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Original Article

In-vitro Quantitative Assay of Interferon Gamma in Serum of Nigerian Indigenous and Exotic Breeds of Chickens

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ABSTRACT

The Nigerian Indigenous breeds of Chicken (NIC) have thrived in harsh tropical environment with little veterinary care and poor nutrition compared with the introduced exotic breeds which performs sub-optimally in the tropics. However, they receive little attention for commercial production in spite of low input required. A comparative assessment of cellular immune response of the indigenous and exotic breeds was carried out to provide scientific explanation for their hardy nature and justify production for economic purposes. Fifteen chickens from each of three indigenous breeds i.e. Frizzled- feathered, Naked-neck and Smooth-feathered, and 8 Isa Brown pullets were 10 weeks old and reared in separate cages. The chickens were stabilized and administered Newcastle Disease Vaccine (NDV), LaSota strain. At 14 and 16 weeks old, all breeds were administered NDV Komarov strain in Freund's adjuvant and in PBS intramuscularly as sensitizing and challenge inoculants, respectively. They were bled for serum 5 days later and concentrations of Interferon-gamma (IFN-gamma) were determined using competitive Enzyme-linked immunosorbent assay. Results showed that the Frizzled-feathered chickens had the highest concentration of IFN-gamma (58±2.8 pg/ml) which was significantly higher than 49±3.2 pg/ml and 44±2.5 pg/ml recorded for Smooth-feathered and Isa brown breeds respectively. Also, concentration in Naked-neck breed was 54±2.9 pg/ml, which was significantly higher than Isa Brown. Isa Brown had the significantly lowest concentration. It was concluded that the three NIC studied, have inherent capacity to mount higher levels of cellular immune response compared with the exotic Isa brown, when challenged.

Keywords: Cellular Immunity, ELISA, Exotic Breed, Interferon-Gamma, Nigerian Indigenous Chickens.

INTRODUCTION

Indigenous poultry are found in rural areas of tropical and sub-tropical countries where they are reared by the rural poor. The Nigerian indigenous poultry constitute eighty-four per cent (FDLPCS, 2006) of the over 192 million poultry population in Nigeria (NBS, 2010). According to the extrapolation of Adene and Oguntade (2006) about eighty per cent of the indigenous poultry population are chickens which are of different breeds. These indigenous breeds of chicken are economically, nutritionally and socially important to the rural and peri-urban dwellers where they mostly exist. Although productivity with regards to eggs and meat per bird per annum is below that of the exotic commercial layers and broilers breeds, respectively: they are able to survive on scavenging in the harsh environment of the tropics where temperature and relative humidity are mostly high and uncomfortable (Oladele et al., 2010). The Nigerian indigenous chickens are believed to be more tolerant to diseases than their exotic counterparts (Akinokun, 1990; Fayeye et al., 2006). They are known to be susceptible to common diseases of poultry such as Newcastle disease, Coccidiosis, Fowl pox, etc., but have thrived commendably in the face of these epizootics (Adene, 1989; Nwosu, 1990). Considering the capability of the indigenous chicken to thrive in the tropics with little input compared with the exotic breeds of chickens which perform sub-optimally due to the harsh weather conditions, it becomes imperative to verify the speculated disease tolerant trait of the indigenous chickens in order to encourage production in commercial quantities.

Interferon-gamma (IFN-gamma) is a multifunc-tional protein initially believed to

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interfere with viral replication (Isaacs and Lindenmann, 1957) but now known to regulate several aspects of the immune response, stimulates bactericidal activity of phagocytes and stimulates antigen presentation through class I and class II Major Histocompatibility Complex (MHC) molecules amongst other activities (Boehm et al., 1997). IFN-gamma was earlier reported to be secreted exclusively by CD4+ T-helper lymphocytes, CD8+ cytotoxic lymphocytes and Natural Killer (NK) cells (Young, 1996; Bach et al., 1997) but there are later evidences of its secretion by B lymphocytes, NKT cells and antigen presenting cells (Gessani and Belardelli, 1998; Flaishon et al., 2000; Jonasch and Haluska, 2001). IFN-gamma secreted by NK cells and probably Antigen Presenting Cells (APCs) could be important in early host defence against infection whereas secretion from T lymphocytes is essential in adaptive immune response (Frucht et al.,2001; Sen, 2001). Assay of IFN-gamma following antigen stimulation is therefore a logical means of assessing cellular immune response and could be employed in characterizing the immune status of the Nigerian indigenous breeds chicken.

This principle was therefore employed in the characterization of the cellular immunity of the different breeds of indigenous chickens in Nigeria and compared the findings with an improved exotic breed.

MATERIAL AND METHODS

Fifteen adult chickens from each of Frizzled-feathered, Naked-neck, Smooth-feathered breeds of Nigerian indigenous chickens and 8 adult exotic Isa brown chickens were used for this experiment. They were acquired as growers at about 10 weeks of age and reared on deep litter in the experimental pen of the Poultry Diseases unit, Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. Feed and water were provided ad libitum. The chickens were treated prophylactically for coccidiosis and helminthosis.

Newcastle disease vaccine LaSota strain was administered orally to the chickens at the time of acquisition, to prime the immune system. After four weeks, one sensitizing dose of inactivated Komarov strain vaccine (Izovac: Izo S.P.A., Italy) mixed 1:1 in Freund's adjuvant was administered intramuscularly followed by a challenge dose in Phosphate buffered saline 2 weeks later. The chickens were bled via the brachial vein 5 days after challenge. Blood was allowed to clot at room temperature and serum was harvested for IFN-gamma assay using a competitive ELISA sskit (NOVATEINBIO Incorporation, USA) as described by Lambrecht et al. (2000).

One hundred µl of the serum sample undiluted was dispensed into each well of the microtiter plate already pre-coated with capture antibody. IFN-gamma standards provided in the kit were also included. Fifty µl of the conjugate (IFN-gamma: Horse-radish peroxidase) was also added to all the wells, this was mixed properly and covered with adhesive before incubating for one hour at 37ºC. The plate was washed manually five times and blot-dried using absorbent paper. Fifty µl each of chromogenic substrate A and B as provided by the manufacturer were added and incubated for 10 minutes at 20ºC after which stop solution was added to stop the reaction. The Optical Density (OD) of reaction solutions were read at 450nm using ELISA reader (Optic Ivymen® system 2100-c).

Concentrations of IFN-gamma in serum samples were derived from the standard curve generated from the IFN-gamma standards provided. Mean concentration for each group was calculated and comparison was made between groups for statistical significance differences using Analysis of Variance (ANOVA) and Dunnett's test of multiple comparisons at $P < 0.05$.

RESULTS AND DISCUSSION

The Frizzled-feathered chickens had the highest mean concentration of IFN-gamma (58±2.8 pg/ml) which was significantly higher than 49±3.2 pg/ml and 44±2.5 pg/ml recorded for the Smooth-feathered and Isa brown breeds respectively ($P<0.05$) (Figure 1). Also, the mean concentration in the Naked-neck breed i.e. 54 ± 2.9 pg/ml was significantly higher than that of Isa Brown (Figure 1). The Isa Brown breed had the lowest value which was statistically significant ($P<0.05$).

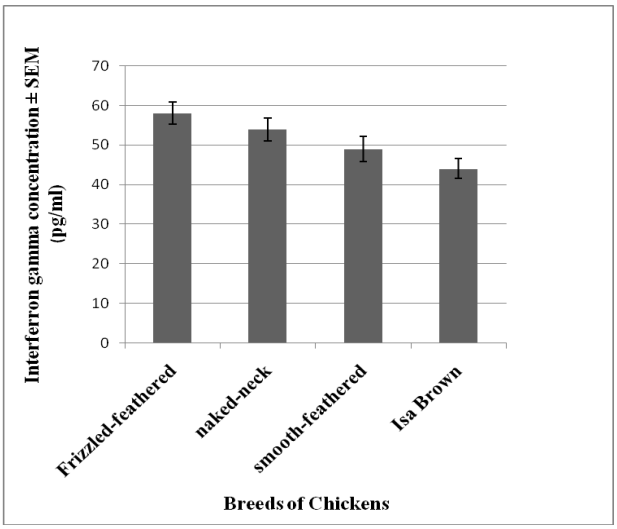

Figure1: Interferon gamma concentrations in Nigerian indigenous and exotic chicken breeds

Results showed that all the three indigenous breeds of chickens had higher concentrations of IFN-gamma than the exotic Isa Brown breed, the difference being statistically significant ($P<0.05$) with the Frizzled-feathered and Naked-neck breeds. This finding corroborates an earlier report by Oladele et al. (2010) that a faster and more intense delayed-type hypersensitivity reaction was observed in the Nigerian indigenous chickens compared with exotic breeds. CD4+ T-helper

lymphocytes and CD8+ cytotoxic lymphocytes have been identified as major players in the secretion of IFN-gamma in the body (Young, 1996; Bach et al., 1997) with attendant effects on adaptive immune response (Frucht et al., 2001; Sen, 2001). The results of this study therefore shows a clear evidence of relatively superior immunocompetence by the indigenous breeds which might account for their disease resistance trait as advanced by some earlier workers (Aire, 1973; Adene, 1990; Akinokun, 1990; Fayeye et al., 2006). Also, a report of significantly higher antibody response to experimental infection with infectious bursal disease virus by the Nigerian indigenous chickens in comparison to an exotic breed had earlier been published (Oladele et al., 2007).

Comparing the indigenous breeds, the Frizzled-feathered chickens had the highest concentration of IFN-gamma (58±2.8 pg/ml) while 54±2.9 pg/ml and 49±3.2 pg/ml were recorded for the naked neck and smooth-feathered, respectively. The feather structure and the feather distribution genes i.e. frizzle (F) and naked (Na) genes respectively, have been associated with the ability to cope with heat stress due to improvement in convectional heat loss (Ajayi, 2010). These genes have been linked to superior production characters in the tropics such as body weight, age at attainment of sexual maturity and egg production in number and weight (Horst, 1988; 1989; Mathur and Horst, 1990; Ibe, 1993). Heat stress has been reported to reduce immunocompetence thereby increasing disease susceptibility in animals (Aggarwall and Upadhyay, 2013). Thus, the higher concentrations of serum IFN-gamma in the Frizzled-feathered and naked-neck breeds compared with the Smooth-feathered breed is an indication of superiority in immunocompetence even with the indigenous breeds.

This study has shown that the three Nigerian indigenous breeds of chickens studied, have inherent capacity to mount higher levels of cellular immune response compared with the exotic Isa brown, when challenged. This fact is a valuable justification for the commercial production of the Nigerian indigenous breeds of chicken even in the face of prevailing diseases and environmental challenges.

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