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Immune response kinetics in Nigerian indigenous chickens challenged with attenuated *Salmonella*

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Abstract

Salmonella infection is a major cause of death in chickens and this disease can be transferred through meat and egg to humans. The use of antibiotics and vaccines in the prevention and control of this infection are not totally efficient and also have their side effects. Therefore, genetic control is a better approach in finding a lasting solution. One hundred and ten chickens (4th generation) comprising the three genotypes of Nigerian indigenous chickens (NIC) that had shown divergent response (high vs low) to sheep red blood cells antigens were used in this study to investigate the immune response kinetics in NIC after challenged with attenuated Salmonella. There were six classes namely, high titre normal, low titre normal, high titre frizzle, low titre frizzle, high titre naked neck and low titre naked neck. Haematological parameters: packed cell volume (PCV), haemoglobin concentration (Hb), red blood cells count (RBC), white blood cells count (WBC), heterophils (H), lymphocytes (L), eosinophils (E), basophils (B), monocytes (M) and heterophils-lymphocytes ratio (HLR) were used to assess the immune responses of the chickens and data collected were subjected to General Linear Model procedure of SAS 9.2 version. The results showed that genotype-antibody titre had significant effect ($p<0.05$) on the immune response parameters but sex had no significant effect ($p>0.05$), while sex by genotype-antibody titre interaction had significant effect ($p<0.05$). The immune response kinetics of the chickens after challenge with attenuated Salmonella was shown by the significance of the effect of days of blood collection after challenge. High titre naked neck chickens recorded the highest PCV, Hb and RBC values while genotype-antibody titre had no significant effect ($p>0.05$) on the rest of the parameters. Low titre naked neck males recorded the highest PCV, HB and RBC values while the rest of the parameters were not significant ($p>0.05$). Generally, days had significant effect ($p<0.05$) on all the immune response parameters except on H, L and HLR. The study showed that the high titre naked neck males were least affected by the Salmonella vaccine in terms of decrease in packed cell volume, haemoglobin concentration and red blood cell count while the low titre normal females were most affected.

Keywords: Salmonella, immune response, Nigerian indigenous chickens; Animal breeding and genetics

Introduction

The chicken has long been used as a model organism for developmental and immunological studies (Burt and White, 2007), and improving animal health is a major goal in the current animal breeding industry (Soller and Andersson, 1998). Antibody response in chickens, which has low to medium heritability, is controlled by some quantitative trait loci (QTL) (Schmid *et al.,* 2000). Disease-related costs and losses are expected to be reduced by genetic control of resistance to pathogens and improvement of the immune capacity of

animals *(*Lamont*,* 1998*)*. *Salmonella* contamination of poultry meat and eggs continues to be a global threat to public health (Barrow and Duchet-Sochaux,1997). *Salmonella enteritidis* is an enteric bacterium that is a zoonotic intracellular pathogen of poultry and humans as well as other species (McIlroy et al., 1989). *S. enteritidis* is one of the major causes of human food-borne illness and is the most frequent serovar detected in outbreaks of human salmonellosis *(*Lu *et al.,* 1999*)*. In the advent of *S. enteritidis* infection, some susceptible animals die, but others can host the bacteria for several weeks or months without presenting any particular symptoms. These healthy carriers are therefore an obstacle to the eradication of *S. enteritidis* and are responsible for the transmission to humans. Different kinds of prophylactic measures to control *S. enteritidis* infection in poultry have been studied, including competitive exclusion and vaccination. The use of antibiotics in domestic livestock has been questioned due to the possible creation of antibiotic-resistant bacteria and the possibility of antibiotic residues being consumed by humans (White *et al*., 2001). Vaccination and the use of antibiotics are often insufficient to eradicate the disease fully in poultry. Selective breeding, therefore, has been considered a valuable alternative (Connell *et al*., 2012). Early selective breeding efforts focused on reducing the incidence of the disease in poultry production systems. Later studies gave more emphasis to selection for resistance to the carrier state ability or bacterial colonisation of the birds, to reduce asymptomatic propagation of the pathogen (Calenge and Beaumont, 2012). The use of antibiotics and vaccines to control *Salmonella* infection in chickens has been criticized because of the possible development of antibiotic-resistant bacteria, and the potential dangers of antibiotic and vaccine residues in animal-derived food products for human consumption. The enhancement of natural genetic resistance is, therefore, an alternative approach to control *Salmonella* in poultry (Kramer *et al*., 2003). Thus, we aimed to investigate the kinetics of the immune response in Nigerian indigenous chickens after challenge with attenuated *Salmonella* and to check if sex and its interaction have an effect on the response of the chickens to the attenuated *Salmonella*.

Materials and methods

The experiment was conducted at the Poultry Unit, Directorate of University Farms (DUFARMS) Federal University of Agriculture (FUNAAB), Abeokuta, Ogun State and Department of Animal Breeding and Genetics Biotechnology Laboratory, FUNAAB. Three genotypes of NIC: Normal feather, Frizzle feather and Naked-neck separated into two distinct lines, High antibody titre (HAT) and Low antibody titre (LAT) that went through four generations of selection since 2014 based on the response to sheep red blood cell (SRBC) were used. Briefly, SRBC preparation and inoculation involved the collection of blood sample (5 mL) from sheep via the jugular vein. The sheep was restricted and the jugular was palpated. The site of collection was cleaned with methylated spirit and using 5 mL syringe, blood sample was obtained and emptied into EDTA (Ethylene Diamine Tetra Acetic acid) bottle to disallow clotting. Blood was centrifuged at 3000 rpm for 10 minutes at 4 ºC. The supernatant was removed subsequently. The settled red blood cells were washed three times using phosphate buffered saline. 1 mL SRBC was intravenously administered to the chicks via jugular vein at six weeks of age. Blood was collected at the seventh day post-inoculation. The blood was collected in

plain tube and slanted 45 º for the blood to clot and for serum separation. Serum was separated subsequently in the laboratory and kept at -20 ºC in the refrigerator for haemaglutinnation inhibition (HI) assay. This was carried out by making serial dilutions of serum (50 µL) obtained from chicken blood samples and with a combination of PBS (50 µL) and 1% SRBC (50 µL). Based on HI titre, chickens were classified as HAT and LAT chicken lines. At the fourth generation, one hundred and twenty-day-old chicks were selected at random from the total of chicks hatched. The chicks from each genotype were brooded for two weeks before been transferred to separate pens based on genotype and antibody titre. The chickens were managed on deep litter system in pens having natural ventilation. The separation of the chicks gave six classes: Normal feather high, Normal feather low, Frizzle high, Frizzle low, Naked-neck high and Naked-neck low. All the birds were given 0.5 mL/bird of *Salmonella* vaccine subcutaneously with needle and syringe at eight weeks old. A day pre-challenge with attenuated *Salmonella*, blood samples were collected from each bird with needle and syringe via the brachial vein into EDTA (Ethylene Diamine Tetra Acetic acid) bottles. This was used to determine the haematological parameters prior to challenge which in turn served as the baseline for comparison with the haematological parameters that was determined post-challenge. After challenge, blood sample for haematological evaluation were collected on weekly basis for three weeks from the brachial vein of all the chickens into EDTA bottles. The EDTA bottles were labelled according to the tag number of each bird. Variation in the levels of different haematological parameters checked over the weeks showed the immune response of the chickens to the attenuated *Salmonella*.

The immune response of the chickens was assessed by analysing different haematological parameters: packed cell volume (PCV), red blood cells count (RBC), haemoglobin concentration (HB), white blood cells count (WBC), heterophils (H), lymphocytes (L), monocytes (M), eosinophil (E), basophil (B) and heterophils-lymphocytes ratio (HLR).

The data for the haematological parameters collected were transformed with square-root (PCV, H, L, E, B and M) and logarithm (Hb, RBC and WBC) so as to follow the normal distribution curve.

Statistical analysis

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

$Y_{ijkl} = \mu + G_i + S_j + D_k + (GS)_{ij} + \varepsilon_{ijkl}$

Where,

Y_{ijkl} = immune response variables

μ = Overall mean

G_i = fixed effect of i^{th} titre levels within chicken genotypes (NkL, NkH, NmH, NmL, FL, FH)

S_j = fixed effects of j^{th} sex (male and female)

D_k = fixed effects of k^{th} day of blood collection pre and post challenge with attenuated *Salmonella* (1, 8, 15 and 22)

$(GS)_{ij}$ = effect of i^{th} titre and j^{th} sex interaction

ε_{ijkl} = residual error.

Results and discussion

Effect of genotype-antibody titre on the haematological parameters of Nigerian indigenous chickens challenged with attenuated Salmonella

There were significant differences ($p < 0.05$) in some of the haematological parameters used in assessing the immune

response in Table 1 due to the effect of genotype-antibody titre. Packed cell volume, haemoglobin concentration and red blood cells count were significantly different with high titre naked neck (NNH) chickens recording the highest values (32.80 ± 0.92 and 10.85 ± 0.30 for packed cell volume and haemoglobin concentration respectively) and high titre frizzle having the highest value for red blood cells count (2.71 ± 0.05) while low titre normal (NL) chickens recorded the least values (30.38 ± 0.33, 10.12 ± 0.11 and 2.53 ± 0.03, respectively). The rest of the parameters (white blood cell count, heterophils, lymphocytes, eosinophils, basophils, monocytes and heterophils lymphocytes ratio) had no significant difference among the different genotype-antibody titres. Despite no statistical difference, high titre normal (NH) chickens had the highest value (13.29 ± 0.26) of white blood cells count while high titre naked neck (NNH) chickens had the least value (12.28 ± 0.47). High titre frizzle had the highest value (31.29 ± 0.74) for heterophils while high titre naked neck chickens had the least value (30.70 ± 1.52); high titre naked neck chickens had the highest value (67.95 ± 1.48) for lymphocytes while low titre naked neck chickens had the least value (66.75 ± 1.64); low titre naked neck (0.70 ± 0.18), low titre frizzle (0.60 ± 0.10) and high titre naked neck chickens (0.95 ± 0.28) had the highest values for eosinophils, basophils and monocytes respectively while high titre frizzle (0.38 ± 0.08), low titre naked neck (0.35 ± 0.13) and low titre frizzle chickens (0.63 ± 0.12) had the least values for the aforementioned parameters respectively. High titre naked neck, low titre frizzle, high titre normal and low titre normal chickens had the same value (0.47) which was collectively the least while low titre naked neck chickens (0.49 ± 0.04) had the highest.

Effect of sex on the haematological parameters of Nigerian indigenous chickens challenged with attenuated Salmonella

There was no significant difference ($p > 0.05$) in all of the haematological parameters used in assessing the chicken immune response in Table 2 due to the effect of sex. Although both sexes were not statistically different, males had the higher values for packed cell volume (31.53 ± 0.31), haemoglobin concentration (10.48 ± 0.11), red blood cells count (2.62 ± 0.03) and heterophils-lymphocytes ratio (0.48 ± 0.01) while females were higher for white blood cells count (13.10 ± 0.17), heterophils (31.25 ± 0.49), lymphocytes (67.47 ± 0.36), eosinophils (0.48 ± 0.04), basophils (0.47 ± 0.04) and monocytes (0.78 ± 0.05).

Effect of sex and genotype-antibody titre interaction on the haematological parameters of Nigerian indigenous chickens challenged with attenuated Salmonella

There were significant differences ($p < 0.05$) in some of the haematological parameters used in assessing the immune response in Table 3 due to the effect of sex and genotype-antibody titre interaction. Packed cell volume, haemoglobin concentration and red blood cells count were significant, high titre naked neck males (NNHM) had the highest values (34.50 ± 2.90, 11.60 ± 0.91 and 2.90 ± 0.27 respectively) while low titre normal females (NLF) had the least values (29.89 ± 0.44, 9.97 ± 0.15 and 2.49 ± 0.04 respectively). The rest of the parameters (white blood cell count, heterophils, lymphocytes, eosinophils, basophils, monocytes and heterophils lymphocytes ratio) had no significant difference among the different interactions of sex and genotype-antibody titres. For white blood cells count, high titre naked neck males

Table 1: Effect of genotype-antibody titre on the haematological parameters of Nigerian indigenous chickens challenged with attenuated Salmonella

Parameter	Genotype-Antibody titre					
	FH	FL	NH	NL	NNH	NNL
Packed cell volume (%)	32.49±0.60^{a}	32.52±0.69^{a}	31.72±0.38^{ab}	30.38±0.33^{b}	32.80±0.92^{a}	31.85±1.21^{ab}
Haemoglobin concentration (g/dl)	10.77±0.20^{ab}	10.78±0.21^{a}	10.53±0.13^{ab}	10.12±0.11^{b}	10.85±0.30^{a}	10.60±0.39^{ab}
Red blood cells count ($\times 10^{12/L}$)	2.71±0.05^{a}	2.68±0.56^{ab}	2.64±0.03^{ab}	2.53±0.03^{b}	2.69±0.09^{a}	2.66±0.10^{ab}
White blood cells count ($\times 10^{9/L}$)	13.17±0.32	12.63±0.31	13.29±0.26	13.14±0.21	12.28±0.47	12.55±0.56
Heterophils (%)	31.29±0.74	30.87±0.95	30.89±0.52	30.98±0.48	30.70±1.52	31.25±1.62
Lymphocytes (%)	67.07±0.71	67.27±0.95	67.51±0.51	67.43±0.47	67.95±1.48	66.75±1.64
Eosinophils (%)	0.38±0.08	0.63±0.11	0.42±0.05	0.42±0.05	0.50±0.15	0.70±0.18
Basophils (%)	0.51±0.09	0.60±0.10	0.43±0.06	0.47±0.05	0.30±0.13	0.35±0.13
Monocytes (%)	0.74±0.11	0.63±0.12	0.74±0.07	0.68±0.07	0.95±0.28	0.85±0.23
Heterophils-lymphocytes ratio	0.48±0.02	0.47±0.02	0.47±0.01	0.47±0.01	0.47±0.04	0.49±0.04

Table 2: Effect of sex on the haematological parameters of Nigerian indigenous chickens challenged with attenuated Salmonella

Parameter	Sex	
	Female	Male
Packed cell volume (%)	31.42±0.29	31.53±0.31
Haemoglobin concentration (g/dl)	10.44±0.09	10.48±0.11
Red blood cells count ($\times10^{12/L}$)	2.61±0.03	2.62±0.03
White blood cells count ($\times10^{9/L}$)	13.10±0.17	13.04±0.19
Heterophils (%)	31.25±0.49	30.80±0.36
Lymphocytes (%)	67.47±0.36	67.25±0.47
Eosinophils (%)	0.48±0.04	0.41±0.04
Basophils (%)	0.47±0.04	0.46±0.05
Monocytes (%)	0.78±0.05	0.63±0.06
Heterophils-lymphocytes ratio	0.47±0.01	0.48±0.01

(NNHM) had 13.43 ± 1.47 which was the highest while high titre naked neck females (NNHF) had the least value (11.99 ± 0.47). Low titre naked neck males (NNLM) (32.50 ± 3.47), high titre naked neck males (68.00 ± 1.35), both low titre frizzle females (FLF) and low titre naked neck female (NNFL) (0.75), high titre naked neck males (0.75 ± 0.48 and 1.75 ± 0.48) were the highest for heterophils, lymphocytes, eosinophils, basophils and monocytes respectively while high titre naked neck males (29.25 ± 1.11), low titre naked neck males (65.63 ± 3.32), high titre naked neck males (0.25 ± 0.25), high titre naked neck females (0.19 ± 0.10) and both low titre frizzle males (FLM) and low titre normal males (0.50) were the least for the aforementioned parameters respectively. Low titre naked neck males (0.52 ± 0.08) had the highest value for heterophils-lymphocytes ratio while high titre naked neck males (0.43 ± 0.02) had the least.

Table 3 a: Effect of sex and genotype-antibody titre interaction on the haematological parameters of Nigerian indigenous chickens challenged with attenuated Salmonella

	Parameter	PCV (%)	HB (g/dl)	RBC ($\times10^{12/L}$)	WBC ($\times10^{9/L}$)
Sex and genotype-antibody interaction	FHF	32.75±0.81^{a}	10.84±0.28^{a}	2.72±0.07^{a}	13.12±0.43
	FLF	31.04±0.79^{ab}	10.30±0.26^{ab}	2.55±0.07^{ab}	12.89±0.46
	NHF	32.33±0.32^{a}	10.75±0.17^{a}	2.70±0.05^{a}	13.34±0.34
	NLF	29.89±0.44^{b}	9.97±0.15^{b}	2.49±0.04^{b}	13.18±0.27
	NNHF	32.38±0.92^{a}	10.66±0.31^{ab}	2.64±0.09^{ab}	11.99±0.47
	NNLF	31.58±1.66^{ab}	10.48±0.53^{ab}	2.62±0.15^{ab}	12.45±0.75
	FHM	32.00±0.84^{ab}	10.65±0.28^{ab}	2.69±0.07^{a}	13.25±0.47
	FLM	34.25±0.96^{a}	11.35±0.32^{a}	2.83±0.08^{a}	12.33±0.41
	NHM	30.68±0.51^{ab}	10.16±0.18^{ab}	2.53±0.05^{ab}	13.21±0.39
	NLM	30.96±0.49^{ab}	10.31±0.16^{ab}	2.57±0.04^{ab}	13.09±0.32
	NNHM	34.50±2.90^{a}	11.60±0.91^{a}	2.90±0.27^{a}	13.43±1.47
	NNLM	32.25±1.83^{ab}	10.79±0.60^{a}	2.71±0.15^{a}	12.69±0.91

PCV- Pack cell volume *Hb*- Haemoglobin concentration *RBC*- Erythrocyte counts *WBC*- White blood cell *H* – Heterophils *L* – Lymphocytes *E* – Eosinophils *B* – Basophils *M* – Monocytes *HLR* – Heterophils Lymphocytes Ratio ^{ab}: means on the same row with different superscript differ significantly ($p<0.05$)

Table 3b: Effect of sex and genotype-antibody titre interaction on the haematological parameters of Nigerian indigenous chickens challenged with attenuated Salmonella

	Parameter	H (%)	L (%)	E (%)	B (%)	M (%)	HLR
Sex and genotype-antibody interaction	FHF	31.25±0.96	67.00±0.92	0.39±0.09	0.55±0.11	0.84±0.12	0.48±0.02
	FLF	30.93±1.44	66.93±1.44	0.75±0.15	0.64±0.13	0.75±0.13	0.48±0.03
	NHF	30.50±0.64	67.90±0.64	0.44±0.07	0.47±0.08	0.70±0.09	0.46±0.01
	NLF	30.87±0.56	67.35±0.66	0.46±0.06	0.45±0.07	0.84±0.10	0.47±0.01
	NNHF	31.06±1.88	67.94±1.34	0.56±0.18	0.19±0.10	0.75±0.31	0.47±0.04
	NNLF	30.42±1.60	67.50±1.71	0.75±0.25	0.25±0.18	0.91±0.19	0.46±0.03
	FHM	31.38±1.17	67.21±1.13	0.42±0.13	0.46±0.15	0.54±0.20	0.48±0.03
	FLM	30.79±1.23	67.67±1.23	0.50±0.15	0.54±0.17	0.50±0.21	0.47±0.03
	NHM	31.57±0.89	66.84±0.87	0.39±0.08	0.36±0.08	0.82±0.12	0.49±0.02
	NLM	31.11±0.82	67.54±0.80	0.38±0.09	0.49±0.07	0.50±0.08	0.48±0.02
	NNHM	29.25±1.11	68.00±1.35	0.25±0.25	0.75±0.48	1.15±0.48	0.43±0.02
	NNLM	32.50±3.47	65.63±3.32	0.63±0.26	0.50±0.19	0.75±0.53	0.52±0.08

PCV- Pack cell volume *Hb*- Haemoglobin concentration *RBC*- Erythrocyte counts *WBC*- White blood cell *H* – Heterophils *L* – Lymphocytes *E* – Eosinophils *B* – Basophils *M* – Monocytes *HLR* – Heterophils Lymphocytes Ratio

Effect of days on the haematological parameters of Nigerian indigenous chickens challenged with attenuated Salmonella

There were significant differences ($p < 0.05$) in some of the haematological parameters used in assessing the immune response kinetics in Table 4 due to the effect of days. Packed cell volume, haemoglobin concentration and red blood cells counts had the highest values on day 1 (33.32 ± 0.46, 11.07 ± 0.15 and 2.79 ± 0.04 respectively) while day 22 had the least value of packed cell volume (29.86 ± 0.33) and day 8 had the least values (9.90 ± 0.11 and 2.47 ± 0.03) for haemoglobin concentration and red blood cells count respectively. White blood cells count had the highest value (14.31 ± 0.29) on day 8 and the least value on day 1 (11.83 ± 0.19).

Table 4: Effect of days on the haematological parameters of Nigerian indigenous chickens challenged with attenuated Salmonella

Parameter	Days			
	1	8	15	22
PCV (%)	33.32±0.46^{a}	29.92±0.31^{b}	32.74±0.48^{a}	29.86±0.33^{b}
HB(g/dl)	11.07±0.15^{a}	9.90±0.11^{b}	10.88±0.16^{a}	9.96±0.11^{b}
RBC ($\times 10^{12/L}$)	2.79±0.04^{a}	2.47±0.03^{b}	2.72±0.04^{a}	2.49±0.03^{b}
WBC ($\times 10^{9/L}$)	11.83±0.19^{c}	14.31±0.29^{a}	13.10±0.27^{b}	13.07±0.20^{b}
H (%)	31.88±0.67	31.65±0.52	30.02±0.57	30.38±0.53
L (%)	67.13±0.68	67.18±0.50	68.46±0.58	66.77±0.52
E (%)	0.31±0.05^{bc}	0.23±0.04^{c}	0.44±0.06^{b}	0.84±0.07^{a}
B (%)	0.32±0.05^{b}	0.31±0.05^{b}	0.35±0.05^{b}	0.88±0.07^{a}
M (%)	0.43±0.06^{c}	0.65±0.07^{bc}	0.70±0.08^{b}	1.10±0.09^{a}
HLR	0.49±0.02	0.48±0.01	0.45±0.01	0.47±0.01

PCV- Pack cell volume *Hb*- Haemoglobin concentration *RBC*- Erythrocyte counts *WBC*- White blood cell *H* – Heterophils *L* – Lymphocytes *E* – Eosinophils *B* – Basophils *M* – Monocytes *HLR* – Heterophils Lymphocytes Ratio. ^{ab}: means on the same row with different superscript differ significantly ($p<0.05$)

The values became highest for eosinophils, basophils and monocytes on day 22 (0.84 ± 0.07, 0.88 ± 0.07 and 1.10 ± 0.09) respectively but the least values were recorded on day 8 for eosinophils (0.23 ± 0.04) and basophils (0.31 ± 0.05) while the least for monocytes (0.43 ± 0.06) was recorded on day 1. Heterophils, lymphocytes and heterophils-lymphocytes ratio were not significantly different (P > 0.05) across all days. Lymphocytes attained the highest value (68.46 ± 0.58) at day 15 and the least (66.77 ± 0.52) at day 22 while heterophils (31.88 ± 0.67) and heterophils-lymphocytes ratio (0.49 ± 0.02) were highest at day 1 and least (30.02 ± 0.57 and 0.45 ± 0.01) respectively on day 15. Clinical signs of the birds vaccinated intramuscularly with *Salmonella* vaccine were observed 3-5 days post-vaccination. The birds showed less activities, reduced appetite and dullness. This is in accordance with the findings of Shah *et al.* (2013) in broiler chickens infected intraperitoneally with *Salmonella gallinarium*, but the birds under study later recovered and returned to full activity 8 days' post-vaccination. The decrease observed in packed cell volume, haemoglobin concentration and red blood cells count from day 1 to 8 post-vaccination corresponds with the reports of Freitas-Neto *et al.* (2007); Shah *et al*. (2013); Chiroma *et al*. (2017) in chickens and Barde (2014) in Japanese quails whom all reported reductions at the early stage post infection of birds with *S. gallinarium*. Unlike Chiroma *et al*. (2017) that reported continuous decrease till day 35, Shah *et al*. (2013) reported an increase in the three aforementioned haematological parameters from day 7 to 14 which is in agreement with the findings of this study. The reduction at the early phase corresponds with the acute phase of the fowl typhoid disease in which anaemia was reported in chickens (Assoku and Penhale, 1978; Prasanna and Paliwal, 2002). Assoku and Penhale (1978) had reported that the anaemia associated with the early phase maybe due to the take up of modified erythrocytes. Christensen *et al*. (1996) posited that the modification of the erythrocytes is associated directly (lipopolysaccharide/outer membrane proteins) or indirectly (induction of antibody) to the number of bacteria present in the tissues. The intensity of the anaemia was significantly highest in normal low birds but least in the naked neck high birds when the genotype-antibody titre is considered and in the interaction between sex and genotype-antibody titre, the normal low females were mostly affected while the naked neck high males were least affected. Leucocytosis was observed at day 8 post-vaccination which is in line with the reports of Berchieri (2000); Freitas-Neto *et al*. (2007); Shah *et al*. (2013) and Chiroma *et al*. (2017) who all reported significant increase in the total white blood cells count and this increase was attributed to fast multiplication of the *Salmonella* inside the phagocytes, with subsequent cell lysis and release of the bacteria into the extracellular compartment which evoked strong immune response. There was no significant difference among the genotypes by antibody titre and its interaction with sex. The differential leukocyte counts (heterophils and lymphocytes) showed slight changes over the days which were not statistically significant. This differs from the reports of Shah *et al*. (2013) and Chiroma *et al.* (2017) which shows considerable increase in the differential leukocyte counts in broilers and layers respectively. This difference may be attributed to variation in breed used. However, the differential leukocytes reports observed in this study corresponds with the findings of Barde (2014) who reported no significant changes in some group of Japanese quails infected with *S*.

gallinarium. Eosinophils and basophils in this study showed no significant changes at the early phase of the experiment which is in accordance with the reports of Allan and Duffus (1972); Cardoso *et al*. (2003); Freitas-Neto *et al.* (2007). However, as the day progresses, significant increases were observed which contradict the reports of the just afore-mentioned authors. There was significant increase in the level of monocytes in the chicken population studied, this increased continuously from day 1 till day 22 post-vaccination. This corroborates the findings of Shah *et al.* (2013) which reported significantly higher monocytes percentage between birds that were intraperitoneally and orally infected with *S. gallinarium*. However, the report of this study differs from the findings of Allan and Duffus (1972); Cardoso *et al.* (2003); Freitas-Neto *et al.* (2007) that all reported no relevant changes for monocytes. In this study, there was no significant difference in the quantity of heterophils produced as there was in monocytes; heterophils phagocytized more Salmonella than monocytes regardless of the presence or absence of antibodies (during opsonization). Therefore, heterophils are considered to be better bactericidal than monocytes (Stabler *et al*., 2002). This current study has shown that the *Salmonella* vaccine administered to the Nigerian indigenous chickens caused an early phase (day 1-8) decrease in some haematological parameters, such as packed cell volume (5% decrease), haemoglobin concentration (5%) and red blood cell count (11%) but later increased in the middle phase (day 8-15) (4.5%, 4% and 10% respectively) while there was a late phase (day 15-22) decrease (4.5%, 4% and 10% respectively). Also, the study showed that the *Salmonella* vaccine administered to the Nigerian indigenous chickens caused an early phase decrease in eosinophils and basophils percentage (25.8% and 10% respectively) which later increased at middle phase (87% and 14% respectively) and late phase (72% and 128% respectively) but monocytes showed a continuous increase across all the phases (47.5%, 3.4% and 45.9% respectively).

Conclusion

The study showed that the high titre naked neck males were least affected by the *Salmonella* vaccine in terms of decrease in packed cell volume, haemoglobin concentration and red blood cell count while the low titre normal females were most affected. However, none was better than the other in terms of other haematological parameters used in checking immune response.

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