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Research article

# Experimental *Salmonella* Gallinarum infection in point of lay pullets: Correlation of hematology and oxidative stress with egg production

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## Abstract

*Salmonella* Gallinarum is a bacterial pathogen linked with drop in egg production in poultry. In this study, hematological and oxidative stress (OS) parameters were evaluated and correlated with egg production in point of lay (POL) pullets infected with *S.* Gallinarum. Fifty 18-week-old POL pullets were randomly assigned to two groups of 25 POL pullets per group. The infected POL pullets were orally inoculated with *S.* Gallinarum ($10^9$ *S.* Gallinarum colony forming units/mL), while the uninfected pullets were the controls. Hematological and OS parameters were determined using standard methods. There were significant ($P < 0.05$) loss of body weight and drop in egg production in the infected POL pullets when compared to the controls. Acute hemolytic anemia of the macrocytic hypochromic type was observed. The total white blood cell (TWBC), absolute heterophil, lymphocyte, eosinophil and monocyte counts, mean serum malondialdehyde (MDA) level, glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities were significantly ($P < 0.05$) higher in the infected POL pullets when compared to the controls. There was a strong association between heterophilia and drop in egg production. An association was also established between significantly elevated GPx, SOD and CAT, which have antioxidant properties, and clinical outcome, including subsequent weight gain and rise in egg production, suggesting that antioxidants may be useful in the treatment of fowl typhoid. It was concluded that *S*. Gallinarum infection of POL pullets caused significant hematological changes, induced OS, and stimulated the body's antioxidant defense mechanism to elaborate GPx, SOD and CAT.

**Keywords**: Antioxidants, Chickens, Correlation coefficient, Fowl typhoid, Hematology, Oxidative stress markers.

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# INTRODUCTION

Poultry products (meat and eggs) are indispensable sources of proteins for humans, and poultry industry has been on rapid expansion in the past years (Hoque et al., 2019). The expansion and advancement in poultry production are, however, hampered by the occurrence of infectious diseases, which underscores further studies or efforts aimed at alleviating this constraint militating against optimum productivity (Haque et al., 2021; Galovicova et al., 2022).

One of the economically essential poultry diseases is fowl typhoid (FT), caused by *Salmonella* Gallinarum, a Gram-negative bacterium of the *Enterobacteriaceae* family (Molbak et al., 2002; Yasmin et al., 2019). It is a severe systemic and septicemic disease of commercially raised poultry and other galliform species, exhibiting a high prevalence rate worldwide (Yasmin et al., 2019; Zhou et al., 2022). The disease has a global distribution especially in developing countries, where antimicrobial resistance in *S.* Gallinarum strain has increased tremendously following indiscriminate use of multiple antibiotics to enhance production in poultry farms, hence posing huge socioeconomic challenge to the poultry industry, in form of high mortality and drop in egg production (Barbour et al., 2015; Seo et al., 2019; Haque et al., 2021). Fowl typhoid remains one of the prominent poultry diseases globally, despite vaccination and various eradication programs put in place to prevent and control its occurrence in commercial poultry farms (Okwori et al., 2013; Zanetti et al., 2019).

Despite the challenges posed by FT, it has been either scantly reported or poorly studied especially in developing countries where only few cases of outbreaks have been documented (Parvej et al., 2016). This study gap may be due to inaccurate diagnosis as most clinical manifestations of FT have striking resemblance of most enteric diseases of chickens, especially Newcastle disease. Moreover, the few reported cases of FT were on laying hens at their peak of egg production or beyond with little or no report on the point of lay (POL) pullets, negating the crucial nature of this age in the production life of commercial egg producers.

In addition, most studies on *S.* Gallinarum infection of chickens did not consider the hematological and blood biochemical studies, especially oxidative stress (OS) even as FT is a septicemic disease, and such studies may be fundamental to better understanding of the pathophysiology of this disease.

The aim of this study, therefore, was to evaluate the effects of *S.* Gallinarum infection on the hematology and oxidative stress parameters of POL pullets, and to correlate some of the measured parameters with egg production.

# MATERIALS AND METHODS

## Ethical Approval

This research was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria (Approval Reference Number: FVM-UNN-IACUC-2020-0339).

## Flock History

Fifty 18-week-old ISA® brown POL pullets with average weight of 1.79 ± 0.01 kg (± Standard error mean-SEM) were used for the study. They were procured at day-old from CHI® Hatchery, Ibadan, Nigeria. Brooding and rearing were carried out in isolation on deep litter with commercial poultry feed (Hybrid® Feeds, Kaduna, Nigeria) and clean drinking water provided *ad libitum*. Adequate biosecurity measures were observed and the pullets were routinely vaccinated against infectious bursal disease (IBD Vaccine, Bio-Med, India), Newcastle disease (ND LaSota and Komarov Vaccines, MSD Animal Health, Rahway, NJ, USA) and fowl

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pox (Fowl Pox Vaccine, National Veterinary Research Institute, Vom, Nigeria). Oral administration of coccidiostat, Amprolium 250 wsp (Kepro-Holland) at 1.5g per liter of drinking water, deworming with Piperazine Di Hcl (ALFASAN-Holland) at 1g per liter of drinking water and delousing with Ivermectin (Kepro-Holland) at 0.25 ml per liter of drinking water were carried out (Chickipidia, 2019). The pullets were housed in the Poultry Experimental Unit of the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

## Bacterial Inoculum

The *S.* Gallinarum strain was sourced locally from an outbreak of FT in a commercially raised poultry flock and was preserved in the Microbiology Laboratory of the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria. The pathogenicity of the *S.* Gallinarum isolate was maintained by passage in young chicks. The grown colonies on MCA were suspended in nutrient broth and the methods described by Beyaz et al. (2010) and Lopes et al. (2016) were adopted in the preparation of the bacterial inoculum.

## Pre-infection Screening for *Salmonella* Infection

Prior to the experimental infection, cloacal swabs from randomly selected POL pullets were cultured in nutrient broth, incubated at 37°C for 24h and plated onto MCA to rule out Salmonella infection in the experimental chickens (Berchieri et al., 2001; Lopes et al., 2016).

## Experimental Infection

At 18 weeks of age, the 50 POL pullets were randomly assigned to two treatment groups, 25 pullets per group, with each treatment replicated five times in a completely randomized design (CRD), spanning a period of 35 days. Each pullet in the challenged group was inoculated with 1 mL of *S*. Gallinarum inoculum containing 1 x $10^9$ colony-forming units (CFU/mL) by direct administration into the crop using oral gavage needle, while each pullet in the control group received 1 mL of phosphate buffered saline (PBS) placebo.

## Clinical Signs and Mortality Records

The chickens were observed twice daily for clinical signs, following experimental infection. Five randomly selected POL pullets from both the uninfected and infected groups were 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 POL pullets showing reluctance to move around freely are tagged weak, and all the pullets exhibiting signs of the disease are considered morbid. The number of dead POL 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 five randomly picked POL pullets per group on days 0, 7, 14, 21, 28 and 35 PI via jugular venipuncture. The pool blood sample per group, on each sampling day, was dispensed into two appropriately labelled sample bottles (one with Na-EDTA anticoagulant for hematology and the other devoid of anticoagulant for harvesting serum needed for the assay of OS markers). The blood sample was collected each day between 9:00 – 11:00 am.

## Hematological Examination

The determination of packed cell volume (PCV) followed the microhematocrit method, whereas, the assay of hemoglobin concentration (HbC) was done using the cyanomethemoglobin method (Thrall and Weiser, 2002; Higgins et al., 2008).

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The hemocytometer method was employed in the determination of red blood cell (RBC) as well as total white blood cell (TWBC) counts (Campbell, 1994). The determination of differential WBC counts was based on making thin air-dried smears stained by the Leishman technique, while the absolute WBC counts, the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae (Campbell, 1994; Thrall and Weiser, 2002).

## Determination of OS Markers

The serum malondialdehyde (MDA) level was determine by performing the modified thiobarbituric acid test using the ElabScience MDA assay kit (ElabScience Biotech Company Limited, South Africa) (Draper and Hadley, 1990). The cumene hydroperoxidase method was employed in the determination of serum glutathione peroxidase (GPx) activity, using the Fortress diagnostic GPx test kit (Antrim, United Kingdom). The serum superoxide dismutase (SOD) activity was determined by performing the hydroxylamine test using the ElabScience SOD assay kit (ElabScience Biotech Company Limited, South Africa), and visible light method was performed to assay for catalase (CAT) by using ElabScience CAT assay kit (ElabScience Biotech Company Limited, South Africa) (Weydert and Cullen, 2010). The readings of MDA, SOD and CAT assays were done with a Diatek blood biochemistry analyzer (Wuxi Hiwell Diatek Instrument Company Limited, China) set at the MDA-ELS, SOD-ELS, 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 obtained from the body weight measurement, percentage egg production, hematology, and assay of OS markers were analyzed using the independent t-test for equality of means on SPSS for Windows Version 23. The erythrocytic, leukocytic and OS parameters were correlated with egg production 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, reduced intake of feed and water, greenish-yellow diarrhea, dehydration, significant weight loss ($P < 0.05$) (Figure 1) and weakness were first observed on day 4 PI and persisted to day 21 PI in the infected group with overall morbidity of 12/25 (48%). First mortality was recorded on day 4 PI and overall mortality was 3/25 (12%). The infected POL pullets maintained significantly ($P < 0.05$) lower egg production compared to the uninfected controls for the duration of the study. There was, however, a steady rise in egg production in the infected POL pullets from week 5 PI (Figure 2). The control group remained healthy for the duration of the experiment.

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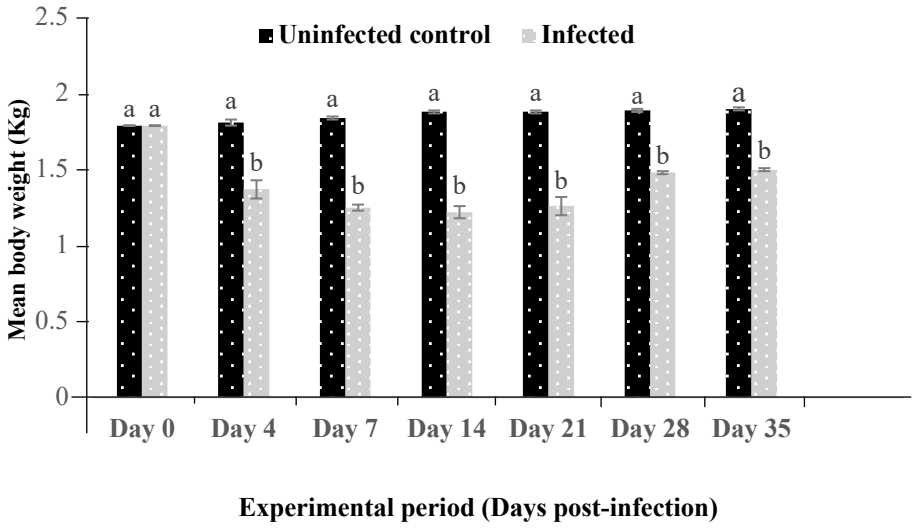



**Figure 1** Mean body weight (kg) of POL pullets infected with *S.* Gallinarum compared with the controls. [a, b] Different alphabets on the error bars indicate significant difference ($P < 0.05$) between the groups.

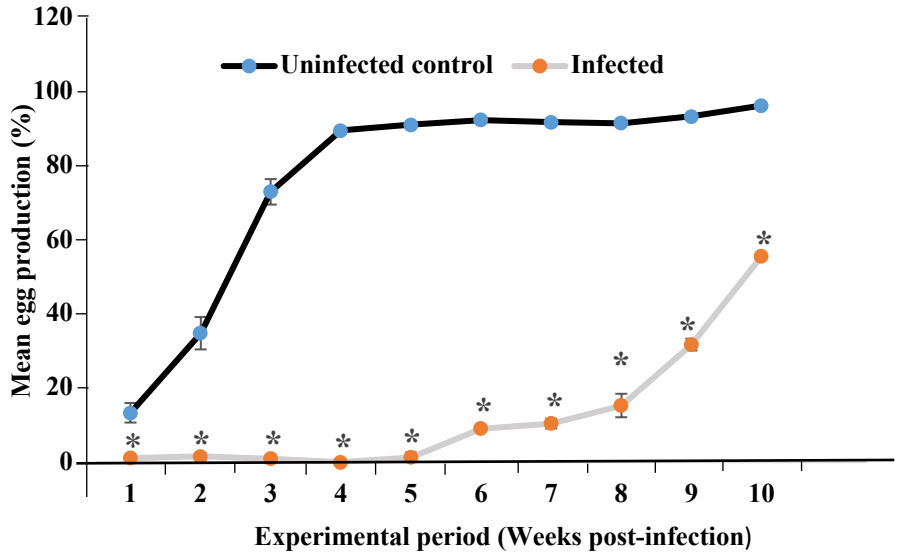



**Figure 2** Percentage (%) egg production. The infected POL pullets maintained significantly lower egg production compared to the uninfected controls for the duration of the study. However, there was a steady rise in production from week 5 PI. * indicates significant difference ($P < 0.05$) between the groups.

## Hematological Alterations

The mean PCV of the POL pullets infected with *S.* Gallinarum was significantly ($P < 0.05$) lower than that of the uninfected POL pullets on days 7 ($P = 0.001$), 14 ($P = 0.011$), 21 ($P = 0.016$), 28 ($P = 0.021$) and 35 ($P = 0.014$) PI (Table 1). There was also significantly ($P < 0.05$) lower mean HbC in the infected POL pullets when compared to that of the uninfected POL controls on days 7 ($P = 0.013$), 14 ($P = 0.001$), 21 ($P = 0.042$), 28 ($P = 0.045$) and 35 ($P = 0.014$) PI (Table 2). Similarly, the mean RBC count of POL pullets infected with *S.* Gallinarum was significantly ($P < 0.05$) lower than that of the uninfected control POL pullets on days 7 ($P < 0.001$), 14 ($P = 0.011$), 21 ($P = 0.019$), 28 ($P = 0.029$) and 35 ($P = 0.049$) PI (Table 3).

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**Table 1** Mean PCV (%) of POL pullets infected with *S.* Gallinarum compared to the uninfected control POL pullets

| Days PI | Uninfected control (n=5) | Infected (n=5) | 95% Confidence Interval | | P-value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Day 0 | 24.62±0.24 | 24.38±0.24 | -0.58 | 1.08 | 0.488 |
| Day 7 | 24.63±0.24[a] | 20.75±0.60[b] | 2.31 | 5.44 | 0.001 |
| Day 14 | 27.00±0.50[a] | 25.13±0.47[b] | -1.74 | 1.99 | 0.011 |
| Day 21 | 29.75±0.88[a] | 23.55±1.63[b] | -6.58 | 8.73 | 0.016 |
| Day 28 | 29.38±1.42[a] | 24.75±0.43[b] | -6.80 | -3.20 | 0.021 |
| Day 35 | 28.25±0.95[a] | 24.88±0.24[b] | -5.76 | -0.99 | 0.014 |

All data are expressed as mean ± standard error mean (SEM) with 95% Confidence Interval.
[(a, b)] Different alphabetical superscripts on mean in a row indicate significant difference (P < 0.05) between the groups. n = number of POL pullets sampled. PCV = Packed cell volume. PI = Post infection.

**Table 2** Mean HbC (g/dL) of POL pullets infected with *S.* Gallinarum compared to the uninfected POL pullets

| Days PI | Uninfected control (n=5) | Infected (n=5) | 95% Confidence Interval | | P-value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Day 0 | 10.23±0.62 | 10.19±0.59 | -1.47 | 2.72 | 0.494 |
| Day 7 | 10.22±0.62[a] | 7.92±0.21[b] | 0.70 | 3.92 | 0.013 |
| Day 14 | 9.36±0.07[a] | 8.66±0.13[b] | 0.44 | 0.97 | 0.001 |
| Day 21 | 10.70±0.35[a] | 7.98±0.99[b] | 0.14 | 5.30 | 0.042 |
| Day 28 | 10.78±0.48[a] | 9.56±0.08[b] | 0.03 | 2.41 | 0.045 |
| Day 35 | 11.06±0.41[a] | 9.10±0.40[b] | 0.56 | 3.36 | 0.014 |

All data are expressed as mean ± standard error mean (SEM) with 95% Confidence Interval.
[(a, b)] Different alphabetical superscripts on mean in a row indicate significant difference (P < 0.05) between the groups. n = number of POL pullets sampled. HbC = Hemoglobin concentration. PI = Post infection.

**Table 3** Mean RBC count ($10^6$/µL) of POL pullets infected with *S.* Gallinarum compared to the uninfected POL pullets

| Days PI | Uninfected control (n=5) | Infected (n=5) | 95% Confidence Interval | | P-value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Day 0 | 3.11±0.04 | 3.06±0.05 | -0.11 | 0.20 | 0.527 |
| Day 7 | 3.10±0.04[a] | 2.34±0.04[b] | 0.63 | 0.90 | 0.000 |
| Day 14 | 3.16±0.09[a] | 2.80±0.02[b] | 0.12 | 0.60 | 0.011 |
| Day 21 | 3.77±0.12[a] | 2.95±0.23[b] | 0.19 | 1.46 | 0.019 |
| Day 28 | 3.42±0.15[a] | 2.96±0.04[b] | 0.06 | 0.85 | 0.029 |
| Day 35 | 3.21±0.18[a] | 2.82±0.01[b] | -0.05 | 0.83 | 0.049 |

All data are expressed as mean ± standard error mean (SEM) with 95% Confidence Interval.
[(a, b)] Different alphabetical superscripts on mean in a row indicate significant difference (P < 0.05) between the groups. n = number of POL pullets sampled. RBC = Red blood cell. PI = Post infection.

The MCV of POL pullets infected with *S.* Gallinarum revealed significantly (P < 0.05) higher value than uninfected controls on day 7 (P < 0.001) PI. The elevation in MCV on days 14 (P = 0.119) and 21(P = 0.476) PI and slight decrease on days 28 (P = 0.674) and 35 (P = 0.907) PI in the infected POL pullets compared to those of the uninfected controls, were not statistically significant (P > 0.05) (Table 4). The MCHC of POL pullets infected with *S.* Gallinarum revealed lower values that were not significantly different, than uninfected controls on days 7 (P = 0.268), 14 (P = 0.772), 21 (P = 0.450) and 35 (P = 0.450) PI. There was an elevation (though not statistically significant) in MCHC in the infected POL pullets compared to uninfected controls on day 28 (P = 0.627) PI (Table 5).

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**Table 4** The MCV (fL) of POL pullets infected with *S.* Gallinarum compared to the uninfected POL pullets

| Days PI | Uninfected control (n=5) | Infected (n=5) | 95% Confidence Interval | | P-value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Day 0 | 78.98±0.60 | 79.62±0.60 | -2.71 | 1.45 | 0.488 |
| Day 7 | 79.32±0.51[a] | 88.63±1.21[b] | -12.52 | -6.09 | 0.000 |
| Day 14 | 85.65±2.01 | 89.92±1.22 | -10.03 | 1.48 | 0.119 |
| Day 21 | 76.42±2.45 | 80.46±4.71 | -17.05 | 8.96 | 0.476 |
| Day 28 | 86.79±6.77 | 83.67±2.04 | -14.18 | 20.42 | 0.674 |
| Day 35 | 89.19±7.40 | 88.29±0.71 | -17.29 | 19.11 | 0.907 |

All data are expressed as mean ± standard error mean (SEM) with 95% Confidence Interval.
[(a, b)] Different alphabetical superscripts on mean in a row indicate significant difference ($P < 0.05$) between the groups. n = number of POL pullets sampled. MCV = Mean corpuscular volume. PI = Post infection.

**Table 5** The MCHC (g/dL) of POL pullets infected with *S.* Gallinarum compared to the uninfected controls

| Days PI | Uninfected control (n=5) | Infected (n=5) | 95% Confidence Interval | | P-value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Day 0 | 39.50±1.90 | 39.10±2.21 | -8.21 | 6.03 | 0.720 |
| Day 7 | 41.47±2.24 | 38.23±1.43 | -3.26 | 9.74 | 0.268 |
| Day 14 | 34.70±0.72 | 34.45±0.42 | -1.78 | 2.28 | 0.772 |
| Day 21 | 36.07±1.68 | 33.60±2.56 | -5.02 | 9.97 | 0.450 |
| Day 28 | 37.07±3.02 | 38.64±0.55 | -9.08 | 5.94 | 0.627 |
| Day 35 | 38.58±1.75 | 36.60±1.71 | -4.01 | 7.96 | 0.450 |

All data are expressed as mean ± standard error mean (SEM) with 95% Confidence Interval. n = number of POL pullets sampled. MCHC = Mean corpuscular hemoglobin concentration. PI = Post infection.

The mean TWBC count was significantly ($P < 0.05$) higher in the infected POL pullets than uninfected controls on days 7 ($P < 0.001$), 14 ($P = 0.001$), 21 ($P = 0.012$), and 35 ($P = 0.047$) PI. There was no significant difference ($P > 0.05$) in the TWBC count of the infected POL pullets and the controls on day 28 PI (Figure 3). The mean absolute heterophil count was significantly higher in the infected POL pullets than uninfected controls on days 7 ($P < 0.001$), 14 ($P = 0.001$), 21 ($P = 0.026$), 28 ($P = 0.020$) and 35 ($P = 0.038$) PI (Figure 4). There was significant elevation in the mean absolute lymphocyte count in the infected POL pullets when compared to the uninfected controls on days 14 ($P = 0.019$), 21 ($P = 0.026$) and 35 ($P = 0.040$) PI. There was no significant difference between the absolute lymphocyte count in the infected POL pullets and the uninfected controls on day 7 ($P = 0.431$) PI. The mean absolute lymphocyte count in the infected POL pullets was, however, significantly lower than that of the uninfected controls on day 28 ($P = 0.048$) PI (Figure 5). The mean absolute eosinophil count was significantly higher in the infected POL pullets than that of the uninfected controls on days 7 ($P = 0.004$), 21 ($P = 0.004$), and 35 ($P = 0.008$) PI. There was no significant difference in the mean absolute eosinophil count between the infected POL pullets and the uninfected controls on days14 ($P = 0.918$) and 28 ($P = 0.791$) PI (Figure 6). The mean absolute monocyte count in the infected POL pullets was significantly lower than that of the uninfected controls on days 7 ($P = 0.019$) and 35 ($P = 0.007$) PI, but was significantly higher in the infected POL pullets when compared to the uninfected controls on day 28 ($P = 0.019$) PI. There was no significant difference between the mean absolute monocyte count of the infected POL pullets and that of the uninfected controls on days 14 ($P = 0.801$) and 21 ($P = 0.723$) PI (Figure 7).

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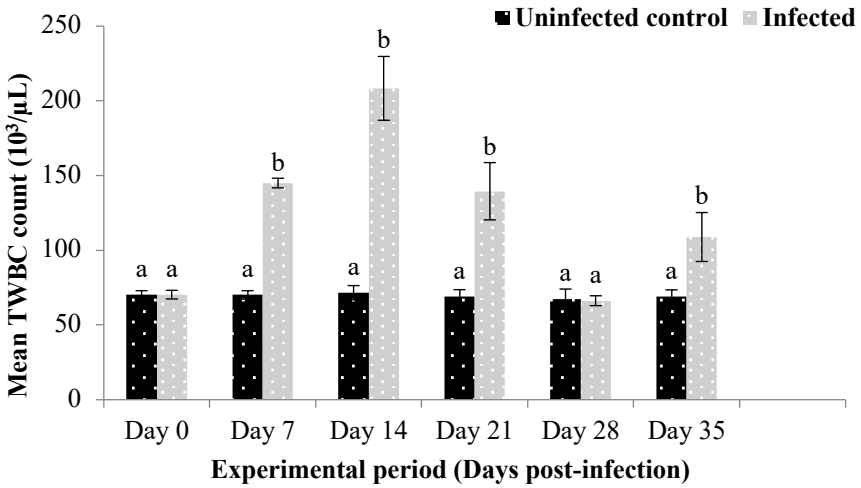



**Figure 3** The mean total white blood cell (TWBC) count ($10^3$/µL) of POL pullets infected with *S.* Gallinarum compared with the controls. [a, b] Different alphabets on the error bars indicate significant difference ($P < 0.05$) between the groups.

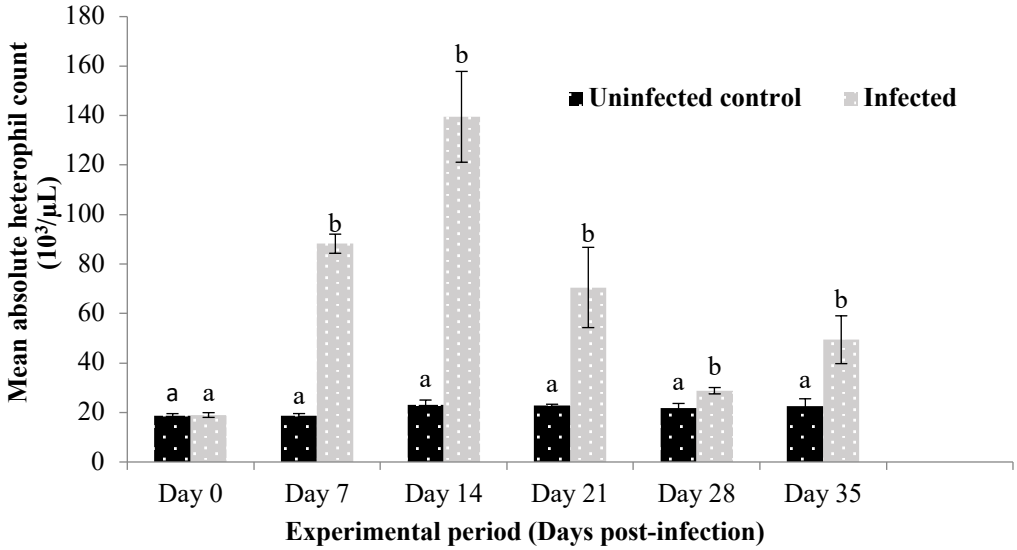



**Figure 4** The mean absolute heterophil count ($10^3$/µL) of POL pullets infected with *S.* Gallinarum compared with the controls. [a, b] Different alphabets on the error bars indicate significant difference ($P < 0.05$) between the groups.

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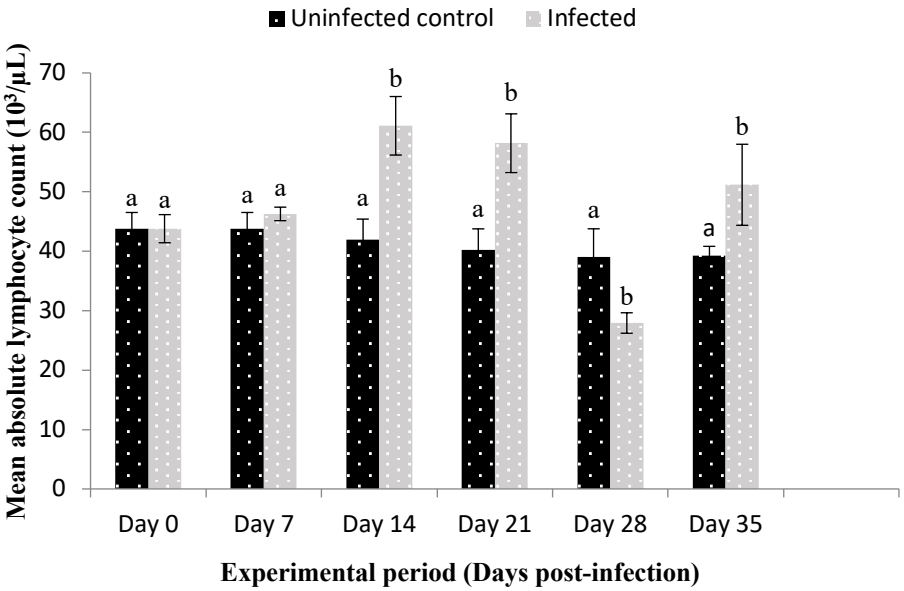



**Figure 5** The mean absolute lymphocyte count ($10^3$/µL) of POL pullets infected with *S.* Gallinarum compared with the controls. [a, b] Different alphabets on the error bars indicate significant difference ($P < 0.05$) between the groups.

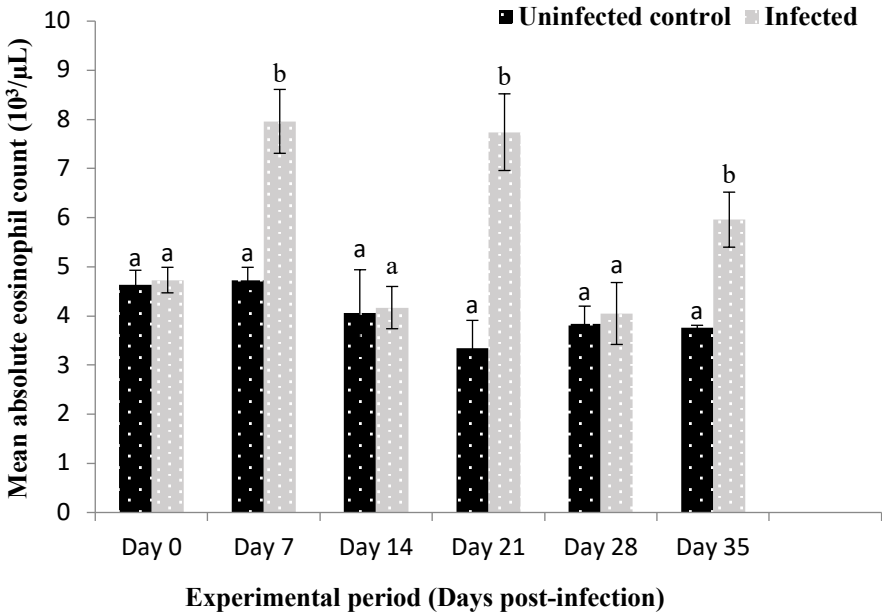



**Figure 6** The mean absolute eosinophil count ($10^3$/µL) of POL pullets infected with *S.* Gallinarum compared with the controls. [a, b] Different alphabets on the error bars indicate significant difference ($P < 0.05$) between the groups.

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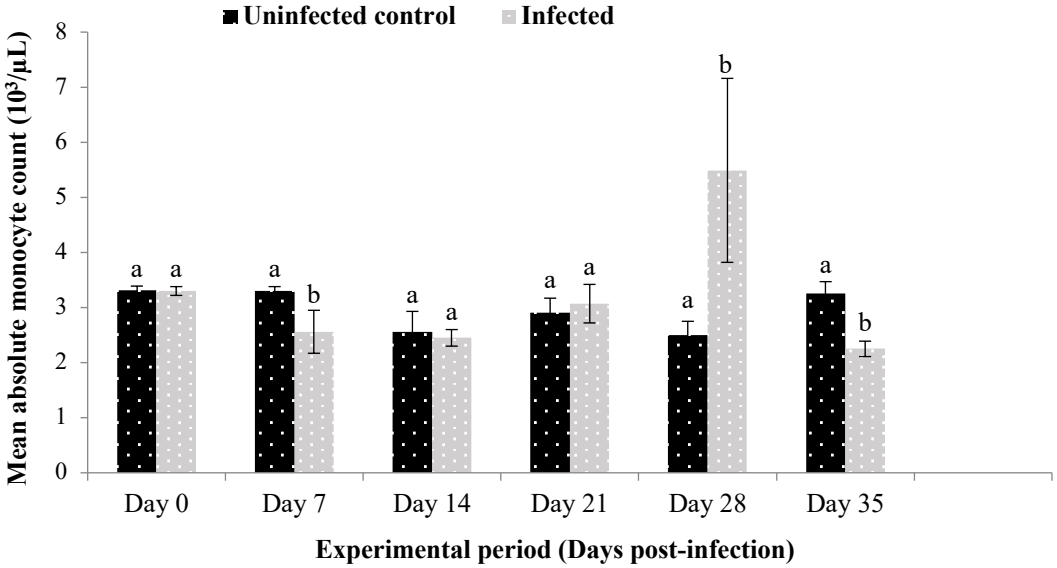



**Figure 7** The mean absolute monocyte count ($10^3$/µL) of POL pullets infected with *S.* Gallinarum compared with the controls. [(a, b)] Different alphabets on the error bars indicate significant difference (P < 0.05) between the groups.

## Alterations in the Oxidative Stress Markers

The mean serum MDA level was significantly higher in the infected POL pullets when compared to the uninfected controls on days 7 (P = 0.027), 14 (P = 0.007), 21 (P = 0.020), 28 (P = 0.049) and 35 (P = 0.018) PI (Table 6). The mean serum GPx activity was also significantly higher in the infected POL pullets when compared to the uninfected controls on days 7 (P < 0.001), 14 (P = 0.010), 21 (P = 0.047), 28 (P = 0.049) and 35 (P = 0.044) PI (Table 7). There was significant elevation in the mean serum SOD activity of the infected POL pullets when compared to uninfected controls on days 7 (P = 0.001), 14 (P = 0.020), 21 (P = 0.012), 28 (P = 0.036) and 35 (P = 0.002) PI (Table 8). In the same vein, the mean serum CAT activity was significantly higher in the infected POL pullets when compared to the uninfected controls on days 7 (P < 0.001), 14 (P = 0.047), 21 (P = 0.008), 28 (P < 0.001) and 35 (P = 0.003) PI (Table 9).

**Table 6** Mean serum MDA (nmol/mL) level of pullets infected with *S.* Gallinarum compared to the uninfected controls

| Days PI | Uninfected control (n=5) | Infected (n=5) | 95% Confidence Interval | | P-value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Day 0 | 8.37±1.19 | 9.37±1.02 | -4.83 | 2.83 | 0.546 |
| Day 7 | 8.36±1.19[a] | 12.74±0.93[b] | -8.05 | -0.68 | 0.027 |
| Day 14 | 8.31±0.16[a] | 15.44±1.78[b] | -11.50 | -2.75 | 0.007 |
| Day 21 | 8.21±0.41[a] | 12.12±1.17[b] | -6.95 | -0.88 | 0.020 |
| Day 28 | 8.57±0.27[a] | 9.35±0.18[b] | -1.57 | -0.01 | 0.049 |
| Day 35 | 8.65±0.22[a] | 9.91±0.32[b] | -2.21 | -0.31 | 0.018 |

All data are expressed as mean ± standard error mean (SEM) with 95% Confidence Interval.
[(a, b)] Different alphabetical superscripts on mean in a row indicate significant difference (P < 0.05) between the groups. n = number of pullets sampled. MDA = Malondialdehyde. PI = Post infection.

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**Table 7** Mean serum GPx (IU/L) activity of pullets infected with *S.* Gallinarum compared to the uninfected controls

| Days PI | Uninfected control (n=5) | Infected (n=5) | 95% Confidence Interval | | P-value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Day 0 | 181.06±8.62 | 189.70±9.96 | -40.85 | 23.61 | 0.537 |
| Day 7 | 181.07±8.62[a] | 422.49±16.51[b] | -287.00 | -195.84 | 0.000 |
| Day 14 | 202.63±8.26[a] | 362.14±42.24[b] | -264.83 | -54.20 | 0.010 |
| Day 21 | 181.07±8.62[a] | 258.67±29.87[b] | -153.66 | -1.53 | 0.047 |
| Day 28 | 189.70±9.96[a] | 224.18±9.95[b] | -68.93 | -0.04 | 0.049 |
| Day 35 | 198.31±8.62[a] | 232.80±16.51[b] | -80.07 | 11.09 | 0.044 |

All data are expressed as mean ± standard error mean (SEM) with 95% Confidence Interval.
[(a, b)] Different alphabetical superscripts on mean in a row indicate significant difference ($P < 0.05$) between the groups. n = number of pullets sampled. GPx = Glutathione peroxidase. PI = Post infection.

**Table 8** Mean serum SOD (IU/mL) activity of pullets infected with *S.* Gallinarum compared to the uninfected controls

| Days PI | Uninfected control (n=5) | Infected (n=5) | 95% Confidence Interval | | P-value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Day 0 | 579.17±9.28 | 586.16±7.82 | -36.67 | 22.72 | 0.586 |
| Day 7 | 579.18±9.28[a] | 632.07±1.81[b] | -76.03 | -29.74 | 0.001 |
| Day 14 | 577.87±6.98[a] | 602.93±3.93[b] | -44.65 | -5.47 | 0.020 |
| Day 21 | 579.78±3.81[a] | 599.44±4.06[b] | -33.29 | -6.04 | 0.012 |
| Day 28 | 584.40±3.64[a] | 593.26±3.64[b] | -21.45 | 3.73 | 0.036 |
| Day 35 | 584.85±1.27[a] | 599.44±2.62[b] | -21.70 | -7.47 | 0.002 |

All data are expressed as mean ± standard error mean (SEM) with 95% Confidence Interval.
[(a, b)] Different alphabetical superscripts on mean in a row indicate significant difference ($P < 0.05$) between the groups. n = number of pullets sampled. SOD = Superoxide dismutase. PI = Post infection.

**Table 9** Mean serum CAT (IU/mL) activity of pullets infected with *S.* Gallinarum compared to the uninfected controls

| Days PI | Uninfected control (n=5) | Infected (n=5) | 95% Confidence Interval | | P-value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Day 0 | 17.96±2.65 | 19.83±2.22 | -10.32 | 6.59 | 0.608 |
| Day 7 | 17.96±2.65[a] | 41.44±0.95[b] | -30.37 | -16.60 | 0.000 |
| Day 14 | 24.38±1.93[a] | 35.55±4.05[b] | -22.15 | -0.19 | 0.047 |
| Day 21 | 25.91±0.63[a] | 31.35±1.24[b] | -8.85 | -2.03 | 0.008 |
| Day 28 | 17.15±0.16[a] | 24.62±0.46[b] | -9.76 | -5.19 | 0.000 |
| Day 35 | 19.18±0.56[a] | 23.57±0.71[b] | -6.61 | -2.17 | 0.003 |

All data are expressed as mean ± standard error mean (SEM) with 95% Confidence Interval.
[(a, b)] Different alphabetical superscripts on mean in a row indicate significant difference ($P < 0.05$) between the groups. n = number of pullets sampled. CAT = Catalase. PI = Post infection.

## Correlation between Absolute Heterophil Count and Oxidative Stress Markers

In the infected POL pullets, the absolute heterophil count correlated very strongly, positively and significantly with MDA ($r = 0.893$; $P = 0.000$), GPx ($r = 0.815$; $P = 0.000$), SOD ($r = 0.626$; $P = 0.000$) and CAT ($r = 0.780$; $P = 0.000$) (Table 10).

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**Table 10** The correlation coefficient of oxidative stress markers POL pullets infected with *S.* Gallinarum and absolute heterophil count

| | MDA (nmol/mL) | GPx (IU/L) | SOD (IU/mL) | CAT (IU/mL) |
|---|---|---|---|---|
| Abs. heterophil count | r = 0.89 | r = 0.82 | r = 0.63 | r = 0.78 |
| Sig. (2-tailed) | P = 0.01 | P = 0.01 | P = 0.01 | P = 0.01 |

The absolute heterophil count correlated very strongly, positively and significantly with MDA, GPx, SOD and CAT
MDA = Malondialdehyde. GPx = Glutathione peroxidase. SOD = Superoxide dismutase. CAT = Catalase.

## Correlation of Erythrocytic Parameters and Egg Production

The correlation data of erythrocytic parameters of POL pullets infected with *S.* Gallinarum and egg production is presented in Table 11, and showed that PCV (r = 0.310; P = 0.051) and MCHC (r = 0.262; P = 0.103), related fairly and positively with egg production but the relationships were not statistically significant. Hemoglobin concentration correlated fairly, positively and significantly with egg production (r = 0.321; P = 0.044), while RBC count had a moderate, positive and significant relationship with egg production (r = 0.453; P = 0.003). The MCV correlated moderately, negatively and significantly (r = -0.474; P = 0.002) with egg production.

**Table 11** The correlation data of erythrocytic parameters of POL pullets infected with *S.* Gallinarum and egg production

| | PCV (%) | HbC (g/dL) | RBC ($10^6$/µL) | MCV (fL) | MCHC (g/dL) |
|---|---|---|---|---|---|
| Egg production | r = 0.31 | r = 0.32 | r = 0.45 | r = -0.47 | r = 0.26 |
| Sig. (2-tailed) | P = 0.05 | P = 0.04 | P = 0.01 | P = 0.01 | P = 0.10 |

The PCV related fairly and positively with egg production but the relationship was not statistically significant. Hemoglobin concentration correlated fairly, positively and significantly with egg production, while RBC count correlated moderately, positively and significantly with egg production. The MCV correlated moderately, negatively and significantly with egg production, while MCHC had fair and positive correlation with egg production. PCV = Packed cell volume. HbC =Hemoglobin concentration. RBC = Red blood cell. MCV = Mean corpuscular volume. MCHC = Mean corpuscular hemoglobin concentration.

## Correlation of Leucocytic Parameters and Egg Production

The correlation coefficient of leucocytic parameters of POL pullets infected with *S.* Gallinarum and egg production is presented in Table 12, and showed that TWBC (r = -0.536; P = 0.000), absolute heterophil (r = -0.529; P = 0.000) and absolute eosinophil counts (r = -0.537; P = 0.000) correlated moderately to strongly, negatively and significantly with egg production. The absolute lymphocyte count (r = -0.355; P = 0.025), correlated fairly, negatively and significantly with egg production, whereas, absolute monocyte count (r = -0.117; P = 0.474) had a poor and negative correlation with egg production.

**Table 12** The correlation coefficient of leucocytic parameters of POL pullets infected with *S.* Gallinarum and egg production

| | TWBC count ($10^3$/µL) | Abs. heterophil count ($10^3$/µL) | Abs. lymphocyte count ($10^3$/µL) | Abs. eosinophil count ($10^3$/µL) | Abs. monocyte count ($10^3$/µL) |
|---|---|---|---|---|---|
| Egg production | r = -0.54 | r = -0.53 | r = -0.36 | r = -0.54 | r = -0.12 |
| Sig. (2-tailed) | P = 0.01 | P = 0.01 | P = 0.03 | P = 0.01 | P = 0.47 |

The TWBC, absolute heterophil and absolute eosinophil counts correlated moderately to strongly, negatively and significantly with egg production. The absolute lymphocyte count correlated fairly, negatively and significantly with egg production, whereas, absolute monocyte count had a poor and negative correlation with egg production. TWBC = Total white blood cell.

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## Correlation of Oxidative Stress Markers and Egg Production

The correlation coefficient of oxidative stress markers of POL pullets infected with *S.* Gallinarum and egg production is presented in Table 13, and showed that serum MDA (r = -0.513; P = 0.001), GPx (r = -0.521; P = 0.001), SOD (r = -0.489; P = 0.001) and CAT (r = -0.540; P = 0.001), correlated moderately to strongly, negatively and significantly with egg production.

**Table 13** Correlation coefficient of oxidative stress markers of POL pullets infected with *S.* Gallinarum and egg production

| | MDA (nmol/mL) | GPx (IU/L) | SOD (IU/mL) | CAT (IU/mL) |
|---|---|---|---|---|
| Egg production | r = -0.51 | r = -0.52 | r = -0.49 | r = -0.54 |
| Sig. (2-tailed) | P = 0.01 | P = 0.01 | P = 0.01 | P = 0.01 |

MDA (r = -0.513; P = 0.001), GPx (r = -0.521; P = 0.001), SOD (r = -0.489; P = 0.001) and CAT (r = -0.540; P = 0.001), correlated moderately to strongly, negatively and significantly with egg production. MDA = Malondialdehyde. GPx = Glutathione peroxidase. SOD = Superoxide dismutase. CAT = Catalase.

# DISCUSSION

Fowl typhoid (FT) caused by *Salmonella* Gallinarum is taunted as one of the major causes of drop in egg production and by extension, an overall low productivity in the commercial poultry industry. The disease has been listed in the league of the notifiable diseases of the World Organization for Animal Health (OIE), taking into consideration its obvious economic importance (OIE, 2019). The results of this study revealed manifestation of clinical signs of fowl typhoid such as depression, weakness, loss of appetite, greenish-yellow diarrhea, dehydration, and loss in body weight with incubation period of 4 days in the infected POL pullets. These findings agree with those of earlier reports (Freitas Neto et al., 2007; Garcia et al., 2010; Chiroma et al., 2017). The delay in the commencement and progression of egg lay which culminated in decreased egg production, however, has not been reported as this appears to be the first study of *S*. Gallinarum infection in POL pullets. The evident loss in body weight in the infected chickens when compared to the uninfected controls could be attributed to inappetence, diarrhea, dehydration and emaciation observed in the infected chickens. Ezema et al. (2009) reported that loss of intestinal villi and villi atrophy which often results in malabsorption might have been responsible for the loss in body weight or emaciation observed in that outbreak of fowl typhoid in layers. Shah et al. (2013) reported a positive correlation between loss in body weight and severity of infection. The trend in the declined egg production in this study differed greatly from those of other reports on FT in layers (Chiroma et al., 2017; Okafor et al., 2023). There was a complete cessation of lay by the infected POL pullets which recorded an egg on day 0 PI, coupled with delay in the commencement and progression of egg lay. The infected POL pullets also maintained significantly lower egg production compared to the uninfected controls for the 10 weeks duration of egg production records in this study. This portends a great danger for the poultry farmer whose flock of potential layers are infected with *S*. Gallinarum at this critical age in the production cycle.

The low mean values of PCV, HbC and RBC count in the infected POL pullets when compared to the controls are indicative of anemia. This finding agrees with previous reports of anemia in acute fowl typhoid (Prasanna and Paliwal, 2002; Shah et al., 2013; Chiroma et al., 2017; Okafor et al., 2024). The anemia was reportedly attributed to heightened ability of reticulo-endothelial cells to engage in extravascular engulfment of red blood cells (Assoku and Penhale, 1978), cytopathic effect of outer membrane proteins of *S.* Gallinarum on red blood cells leading to their modification and subsequent destruction (Christensen et al., 1996), or vitamin

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$B_{12}$ deficiency due to its intestinal malabsorption, poor liver storage following liver dysfunction (Feldman et al., 2000) or excessive hemolysis (Okafor et al., 2024). The finding in the present study suggests a hemolytic anemia following destruction of RBCs; FT being a septicemic disease with chances of elaboration of substances which may have caused lysis of RBCs. In addition, the anemia may also have been induced by oxidative stress following elevated oxidative stress markers in this study. Gutteridge and Halliwell (2000) reported that products of lipid peroxidation in biological membranes indulge in degradation of polyunsaturated fatty acids, compromising the structural and functional integrity and resultant rupture of the membrane and release of cell contents. Taiwo et al. (2003) also reported that elevated polyunsaturated fatty acids, incessant exposure to increased oxygen level as well as presence of iron subject the red blood cells to increased fragility and vulnerability to lipid peroxidation induced lysis.

Morphologically, the class of anemia reported in the acute phase (particularly on day 7 PI) in this study was macrocytic hypochromic, depicted by significantly higher MCV and lower MCHC (though not statistically significant) values in the infected group compared to the uninfected controls. This also suggests a responsive type of anemia (English, 2008), with bone marrow response elicited by the significant decrease in the RBC counts and HbC, and subsequent release of immature and larger sized RBCs and the reticulocytes into blood circulation. This finding is consistent with the reports of Feldman et al. (2000), Chiroma et al. (2017) and Okafor et al. (2023) but contrasts the reported microcytic hypochromic anemia during acute fowl typhoid infection by Christensen et al. (1996) and Mdegela et al. (2002).

Leukocytosis characterized by heterophilia, lymphocytosis, eosinophilia and monocytosis was observed in the infected groups with heterophilia contributing more to the development of leukocytosis. Chitty (2018) reported that leukocytosis can occur following stress, infection, inflammation, tissue damage or neoplasia, however, differential WBC count is essential in distinguishing between these causes of leukocytosis (Baldrey, 2012). The leukocytosis in this study was mainly as a result of significant elevations in the mean heterophil and lymphocyte counts. These findings are consistent with the report of Shah et al. (2013) in experimental fowl typhoid infection in young broiler chickens, and that of Chiroma et al. (2017) and Okafor et al. (2023) in layers. Berchieri et al. (2000) attributed the leukocytosis to multiplication of *S.* Gallinarum inside the phagocytes, with resultant cell lysis and release of the organism into the extracellular compartment, evoking strong blood cellular response.

The significantly higher heterophil counts in the infected POL pullets compared to the controls in the present study reflects acute inflammatory response coupled with degenerative changes occasioned by the invasion of tissues of the target organs by *S.* Gallinarum. The heterophilia recorded in this study was massive, suggesting that the infection was acute and severe, resulting in massive tissue destruction and subsequent release of large numbers of heterophils, which are usually the first line of phagocytic defense against bacterial infection (Feldman et al., 2000; Thrall and Weiser, 2002). Campbell and Ellis (2007) reported that heterophilia with left shift can be seen in more severe inflammatory diseases such as bacterial sepsis.

The lymphocytosis observed in this study reflected host's immune response evoked by assaults of the invading organism on the target organs. Lymphopenia was, however, observed on day 28 PI in this study, which may be attributed to extreme stress occasioned by egg production at that stage of the infection. Jones (2015) reported that lymphopenia can be observed as part of stress response in the avian species.

The eosinophilia as well as monocytosis observed in the current study suggest a compensatory response to the corresponding heterophilia and lymphocytosis as all the cells originate from the same cell line or precursor in the

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bone marrow (pan-leukocytosis) (Thrall and Weiser, 2002). The significant decline in the mean monocyte count on day 28 PI, on the other hand, could be as a result of excess consumption/use up of monocytes following their migration into the tissues for phagocytosis (Thrall and Weiser, 2002).

The infection of POL pullets with *S.* Gallinarum in this study induced OS. The significant ($P < 0.05$) increase in the level of serum MDA in the infected POL pullets when compared to the uninfected controls in this study suggests excessive production of free oxygen radicals, the reactive oxygen species (ROS), which causes lipid peroxidation with the unstable lipid peroxides decomposing to form MDA whose measurement is widely used as a marker of OS (Patterson and Leacke, 1998; Simsek et al., 2006). The significantly ($P < 0.05$) higher serum GPx activity in the infected group suggests excessive production of hydrogen and organic peroxides (another ROSs) by this bacterial infection which in turn increased the production of the antioxidant, GPx. The antioxidant defense mechanism of the host elaborates GPx, which is an enzyme with essential antioxidant function that facilitated the destruction of hydrogen and organic peroxides (Kidd, 1997; Simsek et al., 2006). Similarly, the significant ($P < 0.05$) elevation in the activity of serum SOD in the infected group when compared to the controls could be attributed to excessive production of superoxide radicals as a result of the infection. SOD, another essential antioxidant and first line of defense against ROS, detoxifies superoxide radicals forming hydrogen and water (Gonzales et al., 1984; Ivanov et al., 2016). In addition, the significantly ($P < 0.05$) higher serum CAT activity in the infected POL pullets in the present study could be tied to excessive generation of another oxidant, hydrogen peroxides. Catalase (CAT), another enzyme with antioxidant function, is involved in the catalysis of hydrogen peroxide breakdown to oxygen and water (Gonzales et al., 1984; Ivanov et al., 2016).

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, SOD 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 defense mechanism of the host, hence, improving weight gain and survival rate even without medication in an acute disease like FT.

The fair to moderate and positive correlation between egg production and the values of the recorded erythrocytic parameters in this study suggests that anemia has direct relationship with the drop in egg production caused by FT, whereas, leukocytosis and OS markers which had moderate to very strong and negative correlation coefficient were inversely related to the drop in egg production which are in agreement with previous reports (Bizoń et al., 2023; Okafor et al., 2023).

# CONCLUSIONS

Infection of POL pullets with *S*. Gallinarum brought about significant perturbations in the PCV, HbC, RBC count, TWBC count, and absolute heterophil, lymphocyte, eosinophil and monocyte counts. The MCV, MDA level as well as serum activities of GPx, SOD and CAT also had significant alterations. The significant increase in the level of serum MDA suggests that *S.* Gallinarum infection of POL pullets induced OS. There was a strong association between significantly elevated GPx, SOD and CAT, which have antioxidant function, and improved weight gain, improvement in daily egg production and self-recovery. Hence, the inclusion of hematinic in the therapeutic regimen to facilitate erythropoiesis, as well as early administration of antioxidants could ameliorate the pathology and by

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extension, the mortality of FT-infected POL pullets, and is highly recommended. This gives room for an alternative mode of treatment of FT as incessant vaccine failures and antimicrobial resistance have constituted a major challenge in the poultry industry.

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## AUTHOR CONTRIBUTIONS

**Simeon Chibuko Okafor:** Conceptualization and design of the experiment, investigation, methodology, formal analysis, manuscript preparation, editing and finalization.
**John Ikechukwu Ihedioha, Wilfred Sunday Ezema:** Conceptualization and design of the experiment, investigation, formal analysis and supervision.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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