ANALYSES OF IMMUNE RESPONSE TO Salmonella typhimurium VACCINE OF THREE BROILER CHICKEN BREEDS RAISED UNDER HARSH ENVIRONMENT OF THE TROPICS

Igene, F.U., *Oguntade, D.O., Isidahomen, C.E. and Loyinmi, I.
Department of Animal and Food Sciences, Ambrose Alli University, Ekpoma Edo State
*Corresponding author: oguntadedo@aauekpoma.edu.ng

ABSTRACT
This study examined the body weights and immune response to Salmonella typhimurium vaccine of three popular breeds of broiler chickens (Arbor acre, Ross 308 and Cobb 500) reared in the tropics. A total of 75-day old chicks comprising 25 each of Arbor acre, Ross 308 and Cobb 500 broiler chickens were used for the study. The birds were inoculated with Salmonella typhimurium vaccine on day 14. Body weights were taken on days 7, 14, 21 and 28 from 12 birds from each breed while the blood samples were collected on day 14, 17 and 21 for immunological studies. Cobb 500 showed superiority in terms of body weight at day 7 (147.60±3.16 g) (before inoculation) and 14 (553.07±13.68 g), 21(842.79±20.86 g) and 28 (1340.25±55.71 g). The IgA and IgG levels of the three breeds of broiler chickens were not affected by breed before inoculation with Salmonella vaccines. However, on the 3rd day post inoculation when the birds were 17 days old, the IgA level was significantly (P<0.05) at the highest in Cobb 500 chickens (0.92±0.15µg/ml) while Arbor acre and Ross 308 broilers had IgA levels of 0.38±0.06 µg/ml and 0.48±0.09 µg/ml respectively. The results revealed that there are breed differences in the immune response to Salmonella typhimurium vaccine across three broiler breeds
Keywords: Arbor acre broiler, Ross 308 Broiler, Cobb 500 Broiler, *Salmonella*, IgA, IgG

INTRODUCTION
The major challenge to the overall success of broiler production in Nigeria is the high rate of infectious diseases. One of the infectious diseases of broiler chickens with high economic impact is *Salmonellosis* caused by *Salmonella* bacteria (Oguntade *et al.,* 2021). *Salmonella enterica serovars Enteritidis* and *Typhimurium* are a common cause of human foodborne illness globally (Knodler and Elfenbein, 2020). The fatality rate from invasive salmonellosis in African adults and children is estimated to be 22-25% (Hohmann, 2001) Contaminated poultry products, including chicken meat and eggs, are a frequent cause (Bearson *et al*., 2017). *Salmonella enterica* infection rarely causes clinical symptoms in poultry (Barrow *et al.,* 2012), and chickens are reported to be asymptomatic carriers of *Salmonella* (Shanmugasundaram *et al*., 2019). However, subclinical physiological and behavioral indicators may exist as *Salmonella* infection can induce immune stress in chickens, impacting welfare and performance. Thus, the prevalence of *Salmonella* in broiler flocks and other products is an important production, food safety, and human health concern, but little research has focused on the pathogen resistance of broiler breeds that differ in growth rate (Shanmugasundaram *et al*., 2019).

Both innate and adaptive immune responses are stimulated by *Salmonella* infection within the chicken gastrointestinal system, involving both cytokine action (Brisbin, 2011) and antibody responses. Dar *et al*. (2019) orally challenged 4-day old Cobb broiler chicks with *S. typhimurium* and reported elevated serum levels of immunoglobulins (Ig) M and IgG as well as increased mRNA expression of the cytokines such as interferon-gamma (*IFN-γ*), interleukin (*IL*-12, and *IL*-18). In a study evaluating the susceptibility to intra-abdominal *Salmonella enteritidis* infection of two commercial broiler breeds and their crosses, Swaggerty *et al*. (2005) found that one parent breed and one crossbred breed had a greater number of heterophils at the infection site and increased mRNA cytokine expression. This study therefore was set to determine the immune response of three breeds of broiler chickens to *Salmonella typhimurium* vaccine as this will help in formulating treatment strategies for management of salmonella infections in poultry

MATERIALS AND METHODS
The research was carried out at the Poultry Unit of the Livestock Teaching and Research Farm of Ambrose Alli University, Ekpoma, Edo State.
A total of 75 broiler chickens were used for this study. These consisted of 25 birds each of Ross 308, Arbor acre and Cobb 500 broiler chickens. The experiment lasted for 4 weeks. All the experimental birds were wing tagged for proper identification and subjected to the same management practices

throughout the experimental period. The chicks were brooded for three weeks and medication and vaccinations were carried out accordingly. The birds were fed commercial broiler diets *ad libitum* and provided with clean water.*ad libitum*.

Qualitative Bacteriology of *Salmonella* spp

Qualitative bacteriology and *Salmonella typhimurium* assessment was performed at day-old and 1 day before inoculation at day 13 to ensure that the birds were free of *Salmonella typhimurium* before inoculation. At day1, 15 birds (n = 5 birds/breed) were randomly selected and their vents aseptically swabbed. Swabs were individually incubated in 10 mL trytpic Soy Broth (TSB) diluted with phosphate-buffered saline (PBS) for 24 h at 37 °C. Swabs were plated on bismuth sulfite agar (BSA) plates and incubated at 37 °C for 24 h. Qualitative bacteriology of *Salmonella spp* was repeated on day 13 with 24 birds (n = 8 birds/breed) using the same protocol as done at day-old. Each swab from day 13 was plated on BSA as well as BSA + NAL plates to confirm birds are negative for *Salmonella spp*. and NAL-resistant *Salmonella typhimurium*, respectively.

Inoculation with *Salmonella typhimurium* vaccine

Salmonella vaccine (AviPro ® 109 ST4 Concentration, Elanco *Salmonella* control solutions, USA by/Fabricado en los EE.UU.por: Elanco US Inc.) was purchased from reputable Veterinary source in Ibadan, Oyo state. At day 14, the experimental birds were inoculated with *Salmonella* vaccine. This was done via subcutaneous injection of *Salmonella* vaccine at 0.5mL (LD_{50}) per bird as recommended by the manufacturer.

Data collection

Mortality was recorded daily. Body weight was taken on day 7, 14, 21, and 24 from 12 birds from each breed were selected while the blood samples were collected on day 14, 17 and 21 for immunological analyses. Approximately 2 mL blood samples were collected through the wing vein by using sterile needle and syringe and decanted into plasma separation tubes. Blood was centrifuged for 10 min at 2,000 × g and 15 °C to separate plasma. The body weight was measured using sensitive scale (Camry digital scale @ Echas Scientific Nigeria limited).

Determination of Plasma IgA and IgG

Plasma was stored at −80° C for immunological analyses. An ELISA kit (Bethyl Laboratories Inc., Montgomery, TX, USA) was used to determine immunoglobulin A (IgA) and immunoglobulin G (IgG) concentrations. The manufacturer protocol was religiously followed using the recommended sample dilutions (1:1000 for IgA, and 1:100,000 for IgG). Absorbance was measured on a plate reader at 450 nm. A standard curve was generated for each plate to calculate sample IgA and IgG concentrations (μg/ml).

Statistical analysis

Data on body weights and immunoglobulin were subjected to One-way analysis of variance using the procedure of SAS (2014) for the fixed effect of breed. The mean differences were separated using Duncan's multiple range test of the same software. The below model was adopted:

Yij = μ + bi + eij; Where, Yij = individual observation; μ = overall mean; bi = fixed effect of ith; breeds (1, 2, 3,); eij= experimental errors which is evenly distributed.

RESULTS AND DISCUSSION

Body weights of broiler chickens challenged with *Salmonella typhimurium* vaccine as affected by breeds: The body weights of three breeds of broiler chickens (Arbor acre, Ross and Cobb 500) inoculated with *Salmonella typhimurium* vaccine is showed in Table 1. The results showed that at day 7 before inoculation, Cobb 500 broiler chickens had significantly ($P<0.05$) highest body weight (147.60±3.16 g) followed by Ross (133.80±4.89 g) and Arbor acre (9123.80±3.66 g) also, Cobb 500 broiler chickens showed significant ($P<0.05$) superiority in terms of body weights (553.07 g) at the day of inoculation (day 14) higher body weight in Ross 308 (535.33±17.16 g) and arbor acre (498.67±13.02 g). Cobb 500 broiler chickens recorded significantly ($P<0.05$) highest body weight (842.79 g) at day 21 (7^{th} day post inoculation) than body weight (801.80±27.89 g) obtained for Ross 308 while Arbor acre broiler chickens recorded the lowest body weight (731.93±22.38 g). At Day 28 (14^{th} after inoculation), the body weight (1340.25±55.71 g) of Cobb 500 broiler chicken was significantly ($P<0.05$) outwit those

of Arbor acre and Ross 308 broiler chickens which were not significantly (P>0.05) different from each other.

Table 1 Body weights of broiler chickens challenged with *Salmonella typhimurium* vaccine as affected by breed

Breed	Body weights (Days)			
	Day 7	Day 14 (day 0 PI)	Day 21 (day 7 PI)	Day 28 (day 14 PI)
Arbor acre	123.80±3.66^{b}	498.67±13.02^{b}	731.93±22.38^{b}	1110.20±64.20^{b}
Ross 308	133.80±4.89^{b}	535.33±17.16^{ab}	801.80±27.89^{a}	1057.25±94.60^{b}
Cobb 500	147.60±3.16^{a}	553.07±13.68^{a}	842.79±20.86^{a}	1340.25±55.71^{a}

Note: a, b Means within the same column with different superscripts are significantly different (P<0.05)
PI: Post inoculation

In this study, the significantly heaviest body weight of Cobb 500 compared to Arbor Acre and Ross 308 broiler chickens before inoculation with *Salmonella typhimurium* vaccine could be due to genetic differences in growth rates among these three broiler chicken breeds. In addition, Cobb 500 broiler chickens showed superior body weights at days 0 and 7 post-inoculation with *Salmonella typhimurium* vaccine. Further, on day 14 post-inoculation, when the chickens were 28 days old, Cobb 500 broiler chickens had the heaviest weight, significantly.

The highest body weights of Cobb 500 broiler chickens at days 0, 7 and 14 post-inoculation as compared to Arbor acre and Ross 308 could suggest an improved performance of this breed and thus showcasing breed-specific adaptive immunity. This result is consistent with previous studies reporting on growth rates broiler chickens of different breeds. For instance, Hristakieva *et al.* (2014) observed that Cobb 500 was significantly heavier than Ross 308 by 6.29% under the same experimental conditions. In another experiment by Benyi *et al*. (2015), it was reported that Cobb 500 was outperformed Ross 308 in terms of body weight. In addition, according to earlier research by Petricevic *et al.* (2011), Cobb 500 and Ross 308 were heavier than Hubbard chickens. Thus, the findings of this study align with those of the aforementioned authors regarding the superior body weight of Cobb 500 broiler chickens over Ross 308 and Arbor Acre broiler chickens.

The IgA and IgG of broiler chickens challenged with *Salmonella typhimurium* vaccine as affected by breeds

Table 2 shows the IgA and IgG of broiler chickens challenged with *Salmonella typhimurium* vaccine as affected by breeds. We reported no significant difference in the levels of IgA and IgG across the three breeds of broiler chickens before inoculation with *Salmonella typhimurium* vaccine.

Table 2 The IgA and IgG of broiler chickens challenged with *Salmonella typhimurium* vaccine as affected by breed

Breeds	**Before inoculation (13th day old)**		**Day 3 Post inoculation (17th day old)**		**Day 7 Post inoculation (21st day old)**	
	IgA (µg/ml)	**IgG (µg/ml)**	**IgA (µg/ml)**	**IgG (µg/ml)**	**IgA (µg/ml)**	**IgG (µg/ml)**
Arbor acre	1.09±0.08^{a}	2.33±0.63^{a}	0.38±0.06^{b}	1.40±0.13^{a}	0.28±0.04^{a}	1.03±0.22^{a}
Ross 308	0.70±0.42^{a}	1.03±0.09^{a}	0.48±0.09^{b}	1.63±0.30^{a}	0.35±0.05^{a}	0.72±0.05^{a}
Cobb 500	0.34±0.11^{a}	1.77±0.32^{a}	0.92±0.15^{a}	1.84±0.37^{a}	0.31±0.05^{a}	0.83±0.09^{a}

Note: a, b Means within the same column with different superscripts are significantly different (P<0.05)

This is because the three-broiler chicken breeds were not previously infected before inoculation. However, the levels of IgA were significantly (P<0.05) highest in Cobb 500 broiler chickens (0.92±0.15 µg/ml) followed by Ross 308 (0.48±0.09 µg/ml) and Arbor acre broiler chickens (0.38±0.06 µg/ml) at day 3 post inoculation when the birds were 17th day old. A higher level of IgA in Cobb 500 broiler chicken at day 3 post inoculation suggests its immune capacity to confer protection against infection. Specifically, IgA specializes in protecting the body against bacterial growth and colonization by protecting epithelial surface from infection (Macpherson *et al.,* 2012). Thus, the abundance of IgA in Cobb 500 broiler chickens could suggest immune response potential of this chicken breed to salmonella

infection which aligns with the report by Woof and Mestecky (2005) that immunoglobulin A (IgA), is a major serum immunoglobulin and the predominant antibody class in the external secretions that bathe mucosal surfaces plays key roles in immune protection. Dar *et al.* (2019) orally challenged 4-day old Cobb broiler chicks with *S. typhimurium* and reported elevated serum levels of immunoglobulins (Ig) M and IgG as well as increased mRNA expression of the cytokines

CONCLUSION

This study examined the body weights and immune response to *Salmonella typhimurium* vaccine of three popular breeds of broiler chickens raised in the tropics. The results revealed that Cobb 500 broiler chickens was heaviest in body weights than Arbor acre and Ross 308 broiler chickens both before and post inoculation with *Salmonella typhimurium* vaccine. In addition, Cobb 500 broiler chickens had highest levels of IgA at 3 days post inoculation thus showcasing immune response potential of this breed of chicken against *Salmonella* infection.

REFERENCES

Barrow, P.A., Jones, M.A., Smith, A.L. and Wigley, P. (2012). The Long View: *Salmonella*- the Last Forty Years. *Avian Pathology,* 41: 413–420.

Bearson, B.L., Bearson, S.M.D., Looft, T., Cai, G. and Shippy, D.C. (2017). Characterization of a Multidrug-Resistant *Salmonella Enterica* Serovar Heidelberg Outbreak Strain in Commercial Turkeys: Colonization, Transmission, and Host Transcriptional Response. *Front. Veterinary Science,* 4: 156.

Benyi, K., Tshilate, T.S., Netshipale, A.J. and Mahlako, K.T. (2015). Effects of genotype and sex on the growth performance and carcass characteristics of broiler chickens. *Tropical Animal Health and Production* 47, 1225–1231.

Brisbin J. T. (2011). Regulation of the Chicken Immune Response by the Intestinal Microbiota. [Dissertation]. Ontario, Canada: The University of Guelph.

CDC, (2020). Centers for Disease Control and Prevention. *Salmonella*. Available at: https://www.cdc.gov/salmonella/index.html (Accessed June 23, 2024)

Dar, M.A., Urwat, U., Ahmad, S.M., Ahmad, R., Kashoo, Z.A. and Dar, T.A. (2019). Gene Expression and Antibody Response in Chicken against *Salmonella Typhimurium* Challenge. *Poultry Science,* 98: 2008–2013.

Hohmann, E. L. (2001). Nontyphoidal salmonellosis. Clinical Infectious Diseases, 32(2), 263-269.

Hristakieva, P., Mincheva, N., Oblakova, M. Lalev, M. and Ivanova, I. (2014). Effect of genotype on production traits in broiler chickens. Slovak J. Anim. Sci., 47, 2014 (1): 19-24

Knodler, L. A. and Elfenbein, J. R. (2020). *Salmonella E . Trends Microbiol*ogy, 27 (11): 964–965.

Macpherson, A. J., Geuking, M. B. and McCoy, K. D. (2012). IgA: A bridge between innate and adaptive immunity. *European Journal of Immunology*, 42(10), 2687-2693.

Oguntade, D.O., Ilori, B.M., Durosaro, S.O., Abiona, J.A., Isidahomen, C.E. and Ozoje, M.O. (2021). Genetic Variations in Haematological Indices of Local and Exotic Turkeys Inoculated with Attenuated Salmonella typhimurium Vaccine. *Asian Journal of Biochemistry, Genetics and Molecular Biology,* 8(1): 19-29.

Petricevic, V., Pavlovski, Z., Skrbic, Z. and Lukic, M. (2011). The effect of genotype on production and slaughter properties of broiler chickens. *Biotechnology in Animal Husbandry*, 27(2): 171-181.

Shanmugasundaram, R., Mortada, M., Cosby, D.E., Singh, M., Applegate, T.J. and Syed, B. (2019). Synbiotic Supplementation to Decrease *Salmonella* Colonization in the Intestine and Carcass Contamination in Broiler Birds. *Plos One*, 14: e0223577.

Swaggerty, C.L., Pevzner, I.Y., Lowry, V.K., Farnell, M.B. and Kogut, M.H. (2005). Functional Comparison of Heterophils Isolated from Commercial Broiler Chickens. *Avian Pathology,* 32 (1): 95–102.

Woof, J.M. and Mestecky, J. (2005). Mucosal immunoglobulins Immunological *Reviews,* 206 (1): 64-82