

Determination of Immune Response Traits in Nigerian Indigenous Chickens Challenged with Newcastle Inactive Antigen

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ABSTRACT

Background and Objective: The selection of genotypes and choice of breeding programmes are pivotal determining tools for the improvement and sustain ability of poultry production. This study determines the immune response of Newcastle inactive antigen with immunological traits in the Nigerian Indigenous Chicken (NICs). **Materials and Methods:** Seventy-six chicks consisting of normal feathered high titre, normal feathered low titre, frizzle feathered high titre, frizzle feathered low titre, naked-neck high titre and naked-neck low titre were used to study the immune response traits after being challenged with Newcastle disease vaccine (LaSota) on 7th post challenged. Blood was collected on the 14th, 21th and 28th days Post-Inoculation (PI) to investigate haemagglutination inhibition. Blood samples collected on the 28th day were used for haematological and biochemical parameters. The data collected were analyzed using the general linear model in SAS 9.2. **Results:** Genotype-antibody titre and sex had significant effects ($p < 0.05$) on haemagglutination inhibition at 14th, 21st and 28th days PI. Frizzle feathered high titre, naked-neck high titre and normal feathered high titre chicks (6.95 ± 0.30 , 7.33 ± 1.45 and 7.71 ± 0.47), respectively had higher haemagglutination inhibition than normal feathered low titre chicks across the days of post-inoculation. Haematological parameters were not affected ($p > 0.05$) by genotype-antibody titre and sex except for eosinophils and white blood cells, while biochemical indices were not ($p > 0.05$) affected by genotype-antibody titre and sex on the 28th day old. **Conclusion:** The present study establishes reference values that may contribute to the assessment of chicken health for selection in the breeding programme.

KEYWORDS

Biochemical indices, immune response trait, haemagglutination inhibition, haematological parameters, newcastle disease vaccine, Nigerian indigenous chickens, post inoculation

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INTRODUCTION

In Nigeria, the major genes of indigenous chickens are widely distributed in the rural areas, where they are kept by the natives, principally as a source of protein and income. There are three major genes associated with feather structure in Nigerian indigenous chickens, these are naked-neck, normal and frizzle feather chickens¹. The local chicken has unique adaptive features such as small body size, multicoloured plumage, presence of major genes affecting feather structure and distribution, which predisposes it to

adapt to the local environment better than its exotic counterpart². Tropical adaptation is defined as an animal's ability to survive, grow and reproduce in the presence of endemic stressors of the tropical environment. The concept of adaptability implies that phenotype performance expresses an animal's true genetic capability in its ability to cope with environmental stresses such as heat stress and disease parasites³.

Newcastle disease is a highly contagious and commonly fatal viral poultry disease affecting domesticated and wild avian species⁴. The disease affects birds of all ages. These authors Esaki *et al.*⁵ and Okwor *et al.*⁶ stated that Newcastle Disease (ND) poses serious public health and economic challenges to both commercial and smallholder poultry farmers globally continuously. Reports on seasonal prevalence of Newcastle disease are not uniform as the prevalence may vary depending on the environmental, nutritional and socio-economic condition under which the birds are managed^{7,8}.

The causal agent is the virulent strains of avian paramyxovirus-1, which is a single-stranded non-segmented negative-sense RNA virus. The virus belongs to the family paramyxoviridae and it has ten serotypes designated as APMV-1 to APMV-10^{8,9}. To prevent the disease, various vaccination programmes have been designed depending on the location resulting in varying degrees of success. Newcastle disease symptoms include the following, weight loss, sneezing, paralysis, poor feed conversion ratio and diarrhoea. Current preventive measures predominantly include chemotherapy, conventional vaccines and other in-feed medications. The use of inactivated or attenuated vaccination has been a routine practice. An enhanced capacity to resist infection is inherited and can be passed from parent to offspring usually as a dominant trait¹⁰.

In the face of the development of drug and chemical resistance and increased medication costs, it becomes a challenge for poultry producers to maintain profits. It is estimated that medication costs alone for controlling Newcastle disease may be as high as ₦127 million annually in Nigeria¹¹. Therefore, to economize production and confer long-term disease protection, alternative and sustainable methods of prevention are constantly being sought¹¹⁻¹³. Hence, selecting chickens most resistant to the development of diseases such as Newcastle disease will decrease costs of production and need to be included in the breeding programme.

Therefore, this study focused on assessing the response of the three genotypes of the Nigerian local chicken to the challenge of Newcastle inactive antigen and expands knowledge of the impact of genetics on Newcastle disease resistance by illuminating mechanism regulating the expression of genes related to immune response.

MATERIALS AND METHODS

Experimental site: This experiment was carried out at the Poultry Breeding Unit of the Directorate of University Farms (DUFARMS) of the Federal University of Agriculture Abeokuta Ogun State, Nigeria, Department of Animal Breeding and Genetics Laboratory, FUNAAB and Department of Physiology and Pharmacology Laboratory, FUNAAB.

Two chicken lines, high antibody titre (HAT) (≥ 9) and low antibody titre (LAT) (≥ 7) which originated from Nigerian indigenous chickens, which went through four generations of selection since 2014 based on the response to Sheep Red Blood Cell (SRBC) at 7 weeks of age were used. Seventy-six chicks were selected at random from the total of chicks hatched. The chicks from each genotype were brooded for 5 days before being transferred to separate pens based on genotype and antibody titre. The chickens were managed on a deep litter system in pens having natural ventilation. The separation of the chicks gave six classes: Normal feathered high titre, normal feathered low titre, frizzle feathered high titre, frizzle feathered low titre, naked-neck high titre and naked-neck low titre.

The chicks were challenged with Newcastle disease vaccine 'LaSota' (Lentogenic strain) attenuated freeze-dried which contains $\geq 10^6$ EID₅₀ units Ranikhet Disease LaSota strain virus per dose, manufactured by BIO-MED private limited India, were obtained from the Veterinary Diagnostic Service Shop. The birds were inoculated orally with attenuated vaccine as an antigen according to the manufacturer's instruction for immune response at the 7th day post-challenge. On 14th, 21st and 28th days post-inoculation, the Haemagglutination Inhibition (HI) test was used for detection of the presence of antibodies against Newcastle disease according to the Office International Epizootics. Blood samples were collected from the brachial vein of all the chicks into plain bottles to harvest serum for haemagglutination inhibition test and biochemical indices. On 28 days blood samples were also collected inside plain bottles and EDTA bottles (ethylene diamine tetraacetic acid) for haematological and biochemical parameters:

- **Haematological parameters:** Packed Cell Volume (PCV), Red Blood Cells (RBC), Haemoglobin Concentration (HB), the White Blood Cells Count (WBC), Heterophils (HET), Lymphocytes (LYM), Monocytes (MONO), Eosinophil (EOS), Basophil (BOS)
- **Biochemical indices:** Total Protein (TP), Albumin (ALB), Globulin (GLO), Cholesterol (CHOL), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP)

The data for the haematological and biochemical parameters were collected and transformed with square root (PCV, HET, LYM, EOS, BOS and MONO) and logarithm (Hb, RBC and WBC) to follow the normal distribution curve.

Statistical analysis: Data generated were subjected to statistical analysis using SAS 9.2 software and means were separated using Tukey-Kramer's Honest Significant Difference. The differences were considered to be significant at $p < 0.05$:

$$Y_{ijk} = \mu + G_i + S_j + e_{ij}$$

Where:

Y_{ijk} = Observed values of immune response variables

μ = Overall mean

G_i = Fixed effect of i th genotype-antibody titre (NkL, NkH, NmH, NmL, FL and FH)

S_j = Fixed effects of j th sex (male and female)

e_{ij} = Residual error

The preliminary analysis was carried out and the effect of interaction was not significant. Hence it was removed from further analysis.

RESULTS

Genotype-antibody titre and sex effect on the immune response to Newcastle disease vaccine after challenged: The effect of genotype-antibody titre and sex on the immune response to Newcastle disease vaccine after challenged at 14, 21 and 28 days post-inoculation (PI) is shown in Table 1. Immune response to Newcastle disease vaccine was significantly ($p < 0.05$) affected by genotype-antibody titre and sex of the chicks.

Antibody-titre values for frizzle feathered high titre and normal feathered high titre chicks (6.95 ± 0.30 and 6.94 ± 0.47) were significantly different from normal feathered low titre (4.57 ± 0.41). However, there was no significant difference among frizzle feathered low titre, naked-neck low titre and naked-neck feathered high titre at 14 days PI. Naked-neck high titre and frizzle feathered low titre were significantly higher (7.33 ± 1.45 , 7.08 ± 0.34) than normal feathered low titre (4.81 ± 0.25). However, there was no significant

Table 1: Genotype-antibody titre and sex effect on immune response to Newcastle disease vaccine after challenged

Genotype-antibody titre	N	Antibody immune response (GMT)		
		Day 14	Day 21	Day 28
Frizzle feathered high	19	6.95±0.30 ^a	6.47±0.16 ^{ab}	7.42±0.31 ^{ab}
Frizzle feathered low	12	6.75±0.39 ^{ab}	7.08±0.34 ^a	7.42±0.34 ^{ab}
Normal feathered high	17	6.94±0.47 ^a	6.82±0.46 ^{ab}	7.71±0.47 ^a
Normal feathered low	21	4.57±0.41 ^b	4.81±0.25 ^b	5.52±0.45 ^b
Naked-neck high	3	6.67±0.88 ^{ab}	7.33±1.45 ^a	7.33±1.45 ^{ab}
Naked-neck low	4	6.65±0.63 ^{ab}	6.25±0.75 ^{ab}	7.00±0.71 ^{ab}
Sex				
Female	43	6.70±0.22 ^a	6.66±0.22 ^a	7.52±0.26 ^a
Male	33	5.59±0.40 ^b	5.59±0.28 ^b	6.12±0.33 ^b

N: Number of observations, GMT: Geometric mean time, ^{a,b}Means with different superscripts in the same column for the same age are significantly different ($p < 0.05$)

Table 2: Genotype-antibody titre and sex effect on haematological parameters after challenged at 28 days

Genotype-antibody titre	N	PCV (%)	RBC	WBC	HB	HET (%)	LYM (%)	EOS (%)	BAS (%)	MONO (%)
			($10^9/\mu\text{m}$)	($10^9/\mu\text{m}$)	(g/100 mL)					
Frizzle feathered high	19	4.86±0.23	0.28±0.04	1.09±0.02	8.40±0.68	5.83±0.12	7.95±0.08	0.51±0.16 ^{ab}	0.74±0.17	0.71±0.16
Frizzle feathered low	12	5.35±0.23	0.38±0.02	1.11±0.02	9.89±0.76	5.55±0.14	8.09±0.08	0.82±0.15 ^{ab}	1.07±0.16	0.88±0.16
Normal feathered high	17	5.43±0.09	0.37±0.02	1.12±0.03	9.93±0.36	5.79±0.14	7.86±0.09	1.02±0.14 ^a	1.08±0.11	1.09±0.11
Normal feathered low	21	5.08±0.14	0.34±0.23	1.08±0.02	8.95±0.43	5.39±0.13	8.13±0.09	1.05±0.12 ^a	1.04±0.12	1.08±0.14
Naked-neck high	3	5.15±0.51	0.29±0.08	1.12±0.02	8.85±1.64	5.23±0.24	8.28±0.19	0.60±0.36 ^{ab}	0.85±0.30	1.22±0.41
Naked-neck low	4	4.87±0.79	0.26±0.15	1.15±0.06	8.57±2.16	5.86±0.14	7.87±0.09	0.33±0.33 ^b	1.28±0.14	1.28±0.14
Sex										
Female	43	5.14±0.12	0.32±0.02	1.12±0.01	9.10±0.36	5.68±0.09	7.98±0.06	0.89±0.09	0.78±0.09	1.02±0.08
Male	33	5.17±0.14	0.35±0.02	1.07±0.01	9.33±0.41	5.55±0.09	8.05±0.07	1.11±0.09	0.90±0.09	0.94±0.12

^{a,b}Means with different superscripts in the same column within variable groups are significantly different ($p < 0.05$), N: Number of observation, PCV: Packed cell volume, RBC: Red blood cell, WBC: White blood cell, HB: Haemoglobin concentration, HET: Heterophils, LYM: Lymphocytes, MONO: Monocytes, EOS: Eosinophils and BAS: Basophils

difference among frizzle feathered high titre, naked-neck feathered low titre and normal feathered high titre had antibody immune response at 21 days PI. On 28 days PI, normal feathered high titre (7.71 ± 0.47) had a higher antibody immune response than normal feathered low titre (5.52 ± 0.45) chicks. However, there was no significant difference among frizzle feathered high titre, frizzle feathered low titre, naked-neck high titre and naked-neck low titre. Female chicks had higher values than male counterparts across the ages.

Genotype-antibody titre and sex effect on haematological parameters after challenged at 28 days:

Effect of genotype-antibody titre and sex on haematological parameters (PCV, RBC, ESR, WBC, HB, HET, LYM, MONO, EOS and BAS) of three genotypes of Nigerian indigenous chicken at 28 days after challenge with Newcastle disease vaccine is presented in Table 2. The analysis of variance showed that genotype-antibody titre had no significant ($p > 0.05$) effect on haematological parameters except Eosinophils (EOS). Normal feathered high titre and normal feathered low titre (1.05 ± 0.12 , $1.02 \pm 0.14\%$) were significantly different from naked-neck low titre ($0.33 \pm 0.33\%$) chickens. However, there was no significant difference among frizzle feathered high titre, frizzle feathered low titre and naked-neck high titre. Naked-neck low titre recorded the lower values for RBC and EOS ($0.26 \pm 0.15 \mu\text{m}$, $0.33 \pm 0.33\%$), respectively, frizzle feathered high titre and naked-neck low titre had lower values for PCV (4.86 ± 0.23 , $4.87 \pm 0.79\%$), respectively, frizzle feathered high titre had lower value for HB ($8.40 \pm 0.68 \text{ g mL}^{-1}$), while frizzle feathered high titre had the least values for MONO and BAS (0.74 ± 0.17 , $0.71 \pm 0.16\%$), respectively among the genotype-antibody titre.

Sex had no significant influence ($p > 0.05$) on all the haematological parameters except for WBC. Female chickens ($1.12 \pm 0.01 \mu\text{m}$) were significantly different from male chickens ($1.07 \pm 0.01 \mu\text{m}$). Female chicks recorded higher values for WBC and HET ($1.12 \pm 0.01 \mu\text{m}$, $5.68 \pm 0.09\%$), respectively.

Genotype-antibody titre and sex effect on serum biochemical indices after challenged at 28 days:

The least-squares means with their corresponding standard error for the serum biochemical indices

Table 3: Genotype-antibody titre and sex effect on serum biochemical indices after challenged at 28 days

Genotype-antibody titre	N	TP (g/100 mL)	ALB (g/100 mL)	GLOB (g/100 mL)	CHOL (g/100 mL)	ALP (g/100 mL)	AST (g/100 mL)	ALT (g/100 mL)
Frizzle feathered high	19	4.66±0.31	3.04±0.22	1.61±0.16	86.96±7.36	29.28±1.34	100.61±9.25	25.83±3.86
Frizzle feathered low	12	5.03±0.59	2.67±0.30	2.35±0.39	95.49±8.91	26.36±1.98	81.27±12.80	21.82±4.17
Normal feathered high	17	5.53±0.31	3.37±0.17	2.16±0.26	104.19±5.39	28.32±1.46	92.37±5.87	21.89±2.40
Normal feathered low	21	5.79±0.31	3.37±0.15	2.68±0.17	104.06±5.00	24.85±1.24	106.75±4.24	17.80±1.58
Naked-neck high	3	6.51±0.76	3.38±0.14	2.68±0.62	100.85±10.76	22.75±0.25	115.50±5.20	16.75±2.56
Naked-neck low	4	5.50±0.68	3.65±0.44	1.85±0.31	98.90±19.79	29.75±2.39	108.50±13.79	26.00±2.71
Sex								
Female	43	5.42±0.24	3.21±0.13	2.21±0.17	99.15±4.34	27.30±0.76	96.82±4.37	23.16±1.93
Male	33	5.36±0.25	3.05±0.14	2.31±0.17	97.28±4.36	26.91±1.27	100.97±6.02	19.66±1.77

N: Number of observation, TP: Total Protein, ALB: Albumin, GLO: Globulin, CHOL: Cholesterol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase and ALP: Alkaline phosphatase

namely, total protein, albumin, globulin, cholesterol, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) as shown in Table 3. The analysis of variance showed that genotype-antibody titre and sex had no significant ($p > 0.05$) effect on the serum biochemical indices studied.

Naked-neck high titre had a higher value, while frizzle feathered high titre had the least value for TP (6.51 ± 0.76 , 4.66 ± 0.31 g mL⁻¹), respectively. Naked-neck low titre had a higher value, while frizzle feathered low had the least value for ALB (3.65 ± 0.44 , 2.67 ± 0.30 g mL⁻¹), respectively.

Normal feathered high titre and normal feathered low titre had the greater values, while frizzle feathered high titre had lower values (104.19 ± 5.39 , 104.06 ± 5.00 and 86.96 ± 7.36 g mL⁻¹), respectively for CHOL. Naked-neck high titre had the highest value, while frizzle feathered low titre had a lower value (115.50 ± 5.20 and 81.27 ± 12.80 g mL⁻¹), respectively for AST. Naked-neck low titre had the highest value, while naked-neck high titre had the least value (26.00 ± 2.71 and 16.75 ± 2.56 g mL⁻¹), respectively for ALT.

DISCUSSION

The immune response of the genotype-antibody titre and sex observed in this study against the Newcastle disease vaccine had a significant effect on chicks of all the ages studied. The high immune response to Newcastle disease vaccines recorded in normal feathered high and frizzle feathered high chicks in this study differs from the findings of Pitcovski *et al.*¹⁴, who observed high antibody response in naked-neck chicks. The antibody titre mean values recorded at 21 days post-inoculation (PI) in this study were higher than the observed values recorded by Mpenda *et al.*¹⁵. Sex had significant effects on the immune response to Newcastle disease on chicks of all ages in this study. The findings of Pitcovski *et al.*¹⁴, showed that sex had no significant differences in response to Newcastle disease in chickens, this study showed that sex hormones appear to have a great influence on the immune system. Female chicks had a higher value than the male counterparts in this study, these as a result of sexual dimorphism in immune response in the animal, where females produce more vigorous humoral immune reactions, which are more resistant to certain infectious diseases. Antibody response to the same virus was different among chicken genotypes study according to Mpenda *et al.*¹⁵. The selection of chicken genotypes with high protective antibody titres may have a significant contribution in preventing Newcastle disease outbreaks in chicken populations¹⁶. These findings contribute to the development of chickens that have increased resistance to NDV.

The results obtained from this study on haematological parameters and serum blood indices values can be used for disease diagnosis, which can be used for developing new lines of chickens that are genetically able to resist different diseases. Sex, age and nutrition have been the major factors affecting avian haematology. The variation observed in this study may be as a result of genotypic differences since all the chicks were of the same age, haematological values obtained in the study among the genotype-antibody

titre for PCV, RBC, WBC, HB, HET, LYM, MONO, EOS and BAS were within the normal range proposed for Nigerian indigenous chickens¹⁷ even after being challenged with Newcastle disease vaccine. Genotype-antibody titre had no significant effect ($p > 0.05$) on all the haematological parameters except for EOS. Eosinophil and white blood cells observed were significantly different, this disagrees with the report of Musa *et al.*¹⁸. Decreases in eosinophil counts are associated with stress, steroid exposure and anything that may suppress White Blood Cell (WBC) production generally. The findings of Adeyemo *et al.*¹⁹ and Malik *et al.*²⁰ showed significant differences in all haematological parameters in the three Nigerian indigenous chickens and broilers challenged with Newcastle disease. The WBC could serve as a parameter indicating immunity and inherent resistance to tropical poultry diseases and parasites while haemoglobin values within the reference range of avian could be suitable traits for evaluating and improving the selected population of helmeted guinea fowl for feed conversion efficiency and adequate oxygen supply for maintenance of body function²¹. According to Falcone *et al.*²², lymphocytes which is a fraction of the white blood cells are natural killer cells and can kill cells of the body that do not display MHC class I molecules or display stress markers such as MHC class I polypeptide-related sequence A (MIC-A). The higher the lymphocytes the better and stronger the immune system of the animal, these cells play both an immediate and delayed role in response to infection or inflammation. Increased numbers of lymphocytes are associated with most viral infections and some bacterial infections whereas decreased numbers of lymphocytes are characterized by steroid exposure, some cancers, immunodeficiency and renal failure. The test for WBC levels is very important to detect and measure the ability of the body to destroy diseases. A high level of white blood cells may be due to immunological challenges, which may be largely informed by the associated inherent resistance to tropical diseases²². Haemoglobin concentration, basophils and lymphocytes values observed in this study were consistent with the findings of Malik *et al.*²⁰. Haematological parameters values recorded in this study could be due to the source of experimental birds, where they had undergone previous selection and improvement. Sex had no significant influence ($p > 0.05$) on all the haematological parameters except for WBC where females had the higher values. The findings of Adewole *et al.*²³, reported that sex had no significant influence on all haematological parameters except for red blood cell count that was significantly affected in broiler where the male had higher values. This result is consistent with those of Schmidt *et al.*²⁴, who reported that sex did not affect RBC, WBC and Hb. Similarly, sex did not affect PCV, Hb, WBC and RBC in yellow-legged gulls Garcia *et al.*²⁵ and helmeted guinea fowl Adedibu *et al.*²¹. However, other studies on indigenous chickens and helmeted guinea fowl found that sex affected haematological parameters. These authors Abdi-Hachesoo *et al.*²⁶ and Sanda *et al.*²⁷ attributed higher PCV, RBC and Hb in males than females to the increased courting of the females by the males during their breeding season which was when they bled. Reproductive hormones, such as androgen and oestrogen, depress erythropoiesis Oke *et al.*²⁸, whereas androgen and thyroxin stimulate erythropoiesis Pranoto and Nugrahalla²⁹. Thus, the insignificant effect of sex on haematological parameters in this study may be as a result of non-mating and separation of males from females. Therefore, the genotypes-antibody titre chicks investigated in this study can be successfully raised in any environment they find themselves without adverse effect on their health since their haematological values are compared favourably with standard reference values.

A limited study was done on the influence of vaccination against Newcastle disease on blood analysis and utilization of biochemical profiles in avian medicine. The measurement of serum enzymes activities is a very important diagnostic tool of different health disturbances in avian species. Serum analysis and antibody titre determination are the dependable segments of biochemistry and immunology to diagnose and predict a disease, infection and health status of the birds, depicting the pathophysiological response, helping the farmers in improving the productivity of their flocks and reducing the economic losses³⁰. The genotype-antibody titre and sex effect on serum biochemical indices had no significant effect on all the serum biochemical indices in this study. The lack of significant difference in the blood serum biochemical indices reported in this study is consistent with the report of Sola-Ojo *et al.*³¹, who reported no significant

difference among the serum metabolites in guinea fowl. Data obtained from this study showed that serum ALP and ALT (30.10-82.67, 43.94-60.29) were extremely low, while AST (16.58-49.88) were greater compared to the report of Al-Hussary and Kudair³² at 28 days in the broiler. The author also reported that there is a reduction in the serum biochemical indices by age. These changes correspond with the growth and may be attributed to differences in bone formation or physical role in general metabolic activity³³. The main cause for serum ALP reduction is the damage of the intestine³⁴. The predominant ALP isoenzyme in plasma originates in the gut³⁵. Appearances of abnormal amounts of certain enzymes of intercellular origin in the blood reflect damage to an organ or tissue³⁶. The liver is rich in some enzymes such as ALT and AST and its damage often results in releasing these enzymes to the blood³⁷. The challenge of NDV in the present work did not cause any significant changes in the levels of total protein, cholesterol and glucose. These findings are in agreement with those reported by Al-Hussary and Kudair³² and Silva *et al.*³⁸ for cholesterol and Wilson³⁶ for glucose. Inciting in the level of cholesterol in the chicks may reflect the intact liver function as it was postulated from the unalteration in the activity of ALT in this study. The level of albumin and globulin were not significantly influenced compared to the result of Al-Hussary and Kudair³² that reported that they were significantly different. Also, the data size for albumin is greater than those obtained by Al-Hussary and Kudair³² in this work. The findings of Odunitan-Wayas *et al.*³⁹ on Ovambo chickens reported that sex had a significant influence on the biochemical serum, the values obtained from this study were higher than the reported values of Ahmad *et al.*⁴⁰ in the chicken on the biochemical serum. Albumin, a serum protein is synthesized in the liver, it is responsible for transporting insoluble substances in the blood and aids to maintain oncotic pressure⁴¹. A higher concentration of ALB usually denotes dehydration while a lower concentration may be due to the liver not functioning adequately due to factors such as malnutrition and infection⁴². Total proteins in the female birds were higher than the male birds, these agree with the report of Simaraks *et al.*⁴³. This could be attributed to an oestrogen induced increase in globulin in preparation of the female bird's body for egg-laying⁴³. Liver enzymes, namely the alanine Transaminase Albumin (ALT), Alkaline Phosphatase (ALP) and Aspartate Transaminase (AST) are important in the determination of the proper functioning of the liver⁴⁴. These enzymes are present in negligible concentrations. An increase in the concentration of these enzymes may be because of damage or diseased cells which denote the status of the liver functioning. The concentration of cholesterol in these studies at 28 weeks was within the normal range of cholesterol in birds⁴². Cholesterol is synthesized from fats consumed and endogenously synthesized within the cells. A high level of cholesterol is an indication of a high risk of cardiovascular disease.

Chickens with high protective antibody titre can enhance the resistance to Newcastle disease virus outbreak in the chicken population. All the genotype-antibody titre chickens in this study can be incorporated into the breeding programme to develop a broiler strain against the Newcastle disease virus. Thus, the genetic makeup in the indigenous chickens against diseases will provide a background for genetic improvement and diversification to produce strains that can be raised in any environment for the benefit of the farmers to reduce the cost of production in the poultry sector.

CONCLUSION

The effects of genotype-antibody titre and sex observed in this study on immune response traits of chicks challenged with NDV at different ages were significant. Normal feathered high titre and frizzle feathered high titre had higher titre values compared to normal feathered low titre. The female chicks were favoured in the immune response to the Newcastle disease vaccine across the ages. Genotype-antibody titre and sex had no significant influence on haematological parameters except for Eosinophyes (EOS), while normal feathered high titre, normal feathered low titre and naked-neck low titre had the higher values, while the female chickens had a higher value than their male counterparts for WBC. Genotype-antibody titre and sex did not differ significantly with biochemical indices.

SIGNIFICANCE STATEMENT

This study discovered the use of immunological traits that can be a beneficial factor to check the level of the immune system of a chicken to reduce the Newcastle disease virus in poultry production. This study will help the researcher to uncover the disease challenges facing the poultry sector in Nigeria. Thus, a new theory on the immune response to Newcastle disease virus in Nigerian indigenous chickens may be arrived at.

REFERENCES

1. Adenaike, A.S., A.O. Mabunmi, M.I. Takeet, O.D. Adenaike and C.O.N. Ikeobi, 2016. Genetic differences in the body weight and haematological traits of Nigerian indigenous chickens infected with *Eimeria tenella*. Trop. Anim. Health Prod., 48: 1443-1447.
2. Rotimi, E.A., J.O. Egahi and A.A. Adeoye, 2016. Phenotypic characterization of indigenous chicken population in Gwer-West, Benue State, Nigeria. World Sci. News, 53: 343-353.
3. Adediji, T.A., 2012. Effect of some qualitative traits and non-genetic factors on heat tolerance attributes of extensively reared West African Dwarf (WAD) goats. Int. J. Appl. Agric. Apiculture Res., 8: 68-81.
4. Anebo, Z.G., K. Teklemichael, B. Bacha, T. Habte and A. Hunde, 2014. Evaluation of the newcastle disease antibody level after vaccination regimes in chickens in debrezeit agricultural research center, Ethiopia. J. Vet. Med. Anim. Health, 6: 7-12.
5. Esaki, M., A. Godoy, J.K. Rosenberger, S.C. Rosenberger, Y. Gardin, A. Yasuda and K.M. Dorsey, 2013. Protection and antibody response caused by Turkey herpesvirus vector newcastle disease vaccine. Avian Dis., 57: 750-755.
6. Okwor, G.O., A. El-Yuguda and S.S. Baba, 2014. Profile of maternally derived antibody in broiler chicks and *in-ovo* vaccination of chick embryo against newcastle disease. World J. Vaccines, 4: 72-80.
7. Kazeem, O.H.O., D.M. Sadiq, A.A. Sabi, D.M. Gyang and S.W. Yiltawe, 2012. Restrospecific study of Newcastle disease in commercial poultry farms in Ilorin, Kwara State, Nigeria. Vom J. Vet. Sci., 9: 66-70.
8. Hossain, K.M.M., M.Y. Ali and I. Yamato, 2010. Antibody levels against newcastle disease virus in chickens in Rajshahi and surrounding districts of Bangladesh. Int. J. Biol., 2: 102-106.
9. Ashraf, A. and M.S. Shah, 2014. Newcastle disease: Present status and future challenges for developing countries. Afr. J. Microbiol. Res., 8: 411-416.
10. Lillehoj, H.S., S.H. Lee, S.I. Jang, D.K. Kim and K.W. Lee, 2011. Recent progress in understanding host mucosal response to avian coccidiosis and development of alternative strategies to mitigate the use of antibiotics in poultry production. Korean J. Poult. Sci., 38: 275-284.
11. Lee, S.H., H.S. Lillehoj, D.W. Park, S.I. Jang and A. Morales *et al.*, 2009. Induction of passive immunity in broiler chickens against *Eimeria acervulina* by hyperimmune egg yolk immunoglobulin Y. Poult. Sci., 88: 562-566.
12. Innes, E.A. and A.N. Vermeulen, 2006. Vaccination as a control strategy against the coccidial parasites *Eimeria*, *Toxoplasma* and *Neospora*. Parasitology, 133: S145-S168.
13. Jenkins, M.C., 2001. Advances and prospects for subunit vaccines against protozoa of veterinary importance. Vet. Parasitol., 101: 291-310.
14. Pitcovski, J., A. Cahaner, E.D. Heller, T. Zouri, B. Gutter, Y. Gotfried and G. Leitner, 2001. Immune response and resistance to infectious bursal disease virus of chicken lines selected for high or low antibody response to *Escherichia coli*. Poult. Sci., 80: 879-884.
15. Mpenda, F.N., S.L. Lyantagaye and J. Buza, 2020. Immune response following newcastle disease immunization and growth performance of kuroiler, broiler and local Tanzanian chickens. Int. J. Livest. Prod., 11: 1-7.
16. Luo, C., H. Qu, J. Ma, J. Wang and C. Li *et al.*, 2013. Genome-wide association study of antibody response to newcastle disease virus in chicken. BMC Genet., Vol. 14. 10.1186/1471-2156-14-42.
17. Atansuyi, A.J., T.Z. Ogunribido and C.A. Chineke, 2019. Haematological and serum biochemical characteristics of four chicken genotypes in South-Western, Nigeria. Niger. J. Anim. Sci., 21: 9-16.

18. Musa, A.A., M. Orunmuyi, G.N. Akpa, A.K. Olutunmogun and A.J. Shoyombo, 2018. Haematological profile of birds produced from diallel crossing of three genotypes of Nigerian indigenous chickens. *J. Anim. Prod. Res.*, 30: 169-178.
19. Adeyemo, G.O., M.O. Bolarinwa and O. Ehiabhi, 2018. Hematology and external egg quality parameters of three Nigerian indigenous chicken genotypes. *Int. J. Mol. Biol.*, 3: 197-201.
20. Malik, M., M. Sohail, M. Sajid, Hamidullah, M. Shoaib, N. Bano and S.S.A. Shah, 2018. Effects of newcastle disease virus on different haematological parameters in broilers. *Adv. Anim. Vet. Sci.*, 6: 183-186.
21. Adedibu, I.I., K.L. Ayorinde and A.A. Musa, 2014. Identification of hematological markers suitable for improving productivity of helmeted guinea fowl *Numida meleagris*. *Am. J. Exp. Agric.*, 4: 1186-1196.
22. Falcone, F.H., H. Haas and B.F. Gibbs, 2000. The human basophil: A new appreciation of its role in immune responses. *Blood*, 96: 4028-4038.
23. Adewole, F.A., L.T. Egbeyale, D.A. Ekunseitan, K.O. Bello, O.A. Lala and S.A. Famakinde, 2021. Effect of strain and sex on haematological and serum biochemical indices of tropical indigenous chickens. *Niger. J. Anim. Prod.*, 48: 18-26.
24. dos Santos Schmidt, E.M., A.C. Paulillo, E. Santin, R.L. Dittrich and E.G. de Oliveira, 2007. Hematological and serum chemistry values for the ring-necked pheasant (*Phasianus colchicus*): Variation with sex and age. *Int. J. Poult. Sci.*, 6: 137-139.
25. Garcia, M., Y. Hermosa and J. Aguirre, 2010. Does breeding status influence haematology and blood biochemistry of yellow-legged gulls? *Acta Biol. Hung.*, 61: 391-400.
26. Abdi-Hachesoo, B., A. Talebi, S. Asri-Rezaei and M. Basaki, 2013. Sex-related differences in biochemical and haematological parameters of adult indigenous chickens in Northwest of Iran. <https://www.semanticscholar.org/paper/Sex-Related-Differences-in-Biochemical-and-of-Adult-Abdi-Hachesoo-Talebi/7d8b8aef64db48809f882806fca254610a65405f>
27. Sanda, A.J., O.A. Adebambo, O. Olowofeso, M.A. Adeleke, M.O. Akinfenwa, F.C. Nworgu and R.A. Lawal, 2012. Genetic evaluation of Nigerian indigenous crossbred pullets and broilers. *Thai J. Agric. Sci.*, 45: 197-201.
28. Oke, U.K., U. Herbert and A.H. Akinmutimi, 2003. Early lay characteristics and haematology of pearl guinea fowls as influenced by dietary protein and energy levels. *Int. J. Poult. Sci.*, 2: 128-132.
29. Pranoto H. and M. Nugrahalia, 2020. A study on sex-based hematology and biochemistry of lesser whistling duck (*dendrocygna javanica*). *Int. J. Poult. Sci.*, 19: 124-129.
30. Rehman, M.S., A. Mahmud, S. Mehmood, T.N. Pasha, J. Hussain and M.T. Khan, 2017. Blood biochemistry and immune response in aseel chicken under free range, semi-intensive and confinement rearing systems. *Poult. Sci.*, 96: 226-233.
31. Sola-Ojo, F.E., A.A. Annongu, T.R. Fayeye, A.H.A. Badmos, D.I. Ibiwoye and N.A. Furo, 2016. Effects of feeding processed baobab (*Adansonia digitata*) seed on the haematology and serum biochemistry of broiler chicks. *Ife J. Sci.*, 18: 895-902.
32. Al-Hussary, N.A.J. and I.M. Kudair, 2010. Effect of vaccination on some biochemical parameters in broiler chickens. *Iraqi J. Vet. Sci.*, 24: 59-64.
33. Rizvi, F., A.D. Anjum, A. Khan, M. Mohsan and M. Shaszad, 2008. Pathological and serum biochemical effects of salinomycin. *Pak. Vet. J.*, 28: 71-75.
34. Ul-Rahman, A., M.A.B. Shabbir, M. Ahmed and M.Z. Shabbir, 2020. A comparative evaluation of serum biochemistry profile and antigenic relatedness among velogenic and mesogenic *Avian avulavirus 1* infection in chickens and pigeons. *Trop. Anim. Health. Prod.*, 52: 1977-1984.
35. Okoroafor, O.N., C.O. Okorie-Kanu, P.C. Animoke, J.A. Okoye, J.A. Nwanta and B.M. Anene, 2018. Comparison of blood biochemistry responses of cockerels and turkeys experimentally infected with a velogenic newcastle disease virus. *Niger. Vet. J.*, 39: 168-180.
36. Wison, D.D., 2008. *Manual of Laboratory and Diagnostic Tests*. McGraw-Hills, New York, ISBN: 9780071594059, Pages: 666.

37. Kaplan, L.A., A.J. Pesce and S.C. Kazmierczak, 2003. Liver Function. In: Clinical Chemistry, Sherwin, J.E. (Ed.). (Edn. 4th), Elsevier Science, St. Louis, Toronto.
38. Silva, P.R.L., O.C.F. Neto, A.C. Laurentiz, O.M. Junqueira and J.J. Fagliari, 2007. Blood serum components and serum protein test of Hybro-PG broilers of different ages. Braz. J. Poult. Sci., 9: 229-232.
39. Odunitan-Wayas, F., U. Kolanisi and M. Chimonyo, 2018. Haematological and serum biochemical responses of ovambo chickens fed provitamin A biofortified maize. Braz. J. Poult. Sci., 20: 425-434.
40. Ahmad, S., A. Mahmud, J. Hussain and K. Javed, 2019. Morphometric traits, serum chemistry and antibody response of three chicken genotypes under free-range, semi-intensive and intensive housing systems. Braz. J. Poult. Sci., Vol. 21. 10.1590/1806-9061-2018-0921.
41. Fischbach, F. and M.B. Dunning, 2009. A Manual of Laboratory and Diagnostic Tests. 8th Edn., Lippincott Williams and Wilkins, United States, ISBN-13: 978-0781771948, Pages: 1317.
42. Ashour, E.A., M.S. El-Kholy, M. Alagawany, M.E.A. El-Hack and L.A. Mohamed *et al.*, 2020. Effect of dietary supplementation with *Moringa oleifera* leaves and/or seeds powder on production, egg characteristics, hatchability and blood chemistry of laying Japanese quails. Sustainability, Vol. 12. 10.3390/su12062463.
43. Simaraks, S., O. Chinrasri and W. Aengwanich, 2004. Hematological, electrolyte and serum biochemical values of the Thai indigenous chickens (*Gallus domesticus*) in Northeastern, Thailand. Songklanakarin J. Sci. Technol., 26: 425-430.
44. Ambrosy, A.P., T.P. Dunn and P.A. Heidenreich, 2015. Effect of minor liver function test abnormalities and values within the normal range on survival in heart failure. Am. J. Cardiol., 115: 938-941.