PHYSIOLOGICAL RESPONSES OF CHICKEN GENOTYPES INOCULATED WITH ATTENUATED *SALMONELLA ENTERITIDIS* IN THE DERIVED SAVANNA ZONE

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ABSTRACT

The Nigerian Indigenous Chickens (NICs) are characterized with high viability, disease resistance and heat tolerance compared with their exotic counterpart. Hence, this study examined the physiological responses of chickens inoculated with attenuated Salmonella enteritidis. The experiment was conducted at the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso. Seventy-five (75) matured chickens comprising of Naked Neck (NN), Fulani Ecotype (FE) and Kuroiler (Kr) were sourced from pre-existing stock on the farm while mating was done using artificial insemination in a Completely Randomized Design. One hundred and twenty progenies were generated from three crosses (NN × NN – 40 chicks, FE × FE – 40 chicks and Kr × Kr – 40 chicks) and are reared from day old to 24 weeks. Data collected on weekly basis were: Body Weight (BW), Rectal Temperature (RT), Pulse Rate (PR), Respiratory Rate (RR) and Heat Stress Index (HSI). Data collected were analyzed using General Liner Model and mean were separated by Duncan Multiple range test at P<0.05. Genotype significantly influenced (P<0.05) physiological parameters of all chicken genotypes studied. The progenies of Kuroiler had the highest body weight (632.88g), Kuroiler and Fulani ecotype had higher pulse and respiratory rate (50.14 – 50.55beats/min) after inoculation. Naked neck and FE progenies had higher antibody titre (60% and 40%) respectively after inoculation. In conclusion, NN × NN and FE × FE chickens should be selected for high disease resistance and for better adaption to tropical environment while Kuroiler (Kr × Kr) chickens should be considered for high body weight.

Keywords: Physiological, Inoculated, Attenuated, *Salmonella enteritidis*

INTRODUCTION

Indigenous chicken is the word used to describe chickens that are genetically adapted to a geographical location or environmental conditions (Yakubu *et al*., 2010). They are often indigenous to a particular geographical location, kept mainly in an extensive system, scavenging on free range and unimproved (Ajayi, 2010).These indigenous chickens constitute about 60 percent of the 120 million poultry birds found in Nigeria and more than 80 percent of these poultry population are found in the rural household (FAO, 2009; Oke, 2011). Fulani (Savanna), Yoruba (Forest), Nsukka, Owerri and Awgu ecotypes are some of the indigenous ecotypes of chickens found in Nigeria (Yakubu *et al*., 2010). High prevalence of diseases is among the major factors limiting high productivity of the indigenous chickens in the tropics among these diseases are *Salmonella enteritidis*. Although the disease has largely been eradicated from modern poultry in the developed countries of the world, it has increased in incidence in most developing countries of South America, Asia and Africa (Wale and Davies, 2011).

Salmonella serovar Enteritidis is the main causes of food poisoning worldwide. Chickens may be infected with Salmonella at any age, although the clinical signs may not be severe. Therefore, the infection can easily be transferred to humans. Salmonella is a bacteria disease been facultative and Zoonotic in nature. The level of bacterial pathogenesis depends on both the serovar of bacteria and the immunological status of the host. Salmonella engender acute systemic disease in young chicks (Friedman *et al*., 2003). *Salmonella entenritidis* infection is frequently consider as a problem of adult and grower chickens, chicks are also affected (Wales and Davies, 2011). Attenuated *Salmonella enteritidis* is host specific and can cause morbidity of 10-100% in chickens and mortality of up to 100% in an immunocompromised flock (Wales and Davies, 2011). Previous study evaluated the genetic influence on attenuated *Salmonella enteritidis* infection (Ogie *et al*., 2012), this study in turn evaluates the susceptibility of exotic breed and Nigerian indigenous chickens to experimental *Salmonella enteritis* infection.

Physiological response played an important role in immunity, particularly for the production of antibodies. Physiological responses can be assessed by rectal temperature, pulse rate, heart rate and body temperature. When birds are challenged, their physiological activities of their body changed (Adedeji *et al*., 2015). One of the physiological responses of exposing birds to stress is the release of glucocorticoids, causing dissolution of lymphocytes in lymphoid tissues and leading to lymphopenia. However, there is increase in heterophil release by the bone marrow, thus increasing their number in circulation, but their phagocytic and bactericidal activities decreased (Ilori *et al*., 2011 and Ijadunola *et al*., 2020). Therefore, genetic resistance was introduced to eradicate the spreading of the disease and to avoid the use of antibiotics due to residual effect on human health the cost spending to purchase drugs.

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. The site is located on longitude 415 East and latitude 815 North east of the Greenwich meridian. The altitude is between 300 and 600m above sea level. The mean annual rainfall and temperature are 1247 mm and 27°C, respectively (BATC, 2006).

Experimental Birds and Management

The experimental birds consist of two Nigerian indigenous chickens (Naked neck and Fulani ecotype) and an exotic chicken (Kuroiler). The Parent chickens (20 weeks) were sourced from the pre-existing flock at Teaching and Research farm. Total of fifteen (15) sires and sixty (60) dams were 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 × 15m^2. Prior to stocking, the whole pens and cages were washed and disinfected using Lysol® and left for five days before stocking.

Experimental Feeds and Feeding

The birds were fed *ad-libitum* with commercial grower mash containing 15.0% Crude Protein and 2500kcal/kg Metabolizable Energy for cocks 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.* Vaccines were be administered as at when due.

Semen Collection and Mating

Mating of birds was done using artificial insemination (AI) at 24 weeks while hand massage technique was adopted for semen collection.

Mating Design

Pure breeding:

Naked neck (sire) × Naked-Neck (dam)	(NN)
Fulani ecotype (sire) × Fulani ecotype (dam)	(FE)
Kuroiler (sire) × Kuroiler (dam)	(KR)

Egg Collection and Incubation

Total of 50 fertile eggs were collected from each of the Naked Neck, Fulani Ecotype and Kuroiler parents. Eggs were collected every day and arranged in the egg crates with the pointed ends down and pedigreed along sire and dam genotype lines. Eggs were allowed to accrue and stored at room temperature of 32^{o}C. The eggs were set in a cabinet type incubator at a commercial hatchery in Ibadan, Oyo State, Nigeria. The eggs were set along sire and dam genotype lines at a temperature between 37 - 39C and relative humidity of 55 – 56% for eighteen days.

Chick Rearing and Management

Before the arrival of the day old chicks (DOC), the brooder pens were washed with Lysol® and disinfected three days prior to arrival. The dimension of each brooder pen were 8 × 10m^2 and tagged along the sire and dam genotype lines. Wood-shaving was spread on the floor of each pen and heat source were placed at a corner in the brooder pen to provide warmth for the chicks. The heat was supplied for 4 weeks. At the arrival of DOC, they were given multi vitamin drugs which were served as an anti-stress. Commercial chick mash containing 22.0% Crude Protein and 2900Kcal Metabolizable Energy were fed to the chicks from day old till eight weeks of age. Commercial grower mash containing 15.0% Crude Protein and 2500Kcal Metabolizable Energy were offered from 8-12. Clean water was supplied *ad-libitum* to the birds. The litter was changed at 7 days intervals to prevent accumulation of ammonia gas. Spillage of water on the litter will be prevented as much as possible.

Pre-Inoculation: Forty (40) birds from each of the genotype were selected at 7th weeks to different pen.

Physiological Response: Forty (40) birds from each of the genotype were selected for physiological parameters which were determined by measuring the followings; Rectal Temperature (RT), Pulse Rate (PR), Respiratory Rate (HR) and Heat Stress Index (HSI).
Rectal Temperature (RT): The birds were measured during the hot afternoon (1300hrs – 1600hr). It was measured using a digital thermometer. This was done by inserting the digital thermometer 5cm deep into the cloaca of the birds for 1 minute.
Respiratory Rate (PR): This was measured by observing and counting the number of flank movement for a minute and recorded as breadth minute taken/minute.
Pulse Rate (HR): This was determined using a stethoscope which was placed on the flank to listen and count the number of heart beats/minute.
Body Weight (W): This was measured by sensitive scale (g).
Heat Stress Index (HSI): This is the body sensation of heat cause by the temperature and humidity. HIS = $0.5\times\{T61.0+[(T-68.0)\times1.2]+(RH\times0.094)\}$.
Post-Inoculation (Inoculation of attenuated *Salmonella enteritidis*): The cultured Salmonella used was sourced from the Central Research Laboratory, 132B University Road, Oko Erin 240103, Ilorin Kwara State. At eight weeks old, the selected 40 experimental birds were inoculated with 0.5 mL/bird of the attenuated *Salmonella* cultured subcutaneously with sterile needle, (Opoola 2020). All precaution measures were put in place during and after the inoculation process.
Data Analysis
Data collected on antibody titres were normalized using arscine transformation and thereafter were corrected for sex effect. Later, the data were analysed for the fixed effect of genotype using the procedure of SAS (2000). Significant mean differences were separated by Duncan's Multiple Range Test of the same software.
Physiological Response

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

Where;
Y_{ij} = Individual Observations
μ = Population mean
β_i = effect of i^{th} genotype (1,2,3)
e_{ij} = experimental error

RESULTS AND DISCUSSION

Table 1 revealed the mean values of body weight and physiological parameters of chicken at 7 weeks of age before inoculation with *Salmonella* organism. There was a significant effect ($p < 0.05$) of genotype on body weight, pulse rate, respiratory rate and heat stress index. The highest body weight (547.88g) and pulse rate (50.13 beats/mins) were observed in the Kuroiler birds while Naked neck and Fulani ecotype progenies had the highest heat stress index (3.69) and respiratory rate (54.00 breath/min) respectively. Generally, Naked neck had the lowest physiological parameters. Rectal temperatures of the birds were not significantly affected ($p > 0.05$) by genotype. In this study, increase in body weights across the genotypes after inoculation agrees with the works of Ilori *et al*. (2010). The mean values of body weight and physiological parameters of chicken genotypes after inoculation with *Salmonella* are shown in Table 2. There was a significant effect ($p < 0.05$) of genotype on body weight, pulse rate, respiratory rate and heat stress index. Kuroiler had the highest body weight (632.88g) while Naked neck (421.00g) was the least. The highest pulse rate (40.50 beat/min) and respiratory rate (50.50 breath/min) were observed in Kuroiler and Fulani ecotype chickens, respectively while naked neck had the least value. Naked neck (4.64) and Fulani ecotype (4.12) progenies had the highest heat stress index. The rectal temperature of the chickens before and after inoculation with *Salmonella enteritidis* though not significantly affected; however, the values remained within the normal ranges as reported by Oso *et al*. (2013) and Adedeji *et al*. (2015). Pooled mean values of body weight and physiological parameters of chicken genotypes before and after inoculations with Salmonella organism as revealed in Table 3 showed significant differences ($p < 0.05$) in body weight, pulse rate, respiratory rate and heat stress index across each genotype. Body weight and heat stress index increased across each genotype after inoculation with salmonella while pulse and respiratory rate were significantly lowered after inoculation with Salmonella organism. The results in the present study before inoculation are within the normal range reported by Dein *et al*. (2022) and after inoculation was in line with the reports of Babatunde *et al*. (2021).

Table 1: Mean Values of body weight and physiological parameters of chicken genotype before inoculation at 7 weeks of age

		PARAMETERS				
Genotype	**OBS**	**BW(g)**	**RT(^{0}c)**	**PR(beats/min)**	**RR(breaths/min)**	**HSI**
KR	24	547.88 ±104.70^a	41.78 ±0.32	50.13 ±0.92^a	52.88± 8.42^b	3.08±1.02^c
NN	18	389.00±46.57^c	41.95±0.27	39.50±4.51^c	50.25±5.36^c	3.69±1.40^a
FE	15	477.50±15.73^b	42.23±0.27	49.25± 2.50^b	54.00±12.00^a	3.13±1.30^b

^{abc}= Mean occupying the same column having different superscripts are significantly different ($p < 0.05$). OBS: observation, BW: Body Weight, RT: rectal temperature, PR: pulse rate, RR: respiratory rate, HSI: heat stress index.

Table 2: Mean Values of Body Weight and Physiological Parameters of Chickens after Inoculation

Genotype	**OBS**	**BW(g)**	**RT(^{0}c)**	**PR(beats/min)**	**RR(breaths/min)**	**HSI**
KR	24	632.88±61.02^a	41.8875±0.20	40.00±6.78^{ab}	50.13±8.34^b	4.10±1.42^c
NN	18	421.00±46.56^c	41.95±0.57	34.00±4.55^b	47.00±3.46^b	4.64±1.30^a
FE	15	558.00±9.67^b	42.45±0.25	40.50±8.23^{ab}	50.50±9.40^a	4.12±1.10^b

^{abc}= Means occupying the same column having different superscripts are significantly different ($p < 0.05$). OBS: observation BW: Body Weight, RT: Rectal Temperature, PR: Pulse Rate, RR: Respiratory Rate, HSI: Heat Stress Index.

Table 3: Pooled Mean Values of Body Weight and Physiological Parameters of Chicken Genotype before and after Inoculation with *Salmonella enteritidis*

Genotype		**OBS**	**BW(g)**	**RT(^{0}c)**	**PR(beats/min)**	**RR(breaths/min)**	**HSI**
Kuroiler	Before	24	547.88 ±104.7^b	41.78 ±0.32	50.13 ±0.92^a	52.88±8.42^a	3.08±1.020^b
	After	24	632.88±61.02^a	41.8875±0.20	40.00±6.78^b	50.135±8.34^b	4.10±1.42^a
Naked neck							
	Before	18	389.00±46.57^b	41.95±0.27	39.50±4.51^a	50.25±5.36^a	3.69±1.40^b
	After	18	421.00±46.56^a	41.95±0.57	34.00±4.55^b	47.00 ± 3.46^b	4.64±1.30^a
Fulani ecotype							
	Before	15	477.50±15.73^b	42.23±0.27	49.25± 2.50^a	54.00±12.00^a	3.13±1.30^b
	After	15	558.00±9.67^a	42.45±0.25	40.50±8.23^b	50.55±9.40^b	4.12±1.10^a

^{abc}= Mean occupying the same column within each genotype having different superscripts are significantly different ($p<0.05$). BW: Body Weight, RT: Rectal Temperature, PR: Pulse Rate, RR: Respiratory Rate, HSI: Heat Stress Index. OBS: number of observation.

CONCLUSION AND RECOMMEDATION

There were significant effect of genotypes on body weight, pulse rate, respiratory rate and heat stress index except rectal temperature before and after inoculation with *Salmonella enteritidis*. Kuroiler chicken had the higher body weight followed by Fulani ecotype chickens. However, naked neck and Fulani ecotype chicken had the highest heat stress index and respiratory rate respectively before inoculation. After inoculation, Kuroiler had the highest significant body weight and Pulse rate while Naked neck and Fulani ecotype had the highest respiratory rate and heat stress index. Naked neck had the highest resistance and heat stress index to Salmonella diseases and can be used for further research.

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