Comparative Assessment Of Innate Humoral And Cellular Immunity Of Exotic And Nigerian Indigenous Breeds Of Chickens

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Innate humoral and cellular immunity of the Nigerian indigenous and exotic breeds of chickens were assessed and compared for degree of immunocompetence. Natural antibodies and complement levels of Arbor acres broilers, Nera pullets, Nera cockerels and Nigerian indigenous chickens were assessed using haemolysis-haemagglutination assay. Delayed footpad reaction was also assessed. Results obtained from pullets and cockerels were pooled to constitute the mixed types group.

Natural antibodies and complement titters of indigenous chickens and pullets (7.5 ± 0.62; 3.3 ± 0.21 and 7.5 ± 0.75; 3.0 ± 0.0 respectively) were significantly higher (p<0.05) than those of cockerels and broilers (5.6 ± 0.88; 2.7 ± 0.15 and 4.2 ± 0.17; 2.8 ± 0.09 respectively). Titters in the mixed type group (6.6 ± 0.61; 2.9 ± 0.07) were lower than those of IC (p<0.05). Delayed footpad reaction showed maximum response at 24 hours post challenge in indigenous chickens and broilers and 48 hours post challenge in pullets, cockerels and mixed type groups with indigenous chickens recording the highest delayed footpad reaction value at peak (1.96 ± 0.13 mm).

The study showed higher levels of natural antibodies and complement as well as faster and more intense delayed type hypersensitivity reaction in indigenous chickens than exotic Nera breed both of which are reared for meat and egg.

Key words: Cellular immunity; exotic chickens; humoral immunity; Innate immunity; Nigerian indigenous chickens.

Abbreviations

\text{IC} \quad \text{Indigenous chickens}
\text{DFR} \quad \text{Delayed footpad reaction}
\text{DTH} \quad \text{Delayed-type hypersensitivity reaction}
\text{MT} \quad \text{Mixed type}
\text{NAbs} \quad \text{Natural antibodies}
\text{IBD} \quad \text{Infectious bursal disease}
\text{PEG} \quad \text{Polyethylene glycol}

Introduction

The Nigerian indigenous chickens which constitute majority (84%) of chickens reared in Nigeria (FDLPCS, 1992) have been able to thrive for centuries in the harsh tropical environment. They are more adapted to tropical conditions like high environmental temperature and humidity as well as poor nutrition than the introduced exotic breeds which perform sub-optimally in the tropics (Marks et al., 1969) since they were not bred to be reared in the tropics. The indigenous chickens are scavengers making them less competitive with humans for grains; they are self-breeding and are produced very economically by rural dwellers with little or no housing, feeding and veterinary care (Okoye and Aha-Adulugba, 1998). They are however characterized by small body size, slow
growth, low egg production and late maturity (Nwosu, 1979; Akinokun, 1990). The Nigerian indigenous chickens have been alleged to be hardy and more resistant to diseases than exotic breeds (Akinokun, 1990). Aire (1973) observed that the bursa of Fabricius of Nigerian indigenous cockerels attained a greater relative organ to body weight than White Leghorn cockerels. Glick (1955) had earlier reported that birds with bigger bursa of Fabricius have greater resistance to disease. Although the indigenous chickens are susceptible to a number of diseases including avian pox, Newcastle disease, coccidiosis and ectoparasitism (Adene, 1989; Nwosu, 1990), they have thrived well in the face of these epizootics resulting in the speculation that the indigenous chicken is naturally endowed with disease resistant trait (Adene, 1990; Akinokun, 1990). However, this assumption is yet to be proven.

Constitutive innate immunity provides the first-line of protection against invading microbes. Natural antibodies (NAbs) and complement are two interrelated humoral components amongst these defenses (Matson et al., 2005). NAbs serve as recognition molecules capable of opsonizing invading microorganisms and initiating the complement enzyme cascade, which ends in cell lysis. NAbs are unique immunoglobulin molecules because their presence do not require previous exposure to a particular antigen. The interaction of NAbs and complement is an important link between innate and adaptive immunity (Carroll and Prodeus, 1998; Ochsenbein and Zinkernagel, 2000). Complement deficiencies have been associated with a range of infectious and non-infectious diseases in humans (Schur, 1983). On the other hand, cellular immunity can be assessed by the delayed-type hypersensitivity (DTH) which is a hypersensitive response mediated by sensitized TDTH cells that release various cytokines. The response generally takes 2 to 3 days to develop during which time TDTH cells are activated to secrete cytokines which induce localized influx of macrophages and the subsequent release of their lytic enzymes (Kuby, 1994).

An earlier study conducted by Aire and Ojo (1974) reported that Nigerian indigenous cockerels were more resistant to Salmonell gallinarum infection than White Leghorn cockerels judged by values of haemoglobin, haematocrit, erythrocyte and leucocyte counts. Oladele et al. (2007a) also observed a higher infectious bursal disease (IBD) virus antibody titer in response to experimental infection in Nigerian indigenous chickens than in exotic breed.

This study was therefore conducted to assess and compare innate humoral and cellular immunity in the Nigerian indigenous and exotic breeds of chickens using haemolysis-haemagglutination assay and delayed-type hypersensitivity reaction. This is in order to substantiate the assumption that the indigenous chickens are more resistant to diseases than exotic breeds.

Materials and Methods

Experimental chickens

Day-old Arbor acres broilers, Nera pullets and Nera cockerels were purchased from a commercial hatchery in Ibadan, Nigeria while fertile eggs of indigenous chickens were sourced and also hatched in a commercial hatchery. 60 viable chicks each of broilers, pullets, cockerels and indigenous chicks were selected for the experiment and reared in the experimental animal unit of the Department of Veterinary Medicine, University of Ibadan, Nigeria which is between latitude 15°N and 30°S with relative humidity ranging from 50-85%, rainfall is approximately 70 inches per annum and temperatures ranges between 28°C and 34°C.

Brooding was done for the first two weeks of life while commercially prepared chick mash/broiler mash (Livestock feed®) and water were provided as appropriate ad-libitum. Newcastle disease vaccine (Hitcher B-1 strain) was administered intraocularly at day-old, while infectious busal disease vaccine (IBDV) was administered orally at day 11. Newcastle disease vaccine LaSota strain was administered at 21 day-old. Necessary veterinary attention were given as and when due throughout the duration of the experiment.

Assessment of innate humoral immunity

Harvesting of plasma: Twenty broilers and ten each of pullets, cockerels and indigenous chickens at 4 weeks of age were bled into vacutainer tubes containing sodium citrate and kept on ice pending centrifugation. Blood samples were centrifuged at 2,500 revolution per minute (rpm), plasma samples were harvested into well labelled eppendorf tubes and immediately subjected to hemolysis-heagglutination assay while the remaining samples were frozen for future use.

Positive control immunoglobulin M (IgM): Three adult chickens were injected with 50μl of whole rabbit blood in phosphate buffered solution (PBS) into four (4) different places in the pectoral region. The chickens were bled 90 hours post inoculation, plasma was harvested and pooled. This served as positive control IgM and was frozen for future use.
Hemolysis-haemagglutination assay: This assay was carried out as described by Matson et al. (2005). In 96-well ‘U’ bottom microtiter plates, 25 ±1 of plasma samples were pipetted into columns 1 and 2 of a U-bottom microtiter plate. Twenty five ±1 of 0.1M PBS was pipetted into columns 2-12 using a multichannel pipette. The contents of column 2 wells were serially diluted (1:2) through column 11 while column 12 served as the negative control. This resulted in dilution ranging from 1 to 1:1024. Twenty five ±1 of a 1% rabbit blood cell suspension was added to all wells; the plates were sealed with parafilm M and covered with a polystyrene plate lid. Positive control plasma was also included. Plates were gently vortexed for 10 seconds and incubated by floating in a water bath at 37°C for 90 minutes. After incubation, the long axis of each plate was tilted to an angle of 45° for 20 minutes at room temperature in order to enhance visualization of agglutination. Agglutination titers i.e last well showing agglutination was noted for each sample. Afterwards, plates were kept at room temperature for additional 70 minutes and observed for haemolysis. The last well showing lytic activity was noted for each sample. Half score between two titers were recorded where the termination of agglutination or lysis was intermediate or ambiguous. Results obtained from the pullets and cockerels were pooled to represent the fifth group in order to best simulate the Nigerian indigenous chickens for a more appropriate comparison.

Assessment of innate cellular immunity

Delayed-type hypersensitivity reaction: Delayed-type hypersensitivity reaction was carried out using the delayed foot pad reaction (DFR) method described by Xhu et al. (1999). Forty chickens from each group were used for this experiment. Each group was further divided into 2 subgroups of 20 chickens each and were reared in separate confinements. Chickens in subgroup A of each group were sensitized at 3 and 4 weeks of age by subcutaneous injection of 0.2 ml of killed S aureus (150±g/bird) diluted 1:1 (vol:vol) with polyethylene glycol (PEG) at the neck region while subgroup B were administered 0.2 ml of PEG only and served as control groups. At 6 weeks of age, both sensitized and control birds of the different groups were injected intradermally at the right footpad with 0.1ml of killed S aureus (75±g/bird) diluted (1:1) with PBS as the eliciting challenge. The left foot pads were injected with 0.1ml of PBS alone. The thickness of both footpads at the site of challenge were measured to the nearest 0.01mm using a digital Venier caliper at the time of challenge i.e. 0 hour and at 4, 12, 24, 48 and 72 hours post challenge. Difference in thickness of the foot pads between the right and left footpads of each chicken was referred to as DFR. Results obtained from the Nera pullets and cockerels groups were also pooled to represent the fifth (MT) group.

Histopathology: Representative chickens from the different subgroups were euthanized in CO2 chamber, their footpads were excised and fixed in 10% formalin. Tissue sections were cut at 5 ±m, stained with haematoxylin-eosin and evaluated by light microscopy.

Statistical Analysis

Comparison of mean DFR values obtained from sensitized and unsensitized subgroups of each group of chickens was made using the Student’s t-test while mean DFR values obtained from all sensitized groups and mean agglutination and lysis titers were compared using the Least significant difference (LSD) method of Multiple comparison.

Results

Mean agglutination and lysis titers of broilers, pullets, cockerels, MT and indigenous chickens groups are presented in Table 1. While the pullets and the indigenous chickens had the highest agglutination titers of 7.5 ± 0.75 and 7.5 ± 0.62 respectively, which were significantly higher (p<0.05) than 4.2 ± 0.17 obtained for the broiler group and 5.6 ± 0.88 obtained for the cockerels, the MT group had an agglutination titer of 6.55 ± 0.61 which was

<table>
<thead>
<tr>
<th>Chicken Types</th>
<th>Agglutination titer (log2)</th>
<th>Lysis titer (log2)</th>
<th>Lysis / Agglutination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broilers</td>
<td>4.2 ± 0.17c</td>
<td>2.8 ± 0.09b</td>
<td>67</td>
</tr>
<tr>
<td>Pullets</td>
<td>7.5 ± 0.75a</td>
<td>3.0 ± 0a</td>
<td>40</td>
</tr>
<tr>
<td>Cockerels</td>
<td>5.6 ± 0.88b</td>
<td>2.7 ± 0.15b</td>
<td>48</td>
</tr>
<tr>
<td>Mixed Types (MT)</td>
<td>6.6 ± 0.61ab</td>
<td>2.9 ± 0.07b</td>
<td>44</td>
</tr>
<tr>
<td>Indigenous</td>
<td>7.5 ± 0.62a</td>
<td>3.3 ± 0.21a</td>
<td>44</td>
</tr>
</tbody>
</table>

* Figures in the same column with different superscript are statistically significantly different (p<0.05)
significantly higher (p<0.05) than the values obtained for broilers but not significantly higher than that of the cockerels. Also, the highest lysis titer of 3.3 Ø 0.21 was obtained for the indigenous chickens group which was significantly higher (p<0.05) than those of broilers, cockerels and MT groups. The amount of complement (lysis) relative to NAbs (agglutination) ranged from 40% to 67% in all the groups.

Assessment of DFR in all groups showed higher values in all sensitized sub-groups than in unsensitized control sub-groups which were mostly significant (p<0.05). In sensitized broilers, DFR increased from a mean value of 0.06 Ø 0.01 mm immediately post challenge (pc) to a maximum of 2.45 Ø 0.23 mm at 24 hours pc and decreased to 1.48 Ø 0.13 mm at 72 hours pc. In pullets and cockerels, DFR increased from 0.3 Ø 0.06 mm and 0.3 Ø 0.08 mm respectively immediately pc to peaks of 1.66 Ø 0.23 mm and 1.47 Ø 0.25 mm respectively at 48 hours pc and later decreased to 1.02 Ø 0.17 mm and 0.75 Ø 0.14 mm respectively at 72 hours pc. The indigenous chickens group showed an increase in DFR from 0.29 Ø 0.033 mm immediately pc to a maximum of 1.96 Ø 0.13 mm at 24 hours pc decreasing to 0.69 Ø 0.11 mm at 72 hours pc. The MT group showed a maximum DFR of 1.55 Ø 0.17 mm at 48 hours pc (Figures 1 and 2). DFR value obtained immediately pc for the broilers was significantly lower (p<0.05) than the values from the other four groups. At 4 hours pc DFR value obtained for the cockerels was significantly lower (p<0.05) than those of the broilers, pullets and indigenous group but not that of the mixed types group. At 12 and 24 hours pc, broilers and indigenous chickens had significantly higher (p<0.05) DFR than pullets, cockerels and MT group while at 72 hours pc broilers had significantly higher value than the other four groups.

Sections of the right footpads of sensitized sub-groups particularly the indigenous chickens revealed mild oedema, congested blood vessels as well as marked perivascular infiltration with mononuclear cells which comprised mostly lymphocytes, plasma cells and macrophages. The adipose cushion, dermis and connective tissue layer were also infiltrated. Sections of the left footpads of sensitized chickens and both footpads of unsensitized sub-groups showed moderate to no mononuclear cell infiltration.

**Discussion**

Innate humoral and cellular immunity in Arbor acres broilers, Nera pullets and cockerels as well as the Nigerian indigenous chickens were assessed and compared in this study. In hemolysis-haemagglutination assay, agglutination reflects the presence and titer of NAbs whereas lysis reflects the interaction of complement and NAbs (Matson et al. 2005). Thus, agglutination titers of 7.5 Ø 0.75 and 7.5 Ø 0.62 and the lysis titers of 3.0 Ø 0.0 and 3.3 Ø 0.21 obtained for Nera pullets and indigenous chickens respectively which were significantly higher (p<0.05) than the titers obtained for broilers and cockerels shows higher NAbs and complement titers in these groups. Although the agglutination titer of 6.6 Ø 0.61 obtained for the Nera MT group was not significantly different (p>0.05) from those of pullets and indigenous chickens, the titer is lower and could be significant with regards to immunocompetence. Also, the lysis titer of 2.9 Ø 0.07 obtained for Nera MT group which was significantly lower than those of Nera pullets and indigenous chickens groups shows lower complement level in this group. It was observed that lysis titers were always lower than agglutination titers which indicate that immunoglobulin was not limiting for the measurement of complement levels (Matson et al., 2005) since NAbs are responsible for initiating the complement enzyme cascade, which ends in cell lysis (Carroll and Prodeus, 1998). The amount of complement relative to NAbs was observed to vary ranging from 40% in pullets to 67% in broilers. Matson et al. (2005) suggested that differences in pattern of immune defenses might reflect differences in life history and ecology of species.

It should be noted that broilers are basically meat type chickens and therefore different from the other groups which are reared for meat and eggs. Thus, the results obtained for broilers cannot be compared with the other groups. A combination of pullets and cockerels best simulates the Nigerian indigenous chicken which has the male and female being reared together for the purpose of meat and egg yield. With regards to assessment of NAbs and complement components of innate humoral immunity, higher levels were observed in the Nigerian indigenous chickens than the Nera breed (MT). Earlier workers have observed that chickens artificially selected for high and low primary antibody responses exhibited parallel changes in NAb levels and disease resistance (Pinard et al., 1993; Permentia et al., 2001; 2004). Thus the higher level of IBD virus antibody observed by Oladele et al.(2007a) in Nigerian indigenous chickens than exotic chickens is in concurrence with the higher level of NAb observed in this study. Significantly higher NAb and complement levels observed in the pullets than the cockerels represents sex variation in disease resistance or susceptibility in chickens which requires further investigation.
Figure 1: Delayed footpad reaction in exotic breeds/types and Nigerian indigenous chickens

Figure 2: Delayed footpad reaction in Nera Mixed Types and Nigerian indigenous chickens
The results of elicitation of DFR in this study showed maximum response to S.aureus antigen at 24 hours pc in broilers and indigenous chickens and 48 hours pc in pullets, cockerels and MT groups. Earlier studies in chickens have shown that maximum response in delayed wattle reaction (Toubler, 1968; Cotter et al., 1987) and delayed-footpad reaction (Oladele et al., 2007b) occurred 24 to 48 hours pc which are in agreement with the findings of this study. Comparison of the responses in indigenous chickens and MT group showed not only an earlier response in the indigenous chickens but also a more intense response as reflected by a higher DWR value of 1.96 ± 0.13 mm. Histopathological examination of footpads showed a more severe reaction in the indigenous chickens compared with the other groups. The marked lymphocytic infiltration at challenge site is one of the most characteristic features of the DTH reaction (Anderson, 1971; Klessius et al., 1977; Stites, 1994).

The results of this study showed higher levels of NAbs and complement as well as a faster and more intense DTH reaction in the Nigerian indigenous chickens than the exotic Nera breed which is an indication of a more efficient innate humoral and cellular immunity in this breed of chickens. This study has, to some extent, substantiated the assumption that the Nigerian indigenous breed is relatively more resistant to diseases. As earlier suggested by Matson et al. (2005) life history and ecology might play important roles in these findings apart from genetics. However, it is necessary to conduct more in-depth studies to further characterize the innate immunity of the Nigerian indigenous chickens for scientific justification of its intensive production for commercial purpose.

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**References**


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**International Award For Veterinary Researcher**

Dr Dan Tucker, Senior lecture in veterinary public health at Cambridge Veterinary School, was recently named as the winner of the 2009 Dieter Lattice award. The award, worth 20,000 pounds, recognizes the development of alternative to animal testing for veterinary medicines.

Dr Tucker was chosen for his development of physiologically relevant *in vitro* bovine respiratory organ culture system. Working in collaboration with Duncan Maskell, also of Cambridge Veterinary school, and Josh Slater of the Royal Veterinary College, Dr Tucker has developed a model that allows host-pathogen interactions to be analyzed after either single or mixed infections with *Mannheimia haemolytica* and bovine herpesvirus type 1. The model has replaced the use of animals in some studies of respiratory disease and could be used in developing new vaccines.