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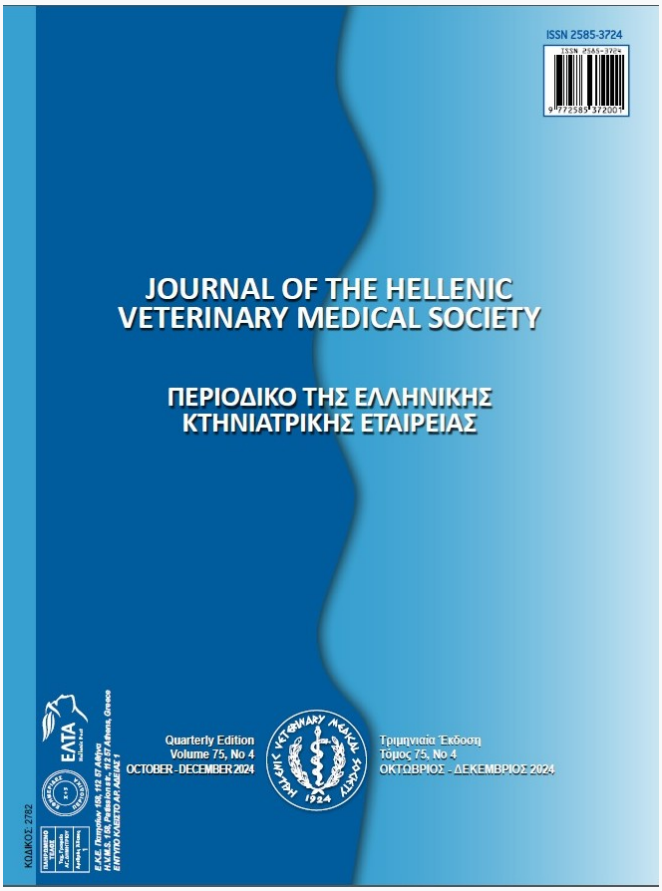

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Cardiac effusion and serum biochemical abnormalities of *Salmonella gallinarum* infection in point of lay pullets

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ABSTRACT: The aim of this study was to evaluate some lesions and biochemical abnormalities of fowl typhoid (FT) in point of lay (POL) pullets. Fifty POL pullets were randomly assigned to two groups of 25 infected orally with *S. gallinarum* (10^9 *S. gallinarum* colony forming units (CFUs)/mL), and 25 uninfected controls. Blood samples were collected from four randomly selected pullets in each group weekly for 35 days post infection (PI), and the harvested serum used for biochemical evaluations, following standard techniques. Relevant tissues were processed for histopathology. The data on biochemical parameters and correlation coefficient were analyzed using the Independent Sample t-test and Pearson's correlation statistic, respectively, on SPSS for Windows Version 23. There was a significant ($P < 0.05$) loss of body weight, 48% morbidity, 12% overall mortality, significant drop in egg production and a severe pericardial effusion in the infected pullets when compared to the uninfected controls. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), superoxide dismutase (SOD) and catalase (CAT) activities, malondialdehyde (MDA), serum total protein, globulin, total cholesterol, total bilirubin, uric acid, and creatinine levels were significantly ($P < 0.05$) higher in the infected pullets. Conversely, serum albumin level was significantly ($P < 0.05$) lower in the infected POL pullets when compared to the uninfected controls. The assayed biochemical parameters variably correlated with egg production, with MDA, SOD and CAT highly significant ($P < 0.01$). There were inflammatory, degenerative and necrotic changes in the affected body organs. An association was established between significantly elevated SOD and CAT, which have antioxidant properties, and clinical outcome, including survival/health improvement indices such as improved weight gain and egg production. It was concluded that *S. gallinarum* infection of POL pullets caused significant alterations in the biochemical indices, induced oxidative stress (OS), and stimulated the body's antioxidant defense mechanism to elaborate SOD and CAT. This may suggest the use of antioxidants in the treatment of fowl typhoid.

Keywords: Antioxidants; biochemical analysis; chickens; correlation coefficient; fowl typhoid; oxidative stress markers

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INTRODUCTION

Salmonella gallinarum is a Gram-negative bacterium of the *Enterobacteriaceae* family, and causes a severe systemic and septicemic disease known as fowl typhoid (FT) in commercially raised poultry and other galliform species, with a high global prevalence (Shivaprasad and Barrow, 2008; Yasmin et al., 2019; Zhou et al., 2022). The disease has worldwide distribution especially in developing nations, where antimicrobial resistance in *S. gallinarum* strain has increased tremendously, and poses huge socioeconomic challenge to the poultry industry, in form of high mortality and drop in egg production (Barbour et al., 2015; Haque et al., 2021). It 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 enormous challenges posed by FT, it has been either scantly reported or poorly studied especially in developing countries including Nigeria where only few cases of outbreaks have been documented (Parvej et al., 2016; Okafor et al., 2023). This study gap may be due to wrong diagnosis as most clinical manifestations of FT resemble those of Newcastle disease (NCD) and other enteric diseases of chickens. Moreover, the few reported cases 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 *S. gallinarum* infection of chickens and turkeys, the heart is one of the major organs believed to be the focal point of infection, as heart blood is one of the recommended samples for isolation of the organism following infection. Surprisingly, cardiac lesions of FT have been poorly documented. Paul et al. (2015) in their investigation on *S. gallinarum* infected layers observed only pericardial adhesions grossly, with mild pericarditis and myocarditis histologically. Moreover, most studies on *S. gallinarum* infection of chickens, did not consider the blood biochemical evaluation, especially oxidative stress (OS) even as FT is a septicemic disease. Elevations in serum enzymes were reported in young pullets, and laying hens (Shah et al., 2013; Chiroma et al., 2017b). However, these elevations were attributed to liver damage, which may not be exhaustive. Furthermore, the few reports on the biochemical abnormalities neither factored in relationship with organ pathology nor correlation with egg production. The importance of poultry production cannot be overemphasized, and any study or efforts made towards alleviating any constraint militating against optimum productivity should be encouraged (Galovicova et al., 2022).

This study, therefore, evaluated the effects of S. gallinarum infection on some selected body organs as well as serum biochemical indices, and correlated these biochemical parameters to egg production in POL pullets.

MATERIALS AND METHODS

Animals and Bacteria

Fifty ISA® brown POL pullets aged 18 weeks 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 done 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 pox (FP 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.

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 POL pullets were screened for *Salmonella* by culturing the cloacal swabs of randomly selected POL pullets in nutrient broth, incubating at 37^0C for 24h and further plating on MacConkey agar (MCA) before the experimental infection. This study 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).

Study Design and Sampling

This study was designed in November 2021, but the actual experimental study commenced in April 2022 with the procurement of day-old-chicks and was terminated in December 2022. The POL pullets used for the study were randomly assigned to two groups of 25 pullets each. Baseline values of average body weight, basic serum biochemistry and oxidative stress markers were determined prior to infection and were considered day 0 values. Each pullet in the infected group received 1 mL of the inoculum containing 1 x 10^9 *S. gallinarum* colony forming units (CFU)/mL, once, into the crop using oral gavage needles, while each pullet in the control group had 1 mL of buffered formal saline (BFS) administered as placebo (Lopes et al., 2016). The two experimental groups were given feed and water throughout the experimental period.

About 2 mL of blood was collected from the jugular vein of each of the four POL pullets randomly selected per group, every 7 days for 5 weeks. The blood samples were dispensed into clean glass test tubes with no anticoagulant and allowed for 30 minutes at room temperature to clot. The samples were centrifuged afterwards for 10 minutes at 3000 revolutions/minute using a table centrifuge, and serum from the centrifuged blood samples were harvested and used for biochemical analyses. Sample collection was consistently done between 9.00 and 11.00 am each day and analyses completed within 48 hours of sample collection.

Clinical Signs and Gross Pathology

The POL pullets were observed for clinical signs of FT, and egg production was recorded for 70 days post infection (PI). Five (5) randomly selected pullets from each group were weighed on days 4, 7, 14, 21, 28 and 35 PI. On day 4, 7 and every 7 days for 35 days, three (3) dead or randomly selected euthanized POL pullets from each group were necropsied, gross lesions observed, and pericardial fluid aspirated with 5 mL syringe for fluid biochemical analysis.

Determination of the Basic Serum and Pericardial Fluid Biochemical Parameters.

The earlier harvested serum and the pericardial fluid were used for the biochemical analysis. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed following Reitman-Frankel Colorimetric method (Colville, 2002), using commercially available Quimica Clinica Aplicada ALT/AST test kits (QCA, Spain). The activity of serum alkaline phosphatase (ALP) was determined using TECO ALP test kit (TECO Diagnostics, Anaheim, California, USA) by Thymolphthalein monophosphate method (Colville, 2002). The serum total protein (TP) assay was done following direct Biuret method using Randox total protein test kit (Randox Laboratories Ltd., County Antrim, UK), serum albumin assay was performed using Randox albumin test kit (Randox Laboratories Ltd., County Antrim, UK), by the bromocresol green method, while serum globulin was gotten by calculation (serum total protein-serum albumin) (Doumas and Peters, 1997; Johnson, 2008). The serum total bilirubin (TB) determination was carried out using Randox bilirubin test kit (Randox Laboratories Ltd., County Antrim, UK) according to modified Jendrassik-Grof method (Higgins et al., 2008b). The assays of serum total cholesterol (TC) and uric acid (UA) were performed using Quimica Clinica Aplicada cholesterol test kit (QCA, Spain) and Randox uric acid test kit (Randox Laboratories Ltd., County Antrim, UK) respectively, following enzymatic colorimetric method (Lamb and Price, 2008; Rifai et al., 2008). Serum creatinine was determined using Quimica Clinica Aplicada creatinine test kit (QCA, Spain), following the modified Jaffe method (Lamb and Price, 2008). Similar methods were employed in the pericardial fluid biochemical analysis. The reading of all the assays was done with Diatek® blood biochemistry analyzer (Wuxi Hiwell Diatek Instrument co. Ltd., China).

Oxidative Stress Markers Assay

The modified thiobarbituric acid, hydroxylamine and visible light methods were employed in the determination of the serum malondialdehyde (MDA) level, superoxide dismutase (SOD) and catalase (CAT) activities, respectively (Draper and Hadley, 1990; Weydert and Cullen, 2010). ElabScience MDA, SOD and CAT assay kits sourced from ElabScience Biotech Co. Ltd., South Africa, were used according to the manufacturer's instructions. The reading of MDA, SOD and CAT was done using Diatek® blood biochemistry analyzer (Wuxi Hiwell Diatek Instrument co. Ltd., China) set at the MDA-ELS, SOD-ELS and CAT-ELS assay program mode, respectively.

Histopathological Study

The heart, liver, spleen, kidney, lung, intestine (duodenum and ileum) and ovary were fixed in 10% neutral buffered formalin. The fixed organs were processed routinely and sectioned at 5µm thickness after fixation, and stained with hematoxylin and eosin (OIE, 2012; Sikandar et al., 2017). The slides were viewed under a light microscope and then photographed with Axiocam® digital camera.

Data Analysis

Data from the biochemical assays were subjected to the Independent Sample t-test for equality of means on IBM Statistical Package and Service Solution (SPSS) for Windows version 23. Correlation between the biochemical data and egg production was performed using Pearson's correlation statistic. The level of significance was accepted at $P < 0.05$.

RESULTS

Clinical and Postmortem findings

Depression, low feed and water intake, and greenish-yellow diarrhea were first observed on day 4 PI in the infected POL pullets and persisted to day 14 PI. There was a significant ($P < 0.05$) loss of body weight (Figure 1a), and delay in commencement of egg lay which culminated in significantly ($P < 0.05$) lower egg production (Figure 1b). Morbidity was 48% (12/25) on day 4 post-infection (PI) with an overall mortality of 12% (3/25), whereas, the control group remained healthy for the duration of the experiment.

In the infected POL pullets, 3 out of 25 died on days 6, 7 and 9 PI. No mortalities were recorded in the

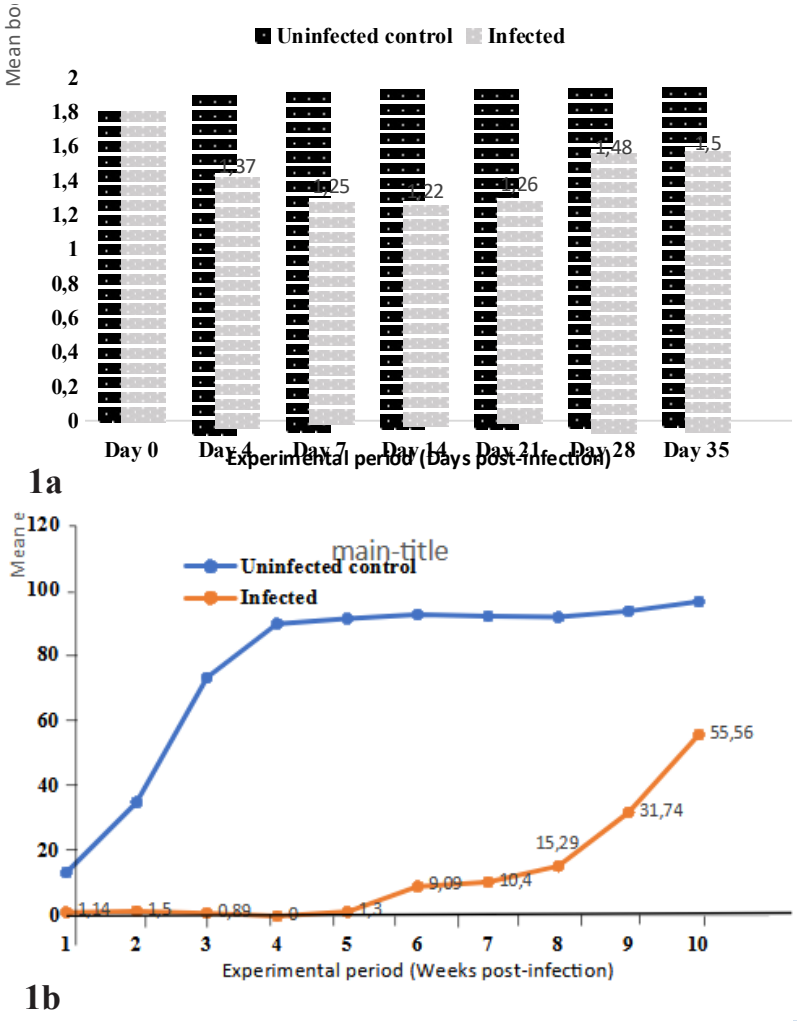

Figure 1. Mean body weight and percentage egg production of POL pullets infected with *S. gallinarum* compared with uninfected controls. (1a) Mean body weight (Kg). Infected POL pullets had significantly lower body weight compared to the uninfected controls. (1b) Percentage egg production. The infected pullets maintained significantly lower egg production compared to the uninfected controls for the duration of the study. * indicates significant difference ($P < 0.05$) between the groups.

uninfected controls. The carcasses of the dead infected pullets were dehydrated and pale. The most prominent postmortem findings were white nodular lesions on the epicardium with pronounced pericardial effusion (Figure 2). The liver of the infected pullets was slightly enlarged and hyperemic and changed to slight bronze color on exposure to air following necropsy. Similarly, the spleen was also slightly enlarged, with discrete white necrotic spots on the surface, compared to that of the uninfected controls. The lungs of the infected pullets had moderate emphysema and were congested, while the kidneys were markedly enlarged. The oviducts were inflamed and contained caseous exudate, and ovaries were small and nodular with regressing follicles, cystic ovarian follicles, as well as multiple misshapen (deformed) grey follicles.

Basic Biochemical Changes

The mean serum ALT activity was significantly higher in the infected POL pullets than uninfected controls on days 7 ($P = 0.030$), 14 ($P = 0.010$), 21 ($P = 0.005$), 28 ($P = 0.014$) and 35 ($P = 0.001$) PI (Table 1). Similarly, the mean serum AST activity was significantly higher in the infected POL pullets than in the uninfected controls on days 7 ($P = 0.008$), 14 ($P < 0.001$), 21 ($P = 0.018$), 28 ($P = 0.003$) and 35 ($P = 0.049$) PI (Table 1). In the same vein, the mean serum ALP activity was significantly higher in the infected POL pullets than uninfected controls on days 7 ($P = 0.007$), 14 ($P = 0.002$), 21 ($P = 0.039$), 28 ($P = 0.006$) and 35 ($P = 0.002$) PI (Table 1)..

The mean serum TP level of the infected POL pullets was significantly higher than that of the uninfected controls on days 7 ($P = 0.040$), 14 ($P = 0.010$), 21 ($P = 0.011$), 28 ($P < 0.001$) and 35 ($P = 0.014$) PI (Table 2). Conversely, the mean serum albumin level of the POL pullets was significantly lower in infected POL pullets than that of the uninfected POL pullets on days 7 ($P = 0.017$), 14 ($P = 0.015$), 21 ($P = 0.002$), 28 ($P = 0.044$) and 35 ($P = 0.041$) PI (Table 2). The mean serum globulin level was also significantly higher in the infected POL pullets when compared to the uninfected controls on days 7 ($P = 0.006$), 14 ($P = 0.009$), 21 ($P = 0.007$), 28 ($P = 0.001$) and 35 ($P = 0.008$) PI (Table 2).

Table 1. Mean serum ALT (IU/L), AST (IU/L) and ALP (IU/L) activities of POL pullets infected with *S. gallinarum* compared to the uninfected control

Days PI	ALT		AST		ALP	
	Uninfected control (n=4)	Infected (n=4)	Uninfected control (n=4)	Infected (n=4)	Uninfected control (n=4)	Infected (n=4)
0	7.21±0.68	7.39±0.69	118.99±2.91	121.33±2.47	21.33±0.52	21.53±0.64
7	7.31±0.68^{a}	12.33±1.68^{b}	118.99±2.91^{a}	132.90±2.08^{b}	21.53±0.64^{a}	25.57±0.77^{b}
14	11.22±1.29^{a}	17.27±0.99^{b}	117.45±2.46^{a}	147.98±1.15^{b}	19.53±0.11^{a}	21.02±0.25^{b}
21	11.40±0.26^{a}	14.92±0.79^{b}	98.58±3.34^{a}	110.54±1.63^{b}	17.45±0.03^{a}	20.85±1.30^{b}
28	18.32±0.58^{a}	23.13±1.28^{b}	93.36±1.28^{a}	102.67±1.36^{b}	27.72±0.15^{a}	29.03±0.28^{b}
35	18.54±0.94^{a}	24.02±0.16^{b}	109.93±5.96^{a}	126.70±3.28^{b}	25.85±0.19^{a}	26.90±0.09^{b}

All data are expressed as mean ± standard error mean (SEM). $^{(a, b)}$ Different alphabetical superscripts on mean in a row indicate significant difference ($P < 0.05$) between the groups. n = number of pullets sampled. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. ALP: Alkaline phosphatase

Table 2. Mean serum TP(g/L), Albumin (g/L) and Globulin (g/L) levels of POL pullets infected with *S. gallinarum* compared to the uninfected control

Days PI	TP		ALB		GBN	
	Uninfected control (n=4)	Infected (n=4)	Uninfected control (n=4)	Infected (n=4)	Uninfected control (n=4)	Infected (n=4)
0	45.87±1.15	45.90±1.12	24.58±1.31	24.20±1.43	21.29±1.27	21.71±1.21
7	46.66±1.82^{a}	52.65±1.39^{b}	24.20±1.43^{a}	20.12±0.40^{b}	22.08±2.00^{a}	32.53±1.55^{b}
14	47.01±3.37^{a}	61.77±2.14^{b}	21.59±0.31^{a}	20.27±0.24^{b}	25.43±3.47^{a}	41.50±2.36^{b}
21	47.07±1.28^{a}	62.67±4.13^{b}	23.66±0.32^{a}	20.71±0.44^{b}	23.41±1.03^{a}	41.96±4.56^{b}
28	56.58±0.25^{a}	64.21±0.59^{b}	22.21±0.09^{a}	20.03±0.85^{b}	34.38±0.22^{a}	44.18±1.43^{b}
35	43.23±0.25^{a}	51.67±2.46^{b}	22.03±0.59^{a}	20.50±0.04^{b}	21.20±0.72^{a}	31.17±2.44^{b}

All data are expressed as mean ± standard error mean (SEM). $^{(a, b)}$ Different alphabetical superscripts on mean in a row indicate significant difference ($P < 0.05$) between the groups. n = number of pullets sampled. TP: Total protein. ALB: Albumin. GBN: Globulin

Table 3. Mean serum TB (mg/dL), TC (mg/dL) and UA (µmol/L) levels of POL pullets infected with *S. gallinarum* compared to the uninfected control

	TB		TC		UA	
Days PI	Uninfected control (n=4)	Infected (n=4)	Uninfected control (n=4)	Infected (n=4)	Uninfected control (n=4)	Infected (n=4)
0	0.22±0.03	0.22±0.03	94.08±2.73	96.26±2.32	384.61±67.50	411.67±59.24
7	0.19±0.01^{a}	0.33±0.02^{b}	94.09±2.73^{a}	151.22±13.48^{b}	249.67±14.99^{a}	456.11±70.50^{b}
14	0.13±0.00^{a}	0.22±0.01^{b}	76.58±2.79 ^{a}	142.70±7.37 ^{b}	397.56±35.00^{a}	513.71±19.99^{b}
21	0.17±0.01^{a}	0.22±0.01^{b}	100.55±2.30 ^{a}	173.63±10.24^{b}	198.57±25.66^{a}	313.13±24.01^{b}
28	0.22±0.01^{a}	0.25±0.01^{b}	153.34±0.23 ^{a}	190.89±1.91^{b}	484.96±28.02^{a}	725.04±25.53^{b}
35	0.28±0.03^{a}	0.50±0.02^{b}	95.50±1.64 ^{a}	107.26±2.20 ^{b}	361.25±9.87 ^{a}	618.41±35.82^{b}

All data are expressed as mean ± standard error mean (SEM). $^{(a, b)}$ Different alphabetical superscripts on mean in a row indicate significant difference ($P < 0.05$) between the groups. n = number of pullets sampled. TB: Total bilirubin. TC: Total cholesterol. UA: Uric acid

The mean serum TB level was significantly higher in the infected POL pullets when compared to the uninfected POL pullets on days 7 ($P = 0.001$), 14 ($P < 0.001$), 21 ($P = 0.004$), 28 ($P = 0.017$) and 35 ($P = 0.001$) PI (Table 3). Similarly, the mean serum TC level was significantly higher in the infected POL pullets when compared to the uninfected controls on days 7 ($P = 0.006$), 14 ($P < 0.001$), 21 ($P < 0.001$), 28 ($P = 0.048$) and 35 ($P = 0.005$) PI (Table 3). In the same vein, the mean serum UA level of the infected POL pullets was significantly higher than that of the uninfected ones on days 7 ($P = 0.029$), 14 ($P = 0.028$), 21 ($P = 0.017$), 28 ($P = 0.001$) and 35 ($P = 0.018$) PI (Table 3).

The mean serum creatinine level in the infected POL pullets was significantly higher than that of uninfected controls on days 7 ($P = 0.017$), 14 ($P = 0.038$), 21 ($P = 0.002$), 28 ($P = 0.002$) and 35 ($P = 0.035$) PI (Figure 3).

The Pericardial Fluid

The total protein level of the pericardial fluid was 51.91g/L and that of serum was 56.10g/L giving a fluid/serum ratio of 0.93. Similarly, albumin and total cholesterol levels of the pericardial fluid were 18.93g/L and 78.15mg/dl, giving a serum: fluid albumin gradient of 1.16g/L and fluid/serum cholesterol ratio of 0.56 (Table 4).

Serum MDA Level, SOD and CAT Activities

Serum MDA level was significantly higher in the infected POL pullets than 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 (Figure 4a). The mean serum SOD activity was significantly higher in the infected pullets than 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 (Figure 4b). Similarly, mean serum CAT activity was significantly higher in the infected pullets than 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 (Figure 4c).

Correlation of Biochemical Parameters and Egg Production

The correlation data of ALT, AST, ALP, TP, ALB and GBN of POL pullets infected with *S. gallinarum* and egg production were presented in Table 5 and indicated that serum AST (r = -0.608; $P = 0.000$), TP (r = -0.481; $P = 0.002$) and GB (r = -0.477; $P = 0.002$) had moderate to strong, negative and highly significant relationships with egg production. The relationship between serum ALT (r = -0.100; $P = 0.537$) and egg production was poor and negative and the relationship was not significant, while the relationship between egg production and serum ALP (r = -0.254; $P = 0.114$) was fair, negative and not significant. The relationship between serum ALB (r = 0.470; $P = 0.000$) and egg production was moderate, positive and highly significant.

The correlation coefficient of TB, TC, UA, CTN, MDA, SOD and CAT of POL pullets infected with *S. gallinarum* and egg production was presented in Table 6 indicating that the relationship with serum TB (r = -0.339; $P = 0.032$), TC (r = -0.313; $P = 0.049$) and UA (r = -0.350; $P = 0.027$) was fair, negative and significant ($P < 0.05$). Serum CTN (r = -0.640; $P = 0.000$) related strongly and negatively with egg production, and the relationship was highly significant ($P < 0.01$). Serum MDA (r = -0.513; $P = 0.001$), SOD (r

Table 4. Evaluation of pericardial fluid accumulated in the pericardial sac

Parameter	Pericardial fluid	Serum	Fluid/Serum ratio	Laboratory criteria for exudates
Total protein (g/L)	51.91	56.10	0.93	Fluid/serum ratio > 0.5 Fluid protein > 30g/L
Albumin (g/L)	18.93	20.08	Serum: fluid albumin grad =1.16g/L	Serum: fluid albumin gradient =1.2g/L
T. cholesterol (mg/dl)	78.15	139.50	0.56	Fluid/Serum cholesterol ratio > 0.3 Fluid cholesterol > 60 mg

The total protein level of the pericardial fluid was 51.91g/L and that of serum was 56.10g/L giving a fluid/serum ratio of 0.93. Similarly, albumin and total cholesterol levels of the pericardial fluid were 18.93g/L and 78.15mg/dl, giving a serum: fluid albumin gradient of 1.16g/L and fluid/serum cholesterol ratio of 0.56.

Table 5. The correlation data of ALT, AST, ALP, TP, ALB and GBN of POL pullets infected with *S. gallinarum* and egg production.

	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/L)	ALB (g/L)	GBN (g/L)
Egg production	r = -0.100	r = -0.608	r = -0.254	r = -0.481	r = 0.470	r = -0.477
Sig. (2-tailed)	*P* = 0.537	*P* = 0.000	*P* = 0.114	*P* = 0.002	*P* = 0.002	P = 0.002

Serum AST, TP and GB, had moderate to strong, negative and highly significant ($P < 0.05$) relationships with egg production. The relationship between serum ALT and egg production was poor and negative and the relationship was not significant ($P > 0.05$), while the relationship between egg production and serum ALP was fair, negative and not significant. The relationship between serum ALB and egg production was moderate to strong, positive and highly significant ($P < 0.05$). ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. ALP: Alkaline phosphatase. TP: Total protein. ALB: Albumin. GBN: Globulin

Table 6. The correlation data of TB, TC, UA, CTN, MDA, SOD and CAT of POL pullets infected with *S. gallinarum* and egg production

	TB (mg/dL)	TC (mg/dL)	UA (µmol/L)	CTN (mg/dL)	MDA (nmol/mL)	SOD (IU/mL)	CAT (IU/mL)
Egg production	r = -0.339	r = -0.313	r = -0.350	r = -0.640	r = -0.513	r = -0.489	r = -0.540
Sig. (2-tailed)	*P* = 0.032	*P* = 0.049	*P* = 0.027	*P* = 0.000	*P* = 0.001	*P* = 0.001	*P* = 0.001

The relationship between egg production and serum TB, TC as well as UA was fair, negative and significant ($P < 0.05$). Serum CTN related strongly and negatively with egg production, and the relationship was highly significant ($P < 0.01$). Serum MDA, SOD and CAT had moderate to strong, negative and highly significant relationships with egg production. TB: Total bilirubin. TC: Total cholesterol. UA: Uric acid. CTN: Creatinine. MDA: Malondialdehyde. SOD: Superoxide dismutase. CAT: Catalase.

= -0.489; $P = 0.001$) and CAT (r = -0.540; $P = 0.001$) had moderate to strong, negative and highly significant ($P < 0.01$) relationships with egg production.

Histopathology

Histologically, the heart of the infected POL pullets had massive infiltration of inflammatory cells in the myocardium (myocarditis) with marked degeneration of the myocardial fiber. These lesions were not found in the uninfected controls (Figures 5 and 6). There was massive infiltration of inflammatory cells around the major hepatic vessels (perivascular hepatitis) as well as hepatocellular necrosis in the liver of infected pullets but were absent in the uninfected controls (Figures 7 and 8). Lesions in the kidneys of infected pullets included infiltration of inflammatory cells into the renal interstitial spaces (interstitial nephritis), and in the glomerular tufts and associated tissues (glomerulonephritis), as well as degeneration of renal tubules and glomerular tufts. The kidneys of uninfected pullets were devoid of these lesions (Figures 9 and 10). In the lungs of infected pullets, there was thickening of the interstitial tissue of the lung and bronchiolar interstitial tissues by infiltration of inflammatory cells (interstitial pneumonia), as well as pulmonary edema or emphysema, congestion and exudation of plasma protein into the alveolar space with hyaline membrane formation typical of acute interstitial pneumonia, and these were not found in the lungs of uninfected pullets (Figures 11 and 12). The intestine of the infected POL pullets was characterized by thickening of the intima and infiltration of inflammatory cells (enteritis), as well as sloughing and loss of the intestinal villi (ulceration). The intestine of uninfected pullets had no such lesions (Figures 13 and 14).

DISCUSSION

Cardiac pathology which culminated in marked pericardial effusion appeared to be the most prominent finding in the current study. The cardiac lesions in addition to the disturbances in other organs of the body especially the ovary, may have contributed immensely to the biochemical perturbations as well as delay in commencement of egg lay with resultant low egg production which has almost become the hallmark of FT in laying chickens. Previous report showed no specific gross cardiac lesions with only pericardial adhesions, mild pericarditis and myocarditis observed histologically in an investigation on *S. gallinarum* infection in layers (Paul et al., 2015). The impact of *S. gallinarum* infection on the heart of POL pullets in this study manifested grossly as pericardial fat, whitish nodular epicardial lesions which mimicked tumors of Marek's disease, and marked pericardial effusion, and histologically in form of inflammatory, degenerative and necrotic changes. The total protein level of the pericardial fluid (51.91g/L) and that of serum (56.10g/L) gave a fluid/serum ratio of 0.93; the pericardial fluid albumin (18.93g/L) and total cholesterol (78.15mg/dl) levels gave a serum: fluid albumin gradient of 1.16g/L and fluid/serum cholesterol ratio of 0.56 respectively, all of which met the laboratory criteria for exudates, thereby confirming the pericardial fluid as an exudate (Gregory and Carl, 2001; Anchinmane and Puranik, 2011).

Serum ALT, AST and ALP activities had significant elevations in the infected POL pullets compared to the uninfected controls in the current study, which were opined to arise from liver damage that accompanies *S. gallinarum* infection in chickens (Shah et al., 2013; Chiroma et al., 2017b). However, the findings of this study are suggestive of involvement of organs other than the liver, viz: the lung, kidney, cardiac and skeletal muscles where these enzymes are found in varying degrees in the avian species, according to Harr (2006) and Forbes (2008). The inflammatory changes observed in these tissues may have altered the permeability of hepatocellular and other cellular membranes and caused cytoplasmic ALT, AST and ALP to leak via the lymphatics into the blood stream.

Varied reports on the effects of *S. gallinarum* infection on serum total protein, albumin and globulin levels of infected chickens have been documented. Decreased serum total protein in *S. gallinarum* infected layers and broiler chickens respectively have been reported, which was attributed to catalase produced by *S. gallinarum* that induces proteolysis, liver dysfunction and resultant impaired synthesis of albumin as well as loss of protein following enteritis and renal dysfunction (Kokosharov, 2006; Shah et al., 2013). Initial increase in serum total protein in *S. gallinarum* infected layers, attributed to dehydration has also been reported (Chiroma et al., 2017b). In this study, elevated serum total protein, decreased serum albumin and elevated serum globulin were observed in the infected POL pullets. The inflammatory response that accompanied this disease may have evidently led to the hyperproteinemia which may have occurred following elevation of alpha, beta or gamma globulins or combination of two or the three globulin fractions which usually occur during acute or chronic inflammation as well as infectious disease process (Samour et al., 2015). In addition, positive acute-phase proteins such as alpha 2-macroglobulins are preferentially produced in the liver in response to inflammatory cytokines (Kaneko, 1997). The hypoalbuminemia that occurred in this study may be due to loss as a result of enteritis or intestinal ulcerations, inhibited production of albumin, a negative acute-phase protein, following sepsis, or kidney dysfunction. This agrees with Harr (2006) who reported that decreased albumin level occurs due to reduced production as a result of liver disease, increased loss through kidney dysfunction or gastrointestinal tract disorder, and increased use in chronic inflammation. The resultant effect is a decreased Albumin: Globulin (A: G) ratio.

Hyperbilirubinemia was observed in the infected POL pullets when compared to the uninfected controls in the present study. It was posited that serum total bilirubin level could increase in conditions of cholestasis, hepatocellular diseases and hemolysis (Braun et al., 1995; Davoudi et al., 2013). It was also reported that biliverdin, the major bile pigment in birds, is not metabolized to bilirubin and as such, bilirubin has little value in liver disease diagnosis in birds, unlike in mammals (Samour et al., 2015). The elevated serum bilirubin in the current study may be attributed to intense hemolysis that occurred coupled with liver dysfunction and resultant inability to conjugate excess bilirubin in circulation.

Significantly higher serum total cholesterol level was observed in the infected POL pullets compared to the uninfected controls. Hypercholesterolemia can occur as a result of hepatic lipidosis and other forms of liver disease, biliary obstruction, starvation, atherosclerosis, high fat diets, reproductive and endo-

crine diseases (Harr, 2006; Samour et al., 2015). It was also posited that dyslipidemia, an abnormal concentration of lipids in circulation that mainly manifests by increasing cholesterol level, is often associated with renal and hepatic diseases (Hegele, 2013). The hypercholesterolemia observed in this study may be ascribed to the inflammatory, necrotic and degenerative changes that occurred in the liver, kidney and reproductive system, as well as starvation following anorexia.

Serum uric acid level was significantly elevated in the infected POL pullets compared to the uninfected controls. This finding agrees with the previous reports that hyperuricemia is a usual occurrence in renal disease of birds, as the major product of nitrogen catabolism in birds is uric acid, which is also produced in the liver and pancreas and eliminated by tubular secretion independent of glomerular filtration, water resorption and urine flow rate (Harr, 2006; Baldrey, 2012). The elevation of uric acid level in this study may be due to renal dysfunction following glomerulonephritis and renal tubular degeneration, hence failure of the kidney to excrete excess uric acid.

It was opined that creatinine is of questionable value in evaluating renal function in the avian species due to reported excretion of creatine before its conversion to creatinine, nevertheless, elevations in the level of serum creatinine have been associated with dehydration and renal trauma (Samour et al., 2015), which may be the case in this study.

The infection of POL pullets with *S. gallinarum* in this study induced OS shown by significantly elevated MDA level, compared to the uninfected controls. Excessive production of reactive oxygen species (ROS), free oxygen radicals, in this disease may have caused lipid peroxidation with the unstable lipid peroxides decomposing to form MDA whose measurement is widely used as an indicator of oxidative stress (Patterson and Leacke, 1998; Simsek et al., 2006; Ayala et al., 2014). The SOD, an essential antioxidant and first line of defense against ROS, is involved in the catalysis of superoxide radical detoxification forming hydrogen and water (Gonzales et al., 1984; Ivanov et al., 2016). The elevation in the activity of SOD in this study is in contrast with a previous report in experimental Newcastle disease in broiler chickens where SOD activity was decreased following antioxidant activity and subsequent consumption of SOD, which created redox imbalance (Okoroafor et al., 2021). The significant elevation in SOD activity in the present study suggests that the body's antioxidant defense mechanism was activated, resulting in elaboration of SOD to counter the effects of the oxidants (Palipoch and Koomhin, 2015). Similarly, the significantly higher serum CAT activity in the POL pullets in the present study may be due to excessive generation of another oxidant, hydrogen peroxide. CAT, an 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). An association was established between the dynamics of MDA, SOD and CAT activities in this disease, and recovery indices such as survival, weight gain (from day 21 PI) and improved egg production (from the 5th week PI).

In this study, all the assayed biochemical parameters, save albumin, showed negative correlation with egg production, with creatinine ($r = -0.640$; $P = 0.000$) being the strongest and the association highly significant, closely trailed by AST ($r = -0.608$; $P = 0.000$). The marked myocardial degeneration and hepatocellular injury observed in this study may have heightened the serum creatinine level as well as AST activity, invariably suggesting a link between cardiac and hepatic pathology, and impairment in egg production process associated with FT in POL pullets.

The major limitation of this study is the inability to assay sodium and potassium as well as assessing the sodium-potassium pump, as these were erroneously omitted during study design. This would have been helpful in better understanding the cardiac activities in this disease.

CONCLUSIONS

The present study showed that infection of POL pullets with *S. gallinarum* resulted in severe inflammatory, degenerative and necrotic changes in the heart, liver, kidney, lung, intestine and ovary. The severity of the cardiomyopathy may be responsible for the marked pericardial effusion observed. In addition, the severity of the hepatitis, glomerulonephritis, pericarditis, pneumonia, enteritis and oophoritis may have accounted for the biochemical perturbations, impaired egg production and subsequent mortality in *S. gallinarum*-infected POL pullets in this study. The basic serum biochemical parameters assayed, MDA level as well as serum activity of SOD and CAT of POL pullets variably correlated with egg production, with creatinine and AST topping the chart of strong but negative correlation. Furthermore, the significantly higher MDA level in the infected pullets com-

pared with uninfected controls suggests that OS may play a lead role in the pathology of FT in chickens. The association between the dynamics of SOD and CAT activities and recovery indices such as survival, weight gain and improved egg production, suggests that antioxidants could ameliorate the pathology and mortality caused by FT.

Therefore, it is recommended that veterinarians and poultry consultants should incorporate antioxidants in the treatment of FT in chickens in the face of incessant vaccine failures and antimicrobial resistance.

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All authors contributed in the design, methodology, data analysis and approval of the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare with respect to the research, authorship, and/or publication of this article.

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