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Haematological alterations in laying hens infected with *Salmonella gallinarum,* and their relationship to egg production

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Abstract

This study investigated haematological alterations in laying hens experimentally infected with *Salmonella gallinarum*, and how these alterations relate to drop in egg production in the laying hens. Fifty laying hens (25 infected orally with wild type *S. gallinarum* and 25 uninfected controls) were used for the study. Haematological parameters were assayed following standard procedures. The packed cell volume of the infected hens was significantly ($p < 0.05$) lower on days 7 and 14 post infection (PI), while the haemoglobin concentration was significantly ($p < 0.05$) lower on days 7, 14 and 21 PI, when compared to the uninfected control. The red blood cell (RBC) counts were also significantly ($p < 0.05$) lower on days 7, 14, 21, 28 and 35 PI in the infected hens when compared to the uninfected controls. The total white blood cell (TWBC) counts of the infected hens were significantly ($p < 0.05$) higher than that of the uninfected controls on days 7, 14, 21 and 35 PI, while the heterophil counts were significantly ($p < 0.05$) higher in the infected hens on days 7, 14 and 21 PI, and the lymphocyte counts were also significantly ($p < 0.05$) higher in the infected hens on days 7, 28 and 35 PI when compared to the uninfected control. The weekly mean percentage egg production in the infected hens was significantly ($p < 0.05$) lower than that of the uninfected control on weeks 1, 2, 3 and 4 PI. Red blood cell counts correlated strongly, positively and significantly ($r = 0.61$; $p < 0.01$) with egg production, while the relationship between TWBC counts and egg production ($r = - 0.64$; $p < 0.01$) was strong, negative and significant. It was concluded that haematological parameters were significantly altered in *S. gallinarum* infected laying hens, and that some of the haematological parameters strongly correlated with egg production.

Keywords: Laying hens, Fowl typhoid, *Salmonella gallinarum*; Haematology; Relationship; Egg production.

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Introduction

The poultry industry plays a pivotal role in food security and agricultural/economic development of Nigeria, and has been rapidly expanding in the past years. Poultry production is however constrained by several factors, amongst which is the occurrence of poultry diseases (Haque *et al*. 1991; FAO, 2008). One of the economically important poultry diseases is fowl typhoid (FT), a severe systemic disease that affects chickens and turkeys, primarily, with pheasants, quail, ducks, guinea-fowl and peafowl also being susceptible. FT is caused by *Salmonella gallinarum*, a Gram-negative bacterium belonging to the family *Enterobacterieceae* (Molbalk *et al*. 2002). It is a septicaemic disease with worldwide distribution, attacking commercial poultry with attendant heavy socioeconomic impact due mainly to high mortality rates and accentuated drop in egg production (Shivaprasad, 1997; Berchieri *et al*., 2001).

In spite of efforts aimed at controlling FT through hygienic measures, serological testing and slaughter of positive reactors, the disease still remains one of the leading poultry diseases not only in developing countries but globally (Barrow, 1999; Okwori *et al*., 2013). The adoption of vaccination programs have not practically assuaged the ravaging effect of FT as the disease is still reported in many areas of the world where vaccination is practiced, including Mexico, Central America, South America, India, South Korea and Africa (Berchieri *et al*., 2000; Shivaprasad, 2000; Okwori *et al*., 2013).

Fowl typhoid has been poorly studied in Nigeria, with only few documented cases of outbreaks in commercial laying flocks, despite huge losses recorded in form of mortality and drop in egg production. Admittedly, a lot of work has been done in many parts of the world by several researchers on the pathology of FT (Shivaprasad, 2000; Freitas Neto *et al*., 2007). However, emphasis had not been placed on monitoring the blood picture, even as FT is a septicaemic disease. Moreover, the few reported haematology studies did not factor in a correlation between the haematological findings and drop in egg production associated with FT in laying hens. This present study was, therefore, carried out to evaluate the alterations in haematology of laying hens experimentally infected with *Salmonella gallinarum*, and how these alterations relate to egg production.

Materials and methods

Flock history: Seventy day-old chicks in the line of brown egg layers, ISA® brown, procured from a reputable commercial hatchery in Nigeria (CHI® Hatchery, Ibadan) were used for the study. They were raised on deep litter and fed with balanced commercial feed (Hybrid Feeds®, Hybrid Feeds Ltd., Kaduna, Nigeria) and clean water provided *ad libitum*. Routine vaccination against the infectious diseases endemic in the study area (Infectious bursal disease, Newcastle disease and Fowl pox) was carried out, but not for FT. Coccidiostat (Amprolium®, Kepro BV, Deventer, Holland) was administered as well as routine deworming and delousing using Wormazine® (Alfason Intl., Woerden, Holland), and Kepromec® (Kepro BV, Deventer, Holland), respectively.

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

Bacterial inoculum: The *S. gallinarum* strain used in the current study was an isolate from a local outbreak of FT that occurred in a commercially raised poultry flock in Nsukka Nigeria, which was preserved in the Microbiology Laboratory of the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. Prior to infection, the *S. gallinarum* was passaged in young chicks to ascertain the maintenance of pathogenicity. The organism in a stock culture was plated on MacConkey agar (MCA) and incubated at 37^{o}C for 24h. The grown colonies on MCA were suspended in nutrient broth and the method described by Beyaz *et al* (2010) was adopted in the inoculum preparation.

Pre-infection screening for *Salmonella* infection and baseline haematological evaluation: Prior to the experimental infection, cloacal swabs from randomly selected hens were cultured in nutrient broth, incubated at 37^{o}C for 24h and plated onto MCA to rule out *Salmonella* infection in the experimental birds (Berchieri *et al*., 2001; Lopes *et al*., 2016). After the pre-infection screening, 1 ml of blood sample was collected from the jugular vein of each of four birds randomly selected per group, and dispensed into sample bottles with Na-EDTA anticoagulant. The blood samples were gently mixed with the EDTA to prevent clotting, and transported to the laboratory in cold ice packs for haematological evaluation. Sample collection was done between 9.00 and 11.00 am and laboratory haematological evaluation was carried out within 24 hour of sample collection. The value obtained at baseline was regarded as Day 0 value.

Experimental infection: At their 28th week of age, the 50 laying hens used for the study were randomly assigned into two groups (Infected group and Uninfected Control) of 25 laying hens each. Each hen in the infected group was challenged by administering 1 ml of the inoculum containing 1 x 10^{9} *S. gallinarum* colony forming units (CFU)/ml into the crop, while each hen in the control group received 1 ml of buffered saline as placebo.

Clinical and histopathological examinations: Following experimental infection, the hens were observed for clinical manifestations of FT, and percentage egg production was recorded for 35 days post infection (PI). Five hens were randomly selected from each group and weighed on days 0, 4, 7, 14, 21, 28 and 35 PI.

Further blood collection for haematological evaluation: On days 7, 14, 21, 28 and 35 post-infection, 1 ml of blood sample was collected from each of four hens randomly picked per group. The blood samples were, as formerly done, dispensed into sample bottles with Na-EDTA anticoagulant and transported to the laboratory in cold ice packs for haematological evaluation. As was earlier done too, sample collection was done between 9.00 and 11.00 am and laboratory haematological evaluation was completed within 24 hours of sample collection.

Haematological evaluation methods and procedures: The packed cell volume (PCV) determination was done by the microhaematocrit method (Thrall and Weiser 2002). Red blood cell (RBC) and total white blood cell (TWBC) counts were done following the haemocytometer method (Campbell, 1994). Determination of the haemoglobin concentration (Hb conc.) was based on the cyanomethaemoglobin method (Higgins *et al.* 2008), and differential white blood cell counts were done on thin air-dried smears stained by the Leishman technique (Campbell, 1994). The absolute differential white blood cell counts, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Campbell, 1994; Thrall and Weiser, 2002).

Statistical analyses: Data generated from haematological evaluation were subjected to the independent sample t-test for equality of means on IBM SPSS for Windows version 21. The haematological parameters evaluated were correlated with the egg production using Pearson's correlation statistic. Level of significance was accepted at $p < 0.05$.

Results

Clinical findings: Clinical signs observed in the infected hens included depression, loss of appetite, greenish-yellow diarrhea, significant ($p < 0.001$) loss of weight (Figure 1), significant ($p < 0.001$) drop in egg production (Figure 2), 60% morbidity (15/25). The earliest death of a hen (1/25) was recorded on day 2 post-infection (PI). The clinical signs of ill-health persisted up to day 21 PI with an overall mortality of 28% (7/25). The control group remained apparently healthy for the whole duration of the experiment. At days 4 and 7 PI, the inoculated organism was recovered from the spleen, liver and heart blood of the dead infected hens following standard methods (Parmer and Davies, 2007) supported by biochemical tests (Haider *et al*., 2012).

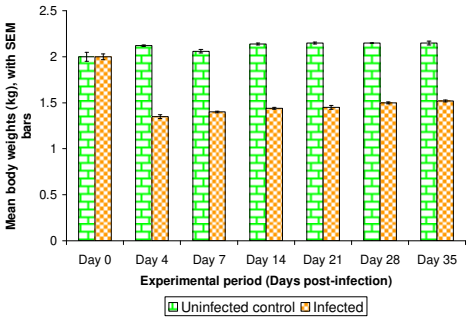

Figure 1: Mean body weight (kg) of laying hens infected with *S. gallinarum*, compared with uninfected control.

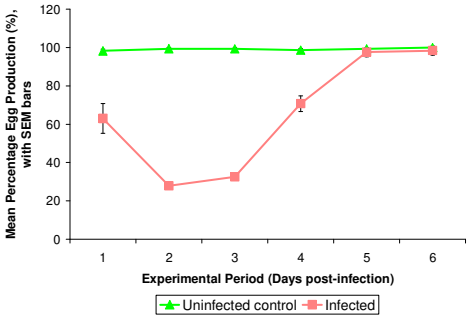

Figure 2: Mean percentage egg production of laying hens infected with *S. gallinarum*, compared with uninfected control.

Haematological alterations: The mean PCV was significantly lower on days 7 (p = 0.006) and 14 (p = 0.023) PI in the infected laying hens when compared to the uninfected controls (Figure 3). The Hb conc. (Figure 4) was significantly lower in the infected hens on days 7 (p = 0.001), 14 (p = 0.022) and 21 (p = 0.025) PI, compared to the uninfected controls. The RBC counts of the infected hens was also significantly lower than that of the controls on days 7 (p = 0.001), 14 (p = 0.012), 21 (p = 0.021), 28 (p = 0.020) and 35 (p = 0.033) PI (Figure 5). The MCV was significantly higher in the infected hens on days 7 (p = 0.045), 14 (p = 0.022) and 28 (p = 0.001) PI when compared to the controls (Figure 6), while the MCHC was significantly lower in the infected hens on days 7 (p = 0.005) and 21 (p = 0.045) PI, when compared to the uninfected control group (Figure 7).

The mean TWBC counts of the infected hens was significantly higher than those of the uninfected control on days 7 (p < 0.001), 14 (p = 0.002), 21 (p < 0.001) and 35 (p = 0.004) PI (Figure 8). The mean absolute heterophil counts of the infected hens was also significantly higher than those of the uninfected control on days 7 (p < 0.001), 14 (p < 0.001) and 21 (p = 0.009) PI (Figure 9), while the mean absolute lymphocyte counts of the infected hens was significantly higher than those of the uninfected control on days 7 (p = 0.019), 28 (p = 0.020) and 35 (p = 0.018) PI (Figure 10). The mean absolute eosinophil counts of the infected hens was significantly higher than those of the uninfected control on days 7 (p = 0.045) and 35 (p = 0.043) PI (Figure 11), but the mean absolute monocyte counts of the infected hens was significantly lower (p = 0.017) than that of the uninfected control on day 28 PI (Figure 12).

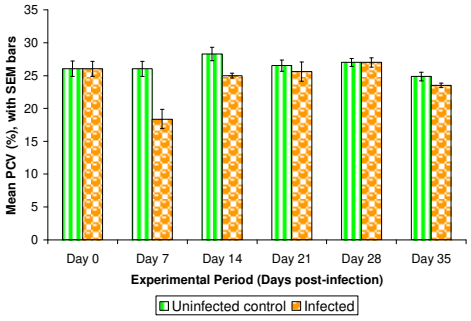

Figure 3: Mean packed cell volume (%) of laying hens infected with *S. gallinarum*, compared with uninfected control.

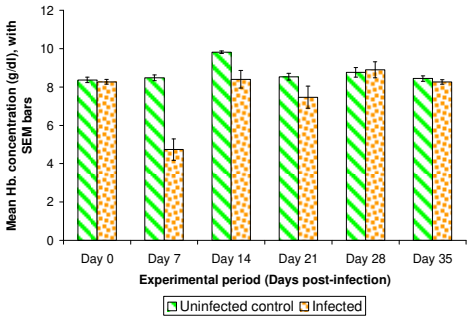

Figure 4: Mean hemoglobin concentration (g/dl) of laying hens infected with *S. gallinarum*, compared with uninfected control.

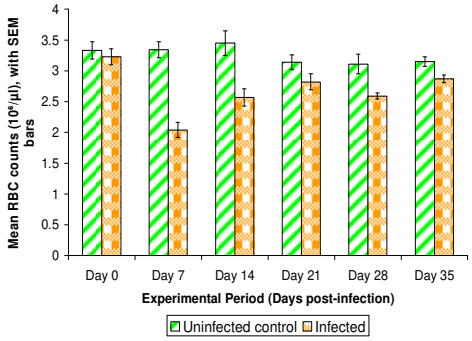

Figure 5: Mean red blood cell counts (10^6/µl) of laying hens infected with *S. gallinarum*, compared with uninfected control.

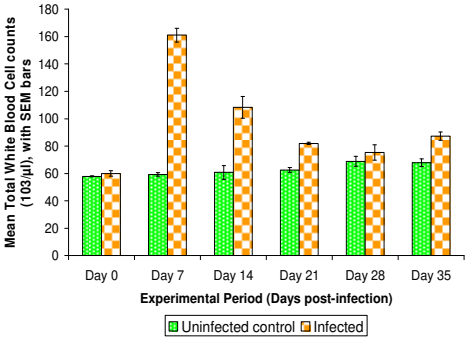

Figure 8: Mean total white blood cell counts (10^3/µl) of laying hens infected with *S. gallinarum,* compared with uninfected control.

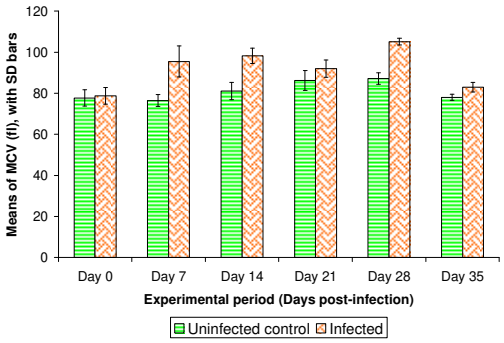

Figure 6: Mean corpuscular volume (MCV) [fl] of laying hens infected with *S. gallinarum,* compared with uninfected control.

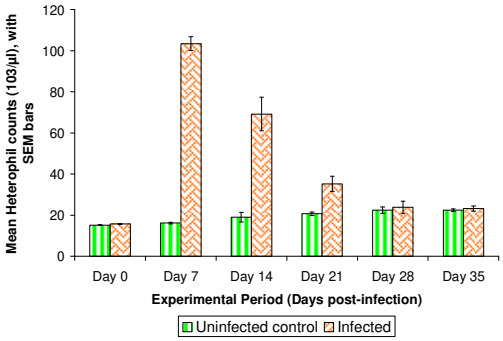

Figure 9: Mean absolute heterophil counts (10^3/µl) of laying hens infected with *S. gallinarum,* compared with uninfected control.

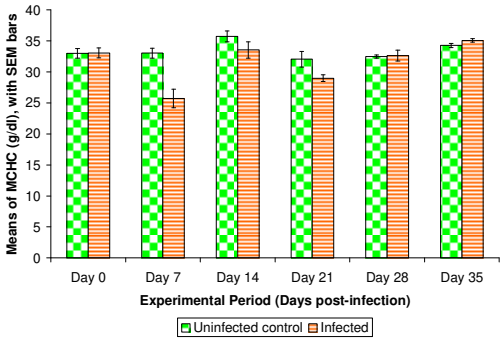

Figure 7: Mean corpuscular haemoglobin concentration (MCHC) [g/dl] of laying hens infected with *S. gallinarum,* compared with uninfected control.

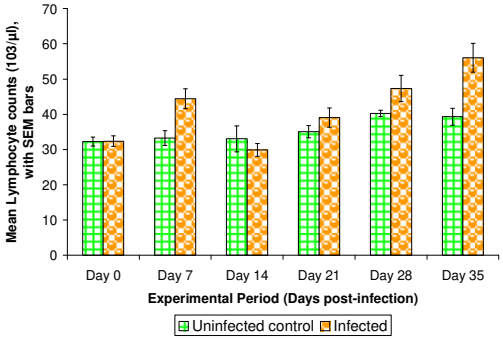

Figure 10: Mean absolute lymphocyte counts (10^3/µl) of laying hens infected with *S. gallinarum,* compared with uninfected control.

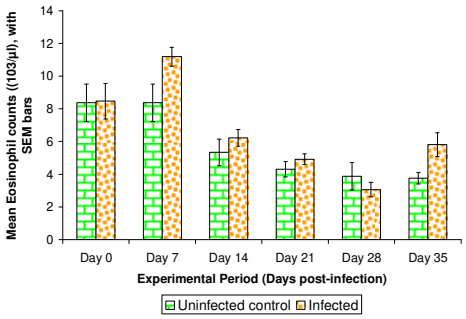

Figure 11: Mean absolute eosinophil counts (10^3/µl) of laying hens infected with *S. gallinarum,* compared with uninfected control.

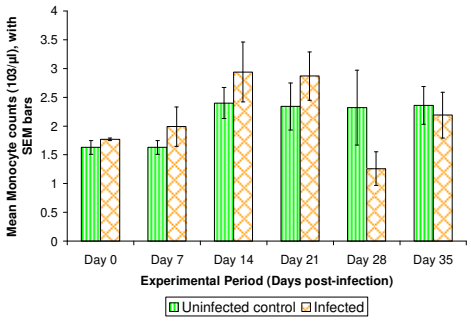

Figure 12: Mean absolute monocyte counts (10^3/µl) of laying hens infected with *S. gallinarum,* compared with uninfected control.

Correlation of the haematological parameters with the egg production: The correlation data of the erythrocytic profile of hens infected with *Salmonella gallinarum* and their egg production is presented in Table 1. The relationships between egg production and PCV (r = 0.409; p = 0.047), Hb conc. (r = 0.462; p = 0.023) and MCHC (r = 0.413; p = 0.045) were moderate, positive and significant, while the relationships between egg production and RBC counts (r = 0.605; p = 0.002) was strong, positive and highly significant. Egg production and MCV (r = - 0.621; p = 0.001) had a strong, negative and highly significant relationship.

The correlation data of egg production versus the leukocytic profile of hens infected with *Salmonella gallinarum* is presented in Table 2. The correlation between egg production and TWBC count (r = - 0.641; p = 0.000) and absolute heterophil counts (r = - 0.675; p = 0.000) was strong, negative and highly significant, while the relationships between egg production and absolute lymphocyte counts (r = - 0.189; p = 0.378), absolute eosinophil counts (r = - 0.151; p = 0.482) and absolute monocyte counts (r = - 0.136; p = 0.527) was poor, negative and not significant.

Table 1. Correlation of egg production and erythrocytic profile of laying hens infected with *Salmonella gallinarum*.

	Correlation coefficient (r) of egg production versus erythrocytic parameters.				
	PCV (%)	Hb. conc. (g/dl)	RBC count (10^6/µl)	MCV (fl)	MCHC (g/dl)
Egg Production	r = 0.409	r = 0.462	r = 0.605	r = - 0.621	r = 0.413
Sig. (2-tailed)	p = 0.047	p = 0.023	p = 0.002	p = 0.001	p = 0.045

Table 2. Correlation of egg production and leukocytic profile of laying hens infected with *Salmonella gallinarum*.

	Correlation coefficient (r) of egg production versus leukocytic parameters.				
	TWBC (10^3/µl)	Lymphocytes (10^3/µl)	Heterophils (10^3/µl)	Eosinophils (10^3/µl)	Monocytes (10^3/µl)
Egg Production	r = - 0.641	r = - 0.189	r = - 0.675	r = - 0.151	r = - 0.136
Sig. (2-tailed)	p = 0.000	p = 0.378	p = 0.000	p = 0.482	p = 0.527

Discussion and Conclusion

The clinical findings recorded in the *S. gallinarum* infected hens in this study concur with findings in previous reports (Shivaprasad, 2000; Freitas Neto *et al*. 2007; Chiroma *et al*., 2017; Garcia *et al*.. 2010). However, the peracute infection with short incubation period of 2 days and first mortality at day 2 PI that was recorded in the current study differed from the 3 days incubation period reported by Garcia *et al.* (2010) and 7 days incubation period reported by Chiroma *et al.* (2017). The 28% mortality recorded in the present study is lower than 40% and 50% reported by Freitas Neto *et al*. (2007) and Chiroma *et al*. (2017), respectively in experimental FT infection in layers, but higher than 25% reported by Ezema *et al*. (2009) in an outbreak of FT in a commercial layer flock in Udi, Enugu State, Nigeria. These differences in clinical findings may be attributed to differences in the strains of *S. gallinarum* used for/encountered in the different studies and also on the breed of the laying hens used for the studies.

The significantly lower mean PCV, Hb conc. and RBC counts in the infected laying hens compared to the controls agrees with previous reports of anaemia in acute FT (Prasanna and Paliwal, 2002; Chiroma *et al*., 2017). The anaemia was reportedly attributed to heightened ability of reticulo-endothelial cells to engage in extravascular engulfment of RBCs (Assoku and Penhale, 1978), cytopathic effect of outer membrane proteins of *S. gallinarum* on RBCs leading to their modification and subsequent destruction (Christensen *et al*., 1996) or vitamin B 12 deficiency due to its intestinal mal-absorption or poor liver storage following liver dysfunction (Feldman *et al*., 2000). The finding in the present study suggests a haemolytic anaemia following destruction of RBCs; FT being a septicaemic disease with chances of elaboration of substances which may have caused lysis of RBCs. The significantly higher MCV and lower MCHC values in the infected group compared to the uninfected controls suggests responsive (macrocytic hypochromic) anaemia, with bone marrow response elicited by the significant decrease in the red blood cell counts and haemoglobin 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) and Chiroma *et al*. (2017), but contrasts with the reported microcytic hypochromic anaemia during acute FT infection by Christensen *et al*. (1996) and Mdegela *et al*. (2002).

The significantly higher TWBC counts recorded in the infected hens in the present study concurs with the reports of Shah *et al*. (2013) in broiler chickens experimentally infected with *S. gallinarum* and that of Chiroma *et al*. (2017) in layers infected with *S. gallinarum*. The leukocytosis which was mainly as a result of significant elevations in the mean heterophil and lymphocyte counts, is believed to be a reflection of acute inflammatory response coupled with degenerative changes occasioned by the invasion of tissues of the target organs by *S. gallinarum* and also host immune response evoked by the assaults on the target organs. 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 defense against bacterial infection (Thrall and Weiser, 2002). The significant elevation of the mean eosinophil count on days 7 and 35 PI is thought to be a compensatory response to the corresponding heterophilia as both cells (heterophils and eosinophils) originate from same cell line or precursor in the bone marrow. 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 (Anosa *et al*., 1999).

The moderate to strong and positive correlation between egg production and the erythrocytic values recorded in the current study suggests that anaemia has direct relationship with the drop in egg production caused by FT, whereas, leukocytosis which had moderate to strong and negative correlation coefficient was inversely

related to the drop in egg production (Chan 2003).

Based on the results of the study, it was concluded that *Salmonella gallinarum* infection in laying hens significantly altered the haematological parameters of the hens when compared with untreated controls, and that some of the haematological parameters significantly correlated with the drop in egg production recorded in the study.

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Conflicts of Interest

The authors declare that no conflict of interest was associated with this work.

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