Polymorphism of *NRAMP1* Gene and Association with Haematological Parameters of Chickens Inoculated with Attenuated *Salmonella enteritidis*

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Abstract
Poultry production is plagued with many severe diseases and one of them is *salmonellosis*. Treatment of this disease with antibiotics often leads to bacteria-resistance strains and accumulation of antibiotics residue in food, hence the need to use a safer method of treatment. NRAMP 1 is a candidate gene for natural immune response associated with *Salmonella enteritidis*. One hundred and twenty pure breed progenies generated from Naked neck, Fulani ecotype and Sasso chickens were reared together from day old to 8 weeks. At 7 weeks 10 birds were randomly selected from each genotype and 2ml of blood sample were obtained from each bird for laboratory analysis. The selected birds were inoculated with attenuated *S. enteritidis* at week 8 and thereafter 2ml of blood was obtained from each bird's jugular vein for laboratory analysis. Data were collected on Packed Cell Volume (PCV), Haemoglobin (HB), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell (WBC), Neutrophils (N), Lymphocytes (L), Monocytes (M), Basophils (B), Eosinophils (E) and Platelet (PLT). The result revealed that genotype and inoculation of Salmonella had significant ($p<0.05$) effect, on all the haematological parameters studied except for the Eosinophilis ($p>0.05$). Sasso chickens had Eosinophilis values before and after (0.00±0.00 and 1.37±0.65), NN (0.50±0.50 and 1.50±0.50) and FE (0.00±0.00). Conclusively, there was a significant effect of genotype on all haematological parameters except eosinophils before and after inoculation. Naked neck had the highest values in all parameters except for mean corpuscular haemoglobin and lymphocytes.

Keywords: *NRAMP 1*, *Salmonella enteritidis*, Salmonellosis, Candidate, Gene

DESCRIPTION OF PROBLEM

Poultry production have played significant role in the economy of Nigeria and these roles are as follows; they are good sources of protein, high level of acceptability as it has no barriers (1). However, poultry production is faced with challenges of inefficient management, disease and parasites (2). Diseases have led to the reduction in the production of poultry related products, and also the input costs like labour and feed get increased. *Salmonella* is an enteric pathogen that can infect almost all animals including humans. Salmonellosis in poultry is caused by Gram-negative bacteria from the genus *Salmonella*. Most serotypes of *Salmonella* can infect several animal species, such as *Salmonella* Typhimurium and *Salmonella enteritidis*. Chicken can get infected with *Salmonella enterica* at any time during their life time. Candidate genes are generally the genes with known biological function directly or indirectly regulating the developmental processes of the investigated traits, which could be confirmed by evaluating the effects of the causative gene variants in an association analysis (3). *NRAMP 1* gene is a candidate gene associated with *Salmonella enteritidis* mediated immune response, and is related to the phagocytosis of *Salmonella enteritidis*. Haemtological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (4). Haematological parameters are those parameters that are related to the blood and blood forming organs (5,6).

MATERIALS AND METHODS

Experimental Sites

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso (LAUTECH), Oyo State, Nigeria. It is located on longitude 4°15′ East and latitude 8°15′ North east of the Greenwich meridian. The altitude is between 300 and 600m above sea level. The mean annual rainfall and temperature are 1247mm and 27°C respectively (7). Ogbomoso is located in a derived savannah zone. The laboratory analysis was carried out at the Central Research Laboratory, Ilorin Kwara State.

Experimental Birds and Management

The experimental birds used consist of two Nigerian indigenous chickens (Naked neck and Fulani ecotype) and an exotic chicken (Sasso). The Parent chickens were sourced from pre-existing flock at LAUTECH Teaching and Research farm at maturity. Total of fifteen (15) sires and sixty (60) dams was used as parent stocks and distributed as 5 cocks to 20 hens

in each genotype. Birds were individually wing tagged for identification purposes. Sires and dams were caged in an open-sided house of 1.161m^2. Each bird was confined in a cell space of 15 × 15 inches for all the birds. Prior to stocking the cage, the whole pens and cages were washed and disinfected using Lysol® and was left for five days before stocking.

Experimental Feeds and Feeding

The cocks were fed *ad-libitum* with commercial grower mash containing 15.0% Crude Protein and 2500kcal/kg metabolizable energy while the hens were fed with commercial layer mash containing 16.0% Crude Protein and 2800kcal/kg metabolizable energy. Clean water was supplied *ad-libitum*. Drugs and vaccines were administered as at when due.

Brooding, Chick Rearing and Management

Before the arrival of the day old chicks, the brooder pens was washed with Lysol® and disinfected. The dimension of each brooder pen was 8 × 10m^2. Wood-shaving was spread on the floor of each pen and heat source was placed at a corner in the brooder pen to provide warmth for the chicks. The heat was supplied for 4 weeks. The chicks were brooded for four weeks. Adequate heat, ventilation, medication and feeding will be provided. The chicks were duly vaccinated and antibiotics were administered as required. The three generated progenies were reared in a deep litter. All the experimental birds were wing tagged for proper identification. At the arrival of the chicks, they were given multi vitamin drugs which served as an anti-stress. Commercial chick mash containing 22.0% crude protein and 2900Kcal/kg metabolizable energy was fed to the chicks from day old till eight weeks of age. Clean water was supplied *ad-libitum* to the birds. The litter was changed as at when due to prevent accumulation of ammonia gas. Spillage of water on the litter was also prevented as much as possible. All chicks remained on deep-litter throughout the period of the experiment.

Duration of the Experiment

The experiment was carried out for twenty-four (24) weeks.

Pre-Inoculation

The following data were collected before inoculating the birds with *Salmonella enteritidis*

Blood Collection

Two (2mls) of blood was collected from the wing veins of randomly selected birds for determination of both haematological and DNA analysis.

Haematological Parameters

Sample of blood (1 ml) was collected from twenty healthy birds randomly selected in each genotype at 7th week of age from both sexes, before the inoculation with attenuated *salmonella* and served as a base line study. The samples of blood was collected by the use of sterile syringes and needles from the wing veins and stored in the EDTA bottles (ethylene diamine tetra acetic acid). Erythrocyte concentration (RBC), haemoglobin (Hb), packed cell volume (PCV) and leucocytes concentration (WBC) counts was measured using an automated cell counter within 24 hours after collection of blood (8, 9). Haemoglobin (Hb) concentration was estimated using hemoglobinometer and Packed Cell Volume (PCV) was determined using the micro haematocrit method. Differential leukocyte count and RBC, WBC counting was carried out using compound microscope. The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were also determined, (10).

The following formulas were used: MCV in femrolitre (fL) = 10 × PCV (%)/RBC counts (millions/µ), MCH in pg/cell = haemoglobin (g/100 ml)/RBC counts (millions/µ), MCHC in g/dl = haemoglobin (g/100 ml) × 100/PCV (%)

Inoculation with attenuated *Salmonella*

The *Salmonella* to be used was sourced from the Central Research Laboratory, Ilorin Kwara State. At 8 weeks, the selected 20 experimental birds were inoculated subcutaneously with 0.5mL/bird of the attenuated *Salmonella* (9).

Post-Inoculation

Sample of blood (1 ml) was collected from twenty healthy birds randomly selected in each genotype at 7th week of age from both sexes, before the inoculation with attenuated *salmonella* and served as a base line study. The samples of blood was collected by the use of sterile syringes and needles from the wing veins and stored in the EDTA bottles (ethylene diamine tetra acetic acid). Erythrocyte concentration (RBC), haemoglobin (Hb), packed cell volume (PCV) and leucocytes concentration (WBC) counts was measured using an automated cell counter within 24 hours after collection of blood (8, 9). Haemoglobin (Hb) concentration was estimated using hemoglobinometer and Packed Cell Volume (PCV) was determined using the micro haematocrit method. Differential leukocyte count and RBC, WBC counting was carried out using compound microscope. The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were also determined, (10).

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Statistical Analysis

Data collected on blood was arcsine transformed to normalize the data and subsequently corrected for sex effect. After the correction, data was subjected to One-Way Analysis of Variance using the procedure of SAS (11). Significant mean differences were separated by Duncan's Multiple Range Test of the same software.

The model adopted is as specified below:

$Y_{ij} = \mu + \beta_i + e_{ij}$

Where;

Y_{ij} = response variable

μ = Overall mean,

β_i = Fixed effect of the i^{th} breed (i =1, 2, 3),

e_{ij} = Experimental error which is evenly distributed

RESULTS AND DISCUSSION

The mean values of haematological parameters of Sasso, Naked Neck and Fulani ecotype chickens before and after inoculation are presented in Table 1. The results of the Table revealed that genotype had significant ($p<0.05$) effect, on packed cell volume (PCV), haemoglobin (HB), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC), lymphocytes (L), basophils (B) and platelet (PLT) except for eosinphils ($p>0.05$) at both before and after. The values for PCV, HB, RBC, MCV, MCH, MCHC, WBC, L, B, M and PLT within each parameter were higher after inoculation when compared to before inoculation except for Sasso (MCV and M). Haematological examination contributes immensely to detection of some changes in the health status of birds that may not be obvious at the time of physical examination but undoubtedly affect the fitness of the birds (12). The haematological parameters observed in this research were genotype dependent and is consistent with the report of (13) strengthening the argument for inherent genetic differences. Red blood cell (RBC) transports oxygen to animal tissues for the oxidation process to release energy and transport carbon-dioxide out of the tissues (14).

CONCLUSION AND APPLICATION

There is a significant effect of genotype on all haematological parameters except eosinophils before and after inoculation. Naked neck had the highest values in all parameters except for mean corpuscular haemoglobin and lymphocytes.

Table 1: Mean Values of haematological parameters of chicken genotypes before and after inoculation

		GENOTYPE		
PARAMETERS (n=17)		**SASSO**	**NAKED NECK**	**FULANI ECOTYPE**
PCV (%)	Before	16.33±2.95^{b}	32.50±2.84^{b}	20.33±7.64^{b}
	After	26.25±1.72^{a}	34.50±2.84^{a}	23.63±4.41^{a}
HB (g/dl)	Before	7.600±1.47^{b}	15.53±1.29^{b}	9.13±2.09^{b}
	After	12.76±0.74^{a}	20.35±1.29^{a}	10.99±0.43^{a}
RBC (x10^{6}/µl)	Before	1.43±0.28^{b}	2.65±0.23^{b}	1.70±0.38^{b}
	After	2.24±0.15^{a}	3.59±0.23^{a}	1.99±0.39^{a}
MCV (fl)	Before	120.18±6.46^{a}	113.11±0.94^{b}	119.20±2.10^{b}
	After	117.49±1.12^{b}	126.35±1.24^{a}	122.59±2.10^{a}
MCH (pg)	Before	53.66±0.64^{b}	58.73±1.34^{b}	54.07±0.38^{b}
	After	56.93±0.89^{a}	61.50±1.34^{a}	55.89±0.38^{a}
MCHC (g/dl)	Before	453.88±19.9^{b}	479.50±14.27^{b}	452.33±9.02^{b}
	After	483.75±5.70^{a}	480.08±7.14^{a}	469.70±5.21^{a}
WBC (x10^{3}/µl)	Before	81.79±18.26^{b}	152.35±7.10^{b}	86.93±23.56^{b}
	After	112.875±3.90^{a}	154.35±7.10^{a}	107.51±23.56^{a}
L (x10^{3}/µl)	Before	84.75±2.76^{a}	69.25±3.90^{b}	88.00±1.00^{b}
	After	71.25±3.90^{b}	70.52±3.90^{a}	92.08±0.58^{a}
N (x10^{3}/µl)	Before	9.50±1.88^{b}	12.00±2.12^{b}	6.67±0.67^{b}
	After	11.50±2.11^{a}	16.85±2.12^{a}	9.14±0.67^{a}
M (x10^{3}/µl)	Before	2.50±0.59^{a}	4.25±1.03^{b}	3.27±0.17^{b}
	After	2.13±0.48^{b}	5.52±1.31^{a}	4.73±1.67^{a}
B (x10^{3}/µl)	Before	3.00±0.86^{b}	4.83±2.42^{b}	2.67±0.17^{b}
	After	13.75±2.62^{a}	16.07±2.42^{a}	11.52±0.07^{a}
E (x10^{3}/µl)	Before	0.00±0.00	0.50±0.50	0.00±0.00

	After	1.375±0.65	1.50±0.50	0.00±0.00
PLT ($x10^3/\mu l$)	Before	24.00±12.14[b]	83.25±44.93[b]	29.33±18.88[b]
	After	43.87±6.04[a]	85.43±22.46[a]	31.29±10.90[a]

[abc]= Mean occupying the same column having different superscripts are significantly different ($p<0.05$). OBS, PCV= Packed Cell Volume, HB= Haemoglobin, RBC= Red Blood Cell, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin MCHC= Mean Corpuscular Haemoglobin Concentration, WBC= White Blood Cell, N= Neutrophils L= Lymphocytes, M= Monocytes, B= Basophils, E= Eosinophils, PLT= Platelet

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