

EVALUATION OF THE EFFECTS OF EXPERIMENTAL *SALMONELLA GALLINARUM* INFECTION ON THE HAEMATOLOGY AND OXIDATIVE STRESS MARKERS IN YOUNG PULLETS

¹OKAFOR, Simeon Chibuko, ¹IHEDIOHA, John Ikechukwu, ¹EZEMA, Wilfred Sunday, ¹ONYEMA, Ifeanyi-chukwu, ¹EZEMA, Arinzechukwu Stephen and ²CHAH, Kennedy Foinkfu

¹Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

²Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

Corresponding Author: Okafor, S.C. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. **Email:** simeon.okafor@unn.edu.ng
Phone: +234 803 506 1920

Received April 10, 2024; Revised April 23, 2024; Accepted April 24, 2024

ABSTRACT

The effects of Salmonella gallinarum infection on the haematology and oxidative stress (OS) in pullets were evaluated in this study. Fifty 7-week-old pullets were randomly assigned to two groups of 25 pullets per group. The infected pullets were inoculated orally with S. gallinarum (10⁹ S. gallinarum colony forming units/mL), while uninfected pullets were the controls. Haematological and OS parameters were determined following standard protocols. There was a significant loss (p<0.05) of body weight. The packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) count and mean corpuscular haemoglobin concentration (MCHC) were significantly lower (p<0.05). In contrast, the mean corpuscular volume (MCV) was significantly higher (p<0.05) in the infected pullets when compared to the controls. The total white blood cell (TWBC), absolute heterophil, lymphocyte, eosinophil and monocyte counts, of the infected pullets, were significantly higher than that of the controls. Serum malondialdehyde (MDA) level, glutathione peroxidase (GPx) and catalase (CAT) activities of the infected pullets were significantly higher (p<0.05) when compared to the uninfected controls. Absolute heterophil count correlated strongly, positively and significantly with MDA. An association was established between significantly elevated GPx and CAT, which have antioxidant properties, and survival/health improvement indices such as improved weight gain and self-recovery. It was concluded that S. gallinarum infection of pullets caused significant alterations in the haematology, induced OS, and stimulated the body's antioxidant defence mechanism to elaborate GPx and CAT. This may suggest the use of antioxidants in the treatment of fowl typhoid.

Keywords: Correlation coefficient, Fowl typhoid, Haematology, Oxidative stress markers, Pullets, *Salmonella gallinarum*

INTRODUCTION

Salmonella gallinarum (Enterobacterales: Enterobacteriaceae) is a Gram-negative bacterium that causes fowl typhoid (FT), an important disease of chickens, turkeys and other galliform species all

over the world, especially in developing countries where increasing antimicrobial resistance in this strain has also become a problem (Mølbak *et al.*, 2002; Barbour *et al.*, 2015; Parvej *et al.*, 2016). Chickens are considered the main host for *S. gallinarum*, but variable susceptibility to FT occurs

amongst lineages (Oliveira *et al.*, 2005). FT is a severe septicemic as well as systemic disease. It is a major cause of huge economic loss to the poultry industry around the globe every year in the form of production losses, mortalities, vaccination and other medication costs (Berchieri *et al.*, 2001).

The epidemiology of FT is quite complex with the route of contact of infection mainly horizontal, although vertical transmission occurs, reportedly associated with vaccination against FT in young chicks (Roa, 2000). Since over a century ago, when FT was first described, a lot of research has been carried out which availed information on its epidemiology, and the introduction of effective measures towards controlling FT in commercial poultry flocks (Barrow and de Freitas Neto, 2011; Shivaprasad *et al.*, 2013). Even though FT has been eradicated in developed countries of the world, the poultry industries in Asia, Latin America, and Africa are still threatened by the disease (OIE, 2015; Zanetti *et al.*, 2019).

Considering the relationship between tissue lesions and metabolic alterations and the fact that *S. gallinarum* infections can cause lesions in a variety of organs (Berchieri *et al.*, 2000; Shivaprasad 2000; de Freitas Neto *et al.*, 2013), the use of laboratory analyses such as haematology, and evaluation of oxidative stress (OS) parameters may be fundamental to better understanding of the pathophysiology of this disease. There is, however, a scarcity of information in the available literature on the blood picture, especially OS markers of *S. gallinarum*-infected chickens, as less emphasis is placed on this aspect of the study, even as FT is a septicemic disease. This study evaluated the effects of *S. gallinarum* infection on the haematology and OS in pullets.

MATERIALS AND METHODS

Ethical Approval: The approval for this study was granted by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria (Approval Number: FVM-UNN-IACUC-0339).

Flock History: Fifty-day-old chicks of a commercial line of brown egg layers, ISA brown, procured from CHI Hatchery, Ibadan, Nigeria, and raised on deep litter, were used for this study.

Balanced commercial feed – Hybrid Feeds (Chick's mash with 18% crude protein, 2550 Kcal/Kg metabolizable energy given from 0 – 4 weeks of age, and grower mash with 15% crude protein, 2350 Kcal/Kg metabolizable energy given from 5 weeks of age, manufactured by Hybrid Feeds Limited, Kaduna, Nigeria) and clean water were provided *ad libitum*. Adequate biosecurity measures were observed and the pullets were routinely vaccinated against infectious bursal disease (IBD) and Newcastle disease (ND) (LaSota) and were dewormed with Wormazine (Chickpedia, 2019).

Bacterial Inoculum: The *S. gallinarum* strain used in this study was isolated from a local outbreak of FT in Nsukka, South East, Nigeria. The organism in a stock culture was plated on MacConkey agar (MCA) and incubated at 37°C for 24 hours. The grown colonies on MCA were suspended in nutrient broth and the methods described by Desmidt *et al.* (1997) and Beyaz *et al.* (2010) were adopted in the inoculum preparation.

Pre-infection Screening: Before the experimental infection, cloacal swabs from randomly selected birds were inoculated into the nutrient broth, incubated at 37°C for 24h and plated onto MCA to rule out infection with *Salmonella spp.* (Berchieri *et al.*, 2001; Lopes *et al.*, 2016).

Experimental Infection: At 7 weeks of age, the 50 pullets were randomly assigned to two treatment groups, with each treatment replicated five times and each replicate having five birds (25 pullets per group) in a completely randomised design (CRD). Each pullet in the challenged group was inoculated with 1 ml of *S. gallinarum* inoculum containing 1×10^9 colony-forming units (CFU/mL) by administration into the crop, while each pullet in the control group received 1 ml of phosphate-buffered saline (PBS) placebo.

Clinical Signs and Mortality: Following experimental infection, the chickens were observed twice daily for clinical signs. Five pullets were randomly selected from both the uninfected controls and infected groups and weighed on days 0, 4, 7, 14, 21, 28 and 35 PI using a standard weighing balance (Avery, United Kingdom) and

body weight in kilogram recorded. The number of dead pullets was recorded daily and the overall percentage mortality was determined by dividing the total number of dead pullets by the total number of infected pullets and multiplying the result by 100.

Blood Sample Collection: Approximately 3 ml of blood was aseptically drawn from each pullet randomly picked from each replicate (five pullets per group) on days 0, 7, 14, 21, 28 and 35 PI via jugular venipuncture and the pool blood sample per group was dispensed into two appropriately labelled sample bottles (one with Na-EDTA anticoagulant for haematology and the other devoid of anticoagulant for harvesting serum for OS markers assay). The time for blood sample collection was between 9:00 – 11:00 am each day.

Haematological Examination: The microhematocrit method was employed for the determination of packed cell volume (PCV) (Thrall and Weiser, 2002), while red blood cell (RBC) count and total white blood cell (TWBC) count were determined using the haemocytometer method (Campbell, 1994). The assay of haemoglobin (Hb) concentration was done using the cyanomethaemoglobin method (Higgins *et al.*, 2008) and the Leishman technique was used for the determination of differential WBC counts (Thrall and Weiser, 2002). The absolute WBC counts, the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Thrall and Weiser, 2002).

Determination of OS Parameters: The modified thiobarbituric acid test was performed to determine serum malondialdehyde (MDA) level using the ElabScience MDA assay kit (ElabScience Biotech Company Limited, South Africa) (Draper and Hadley, 1990). The serum glutathione peroxidase (GPx) activity was determined by the cumene hydroperoxidase method, using the Fortress diagnostic GPx test kit (Antrim, United Kingdom), and catalase (CAT) assay was performed by visible light method using ElabScience CAT assay kit (ElabScience Biotech Company Limited, South Africa) (Weydert and Cullen, 2010). The reading of MDA and CAT assays

were done with a Diatek blood biochemistry analyzer (Wuxi Hiwell Diatek Instrument Company Limited, China) set at the MDA-ELS assay program mode, and CAT-ELS x 325 assay program mode respectively. GPx assay, on the other hand, was read using a CHEM5V3 clinical chemistry analyzer (Erba Diagnostics, Mannheim, Germany) set at an absorbance wavelength of 340 nm.

Statistical Analyses: Data generated from the body weight measurement, haematology, and OS markers assays were analyzed using the independent t-test for equality of means on SPSS for Windows Version 23. The TWBC and absolute heterophil counts were correlated with OS parameters using Pearson correlation statistics. The level of significance for the independent t-test was accepted at $p < 0.05$, while the actual p-value was used for the correlation statistics.

RESULTS

Clinical Manifestations and Mortality: Clinical signs such as depression, decreased feed and water intake, greenish-yellow diarrhoea, droopy wings, prostration, ruffled feathers, dehydration, significant weight loss ($p < 0.05$) (Figure 1) and weakness were first observed on day 4 PI and persisted to day 14 PI in the infected group with overall morbidity of 18/25 (72.00%), whereas, the control group remained healthy for the duration of the experiment. First mortality was recorded on day 4 PI and overall mortality was 9/25 (36.00%) in the infected group (Table 1).

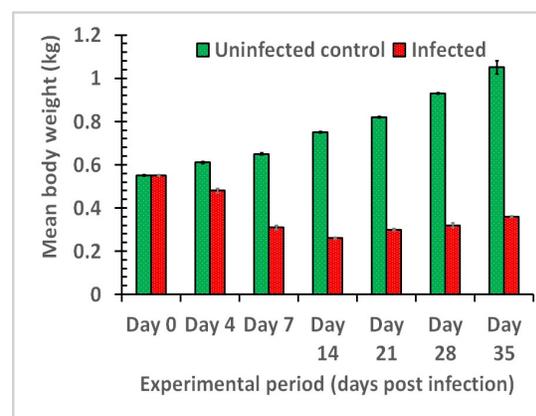


Figure 1: Mean body weight (Kg) of pullets infected with *Salmonella gallinarum* compared with the uninfected pullets

Table 1: Manifestation of clinical signs and mortality of pullets infected with *Salmonella gallinarum*

Days PI	Clinical signs	Infected	
		Morbidity (%)	Mortality (%)
4	Depression, decreased feed intake, ruffled feathers	10(40.00)	1(4.00)
5	Depression, decreased feed intake, ruffled feathers	13(52.00)	1(4.00)
6	Anorexia, ruffled feathers, droopy wings	18(72.00)	1(4.00)
7	Anorexia, greenish-yellow diarrhoea, prostration	18(72.00)	2(8.00)
8	Anorexia, greenish-yellow diarrhoea, prostration	14(56.00)	1(4.00)
9	Anorexia, greenish-yellow diarrhoea, dehydration	12(48.00)	1(4.00)
10	Greenish-yellow diarrhoea, dehydration, weakness	10(40.00)	1(4.00)
11	Dehydration, weakness	8(32.00)	1(4.00)
12	Dehydration	8(32.00)	0(0.00)
13	Dehydration	6(24.00)	0(0.00)
14	Dehydration	6(24.00)	0(0.00)
Total		18(72.00)	9(36.00)

Haematological Alterations: The mean PCV of the pullets infected with *S. gallinarum* was significantly lower ($p < 0.05$) on days 7 ($22.55 \pm 0.32\%$), 14 ($23.13 \pm 1.18\%$), 21 ($26.63 \pm 0.38\%$), 28 ($26.00 \pm 0.35\%$) and 35 ($23.13 \pm 0.47\%$) PI compared to the uninfected pullets (Figure 2).

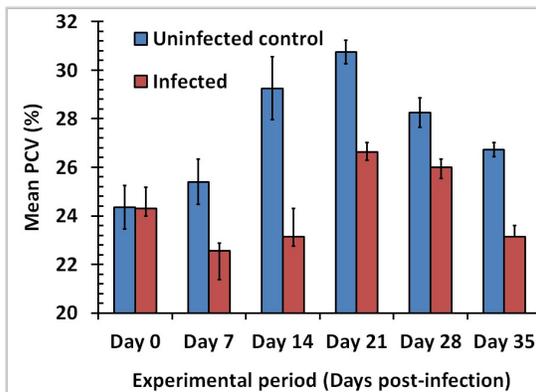


Figure 2: Mean packed cell volume (%) of pullets infected with *Salmonella gallinarum* compared to the uninfected pullets

The mean Hb concentration of the pullets was significantly lower ($p < 0.05$) than that of the uninfected controls on days 7 (7.05 ± 0.37 g/dL), 14 (7.10 ± 0.73 g/dL), 21 (7.36 ± 0.43 g/dL), 28 (7.67 ± 0.13 g/dL) and 35 (8.52 ± 0.18 g/dL) PI (Figure 3). Significantly lower ($p < 0.05$) mean RBC count was recorded on days 7 ($2.25 \pm 0.04 \times 10^6/\mu\text{L}$), 14 ($2.49 \pm 0.24 \times 10^6/\mu\text{L}$), 21 ($2.92 \pm 0.03 \times 10^6/\mu\text{L}$), 28 ($2.87 \pm 0.03 \times 10^6/\mu\text{L}$) and 35 ($2.44 \pm 0.18 \times 10^6/\mu\text{L}$) PI in the infected young

pullets when compared to the uninfected control young pullets (Figure 4).

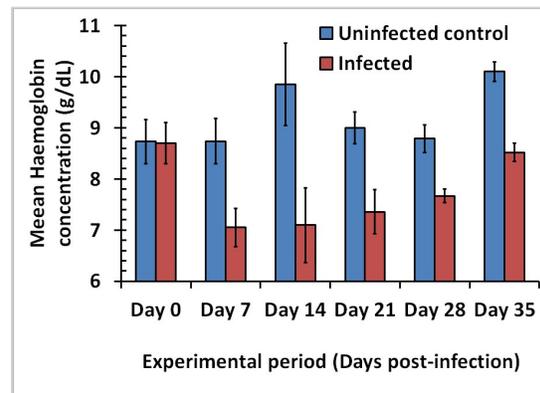


Figure 3: Mean haemoglobin concentration (g/dL) of the pullets infected with *Salmonella gallinarum* compared to the uninfected controls

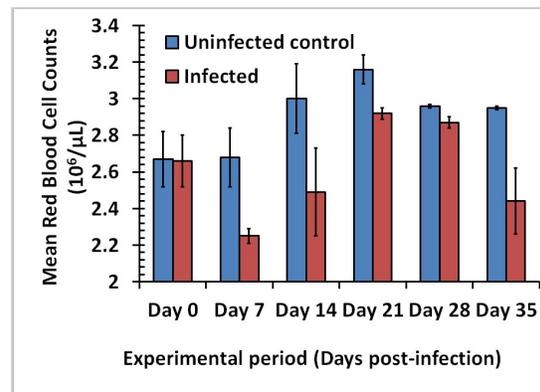


Figure 4: Mean red blood cell count ($10^6/\mu\text{L}$) of the pullets infected with *Salmonella gallinarum* compared to the uninfected controls

The MCV of the pullets infected with *S. gallinarum* was significantly higher ($p < 0.05$) than that of the uninfected controls on days 7 (100.37 ± 0.69 fl) and 35 (95.11 ± 2.41 fl) PI, but significantly lower ($p < 0.05$) on days 21 (91.10 ± 0.52 fl) and 28 (90.60 ± 1.18 fl) PI. The decline in MCV on day 14 PI in the infected young pullets compared to the controls was not statistically significant ($p > 0.05$) (Figure 5).

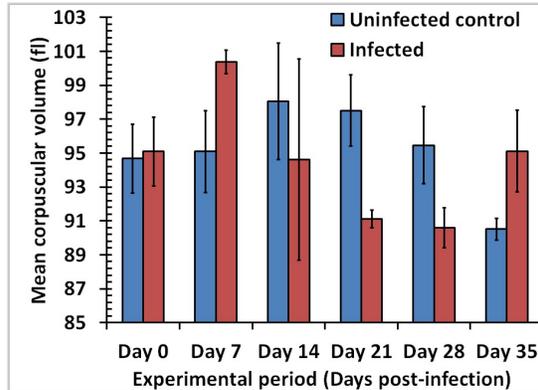


Figure 5: Mean corpuscular volume (fl) of the pullets infected with *Salmonella gallinarum* compared to the uninfected control young pullets

The MCHC value of the infected pullets was significantly lower ($p < 0.05$) than that of the uninfected controls on day 7 (31.21 ± 1.21 g/dL) PI. No significant differences ($p > 0.05$) in MCHC value were recorded in the infected pullets compared to the uninfected controls on days 14 (30.79 ± 3.13 g/dL), 21 (27.60 ± 1.22 g/dL), 28 (29.52 ± 0.47 g/dL) and 35 (36.85 ± 0.14 g/dL) PI (Figure 6).

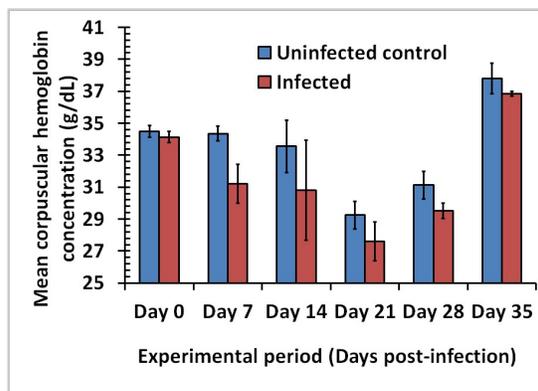


Figure 6: Mean corpuscular haemoglobin concentration (g/dL) of young pullets infected with *Salmonella gallinarum* compared to the uninfected controls

The mean TWBC count was significantly higher ($p < 0.05$) in the infected young pullets when compared to the uninfected controls on days 7 ($115.25 \pm 15.58 \times 10^3/\mu\text{L}$), 14 ($121.88 \pm 16.32 \times 10^3/\mu\text{L}$), 21 ($106.13 \pm 11.47 \times 10^3/\mu\text{L}$), 28 ($94.63 \pm 3.75 \times 10^3/\mu\text{L}$) and 35 ($76.75 \pm 3.19 \times 10^3/\mu\text{L}$) PI (Figure 7).

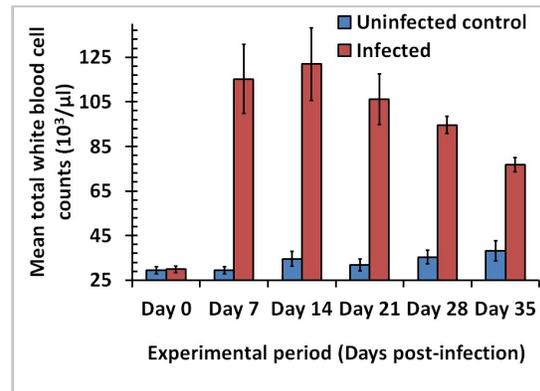


Figure 7: Mean total white blood cell count ($10^3/\mu\text{L}$) of the pullets infected with *Salmonella gallinarum* compared to the uninfected controls

The mean absolute heterophil count of young pullets infected with *S. gallinarum* was significantly higher ($p < 0.05$) than that of the uninfected controls on days 7 ($81.14 \pm 12.97 \times 10^3/\mu\text{L}$), 14 ($73.10 \pm 17.42 \times 10^3/\mu\text{L}$), 21 ($57.29 \pm 9.55 \times 10^3/\mu\text{L}$), 28 ($32.32 \pm 2.51 \times 10^3/\mu\text{L}$) and 35 ($20.93 \pm 1.50 \times 10^3/\mu\text{L}$) PI (Figure 8).

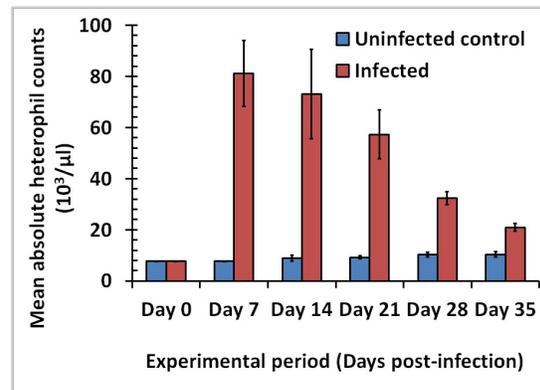


Figure 8: Mean absolute heterophil count ($10^3/\mu\text{L}$) of young pullets infected with *Salmonella gallinarum* compared to the uninfected controls

The mean absolute lymphocyte count was significantly higher ($p < 0.05$) in the infected young pullets than in the uninfected controls on days 14 ($37.97 \pm 1.28 \times 10^3/\mu\text{L}$), 21 (34.74 ± 2.43

$\times 10^3/\mu\text{L}$), 28($53.25 \pm 2.64 \times 10^3/\mu\text{L}$) and 35($39.78 \pm 0.64 \times 10^3/\mu\text{L}$) PI. There was no statistically significant difference ($p > 0.05$) in the mean absolute lymphocyte count between the infected pullets and the uninfected controls on day 7($19.43 \pm 1.81 \times 10^3/\mu\text{L}$) PI (Figure 9).

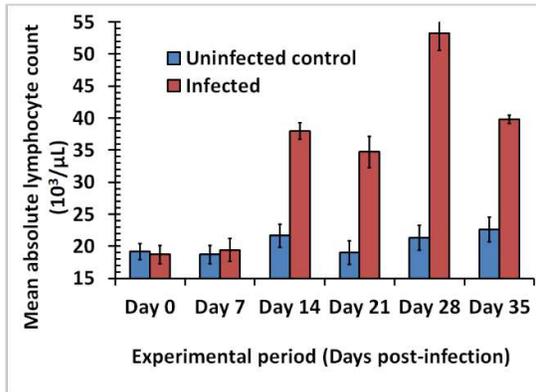


Figure 9: Mean absolute lymphocyte count ($10^3/\mu\text{L}$) of the pullets infected with *Salmonella gallinarum* compared to the uninfected pullets

The mean absolute eosinophil count of the pullets infected with *S. gallinarum* was significantly higher ($p < 0.05$) than that of the uninfected pullets on days 7($5.88 \pm 0.15 \times 10^3/\mu\text{L}$), 14($6.68 \pm 1.14 \times 10^3/\mu\text{L}$), 21($9.84 \pm 0.75 \times 10^3/\mu\text{L}$), 28($5.40 \pm 0.30 \times 10^3/\mu\text{L}$) and 35($8.40 \pm 1.74 \times 10^3/\mu\text{L}$) PI (Figure 10).

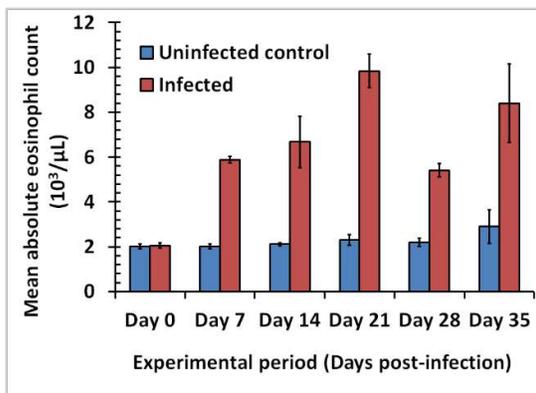


Figure 10: Mean absolute eosinophil count ($10^3/\mu\text{L}$) of the pullets infected with *Salmonella gallinarum* compared to the uninfected pullets

Similarly, the mean absolute monocyte count of the infected pullets was significantly higher ($p < 0.05$) than that of the uninfected controls on days 7($8.56 \pm 1.07 \times 10^3/\mu\text{L}$), 14($4.13 \pm 0.80 \times 10^3/\mu\text{L}$), 21($3.90 \pm 0.57 \times 10^3/\mu\text{L}$), 28($3.67 \pm$

$1.03 \times 10^3/\mu\text{L}$) and 35($7.66 \pm 0.31 \times 10^3/\mu\text{L}$) PI (Figure 11).

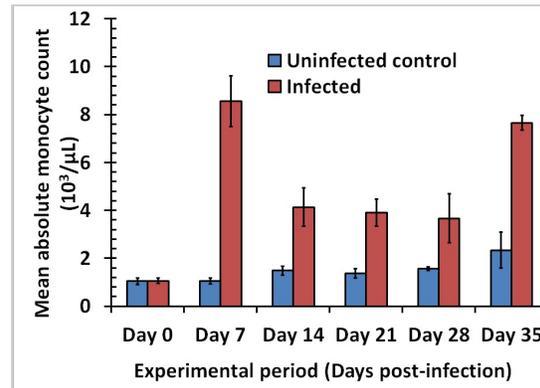


Figure 11: Mean absolute monocyte count ($10^3/\mu\text{L}$) of the pullets infected with *Salmonella gallinarum* compared to the uninfected controls

Alterations in the Oxidative Stress Markers:

The mean serum MDA level of the infected pullets was significantly higher ($p < 0.05$) than that of the uninfected pullets on days 7($13.13 \pm 1.35 \text{ nmol/mL}$), 14($14.16 \pm 1.64 \text{ nmol/mL}$), 21($9.29 \pm 0.94 \text{ nmol/mL}$), 28($7.74 \pm 0.33 \text{ nmol/mL}$) and 35($7.12 \pm 0.12 \text{ nmol/mL}$) PI (Figure 12).

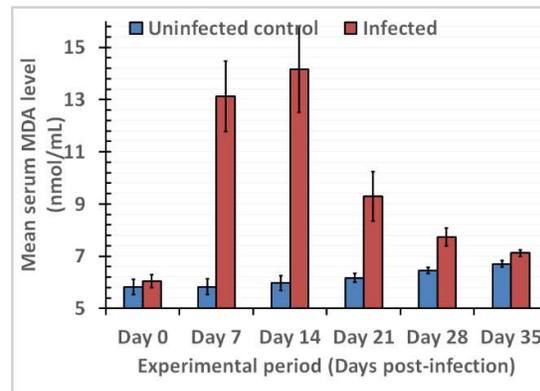


Figure 12: Mean serum MDA level (nmol/mL) of the pullets infected with *Salmonella gallinarum* compared to the uninfected pullets

The mean serum GPx activity of the infected pullets was significantly higher ($p < 0.05$) than that of the uninfected controls on days 7($646.67 \pm 68.07 \text{ IU/L}$), 14($405.25 \pm 16.51 \text{ IU/L}$), 21($284.54 \pm 35.55 \text{ IU/L}$), 28($267.29 \pm 24.39 \text{ IU/L}$), 35($241.43 \pm 12.19 \text{ IU/L}$) PI (Figure 13). In the same vein, the mean serum CAT activity of the infected pullets was significantly higher ($p < 0.05$) than that of the uninfected controls on days 7($52.98 \pm 4.42 \text{ IU/mL}$),

14(47.62 ± 2.04 IU/mL), 21(20.40 ± 3.20 IU/mL) 28(19.50 ± 0.46 IU/mL) and 35(16.50 ± 0.89 IU/mL) PI (Figure 14).

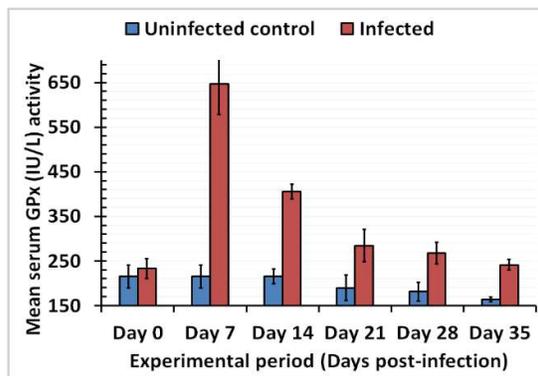


Figure 13: Mean serum GPx (IU/L) activity of the pullets infected with *Salmonella gallinarum* compared to the uninfected controls

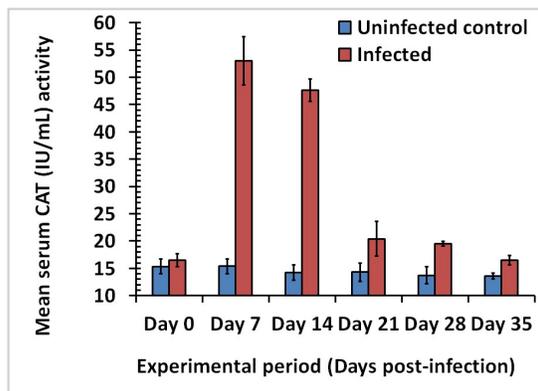


Figure 14: Mean serum CAT (IU/mL) activity of young pullets infected with *Salmonella gallinarum* compared to the uninfected control young pullets

Correlation of Total White Blood Cell Counts with Oxidative Stress Markers:

In the young pullets infected with *S. gallinarum* the TWBC counts correlated very strongly, positively and significantly with MDA (r = 0.82; p = 0.01), GPx (r = 0.72; p = 0.01), and CAT (r = 0.74; p = 0.01) (Table 2).

Table 2: Correlation of total white blood cell counts and oxidative stress markers of pullets infected with *Salmonella gallinarum*

Parameter	MDA (nmol/mL)	GPX IU/L	CAT (IU/mL)
TWBC counts (10³/μl)	r = 0.82	r = 0.72	r = 0.74
Significance (2-tailed)	P = 0.01	P = 0.01	P = 0.01

Correlation between Absolute Heterophil Count and Oxidative Stress Markers:

In the young pullets infected with *S. gallinarum*, the absolute heterophil count correlated very strongly, positively and significantly with MDA (r = 0.89; p = 0.01), GPx (r = 0.82; p = 0.01), and CAT (r = 0.84; p = 0.01) (Table 3).

Table 3: Correlation of absolute heterophil count and oxidative stress markers of pullets infected with *Salmonella gallinarum*

Parameter	MDA (nmol/mL)	GPX (IU/L)	CAT (IU/mL)
Absolute heterophil count (10³/μl)	r = 0.89	r = 0.82	r = 0.84
Significance (2-tailed)	P = 0.01	P = 0.01	P = 0.01

DISCUSSION

The clinical signs including depression, weakness, loss of appetite, greenish-yellow diarrhoea, droopy wings, prostration, ruffled feathers, dehydration and significant weight loss (p<0.05) observed in the present study, agree with previous reports (Shivaprasad, 2000; Shah *et al.*, 2013; Fotouh *et al.*, 2014; Lopes *et al.*, 2016). The total mortality of 36.00% recorded in this study is relatively lower than the 40.00% reported in 5-day-old chicks by Lopes *et al.* (2016), but higher than 22.50% and 24.40% reported by Shah *et al.* (2013) and Fotouh *et al.* (2014) in 7-week-old and one-day-old broiler chickens, respectively, infected with *S. gallinarum*. Lahiri *et al.* (2010) reported that the difference in mortality rates may be due to differences in the strains, infective dose, pathogenicity and virulence of *S. gallinarum* used in the different studies, as well as breed and age of the chickens, coupled with host’s immune response. The findings of this study were in agreement with the findings of Lahiri *et al.* (2010).

The significantly lower (p<0.05) mean PCV, Hb concentration and RBC count in the infected pullets when compared to the uninfected controls in this study indicated that experimental FT infection results in anaemia.

This finding is consistent with the reports of other researchers who observed acute

anaemia in FT-infected chickens. Christensen *et al.* (1996) attributed the anaemia to RBC modification and subsequent destruction following cytopathic effects of *S. gallinarum* outer membrane proteins on RBCs. Feldmann *et al.* (2000) reported that anaemia can be caused by a deficiency in vitamin B₁₂, needed for erythropoiesis, as a result of its intestinal malabsorption or poor liver storage following liver dysfunction. This study, however, observed a haemolytic anaemia following the destruction of the RBCs. The septicemic nature of FT may have led to the elaboration of substances which caused lyses of the RBCs. The significantly higher and lower ($p < 0.05$) MCV and MCHC values respectively in the infected pullets compared to the controls suggest a responsive or regenerative (macrocytic hypochromic) anaemia, as the significant decrease ($p < 0.05$) in the erythrocytic values may have elicited bone marrow response resulting in increased erythropoiesis and release of reticulocytes, which are the immature and larger sized RBCs, into blood circulation. This finding agrees with the report of Shah *et al.* (2013). Microcytosis, however, was reported between days 14 and 28 in this study which may be a result of iron depletion following severe haemolysis and agrees with the report of Mdegela *et al.* (2002).

The significantly higher ($p < 0.05$) TWBC count in the infected pullets when compared to the controls in this study may be a reflection of tissue degeneration and acute inflammatory response, resulting in massive heterophilia, and the host's immune response evoked by assaults of the invading organisms on the target organs resulting in lymphocytosis. These findings are consistent with the reports of Shah *et al.* (2013) and Fotouh *et al.* (2014) in experimental FT infection in young broiler chickens. Chitty (2018) reported that leukocytosis can occur following stress, infection, inflammation, tissue damage or neoplasia. The significant elevation ($p < 0.05$) in the mean eosinophil count, as well as mean monocyte count on days 7, 14, 21, 28 and 35 PI, suggests a compensatory response to the corresponding massive heterophilia, heterophils being the first line of defence against bacterial infection, as they all originate from same cell line

or precursor in the bone marrow (Thrall and Weiser, 2002).

The infection of pullets with *S. gallinarum* in this study induced OS, which has not been reported on FT-infected pullets, in available literature. The significant elevation ($p < 0.05$) in serum MDA in the infected pullets compared to the uninfected controls in this study, suggests excessive production of reactive oxygen species (ROS) which causes lipid peroxidation with the unstable lipid peroxides decomposing to form MDA whose measurement, according to earlier reports, is widely used as an indicator of OS (Patterson and Leake, 1998; Şimşek *et al.*, 2006). The significantly higher ($p < 0.05$) serum GPx activity in the infected pullets compared to the controls, suggests excessive production of hydrogen peroxide and organic peroxides (another ROS) by this bacterial infection which in turn increased the production of the antioxidant, GPx. The destruction of hydrogen and organic peroxides is facilitated by GPx, an enzyme with an essential antioxidant function elaborated by the antioxidant defence mechanism of the host (Kidd, 1997; Ivanov *et al.*, 2017). Similarly, the significantly higher ($p < 0.05$) serum CAT activity in the infected pullets than that of the uninfected controls in this study may be linked to the serum CAT generation above other antioxidants e.g. hydrogen peroxides. CAT, another enzyme with antioxidant function, is involved in the catalysis of hydrogen peroxide to oxygen and water (Gonzales *et al.*, 1984; Ivanov *et al.*, 2017).

The very strong, positive and highly significant correlation between absolute heterophil count and MDA in this study is quite expected as massive heterophilia would induce OS. This suggests that OS may play a significant role in the pathology of FT-infected chickens. In the same vein, the strong to a very strong, positive and highly significant relationship between serum GPx and CAT activities, and absolute heterophil count (Bizoń *et al.*, 2023), suggests that the massive heterophilia elicited OS and by extension, the production of the antioxidants by the antioxidant defence mechanism of the host, hence, improving weight gain and survival rate even without medication in an acute disease like FT.

Conclusion: Infection of pullets with *S. gallinarum* significantly altered the PCV, Hb concentration, RBC count, TWBC count, absolute heterophil, lymphocyte, eosinophil and monocyte counts, MCV, MCHC, MDA level as well as serum activities of GPx, and CAT. The significant elevation in the serum MDA level suggests that *S. gallinarum* infection of pullets induced OS. An association was established between significantly elevated GPx and CAT, which have antioxidant properties, and survival/health improvement indices such as improved weight gain and self-recovery. Hence, the inclusion of hematinics to facilitate erythropoiesis, as well as early antioxidant administration could ameliorate the pathology and by extension, the mortality of FT-infected pullets and is highly recommended, creating an alternative mode of therapeutic intervention as incessant vaccine failures and antimicrobial resistance have become a major constraint in the poultry industry.

ACKNOWLEDGEMENTS

This work was supported by an Institutional Based Research (IBR) grant from the Tertiary Education Trust Fund (Grant number: TETFund/DESS/UNI/NSUKKA/2018/RP/VOL.1).

REFERENCES

BARBOUR, E. K., AYYASH, D. B., ALTURKISTNI, W., ALYAHIBY, A., YAGHMOOR, S., IYER, A., YOUSEF J., KUMOSANI, T. and HARAKEH, S. (2015). Impact of sporadic reporting of poultry *Salmonella* serovars from selected developing countries. *Journal of Infection in Developing Countries*, 9(01): 001 – 007.

BARROW, P. A. and DE FREITAS NETO, A. C. (2011). Pullorum disease and fowl typhoid-new thoughts on old diseases: a review. *Avian Pathology*, 40(1): 1 – 13.

BERCHIERI, Jr. A., MURPHY, C. K., MARSTON, K. and BARROW, P. A. (2001). Observations on the persistence and vertical transmission of *Salmonella enterica* serovars Pullorum and Gallinarum in chickens: effect of bacterial and host

genetic background. *Avian Pathology*, 30(1): 221 – 231.

BERCHIERI, Jr. A., OLIVEIRA, G. H., PINHEIRO, L. A. S. and BARROW, P. A. (2000). Experimental *Salmonella gallinarum* infection in light laying hen lines. *Brazilian Journal of Microbiology*, 31(1): 50 – 52.

BEYAZ, L., ATASEVER, A., AYDIN, F., GUMUSOY, K. S. and ABAY, S. (2010). Pathological and clinical findings and tissue distribution of *Salmonella gallinarum* infection in Turkey poult. *Turkish Journal of Veterinary and Animal Sciences*, 34(2): 101 – 110.

BIZOŃ, A., CHOJDAK-ŁUKASIEWICZ, J., BUDREWICZ, S., POKRYSZKO-DRAGAN, A., & PIWOWAR, A. (2023). Exploring the relationship between antioxidant enzymes, oxidative stress markers, and clinical profile in relapsing-remitting multiple sclerosis. *Antioxidants (Basel, Switzerland)*, 12(8): 1638. <https://doi.org/10.3390/antiox12081638>

CAMPBELL, T. W. (1994). Hematology. Pages 176 – 198. *In*: RITCHIE B. W., HARRISON G. J. and HARRISON L. R. (Eds.). *Avian Medicine: Principles and Application*. Wingers Publishing Incorporated, Lake Worth, Florida, USA.

CHICKIPEDIA (2019). *Health and Biosecurity: What is a Healthy Hen?* Chickipedia.com. Pages 60 – 68. <https://hn-int.com/health-biosecurity/>

CHITTY, J. (2018). Sample taking and basic clinical pathology. Pages 172 – 186. *In*: CHITTY, J. and MONKS, D. (Eds.). *BSAVA Manual of Avian Practice*. British Small Animal Veterinary Association, Gloucestershire, United Kingdom.

CHRISTENSEN, J. P., BARROW, P. A., OLSEN, J. E., POULSEN, J. S. D. and BISGAARD, M. (1996). Correlation between viable counts of *Salmonella gallinarum* in spleen and liver and the development of anemia in chickens as seen in experimental fowl typhoid. *Avian Pathology*, 25(4): 769 – 783.

DE FREITAS NETO, O. C., SETTA, A., IMRE, A., BUKOVINSKI, A., ELAZOMI, A., KAISER,

- P., JUNIOR, A. B., BARROW, P. and JONES, M. (2013). A flagellated motile *Salmonella gallinarum* mutant (SG Fla+) elicits a pro-inflammatory response from avian epithelial cells and macrophages and is less virulent to chickens. *Veterinary Microbiology*, 165(3-4): 425 – 433.
- DESMIDT, M., DUCATELLE, R. and HAESEBROUCK, F. (1997). Pathogenesis of *Salmonella enteritidis* phage type four after experimental infection of young chickens. *Veterinary Microbiology*, 56(1-2): 99 – 109.
- DRAPER, H. H. and HADLEY, M. (1990). Malondialdehyde determination as an index of lipid peroxidation. *Methods in Enzymology*, 186: 421 – 431.
- FELDMANN, B. V., ZINKI, J. G. and JAIN, N. C. (2000). *Shalm's Veterinary Hematology*. 5th Edition, Lea and Fibiger, Philadelphia, USA.
- FOTOUH, A., GAB-ALLAH, M. S., TANTAWY, A. A., SOUFY, H. and NASR, S. M. (2014). Alterations of blood components in broiler chicks experimentally infected with *Salmonella gallinarum*. *Global Veterinaria*, 13(5): 787 – 793.
- GONZALES, R., AUCLAIR, C., VOISON, E., GAUTERO, H., DHERMY, D. and BOIVIN, P. (1984). Superoxide dismutase, catalase and glutathione peroxidase in red blood cells from patients with malignant diseases. *Cancer Research*, 44(9): 4137 – 4139.
- HIGGINS, T., BEUTLER, E. and DOUMAS, B. T. (2008). Measurement of haemoglobin in blood. Pages 514 – 515. *In*: BURTIS, C. A., ASHWOOD, E. R. and BRUNS, D. E. (Eds.). *Tietz Fundamentals of Clinical Chemistry*. 6th Edition, Sanders Elsevier, Missouri, USA.
- IVANOV, A. V., VALUEV-ELLISTON, V. T., IVANOVA, O. N., KOCHETKOV, S. N., STARODUBOVA, E. S., BARTOSCH, B. and ISAGULIANTS, M. G. (2017). Oxidative stress during HIV infection: mechanisms and consequences. *Oxidative Medicine and Cellular Longevity*, 2017: 3496043. <http://dx.doi.org/10.1155/2017/3496043>
- KIDD, P. M. (1997). Glutathione: Systemic protectant against oxidative and free radical damage. *Alternative Medicine Review*, 2(3): 155 – 176.
- LAHIRI, A., IYER, N., DAS, P. and CHAKRAVORTTY, D. (2010). Visiting the cell biology of *Salmonella* infection. *Microbes and Infection*, 12(11): 809 – 818.
- LOPES, P. D., NETO, O. F., BATISTA, D. F. A., DENADAI, J., ALARCON, M. F. F., ALMEIDA, A. M., VASCONCELOS, R. O., SETTA, A., BARROW, P. A. and JUNIOR, A. B. (2016). Experimental infection of chickens by a flagellated motile strain of *Salmonella enterica* serovar *Gallinarum* biovar *Gallinarum*. *The Veterinary Journal*, 214: 40 – 46.
- MDEGELA, R. H., MSOFFE, P. L. M., WAIHENYA, R. W., KASANGA, J. C., MTAMBO, M. M. A., MINGA, U. M. and OLSEN, J. E. (2002). Comparative pathogenesis of experimental infections with *Salmonella gallinarum* in local and commercial chickens. *Tropical Animal Health and Production*, 34(3): 194 – 204.
- MØLBAK, K., GERNER-SMIDT, P. and WEGENER, H. C. (2002). Increasing quinolone resistance in *Salmonella enterica* serotype Enteritidis. *Emerging Infectious Diseases*, 8(5): 514 – 525.
- OIE (2015). *World Information on Animal Diseases – Fact Sheets*. OIE: World Organization for Animal Health, Paris, France. <https://www.woah.org/app/uploads/2021/03/notification-en.pdf>
- OLIVEIRA, G., BERCHIERI, A. and FERNANDES, A. (2005). Experimental infection of laying hens with *Salmonella enterica* serovar *Gallinarum*. *Brazilian Journal of Microbiology*, 36(1): 51 – 56.
- PARVEJ, M. S., NAZIR, K. N. H., RAHMAN, M. B., JAHAN, M., KHAN, M. F. R. and RAHMAN, M. (2016). Prevalence and characterization of multi-drug resistant *Salmonella enterica* serovar *Gallinarum* biovar *Pullorum* and *Gallinarum* from chicken. *Veterinary World*, 9(1): 65 – 70.
- PATTERSON, R. A. and LEAKE, D. S. (1998). Human serum cysteine and histidine

- inhibit the oxidation of low-density lipoprotein less at acidic pH. *Federation of European Biochemical Society (FEBS) Letters*, 434(3): 317 – 321.
- ROA, G. (2000). *A Comprehensive Textbook on Poultry Pathology*. Jaypee Brothers Medical Publisher Limited, New Delhi, India.
- SHAH, S. N., KAMIL, S. A., DARZI, M. M., MIR, M. S. and BHAT, S. A. (2013). Hematological and some biochemical changes in experimental fowl typhoid infection in broiler chickens. *Comparative Clinical Pathology*, 22: 83 – 91.
- SHIVAPRASAD, H. L. (2000). Fowl typhoid and Pullorum disease. *Review in Science and Technology of the Office of International Epizootics*, 19(2): 405 – 424.
- SHIVAPRASAD, H. L., METHNER, U. and BARROW, P. A. (2013). Salmonella infections in the domestic fowl. Pages 162 – 192. *In: BARROW P. A. and METHNER, U. (Eds.). Salmonella in Domestic Animals*. Second Edition, CAB International, Wallingford, Oxfordshire, United Kingdom.
- ŞİMŞEK, S., YÜCE, A. and ÜTÜK, A. E. (2006). Determination of serum malondialdehyde levels in sheep naturally infected with *Dicrocoelium dendriticum*. *FU Sağlık Bil Dergisi*, 20(3): 217 – 220.
- THRALL, M. A. and WEISER, M. G. (2002). Hematology. Pages 29 – 74. *In: HENDRIX, C. M. (Ed.). Laboratory Procedures for Veterinary Technicians*. 4th Edition, Mosby Incorporated, Missouri, USA.
- WEYDERT, C. J. and CULLEN, J. J. (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissues. *Nature Protocols*, 5(1): 51 – 66.
- ZANETTI, N. S., DE CARLI, S., SONZA, M. N., LEHMANN, F. K. M., KIPPER, D., DIAS, K. K. R., FONSECA, A. S. K., LUNGE, V. R. and IKUTA, N. (2019). Molecular detection and characterization of *Salmonella gallinarum* from poultry farms in Brazil. *Journal of Applied Poultry Research*, 28(4): 1335 – 1341.



This article and articles in Animal Research International are Freely Distributed Online and Licensed under a [Creative Commons Attribution 4.0 International License \(CC-BY 4.0\)](https://creativecommons.org/licenses/by/4.0/)