génotype affectait significativement ($P<0,05$) le titre de HA à 5 et 10 dpi. À 5 dpi, les poules à cou nu avaient un titre de 2,11 ± 0,02, ce qui était supérieur ($P<0,05$) à 1,99 ± 0,03 obtenu pour les poules à plumes frisées. À 10 dpi, les poules à cou nu et à plumes normales avaient des valeurs similaires, mais étaient plus élevées ($P<0,05$) que l'estimation pour les poules à plumes frisées. Il y avait un effet d'interaction significatif ($P<0,05$) entre le génotype et le sexe pour la CLS à 5 dpi, où les poules frisées femelles avaient une valeur plus élevée que les poules normales femelles. En conclusion, le titre de HA

pour les poules indigènes nigérianes pourrait être sélectionné à 10 dpi. De plus, les poules à cou nu indigènes nigérianes avec un titre de HA plus élevé et les poules frisées femelles avec une CLS plus élevée pourraient être sélectionnées pour l'amélioration de ces traits.

Mots-clés : hémagglutination, réponse immunitaire, poules indigènes, lysozyme sérique, SRBC.

Introduction

With the great expansion of poultry rearing and farming, infectious diseases and their attendant effects on food security and public health have become an important problem in Nigeria and other parts of the world. There are considerable variations in different chicken lines in the response to viral, bacterial and parasitic pathogens (Kramer *et al*., 2003). Indigenous chickens are generally believed to be resistant to many endemic diseases and stressful environments, and the Nigerian genotypes are not an exception in this regard. This ability to cope with diseases and stress is what is responsible for their higher survival under rural conditions when compared with the exotic strains (Fayeye *et al*., 2010). To harness the potential of indigenous chickens in improving rural livelihood, there is need for selection to identify resistant and susceptible strains, using both the humoral and the cellular immune responses,

Peter (1998) observed that differences were as pronounced within ecotypes just as between ecotypes in the immunocompetence of Tanzanian scavenging indigenous chicken ecotypes. Hassan et al. (2004) showed that there were differences within and between ecotypes in immunocompetence traits for endemic diseases like Newcastle in Egyptian chickens. Basically, selective processes for production parameters such as, early development or egg production often make very uniform strains, lessening thus, population genetic variability (Paludo, 2011). These, therefore, may have contributed indirectly to the selection of more susceptible strains and increased use of antibiotics in poultry production. In addition, the infectious disease of livestock is the most costly and hazardous problem facing the Agrifood industry. This, coupled with consumer concern for both improved food safety and animal well-being, demand alternative approaches to disease prevention which do not rely on extensive use of anti-microbials (Stear *et al.*, 2001)

Selection for enhanced disease resistance or decreased susceptibility by selecting for an immune response induced by a harmless (poly-) antigen such as sheep red blood cells (SRBC) has long been considered as an alternative approach to disease control in poultry. SRBC, besides their non-pathogenic characteristics, are preferred for their multiple antigenic sites, which may stimulate a wide range of immune cells. According to Boa-Amponsem et al. (1997), selection for increased antibody production to an antigen such as SRBC may lead to more disease-resistance in chickens. Based on these reports, and the assumption that indigenous chickens are believed to be resistant to endemic disease and environmental stress, the immune responses of Nigerian indigenous chicken genotypes were evaluated.

Materials and methods

The study was conducted at the Poultry Breeding Unit of the Federal University of Agriculture, Abeokuta, Ogun state, in South-Western Nigeria. A total of 268 indigenous chicken genotypes (naked neck (61), frizzle-feather (70) and normal-feather (137)) were used for the study. The chicken populations were representative from the six South-Western states in Nigeria. All the experimental chickens were wing tagged and maintained under identical management conditions. Water and commercial feed were given to chicken *ad libitum.*

Blood was withdrawn under aseptic conditions from healthy sheep into a heparinised test tube. It was centrifuged at 4000 rpm for 10 minutes at room temperature to settle down the RBCs. After which the supernatant was discarded. The RBCs was then washed three times with phosphate-buffered solution (PBS) pH 7.4 by mixing and centrifuging it to remove other

serum components. Finally, 1% SRBCs suspension was prepared and used for injection of the experimental chickens. 1ml of 1% SRBC suspension was injected into the jugular vein of each chicken with tuberculin syringe at 8 weeks of age. 2mls of blood was collected from the jugular vein of each chicken into sterilized glass tubes at 5, 10 and 15 dpi and allowed to clot for 2 - 3 hours at 37 °C for the hyper immune sera to ooze out of the clotted blood. Sera samples were then collected in 1.5 ml sterile tubes and stored at – 20 ºC till further analysis.

Two-fold serial dilutions of serum were made in 50µl volumes of PBS taken in microtitre plates, to which 50µl of one per cent sheep rbc was added. After mixing, the plates were incubated for 45 minutes at room temperature. HA titre was considered as the reciprocal of highest dilution of antigen which showed complete agglutination.

The serum lysozyme concentration was estimated according the procedure of Choudhary *et al.* (2022) using Lysoplate assay method as follows:

The lysozyme standards were prepared by dissolving 2 mg of standard lysozyme (Sigma USA) in 1 ml of dibasic buffer. Various dilutions were prepared so as to bring the final concentration of lysozyme to 40 µg / mL, 20 µg / mL, 10 µg / mL, 5.0 µg / mL and 2.5 µg/ mL by serial dilution. The agar lysoplate was set up on a horizontal surface. The glass plate was cleaned and sterilized with spirit and air-dried. The size of the gel was determined on the basis of number of samples to be analysed. The borders were prepared placing glass strips on the edges of required area. All the four sides of the borders were sealed with 2% agar. Volume for 0.4 cm thick gel was calculated by the formula: Volume = Length × Width × 0.4 cm

After boiling the Agarose in dibasic buffer it was cooled to about 60 °C and the prediluted *Micrococcus lysodeikticus* (Sigma, USA), 50 µg per ml of dibasic buffer was added into it and mixed thoroughly. Then the whole content was poured onto the sealed glass plate, spread uniformly and was left at room temperature for polymerization. After polymerization of gel, the wells were punched at a distance of approximately 1.5 cm with the help of a gel punch. 15 µl of serum sample was loaded to each well. Lysozyme standard samples were also loaded in the wells at one side (5 dilutions). The plate was incubated at room temperature overnight. The plate was stained with 0.2% Coomassie Brilliant Blue (CBB) for 6 hours and excess stain was removed with destaining solution. The diameters of the lysed zones were measured with digital vernier calliper. The plates were photographed for documentation.

The concentrations of standards were regressed on diameter of the lysed zones. The slope of the curve and intercept were determined. The lysozyme concentration in the sera samples was determined by following regression equation below. Lysozyme concentration (µg/ml) is proportional to the diameter of lysed areas.

$Y = bx + c$

Where,

Y = Concentration of serum sample

b = Slope of regression equation

c = Intercept of regression equation

x = Diameter of the lysed zone around serum sample.

Statistical analysis

Two-way analysis of variance was used to test the effects of genotype and sex on the days post inoculation. Data were analysed using the linear model procedure of R version 4.3.0. (R Core Team, 2023). Non-significant interactions were removed from the model.

Antibody titres and serum lysozyme concentration were expressed as $\log_2$ values prior to analysis. And the statistical model below was used for the analysis.

$Y_{ijk} = \mu + B_i + S_j + (BS)_{ij} + e_{ijk}$

Where Y_{ijk} = value of trait measured on ijk^{th} individual

μ = overall mean

B_i = fixed effect of i^{th} chicken genotype

S_j= fixed effect of j^{th} sex

$(BS)_{ij}$ = effect of ij^{th} interaction

e_{ijk} = residual error

Results

Genotype effect was significant ($P < 0.05$) on HA titre at 5 and 10 dpi. According to Table 1, at 5 dpi, naked neck, normal feather and frizzle feather chickens had 2.11 ± 0.02, 2.06 ± 0.02 and 1.99 ± 0.03 respectively, which were statistically different ($P < 0.05$). At 10 dpi, the values 2.15 ± 0.04, 2.12 ± 0.02 and 2.01 ± 0.04, which were significantly different, were obtained for naked neck, normal feather and frizzle feather, respectively. The effect of sex was not significant ($P > 0.05$) on all the days post inoculation. According to the result, at 5 dpi the male had 2.05 ± 0.02 and female had 2.06 ± 0.02.

Table 1: Effects of genotype and sex on haemagglutination titre on Nigerian indigenous chickens at 5, 10 and 15 days post inoculation (dpi).

Genotype	**Haemagglutination titre**			
	Pre-inoculation	**5 dpi**	**10 dpi**	**15 dpi**
Naked neck	0.19 ± 0.09	2.11 ± 0.02[a]	2.15 ± 0.04[a]	2.01 ± 0.04
Normal-feather	0.13 ± 0.06	2.06 ± 0.02[ab]	2.12 ± 0.02[a]	1.96 ± 0.02
Frizzled-feather	0.21 ± 0.09	1.99 ± 0.03[b]	2.01 ± 0.04[b]	1.91 ± 0.03
Sex				
Male	0.13 ± 0.05	2.05 ± 0.02	2.09 ± 0.02	1.96 ± 0.02
Female	0.21 ± 0.08	2.06 ± 0.02	2.09 ± 0.03	1.97 ± 0.03

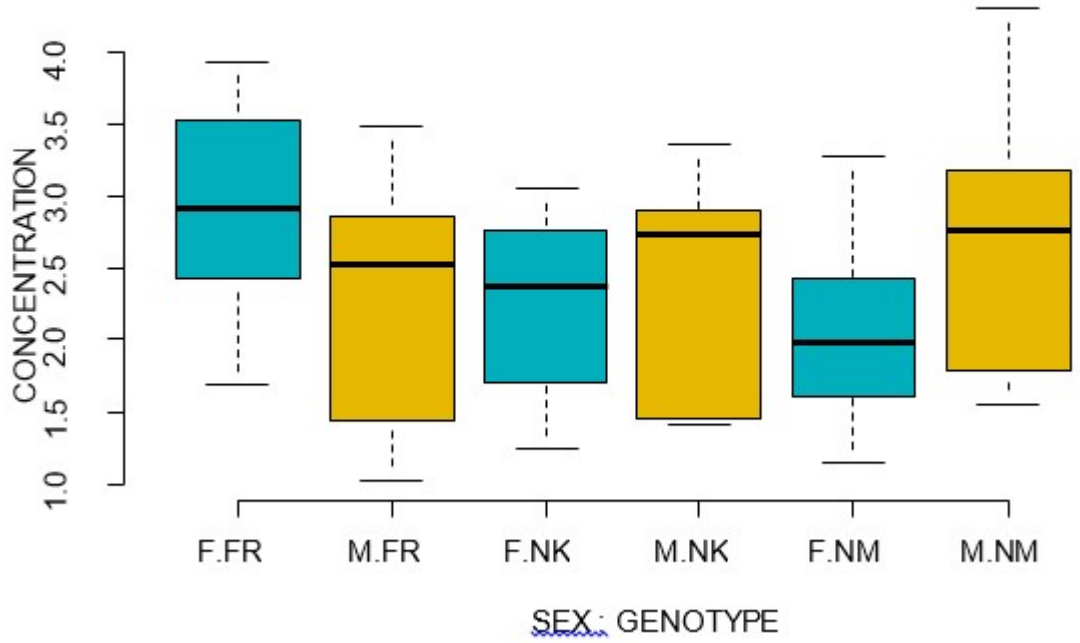
a,b means with different superscript within the same column are significantly ($P < 0.05$) different

Table 2 showed that genotype had no significant ($P > 0.05$) effect on serum lysozyme concentration. Likewise, sex did not significantly ($P > 0.05$) influence the serum lysozyme concentration at 5, 10 and 15 dpi.

Table 2: Effects of genotype and sex on serum lysozyme concentration of Nigerian indigenous chickens at 5, 10 and 15 days post inoculation (dpi).

Genotype	**Serum lysozyme concentration**			
	Pre-inoculation (µg/ml)	**5 dpi (**µg/mL)	**10 dpi (**µg/mL)	**15 dpi (**µg/mL)
Naked neck	2.24 ± 0.18	2.42 ± 0.15	2.63 ± 0.14	2.27 ± 0.23
Normal-feather	2.49 ± 0.21	2.32 ± 0.12	2.47 ± 0.13	2.35 ± 0.17
Frizzled-feather	2.34 ± 0.18	2.55 ± 0.12	2.53 ± 0.17	2.59 ± 0.19
Sex				
Male	2.49 ± 0.16	2.45 ± 0.12	2.61 ± 0.11	2.35 ± 0.13
Female	2.22 ± 0.15	2.41 ± 0.11	2.48 ± 0.13	2.47 ± 0.19

However, there was interaction effect (figure 1) at 5 dpi where the female frizzle feather showed a higher significant ($P < 0.05$) serum lysozyme concentration than the female normal feather.



Where F.FR is female frizzle-feather; M.FR is male frizzle-feather; F.NK is female naked neck; M.NK is male naked neck; F.NM is female normal-feather and M.NM is male normal-feather

Figure 1: Interaction effect between genotype and sex.

Discussion

The HA result of the present study at 5 dpi is higher to the range of 1.2 – 2.8 reported by Boa-Amponsem *et al.* (2000). This could be as a result of differences in breed, the environment or the intensity of selection that has been done on White Leghorn. According to Osei-Amponsah et al. (2013), highly selected chicken populations have less immunity than unselected stocks. Moreover, the high values recorded for the naked chickens could also contribute to their better weight gain than other genotypes, since their immune system can easily fight off common disease such that they are less subjected to environmental stress.

The result obtained for sex, agrees with the report of Kumar and Kumar (2011); Das *et al.* (2014); Radhika *et al.* (2017). However, they also reported higher numerical values for male chickens given sexual dimorphism as a possible reason. But the numerical differences in this report was not apparent.

The low values obtained for the pre-inoculation were expected as the chickens were not inoculated. However, having values for pre-inoculation is an evidence of the presence of natural antibodies in the three genotypes which corresponds to the findings of by Pathak *et al.* (2017).

The observed increase in HA titre from 5 dpi to 10 dpi in all three genotypes of chicken and then, the decline after 10 dpi could be as a result of absence of continuous stimuli and the weaning of antibodies in the blood stream as observed by Radhika *et al.* (2017) in indigenous breeds of chicken. The highest numerical values obtained for antibody response to SRBC measured at 10th dpi is in consonance with those reported in earlier investigations on antibody response to SRBC at various days post inoculation in chicken, where highest antibody titre was observed at 5th dpi in most experiments (Boa-Amponsem *et al*., 2000; Singh *et al.*, 2010; Kumar *et al*., 2011).

The means of serum lysozyme concentration obtained in this study were higher than the range of values 1.33 ± 0.03 and 1.18 ± 0.03 µg/mL obtained by Saxena (1993) for lysozyme

concentration in commercial broilers and guinea fowl and 1.26 ± 0.04 µg/ml in IWJ lines of White Leghorn chickens by Shivakumar (2003). However, the values obtained for this investigation were similar to values by Singh *et al*. (2010) who estimated the mean of serum lysozyme level to be 2.13 ± 0.03 µg/mL in Aseel chicken and at variance with 3.55 to 5.77 µg/ml reported by Nath (1999) for broiler population. The observed differences could be due to the genetic background of the chicken and the environment.

The non-significant difference in the means of the serum lysozyme concentration among the genotypes agrees with report of Das *et al* (2014). Chickens with high HA titre did not also show high serum lysozyme concentration. This is in consonance with the report of Singh and Kumar (2007). It could mean that these traits do not have correlated response and this suggests that the genetic mechanisms responsible for mounting of antibody response to SRBC and regulation of the serum lysozyme might be independent.

The report obtained for this experiment for sex is in agreement with the results of Kumar and Kumar (2011); Das *et al*. (2014) who reported non-significance effect of sex. However, while this study report the presence of interaction, there was no interaction in their reports.

From the result, it can be concluded that the Nigerian indigenous chickens demonstrated high and comparative sensitivity to SRBC inoculation. The evaluation of HA titre for immunocompetent trait gives a better result at 10 dpi. This means that selection for such trait should be done at 10 dpi or Nigerian indigenous chickens. The serum lysozyme concentration was affected by the interaction of genotype and sex. The female frizzle feather expressed more serum lysozyme concentration which makes them a potential preferred choice for serum lysozyme improvement.

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Statements and declarations

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Conflict of interest

The authors declare that there were no conflict of interest.

Ethics approval

Approval or this research was granted by the Ethics Committee of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

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List of Abbreviations

NIC	-	Nigerian Indigenous chickens
SRBC	-	Sheep red blood cells
DPI	-	Days post inoculation
HA	-	Haemagglutination
SLC	-	Serum lysozyme concentration
RBCs	-	Red blood cells
F.FR	-	Female-frizzle feather
M.FR	-	Male-frizzle feather
F.NK	-	Female-naked neck
M.NK	-	Male-naked neck
F.NM	-	Female-normal feather
M.NM	-	Male-normal feather