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# Cellular and Humoral Immune Response in Vertically Transmitted *Salmonella* isolates in Broiler Chickens: A Case Study of Ogun, Oyo and Lagos States, Nigeria

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author RAO designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors DE, AOO and FOO reviewed the experimental design and all drafts of the manuscript. Authors PAA, AOA and DE managed the analyses of the study. Authors RAO and AOO performed the statistical analysis. All authors read and approved the final manuscript.*

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## ABSTRACT

This study investigated the humoral and cellular immune response in vertically transmitted salmonella isolates in broiler chickens in some selected states of South-Western Nigeria and its control through the use of feed additives. Anak 2000 day-old broiler chickens totaling 360 (120 birds from each state) were collected from hatcheries that were positive to *Salmonella* organisms and used for performance testing which lasted for 8 weeks. The birds were laid out in a 3x5 factorial arrangement comprising of 5 dietary treatments including a control and 4 different feed

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additives (mannose oligosaccharide (MOS), arabinoxylose oligosaccharide (AXOS) and *Pediococus acidilactici* (SIML) and antibiotic; Oxytetracycline). Blood samples were collected from the birds and their total and differential white blood cells were determined while humoral antibody titre to *Salmonella* was performed.

Total white blood cells (TWBC) was significantly ($P<0.05$) increased in the control diet across the locations compared to lower values in other dietary treatments applied. Neutrophil was significantly ($P<0.05$) increased across the location and decrease with various dietary treatments while Lymphocyte and Basophil values varied significantly ($P<0.05$) across the locations and the treatments. Monocyte and Eosinophil were not affected by both location of the hatchery and additives. *Salmonella* antibody titre ≤ 1:20 for 'O' and 'H' antigen was observed in all birds from various location after treatment with antibiotic (oxytetracyline) and significant reduction of salmonella antibody among birds fed with MOS, AXOS and SIML.

Inclusion of probiotic and prebiotic additives are effective and safe methods for prevention of *Salmonella* infection in broiler chickens and enhance poultry productivity.

*Keywords: Probiotic; prebiotic; immunity; broiler.*

## 1. INTRODUCTION

*Salmonella* infection, or salmonellosis, is a common cause of mortality of wild birds, affecting many bird species worldwide. *Salmonella* bacteria are more closely associated with poultry than their ubiquitous distribution deserves. *Salmonella* bacteria are not a single entity but exist in a huge range of serotypes (serovars) from dedicated poultry pathogens like *Salmonella pullorum* and *S*. *gallinarum* to zoonotic serotypes [1]. Prevention and clearance of *Salmonella* infection by humoral mechanisms alone is unlikely, as *Salmonella organism* is a facultative intracellular bacterium. There is sufficient evidence from various animal models that cell-mediated immunity plays a major role in controlling *Salmonella* infection [2]. $CD3^+$, $CD4^+$, and $CD8^+$ T cells were observed to proliferate in the reproductive tract of *Salmonella* infected chickens [3,4]. However, T-cell immunosuppression with cyclosporine A showed no significant effect on *S. enterica* serovar Enteritidis infection in chickens [5]. It is therefore unclear whether T cells play a role in immune responses against *S. enterica* serovar Enteritidis in chickens.

The immune system is a naturally existing protective system usually stimulated by pathogens isolates. Though, vaccines stimulate specific immune responses to pathogens that provide animals with protection [6]. In general, the mucosal immune system of the intestine, including mucosal immunoglobulin A (IgA) and mucosa-associated lymphocytes and leukocytes, forms the first line of defence against *salmonella* infection [1]. Systemic immune responses, including humoral and cell-mediated responses, play important roles in the resistance and clearance of *Salmonella* infection. The humoral immune responses of chickens after infection with *Salmonella* have been extensively studied for diagnostic purposes [7]. The fundamental mechanism of mucosal resistance to infection and clearance of *S. enterica* serovar Enteritidis from the gut of chickens through probiotics has received special attention as alternative to antimicrobial use among poultry farmers [8].

Human *salmonella* infection has now been recognised as important food borne diseases with more than 13 million cases of typhoid and paratyphoid infections worldwide [9].

The major route of poultry infection is usually oral, the navel/yolk, transovarian or horizontal by faecal-oral contamination and human infection via consumption of *Salmonella* infected egg or its products which are now fast becoming prevalent, with increasing morbidity of 10 –100% and mortality due to increase pathogenesis and immune-compromise. Mortality in Immuno-compromised flocks could be up to 100% [10]. Therefore, this study was done to assess the humoral and cellular immune responses in *Salmonella* infection vertically transmitted in chicken reared in some states of South-Western, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Location

The *in vivo* studies were carried out at the Teaching and Research Farm Development (TREFAD) of the Federal University of Agriculture, Abeokuta, Nigeria. Abeokuta (7° $10^1$N and 3° $2^1$E) area is 76 m above sea level,

humid and located in the tropical rain forest vegetation zone with an average temperature of 34.7℃.

A total number of 360 day-old broiler birds were purchased from the selected hatcheries within Ogun, Oyo and Lagos State, which have been identified as potentially *salmonella* organism carriers, 120 birds were sourced from each of the locations. Birds from each location were divided into 5 groups of 24 birds each. Each group was further divided into 3 sub-groups of 8 birds each serving as the replicate. Each pen measured 2.7 m by 0.9 m and provided a total floor area of 2.43 $m^2$. The pen was thoroughly washed and disinfected before arrival of the chicks. The birds were raised on deep litter system equipped with separate feeding and water troughs. Water and feed were supplied *ad libitum*, routine vaccination and medications were administered to the birds accordingly. Litter was changed regularly to prevent build up of pathogens. Five experimental diets were formulated such that diet 1 was the control with no test ingredient while diets 2, 3, 4 and 5 had inclusions of an Antibiotic (oxyteraxycline), Prebiotic 1 Mannose oligosaccharide (MOS), Prebiotic 2 Arabinoxylose oligosaccharide (AXOS), and Probiotic Sim® -lac (*Pediococus acidilactici* ) respectively (Tabe1). Recommended levels of inclusion were used for each of the additives.

## 2.2 Cell-mediated Immune Response

A 2.5 ml of blood was collected with syringe from the wing web vein of the birds into tube containing ethylene diamine tetra acetate (EDTA) and stored at 4℃ until it was ready for analysis. After 28 days of feeding the broiler with additives and antibiotics supplemented feed, 2.5 ml of blood samples from each treatment group were collected from the brachial wing vein of four birds per pen (n = 16 per treatment) into vials containing (EDTA) bottles. Haemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method while

**Table 1. Percentage composition of broiler finisher diets (4 -8 weeks)**

| Ingredients % | Experimental diets | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** |
| Maize | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 |
| SBM | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 |
| GNC | 11.00 | 11.00 | 11.00 | 11.00 | 11.00 |
| Fish meal (72%) | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Wheat offal | 19.00 | 19.00 | 19.00 | 19.00 | 19.00 |
| Bone meal | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Oyster shell | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Methionine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Lysine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| ∗Premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| [1].Oxytetracycline | - | + | - | - | - |
| [2].MOS | - | - | + | - | - |
| [3].AXOS | - | - | - | + | - |
| [4].Sim® lac | - | - | - | - | + |
| Total | **100.00** | **100.00** | **100.00** | **100.00** | 100.00 |
| Determined Analysis | | | | | |
| Metabolizable energy (MJ/kg) | 12.43 | 12.43 | 12.43 | 12.43 | 12.43 |
| Crude protein % | 19.90 | 19.90 | 19.90 | 19.90 | 19.90 |
| Crude fibre % | 5.66 | 5.66 | 5.66 | 5.66 | 5.66 |
| Ether extract % | 4.81 | 4.81 | 4.81 | 4.81 | 4.81 |
| Available Ca % | 1.97 | 1.97 | 1.97 | 1.97 | 1.97 |
| Available P % | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 |
| Lysine % | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 |
| Methionine % | 0.56 | 0.56 | 0.56 | 0.56 | 0.56 |

- *Vitamin and mineral premix based on 2.5 kg/ ton; Vit A; 4000000 Iu, Vit D:800000, Vit B12: 25 mg, Niacin:60000 mg, Vit E 40000, Viut k3 800 mg, Vit B3 1000 mg, Vit B2 6000 mg, Vit B6 5000 mg, Panthotenic Acid: 20000, Folic Acid: 200 mg, Biotine 8 mg, Maganese:300000 mg, Iron 80000 mg, Zinc: 20000 mg, Copper: nill, Cobalt: 80 mg, Iodine: 400 mg, Selenium: 40 mg, Choline: 800000 mg*

Packed cell volume (PCV) of blood samples were determined in a Wintrobe haematocrit tube according to the method of [11]. Cellular immune response was determine by total white blood cell count (WBC) by standard method and differential leucocyte cell counts of heterophils, lymphocytes, eosinophils and monocytes were carried out on blood smears stained with May-Grunwald-Giemsa stain and estimated accordingly.

### 2.3 Detection of the Humoral Immune Response

Two millilitres of blood sample was collected via the wing vein puncture using two birds per replicate at day 56 into plain vacutainers. The blood was allowed to clot at room temperature for one hour. The blood was centrifuged at 3000 rpm for 5 mins and the serum carefully collected into clean bijou bottles and stored at –2℃ prior to analysis.

### 2.4 Antibody Titration

Tube agglutination test was performed by antibody titration according to modified method described by [12] on the sera using salmonella polyvalent 'O' and 'H' test kit produced by Cromotest Linear Chemicals, Montgat, Barcelona Spain. Serial dilution of each serum sample was made in sterile normal sodium chloride solution (0.85%), in 1:20, 1:40, 1:60, 1:80, 1:160, 1:320 and 1:640 dilutions. To each 0.1 ml diluted sera, 0.1 ml of salmonella polyvalent 'O' and 'H' antigen was added and thoroughly mixed. All the tubes were incubated at 37℃ for 24 hours. Each tube was microscopically examined for agglutination and the highest dilution showing agglutination was taken as the titre values.

### 2.5 Statistical Analysis

Data obtained were analysed using descriptive test while the analysis of variance was done using [13].

## 3. RESULTS

1. The Interaction effect of location of hatchery and feed additives on haematology and serum profile of finishing broiler is shown in Fig. 1. Total white blood cells (TWBC) was significantly ($P<0.05$) increased in the control diet across the locations compared to lower values in other dietary treatments applied. Decreased count in TWBC was observed among Oyo and Lagos fed with additives.
2. The result of the main effect of location of hatchery and additive on finishing broiler chicken on white blood cell differential measured in this study is presented in Table 3. Value obtained for Neutrophil was significantly ($P<0.05$) increased across the location and decrease with various dietary treatments. The mean values for the various parameters were reduced ($P<0.05$) across the different additives with the control birds having highest ($P<0.05$) value. Location of the hatchery significantly ($P<0.05$) affected the neutrophil count whereby Ogun state location had the highest value and different from the value obtained in Oyo. Lymphocyte, Monocyte, Eosinophil and Basophil which were not significantly ($P>0.05$) affected by location and additives. The interaction effect of location of hatchery and additive on white blood cell differential of finishing broiler is comparatively shown in Table 3. Neutrophil, Lymphocyte, and Basophil values varied significantly ($P<0.05$) across the locations and the treatments. Monocyte and Eosinophil were not affected by both location of the hatchery and additives.
3. *Salmonella* antibody titre in broiler fed different feed additives: Table 4 shows *Salmonella* antibody titre value in broiler chickens fed different feed additives. The result revealed that the control birds showed *Salmonella* antibody titre ≥ 1:40. However, *salmonella* antibody titre ≤ 1:20 for 'O' and 'H' antigen was observed in all birds from various location after treatment with antibiotic (oxytetracyline) except Ogun having 'O' and 'H' 1:40 titre value. *Salmonella* antibody 'O' and 'H' of 1:20 was observed in birds across the locations except Oyo location having 1:40 'H' antigen, thus, a reduction of *salmonella* antibody to MOS. After treatment with AXOS, birds from Oyo location and Ogun location showed reduced titre of 1:20 to *salmonella* 'O'an 'H' antigen, while only Lagos location birds showed an increasing titre of 1:160 and 1:40 to *salmonella* '0' and 'H' antigen. However, *salmonella* antigen 'H' was not present with 0:0 titre recorded for AXOS in Ogun location and 1:40 to *salmonella* 'H' antigen. **Sim® lac** showed a reducing *salmonella* 'O' and 'H' titre of 1:20 in birds from Oyo and Lagos locations, slight increase of 1:40 to *salmonella* 'O' antigen in Ogun location was observed.

## 4. DISCUSSION

The immune response in poultry developed several levels of defense strategies to cope with a wide spectrum of pathogens. This include innate immunity such as physical and chemical barriers that prevent entry of the pathogen, while cellular and soluble components eliminate the pathogen once it has gained entry. Most pathogen often escape innate immune response but recognizing this pathogen as antigen adaptive immunity specifically focus defense mechanisms on that particular pathogen resulting not only in the elimination of the pathogen but also as protection in case of a repeat encounter with the same pathogen [14]. White blood cell count is an indication of body defence mechanism that fights against infection and foreign body, and its increase suggest a challenge for foreign antigen which in order increases its defence abilities. The feeds additives lowered the Neutrophil level which is an indication of positive effect of additives exerted in the birds to suppress any effect of antibodies response from *salmonella* challenge. The lymphocyte, monocyte, eosinophil and basophil did not follow a pattern even though the values were not significant.

Successful probiotic bacteria and prebiotics are usually able to colonize the intestine, at least temporarily, by adhering to the intestinal mucosa and thereby effect antagonistic activity against enteropathogen and modulation of immune system [15]. Protective effects of feed additives antagonism through the production of antimicrobial substances [10] or competition with the pathogen for adhesion site or nutrient sources [16], immunomodulation of the host [17], and inhibition of the production of bacterial toxin [18] are various indicated mechanism of probiotic and prebiotic beneficial effect. This immune reactivity can be modulated by nutritional interventions such as alteration in minerals, vitamins, essential fatty acids or other substances (e.g Oligosaccharide). Animals maintain their immunity by a defense system consisting of various types of white blood cells (leukocytes) which act in concert with a number of biochemical factors in the tissue fluids (humeral factors). The immuno-competent cells can be divided into the antigen specific and antigen-a-specific cell. The antigen-a-specific cells, such as phagocytes, neutrophils and thrombocytes, are responsible for the innate Immunity (The first immune defence line), while the antigen-specific cells: B-cells and the T-cells is responsible for the second defence line at cellular and non-cellular level and the antigen-specificity is then mediated by cell surface receptors. The antigenic variant of different feed additives at different hatchery in *salmonella* antibody titre, in effect, the antibody titre 'O' and 'H' ≥ 1:20 across the locations for oxytetracycline, AXOS and Sim® lac suggest that the birds could have been exposed to *salmonella* antibodies from the hatchery (by vertical transmission) but did not show active infection probably due to effect of the additives on the challenge on the immunity of the birds, thus there was a significant reduction in the titre value at location Oyo and Lagos. Likewise, the birds that showed significant low 'O' titre of 1:20 and 'H' titre 1:40 to MOS indicating increasing response to *salmonella* antigen which could be elicited via lectin complement cascade, where the mannose-binding activity with the complement could initiate active antibody production against *salmonella* antigen through lectin complement pathway, (8). However, lectins are proteins that recognise and bind to specific carbohydrate target mannose binding lectin (MBL) on the surface of pathogen cell by associated serine proteases; MASP-1 and MASP-2 which bind to MBL. The active complex formed by this association causes cleavage and activation of C4 and C2, this further activate the C2-C4 complex to form C5 convertase without need for specific antibody binding which represents an important innate defense mechanisms. The result of this study showed that the titre value for birds on MOS had a lower titre value due to mannose binding activity with, a constituent of the polysaccharide capsule of many pathogenic fungi and yeast which is one of the several polysaccharide substances to which MBL binds via $Ca^{2+}$ dependent interaction [19]. Both MBL and serum ficolins are acute phase reactant, that is, their concentration increases during infection and inflammation. 'H' antibody titre of ≥ 1:20 is significantly low for oxytetraxycline, AXOS and Sim® lac while 'H' antibody titre of 1:40 showed a significant high titre in Oyo Hatchery suggesting long lasting IgA antibody that could confer latent immunity against future challenge and as well suggest a flagellated salmonella infection in the birds. The humoral immune response against *salmonella* (serum IgM and IgA levels) was significantly greater in the feed additives group than in the control; this result is in agreement with (12) who observed significant increase in humoral immune response against salmonella in piglets fed probiotics.

**Table 2. Effect of location and feed additives on white blood cell differentials on broiler chicken**

| Parameters | Hatchery | | | | Additives | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Oyo | Ogun | Lagos | SEM | Control | AB | MOS | AXOS | SIML | SEM |
| Neutrophil | 38.167[b] | 47.933[a] | 39.867[b] | 0.93 | 49.333[a] | 45.056[b] | 37.778[c] | 43.667[b] | 34.111[d] | 1.20 |
| Lymphocyte | 55.500 | 46.400 | 55.200 | 1.41 | 47.167 | 47.889 | 54.000 | 48.111 | 59.667 | 1.82 |
| Monocyte | 1.267 | 1.200 | 0.933 | 0.18 | 0.667 | 1.333 | 1.556 | 1.222 | 0.889 | 0.23 |
| Eoninophil | 2.667 | 3.067 | 4.267 | 0.78 | 2.333 | 5.667 | 2.333 | 3.444 | 2.889 | 1.01 |
| Basophil | 0.667 | 0.467 | 0.533 | 0.12 | 0.667 | 0.556 | 0.556 | 0.556 | 0.333 | 0.15 |

*[abc] means on the same row having different superscript are significantly different (P<0.05), Control=No Additives; AB= Antibiotics (oxytetraxycline); MOS=Mannose oligosaccharide; AXOS=Arabinoxylans Oligosccharide; SIM L=pedicoccus Acidilactis*

**Table 3. Interaction effect of location of hatchery and feed additives on white blood cell differentials on Broiler Chicken**

| | Oyo | | | | | Ogun | | | | | Lagos | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Additives | | | | | Additives | | | | | Additives | | | | | |
| Parameters% | Control | AB | MOS | AXOS | SIML | Control | AB | MOS | AXOS | SIML | Control | AB | MOS | AXOS | SIML | SEM |
| Neutrophil | 41.333[g] | 49.500[d] | 26.000[k] | 26.667[k] | 47.333[e] | 57.333[b] | 47.333[e] | 52.000[c] | 61.000[a] | 22.000[L] | 49.333[d] | 38.333[h] | 35.333[l] | 43.333[f] | 33.000[J] | 2.08 |
| Lymphocyte | 50.500[ab] | 45.000[ab] | 60.000[ab] | 65.000[ab] | 42.000[ab] | 41.000[ab] | 42.333[ab] | 43.000[ab] | 31.000[b] | 74.667[a] | 50.000[ab] | 56.333[ab] | 59.000[ab] | 48.333[ab] | 62.333[ab] | 3.15 |
| Monocyte | 1.333 | 1.000 | 2.000 | 1.333 | 0.667 | 0.667 | 1.607 | 1.000 | 1.667 | 1.000 | 0.000 | 1.333 | 1.667 | 0.667 | 1.000 | 0.39 |
| Eoninoph | 3.333 | 2.333 | 3.000 | 3.333 | 1.333 | 1.667 | 4.667 | 2.000 | 3.000 | 4.000 | 2.000 | 1.000 | 2.000 | 4.000 | 3.333 | 1.75 |
| Basophil | 1.000[a] | 0.000[d] | 1.000[a] | 1.000[a] | 0.333[c] | 0.333[c] | 1.000[a] | 0.000[d] | 0.667[b] | 0.333[c] | 0.667[b] | 0.667[b] | 0.667[b] | 0.333[c] | 0.333[c] | 0.26 |

*[abc] means on the same row having different superscript are significantly different (P<0.05),*
*Control=No Additives; AB= Antibiotics (oxytetraxycline); MOS=Mannose oligosaccharide; AXOS=Arabinoxylans Oligosccharide; SIM L=pedicoccus Acidilactis*

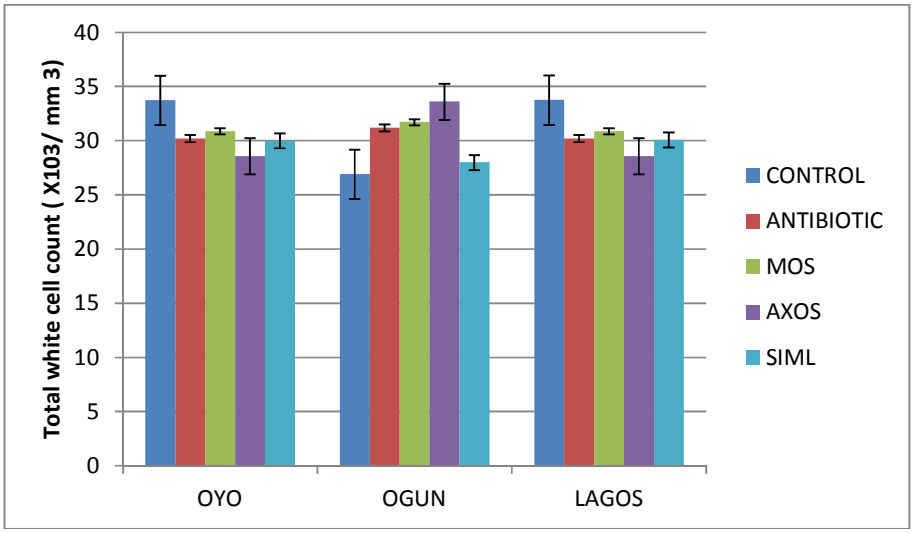


**Fig. 1. Variation in total white cell count in broiler fed with additive and antibiotics**

**Table 4. Average salmonella antibody titre in broiler chickens fed different feed additives from different location/hatchery**

| Treatment locations | Location | | | | | |
|---|---|---|---|---|---|---|
| | Oyo | | Ogun | | Lagos | |
| | Titre values | | Titre values | | Titre values | |
| | O | H | O | H | O | H |
| Control | 1:40 | 1:40 | 1:20 | 1:20 | 1:20 | 1:40 |
| Oxytetracycline | 1:20 | 1:20 | 1:40 | 1:20 | 1:20 | 1:20 |
| MOS | 1:20 | 1:40 | 1:20 | 1:20 | 1:20 | 1:20 |
| AXOS | 1:20 | 1:20 | 1:20 | 0:0 | 1:60 | 1:40 |
| Sim® lac | 1:20 | 1:20 | 1:40 | 1:20 | 1:20 | 1:20 |

*Key 'O' Antigen – somatic cell, 'H' Antigen – flagella;*

Therefore, the positive effect of feeding diet containing probiotic on the immune response indicates the enhancement of adaptive immunity in response to *Salmonella* infection in poultry.

## 5. CONCLUSIONS

Probiotic and prebiotic additives are effective and safe methods for prevention of *Salmonella* infection in broiler chickens.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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