Open Journal of Veterinary Medicine, 2019, 9, 171-184
https://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

Haematological and Serum Biochemistry Profile of Cockerels Experimentally Infected with *Salmonella enterica* Serovar Zega

Paulinus Ekenedilichukwu Emennaa[1], Didacus Chukwuemeka Eze[2], Foinkfu Kennedy Chah[2], John Osita Arinze Okoye[2], John Ikechukwu Ihejioha[2], Milton Nancy Sati[1], Ifeanyi Onyema[2], Christian Okorie-Kanu[2], Asabe Adamu Dzikwi-Emennaa[3], Uchendu Chidiebere[3], Kayode Olorufemi[2], Akuchi Chidiabi Nwamo[1], Jacintha Ngozi Omeke[2], Israel Joshua Barde[1]

[1]National Veterinary Research Institute, Vom, Nigeria
[2]Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
[3]Faculty of Veterinary Medicine, University of Jos, Jos, Nigeria
Email: israelbarde@yahoo.com

How to cite this paper: Emennaa, P.E., Eze, D.C., Chah, F.K., Okoye, J.O.A., Ihejioha, J.I., Sati, M.N., Onyema, I., Okorie-Kanu, C., Dzikwi-Emennaa, A.A., Chidiebere, U., Olorufemi, K., Nwamo, A.C., Omeke, J.N. and Barde, I.J. (2019) Haematological and Serum Biochemistry Profile of Cockerels Experimentally Infected with *Salmonella enterica* Serovar Zega. *Open Journal of Veterinary Medicine*, **9**, 171-184.
https://doi.org/10.4236/ojvm.2019.912015

Received: November 13, 2019
Accepted: December 13, 2019
Published: December 16, 2019

Copyright © 2019 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Salmonellosis is a serious medical and veterinary problem worldwide and causes great concern in the food and livestock industries, especially the poultry industry which occupies a prominent position in the provision of animal protein and accounts for about 25% of local meat production in Nigeria particularly and is identified as a disease of major economic importance causing low performance in poultry production. The study was carried out at the experimental animal farm, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. One hundred (100) five-week old chickens obtained from the Poultry division of National Veterinary Research Institute, Vom, were used for the experiment. The birds were randomly assigned to 4 groups of 25 birds per group (A, B, C, D). Each bird in all the groups received 0.5 ml of PBS containing 1 × 108 cfu/ml of Salmonella enterica serovar Zega as follows: Group A was infected with Salmonella Zega intra-nasally (IN). Group B was infected with Salmonella Zega intra-peritonealy (IP). Group C was infected with Salmonella Zega orally (OR). Group D was the Uninfected control (CT). There was a significant change ($p < 0.05$) in the mean corpuscular volume (MCV) post-infection in all infected groups, however no statistical significant changes ($p > 0.05$) in the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in the infected groups compared across the different days post infection. There was significant increase ($p < 0.05$) in the mean aspartate transferase (AST) and mean alanine transferase (ALT) after infection in all the infected groups between

day 7 and 10 post infection. There was also a marked increase ($p < 0.05$) in the mean serum total protein values in all infected groups following infection. The findings of this study showed that apart from *Salmonella Pullorum* and *Salmonella Gallinarum* that are known to cause pathology in birds *Salmonella Zega* which is none host specific for birds can also cause pathology in them. This is the first report in the study area to the best of our knowledge.

Keywords

Hematological, Serum Biochemistry, Cockerel, Salmonella Zega

1. Introduction

In developing countries especially in Africa, poultry farming is a major source of livelihood [1]. Poultry occupies a prominent position in the provision of animal protein and accounts for about 25% of local meat production in Nigeria [2]. Nebiyu *et al.*, [3] identified disease prevalence as one of the major factors that causes low performance in poultry production.

Salmonella infection is a serious medical and veterinary problem worldwide and causes great concern in the food industry [4]. In poultry, the infection is important both as a cause of clinical disease in poultry and as a source of food-borne contamination in humans [5]. Avian salmonellosis is one of the major disease challenges militating against poultry production in Nigeria. Host-adapted salmonellae *Salmonella Pullorum* and *Salmonella Gallinarum* are responsible for avian salmonellosis, a severe systemic disease characterised by somnolence, inappetence, weight loss and death [6], in spite of efforts at vaccination and medication. There are however other salmonella serotypes that have the capacity to infect and replicate in poultry, usually resident in the gastrointestinal tracts causing negligible or very mild signs of illness [7].

Salmonella enterica serovar Zega, one of the over 3000 identified strains of salmonella were first isolated from dead ducklings in the Belgian Congo (now Democratic Republic of Congo) in 1952 [8]. Recently, it was listed amongst 37 *Salmonella enterica* strains isolated from commercial layer flocks in Southwest Nigeria [9] [10]. This suggests that the agent may be pathogenic for avian species. This strain has the potential of becoming a major factor in the epidemiologic picture of avian salmonellosis in Nigeria. There is therefore a need to formally ascertain the pathogenicity and pathology of the serovar in chickens.

Haematology and serum biochemistry indices of birds and mammals are useful in the evaluation of their physiological and pathological status following infection and assessing the outcome of pathological processes in vital organs [11].

This study was carried out to investigate the haematologic and serum biochemistry values that accompany an experimental infection of Salmonella Zega in chickens as a baseline for assessing its pathogenicity.

2. Materials and Methods

2.1. Experimental Site

The study was carried out at the experimental animal farm, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, South-eastern Nigeria. Nsukka is located between latitudes 5˚50' north and longitude 6˚52' and 7˚54' east [12]. It is an area of fairly high temperature ranging from 21.17˚C to 32.00˚C [12]. Rainfall months' span from March and October while the dry season months are between November and February with a relative humidity of 70% and 20% respectively.

2.2. Experimental Animals

A total of 100, five-week old chickens obtained from the Poultry division of National Veterinary Research Institute, Vom, were used for the experiment. These birds were housed in deep litter system in a concrete pen and uncontaminated quality commercial starter diet was provided for the chicks throughout the experiment which lasted for 21 days. Feed and water were provided *ad-libitum*. The 100 birds were randomly assigned to 4 groups of 25 birds per group (A, B, C and D) in different pens separated by wire mesh and ceiling board from bottom to top of the roof.

2.3. Bacteriological Monitoring before Infection

Before infection, cloacal swabs were collected from the birds on day 2 and 5 post arrival in Nsukka. This was in order to confirm if they were free from *Salmonella* organism. This was done by pre-enrichment of the swab samples in buffered peptone water, followed by plating on MacConkey agar (MCA) using standard laboratory methods [13] [14]. Only *Salmonella* free birds were used for the experiment.

2.4. Bacterial Isolate Used in the Study

Stocked culture of *Salmonella enterica* serovar Zega originally isolated from commercial layers in South-western Nigeria, identified at the Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, South Dakota, USA [9] and maintained at the bacterial bank, Bacterial Research Department, National Veterinary Research Institute, Vom, Nigeria was used in this study.

2.5. Culture and Determination of Bacterial Inoculum

The lyophilized bacterium from the culture bank was reactivated by culturing in peptone water, incubated overnight at 37˚C and sub-cultured on MacConkey agar (MCA). The resulting colonies were examined for their colony characteristics (colour and morphology) and tested for Gram-reaction (Gram-negative). Five colonies were scooped and inoculated into 10 ml of Phosphate Buffered Saline (PBS) and this was incubated for 24 hours at 37˚C after which a ten-fold di-

lution was carried out in test tubes. The colony counts from the test tubes were determined. To obtain the number of organisms that was inoculated into the birds, the number of organisms was multiplied by volume by the dilution factor (CFU = No. of colony × Volume × Reciprocal of Dilution factor) [15].

Salmonella Zega inoculum (in PBS) containing 1×10^8 cfu/ml was used for the experimental infection.

2.6. Experimental Infection

The 100 birds were randomly assigned to 4 groups of 25 birds per group. (A, B, C, D). Each bird in all the groups received 0.5 ml of PBS containing 1×10^8 cfu/ml of *Salmonella enterica* serovar Zega as follows:

(Group A) were infected with Salmonella Zega intra-nasally (IN).

(Group B) were infected with *Salmonella* Zega intra-peritonealy (IP).

(Group C) were infected with *Salmonella* Zega orally (OR).

(Group D) was the Uninfected control (CT).

Note: For group A, 0.25 ml was administered into each nostril of the bird.

2.7. Blood Sample Collection

On days 0, 3, 5, 7 and 10 post-infection (PI), two millilitres of blood were collected from 3 randomly selected birds in each group through the jugular vein following proper restraint using a sterile 2 ml syringe. The samples were collected within 1 minute of capture to ensure that the levels of the monitored parameters were not affected by any stress induced by handling [16] and delivered into sample bottle treated with ethylene diamine tetra-acetic acid for haematological study. The same procedure was employed to obtain serum samples with anti-coagulant free sample bottles [17] [18]. The blood samples were collected in the morning hours to avoid haemolysis of the RBC as a result of high temperature.

2.8. Haematological and Serum Biochemistry Procedures

The anti-coagulated blood was used to determine the packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, Packed cell volume (PCV), haemoglobin (Hb) concentration, Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC). The packed cell volume (PCV) was determined by the micro-haematocrit method [19]; the red blood cell (RBC) count and white blood cell (WBC) count were determined using a manual haemocytometer. The cyanmethaemoglobin method was used to determine the Haemoglobin (Hb) concentration. Consequently calculations to derive the Meancorpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) employed the values of RBC, Hb and PCV as described by Jain [20]. The differential leukocyte count was done on Leishman stained blood films [21]. The stained slides were examined with immersion oil at x100 objective using a

light microscope. Two hundred cells were counted by the longitudinal counting method and each cell type was identified and scored using the differential cell counter. Results for each type of white blood cell were expressed as a percentage of the total count and converted to the absolute value per microliter of blood.

Sera were separated from the clotted blood following centrifugation from which serum metabolites (serum glucose, urea, cholesterol, albumin, globulin and creatinine) were determined by spectrophotometry. Values of all these parameters were analysed using the routine laboratory procedures of Dacie and Lewis [22].

3. Data Analysis

Data obtained were expressed as mean ± S.E.M (standard error of the mean). They were subjected to Repeat Measure One Way Analysis of Variance (ANOVA) to determine the difference in the parameters before infection and post-infection between the groups. Values of $P < 0.05$ were considered significant.

4. Discussion

An analysis of the haematologic parameters across days 0, 3, 5, 7 and 10 post-infection (PI), subsequently compared within the three different routes of infection showed a slight reduction in the mean packed cell volume (PCV), haemoglobin count and red blood cell (RBC) count (**Figures 1-3**). The reduction observed in those parameters at day 3 PI in the infected groups were similar to works done by Freitas-Neto *et al.,* [23], Nwiyi and Omodamiro [24], Barde *et al.,* [25]; they all reported an acute onset in experimental infections of salmonella gallinarum in chickens and quails. The anemia associated with acute fowl typhoid in chicken has been attributed to an increased ability of the reticuloendothelial cells to take up erythrocytes as reported by Assoku and Penhale [26].

Results of the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concerntration (MCHC) were varied across the days post infection and routes of inoculation (**Figure 4** and **Figure 5**). There was no statistical significant changes ($p > 0.05$) in the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in the infected groups compared across the different days post infection as compared to the corresponding mean values obtained in the control group (CT) (**Figure 4** and **Figure 5**). As alluded to by Barde *et al.,* [25] in his study with *salmonella enterica* serovar Gallinarum in quails, these may be as a result of haemorrhages and congestion of organs observed in the infected birds. The intra-peritoneal group (IP) showed a significant increase ($p < 0.05$) in the mean heterophil count as compared to the other infected groups and the control (**Figure 6**) same with mean white blood cells count and mean lymphocytes count (**Figure 7** and **Figure 8**). This increase according to Brar *et al.,* [27] and Morgulis, [28] is common in general infections due to septicaemias caused by infectious agents such as sal-

monella. Monocyte,, and basophil percentage values in chickens in this study were varied and showed no relevant changes (Figure 9 and Figure 10), and were consistent with a previous report [29].

There was significant increase ($p < 0.05$) in the mean aspartate transferase (AST) and mean alanine transferase (ALT) after infection in all the infected groups between day 7 and 10 post infection (Figure 11 and Figure 12). There was also a marked increase ($p < 0.05$) in the mean serum total protein values in all infected groups following infection (Figure 13) but no significant changes in albumin, gbobulin cholesterol and bilirubin values (Figures 14-17). This increase in mean aspartate transferase (AST) agrees with Brar *et al.,* [27] and Freitas-Neto *et al.,* [23] that interprete it as a consequence of an incapacity of the liver to synthesize protein due to the lesion intensity resulting from hepatic disease.

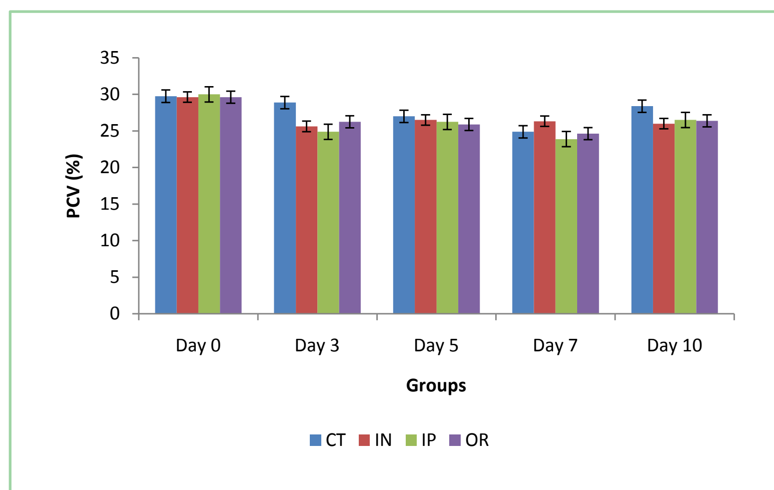

Figure 1. Mean Packed Cell Volume Count of Chickens at Day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra—nasal, IP—Intra—peritoneal, OR—Oral.

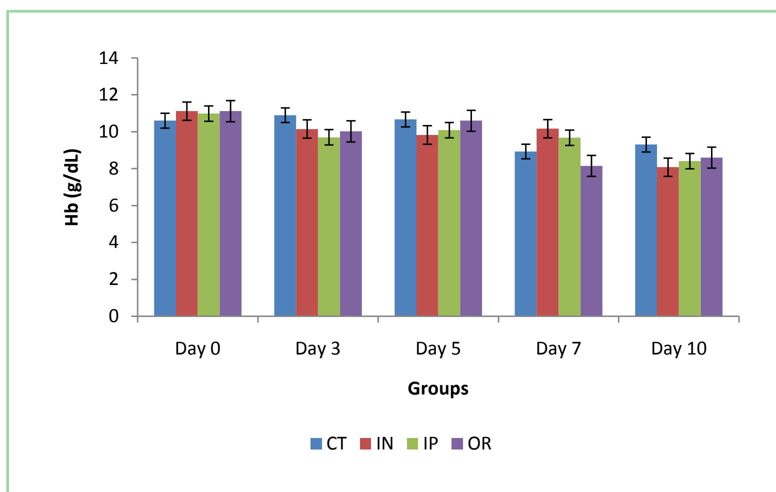

Figure 2. Mean Haemoglobin Concentration of Chickens at Days 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra—nasal, IP—Intra—peritoneal, OR—Oral.

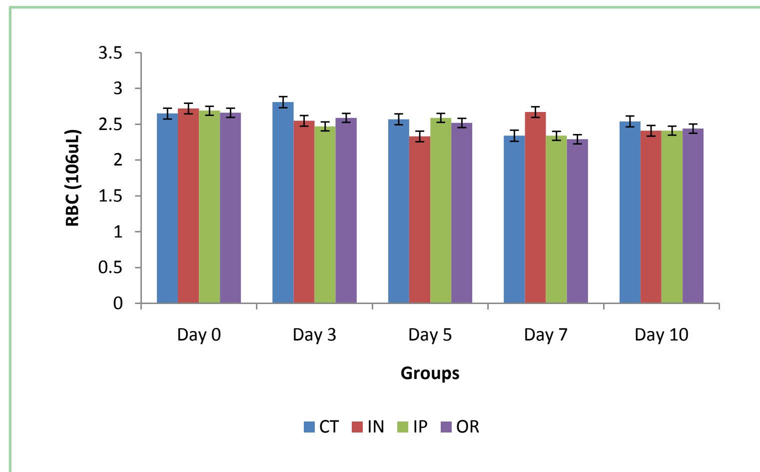

Figure 3. Mean Red Blood Cell Count of Chickens at Days 0, 3, 5, 7 and 10 PI. CT—, IN—Intra—nasal, IP—Intra-peritoneal, OR—Oral.

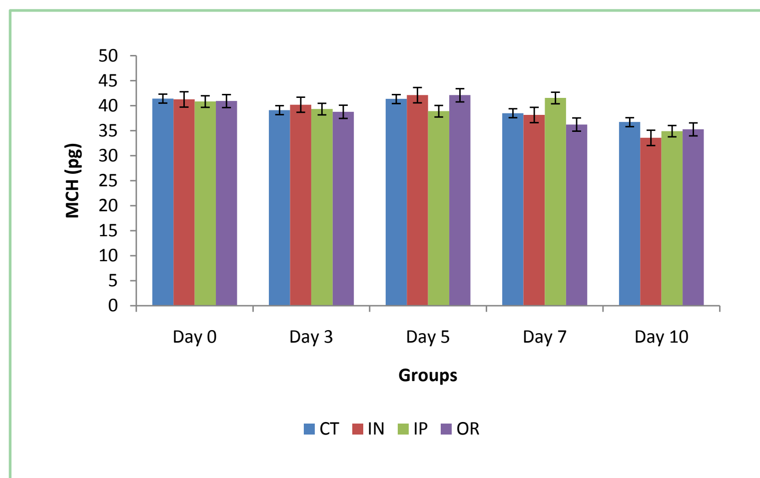

Figure 4. Mean Corpuscular Haemoglobin count of Chickens at days 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

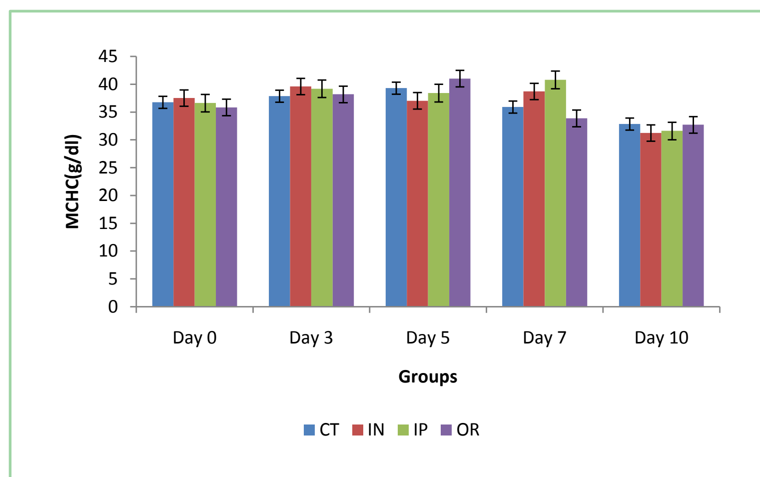

Figure 5. Mean Corpuscular Haemoglobin Concentration at days 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

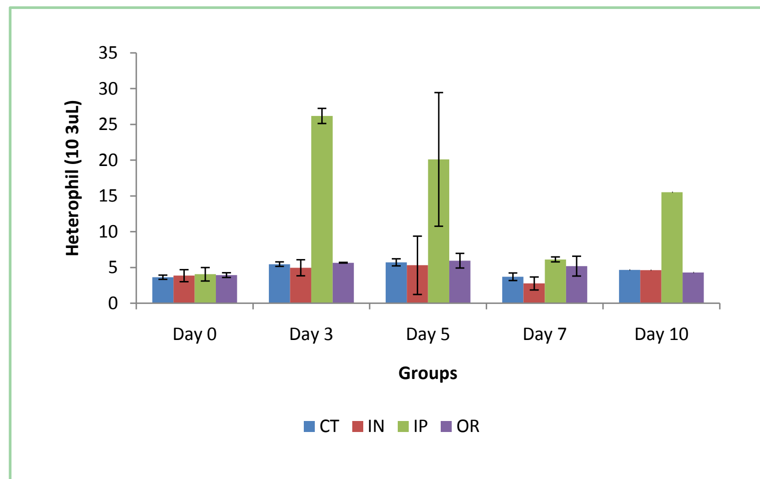

Figure 6. Mean Heterophil count of chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

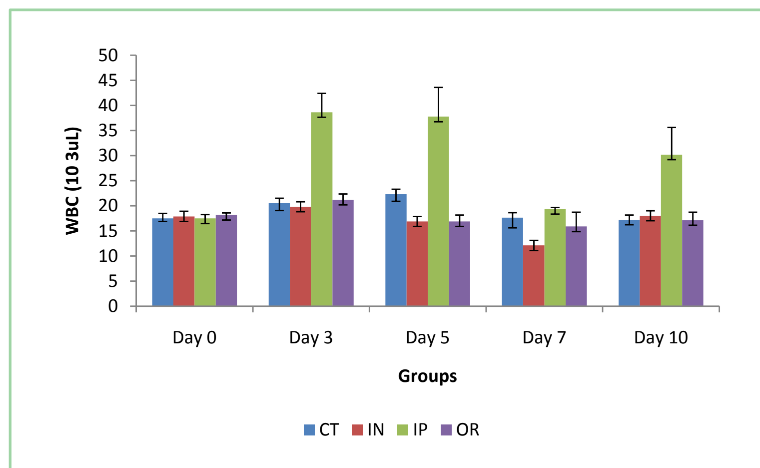

Figure 7. Mean White Blood Cell count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

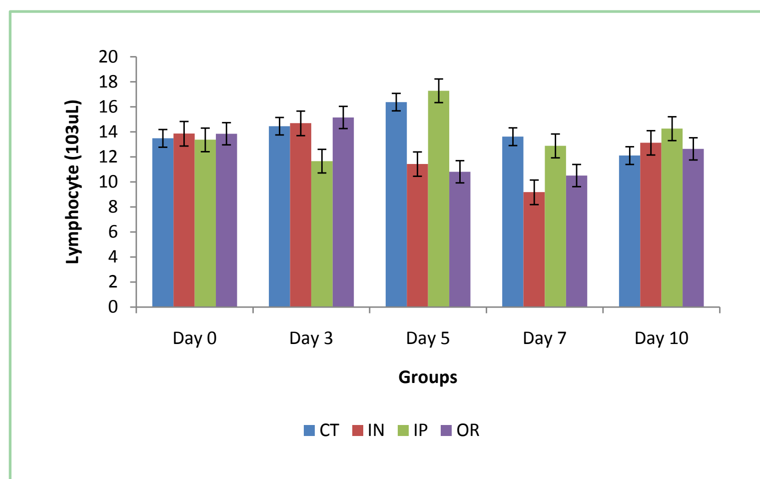

Figure 8. Mean Lymphocyte count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

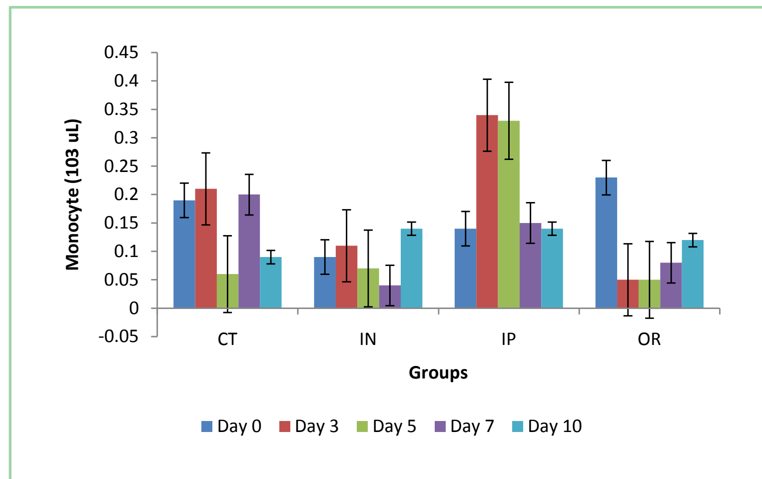

Figure 9. Mean Monocyte count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

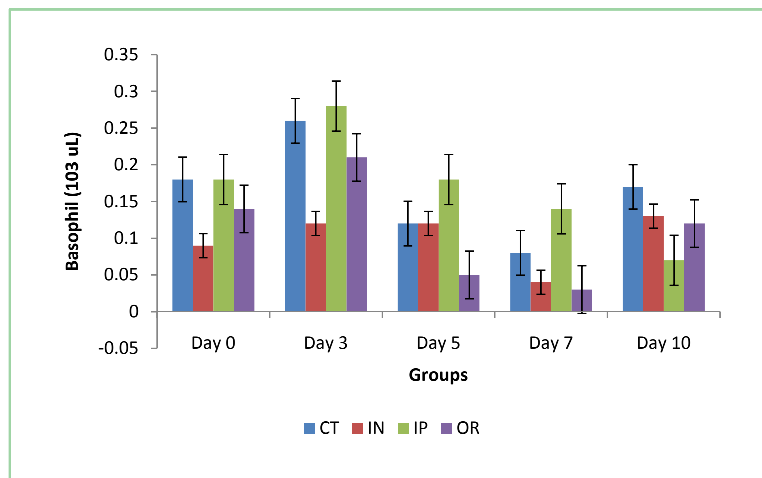

Figure 10. Mean Basophil count of chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

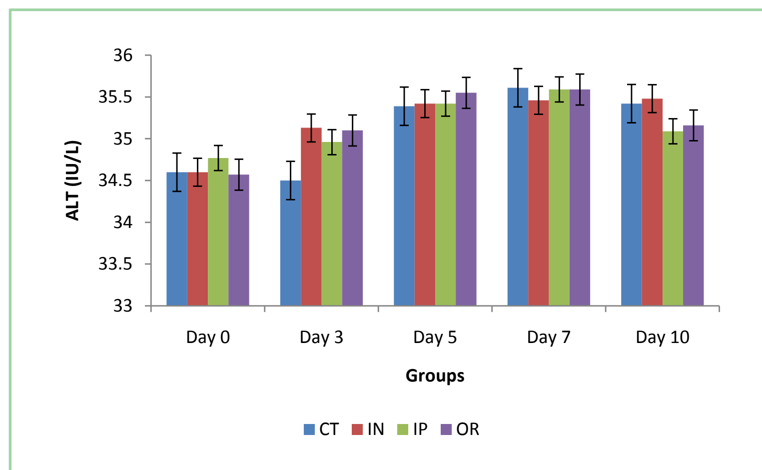

Figure 11. Mean serum alanine transferase (ALT) count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

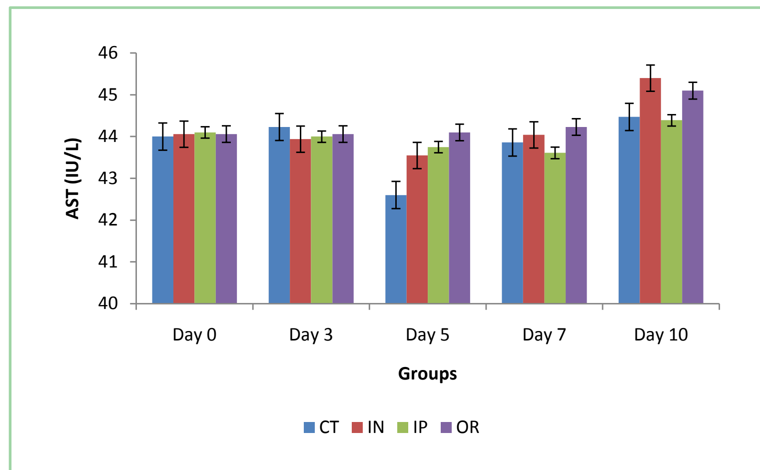

Figure 12. Mean serum aspartate transferase (AST) count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

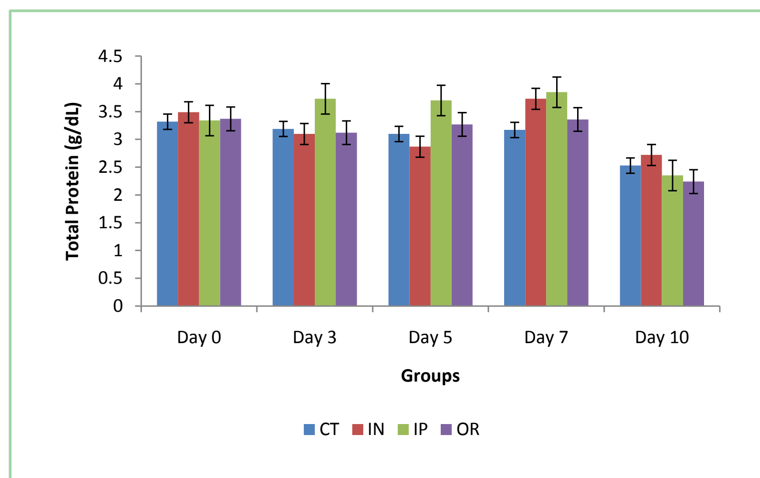

Figure 13. Mean Total Protein count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

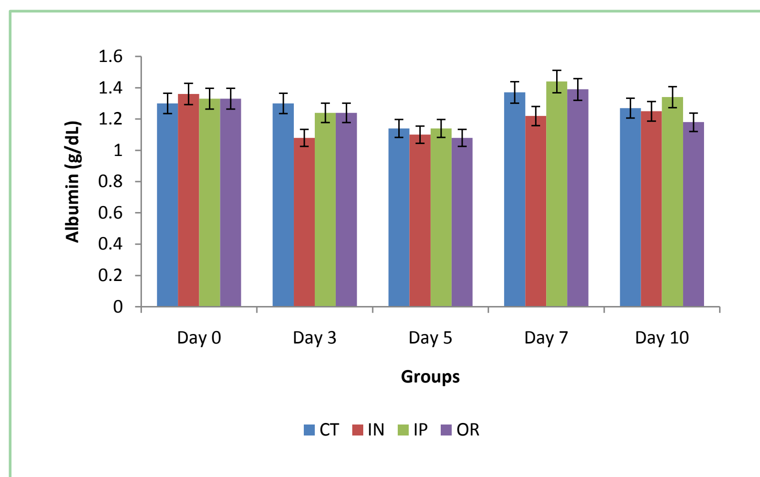

Figure 14. Mean Albumin count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

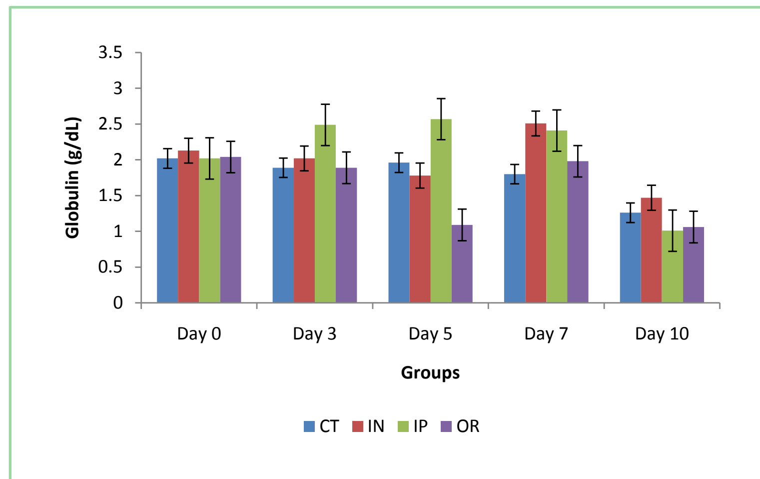

Figure 15. Mean Globulin count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

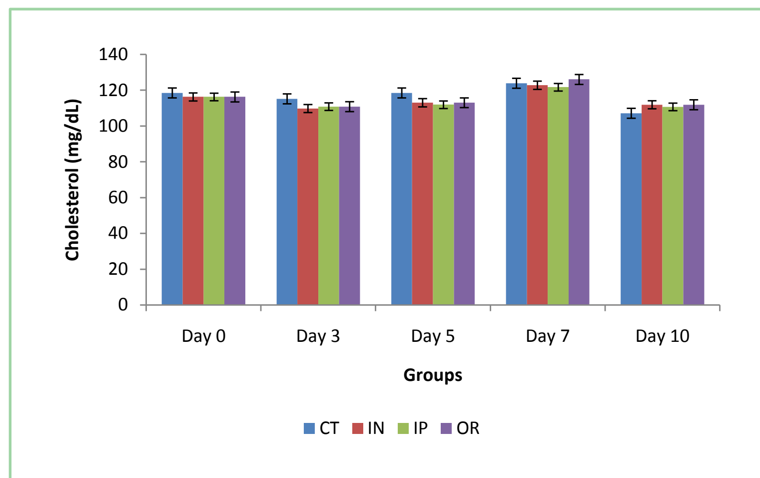

Figure 16. Mean Cholesterol count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

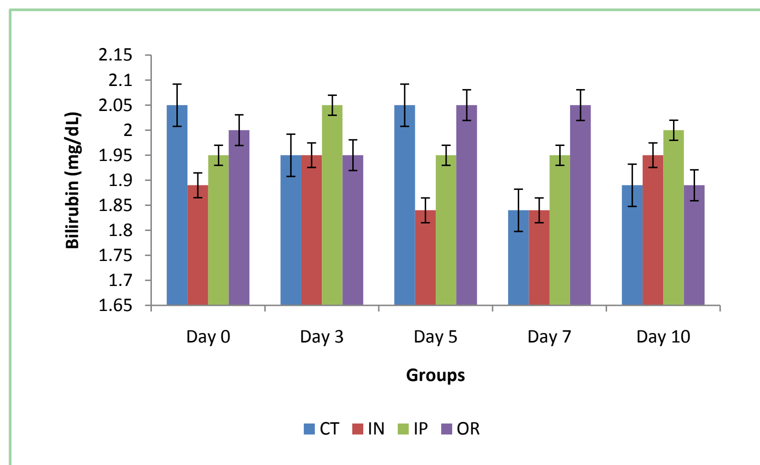

Figure 17. Mean Bilirubin count of Chickens at day 0, 3, 5, 7 and 10 PI. CT—Control, IN—Intra-nasal, IP—Intra-peritoneal, OR—Oral.

5. Conclusion

There was a significant change ($p < 0.05$) in the mean corpuscular volume (MCV) post-infection in all infected groups, however no statistical significant changes ($p > 0.05$) in the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in the infected groups compared across the different days post infection. There was significant increase ($p < 0.05$) in the mean aspartate transferase (AST) and mean alanine transferase (ALT) after infection in all the infected groups between day 7 and 10 post infection. There was also a marked increase ($p < 0.05$) in the mean serum total protein values in all infected groups following infection.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Cardinale, E., Tall, F., Gueye, E.F., Cisse, M. and Salvat, G. (2004) Risk Factors for *Salmonella enterica subspecie enterica* Infection in Senegalese Broiler-Chicken Flocks. *Preventive Veterinary Medicine*, **63**, 151-161. https://doi.org/10.1016/j.prevetmed.2004.03.002

[2] Agbaje, M., Davies, R., Oyekunle, M.A., Ojo, O.E., Fasina, F.O. and Akinduti, P.A. (2010) Observation on the Occurrence and Transmission Pattern of S.G in Commercial Poultry Farm in Ogun State, South Western Nigeria. *Africa Journal of Microbiology Research*, **4**, 776-800.

[3] Nebiyu, Y., Brhan, T. and Kelay, B. (2013) Characterization of Village Chicken Production Perfomance under Scavenging Systems in Halaba District of Southern Ethiopia. *Ethiopia Veterinary Journal*, **17**, 69-80. https://doi.org/10.4314/evj.v17i1.6

[4] Begum, F., Adachi, Y. and Kham, H.S.R. (2008) Immunodominant Surface Protein of Some Salmonella Serovars. *Bangladesh Journal of Veterinary Medicine*, **6**, 145-151. https://doi.org/10.3329/bjvm.v6i2.2327

[5] Waltman, W.D., Gast, R.K. and Mallinson, E.T. (1998) Salmonellosis, a Laboratory Manual for the Isolation and Identification of Avian Pathogens. In: Swayne, D.E., Glisson, J.R., Jackwood, M.W., Pearson, J.E. and Reed, W.M., Eds., *American Association of Avian Pathologists*, 4th Edition, University of Pennsylvania, Philadelphia, PA, 4-13.

[6] Okoye, J.O.A. (2010) Pathology of Major Viral and Bacterial Diseases of Poultry. Veterinary Diagnostic Pathology Certificate Course Presentations, National Veterinary Research Institute, 255-261.

[7] Berchieri Jr., A., Murphy, C.K., Marston, K. and Barrow, P.A. (2001) Observations on the Persistence of Vertical Transmission of 7 *Salmonella enterica* Serovars Pullorum and Gallinarum in Chickens: Effect of Bacteria and High Genetic Background. *Avian Pathology*, **30**, 221-231. https://doi.org/10.1080/03079450120054631

[8] Fain, A., Kauffmann, F. and Schoetter, M. (1952) A New Salmonella Type (S. Zega) from the Belgian Congo. *Acta Pathologica Microbiologica Scandinavica*, **31**, 325. https://doi.org/10.1111/j.1699-0463.1952.tb00196.x

[9] Useh, N.M., Ngbede, E.O., Akange, N., Thomas, M., Foley, A., Keena, M.C., Nelson,

E., Christopher-Hennings, J., Tomita, M., Suzuki, H. and Scaria, J. (2016) Draft Genome Sequences of 37 Salmonella Enteric Strains Isolated from Poultry Sources in Nigeria. *Genome Announcement*, **4**, e00315-e00316. https://doi.org/10.1128/genomeA.00315-16

[10] Mshelbwala, F.M., Ibrahim, N.D.-G., Saidu, S.N., Azeez, A.A., Akinduti, A.A., Kwanashie, C.N., Fakilahyel Kadiri, A.K., Muhammed, M., Fagbamila, I.O. and Luka, P.D. (2017) Motile Salmonella Serotypes Causing High Mortality in Poultry Farms in Three South-Western States of Nigeria. *Veterinary Record Open*, **4**, e000247. https://doi.org/10.1136/vetreco-2017-000247

[11] Stockhan, S.L. and Scott, M.A. (2008) Fundamentals of Veterinary Clinical Pathology. 2nd Edition, Blackwell Publishing, Ames, IA, 133-140.

[12] Federal Ministry of Agriculture and Natural Resources (1999) Geographic Data. Enugu, Nigeria, 2-18.

[13] Wigley, P., Berchieri Jr., A., Page, K.L., Smith, S.L. and Barrow, P.A. (2001) *Salmonella enterica* Serovar Pullorum Persists in Splenic Macrophages and in the Reproductive Tract during Persistent, Disease-Free Carriage in Chickens. *Infectious Immunology*, **69**, 7873-7879. https://doi.org/10.1128/IAI.69.12.7873-7879.2001

[14] Parma, D. and Davies, R. (2007) Fowl Typhoid in a Small Backyard Laying Flock. Veterinary Research, **160**, 348. https://doi.org/10.1136/vr.160.10.348

[15] Miles, A.A. and Misra, S.A. (1938) The Estimation of Bacterial Power of the Blood. *Journal of Hygiene*, **38**, 732-749. https://doi.org/10.1017/S002217240001158X

[16] Chloupek, P., Voslarova, E., Chloupek, J., Bedanova, I., Pistekova, V. and Vecerek, V. (2009) Stress in Broiler Chickens Due to Acute Noise Exposure. *Acta Veterinaria Brno*, **78**, 93-98. https://doi.org/10.2754/avb200978010093

[17] Oladele, S.B., Enoch, I. and Ibrahim, N.D.G. (2008) Changes in Histopathology, Haematocrit, Haemoglobin, Haemagglutination Inhibition Antibody Titre and Total Protein of Japanese Quails (*Coturnix coturnix japonica*) Administered Different Doses of Newcastle Disease Virus. *Journal of Animal and Veterinary Advances*, **7**, 418-424.

[18] Samour, J.H., Naldo, J.L., Rahman, H. and Sakkir, M. (2010) Haematologic and Plasma Biochemical Reference Values in Indian Peafowl (*Pavo cristatus*). *Journal of Avian Medicine and Surgery*, **24**, 99-106. https://doi.org/10.1647/2008-019.1

[19] Thrall, M.A. and Weisere, M.G. (2002) Haematology. In: Hendrix, C.M., Ed., *Laboratory Procedures for Veterinary Technician*, 4th Edition, Mosby Inc., Maryland Heights, MO, 29-74.

[20] Jain, N.C. (1986) Schalm's Veterinary Haematology. 4th Edition, Lea and Febiger, Philadelphia, PA, 1221.

[21] Schalm, O.W., Jain, N.C. and Caroll, E.J. (1975) Veterinary Haematology. 3rd Edition, Lea and Fabriger, Philadelphia, PA, 19-25.

[22] Dacie, J.V. and Lewis, S.M. (2007) Practical Haematology. 7th Edition, Livingstone, Churchill, London, 88-113.

[23] Freitas Neto, O.C., Arroyave, W., Alessi, A.C., Fagliari, J.J. and Berchieri, A. (2007) Infection of Commercial Laying Hens with Salmonella Gallinarum: Clinical, Anatomopathological and Haematological Studies. *Brazilian Journal of Poultry Science*, **9**, Article ID: 133141. https://doi.org/10.1590/S1516-635X2007000200010

[24] Nwiyi, P. and Omadamiro, O. (2012) Seroprevalence and Isolation of Ch f Animal and Feed Research. *Journal of Animal and Feed Research*, **2**, 483-487.

[25] Barde, I.J., Bale, J.O.O., Oladele, S.B., Fatihu, M.Y., Kumbish, P.R., Chukwu, D.I.,

Rimfa, A.G., Akanbi, B.O., Moses, G.D., Ahmed, J.S. and Okewole, P.A. (2015) Gross and Histo-Pathological Changes in Japanese Quail (Coturnix Coturnix Japonica) Experimentally Infected with Salmonella Enterica Serovar Gallinarum. *Animal and Veterinary Sciences*, **3**, 84-88. https://doi.org/10.11648/j.avs.20150303.12

[26] Assoku, R. and Penhale, W.J. (1974) The Pathogenesis of FT: The Influence of Anaemia and Erythrocyte Clearance and the Susceptibility of the Fowl to Bacteria Endotoxin. *Journal of Comparative Pathology*, **84**, 443-453. https://doi.org/10.1016/0021-9975(74)90037-1

[27] Brar, R.S., Sandhu, H.S. and Singh, A. (2000) Veterinary Clinical Diagnosis by Laboratory Methods. Kalyani Publishers, New Delhi, 146-150.

[28] Morgulis, M. (2002) Imunologia aplicada. In: Macari, M., Furlan, R.L. and Gonzalez, E., Eds., *Fisiologia aviária aplicada a frangos de corte*, FUNEP/UNESP, Jaboticabal, 375-429.

[29] Cardoso, A., Castro, A. and Tessari, E. (2003) Estudo hematológico em aves inoculadas com Salmonella Gallinarum. *Arquivos do Instituto Biológico*, **70**, 35-42.