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HAEMATOLOGICAL CHANGES IN LAYERS EXPERIMENTALLY INFECTED WITH *PASTEURELLA MULTOCIDA*

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ABSTRACT

This study investigated the haematological changes associated with infection of layers with Pasteurella multocida. A total of 20 ISA Brown layers aged 18 weeks were used in this study. The birds were assigned into two groups (infected and control) of 10 layers each. Each bird of the infected group was challenged by intra nasal (0.1 ml) and intramuscular (0.4 ml) administration of Pasteurella multocida inoculum containing $4.5x10^8$ cfu. Blood samples (2.5 ml) were collected from each layer in both groups and used for haematological analyses on days zero (Day 0), 2, 4, 7, 14, 21, 28, 35, 42, post-infection. The results showed that by day 5 pi, layers in the infected group manifested ruffled feathers, sneezing, greenish-yellowish diarrhoea, weight loss, drop in egg production and 20% mortality. Compared to the uninfected control group, the infected layers had significantly lower ($p < 0.05$) packed cell volume, haemoglobin concentration and red blood cell counts, with no significant changes ($p > 0.05$) in their mean corpuscular values. Also infected layers had significantly higher ($p < 0.05$) total leukocyte, heterophil and lymphocyte counts when compared to the un-infected group. From the result of our finding, it was concluded that the experimental infection with Pasteurella multocida in layers is associated with normocytic, normochromic anaemia, leukocytosis, heterophilia and lymphocytosis.

Keywords: Fowl cholera, *Pasteurella multocida*, Experimental infection, Haematology.

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INTRODUCTION

Fowl cholera (avian pasteurellosis) is a contagious and economically important disease of poultry caused by a Gram negative, non-motile fermentative organism, *Pasteurella multocida* [1]. Besides chickens, turkeys, ducks, geese and many other types of birds are also susceptible to the disease [2,3]. The disease

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affects birds of all ages, but rarely occurs in commercial poultry of less than 8 weeks of age [4]. Currently the genus *Pasteurella* includes *Pasteurella multocida subspecies multocida, P. multocida subspecies septic* and *P. multocida subspecies gallicida* [5].

Three clinical forms of the disease have been identified in poultry; per- acute, acute and chronic. The per-acute form is usually due to infection with the most virulent and highly infectious organism where birds in good condition would suddenly die without any premonitory signs. In the acute form, chickens will show anorexia, mucus discharge from the beak, high fever, loss of weight, drop in egg production, cyanosis of wattles and comb and green foetid diarrhoea [6]. The chronic form of the disease is associated with conjunctivitis, swollen wattles, tracheitis, lameness, dyspnoea, swelling of joints and tendon sheaths of legs and wings as well as torticollis [7].

Gross lesions of fowl cholera in chickens include petechial and ecchymotic haemorrhages on coronary fats of the heart, epicardium, proventriculus, gizzard, peritoneum, intestines, and abdominal fats. The liver is frequently enlarged, congested, streaked with multiple pinhead greyish necrotic foci and there is splenomegaly and congestion of ovarian follicles [8].

Fowl cholera causes devastating economic losses to the poultry industry through weight loss, condemnation of carcasses and death world-wide [9]. It has been reported to cause 1.8-21% mortality and a decline in egg production by 15-20% and resulted in shorter egg laying period leading to infected flocks being culled at an earlier stage [10, 11]. As a result of associated losses, the disease remains a significant obstacle to commercial poultry production in most parts of tropical Africa and Asia. It usually occurs as a fulminating disease with massive bacteraemia, high morbidity and mortality [12].

The disease is transmitted mechanically through vectors, aerosols and the ingestion of contaminated feed and water. Most farm animals may be carriers of *Pasteurella multocida* [13].
In general, despite available literature on the haematological changes associated with experimental infection of layers with *P. Multocida*, there is still need for more studies to have a better understanding of the disease. This study, therefore, evaluated the haematological changes in layers experimental infected with *Pasteurella multocida*

MATERIALS AND METHODS

Area of Study

This study was carried out in Zaria, Kaduna State, which is located within the Northern Guinea Savannah zone of North western Nigeria. It lies between latitude 7^{o} and 11^{0} N, and longitude $7^{o}$and 44^{0}E. It has an average rainfall between 1,000 and 1,250 mm and an average temperature between 17 ^{0}C and 33 ^{0}C with a vegetation cover of predominantly trees and grasses [14].

Experimental Birds

A total of 20 ISA Brown layers aged 18 weeks were acquired from a reputable farm that brood poultry for research purposes at Kujama, Kaduna State. The birds were routinely immunized against all endemic diseases other than fowl cholera. On arrival at the Poultry Research Unit of the Department of Pathology, Faculty of Veterinary medicine, Ahmadu Bello University, Zaria, Nigeria, they were housed and managed intensively in pens that were thoroughly washed with detergent and sprayed with formalin at a concentration of 4 ml/litre of water. The birds were kept for 7 weeks to acclimatize to the new environment and also reach peak of egg production. They were fed standard commercial layers mash (Hybrid feeds®) with drinking water provided *ad libidum* throughout the study period.

Source of bacterial organism and pre-infection of experimental birds

The challenge bacterium, *Pasteurella multocida* serotype A: 1, used in this study was provided by the Department of Bacteriology, National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Nasal swabs were collected from experimental birds in both groups and inoculated into blood agar supplemented with 10% sheep blood and McConkey agar. The plates were incubated for 24-48 h at 37°C and were checked every day for bacterial colonies [15].

Challenge of the birds with *Pasteurella multocida*

After reaching peak of egg production (80%) at 26 weeks old, the birds were randomly assigned into two groups (infected and uninfected) of 10 layers each. On the day of infection (Day 0) each of the birds in the infected group was challenged with a dose of 0.5ml of the inoculum containing $4.5x10^8$cfu of *Pasteurella multocida.* One tenth (0.1ml) of the total infection dose volume was administered intra-nasally [16], while the remaining 0.4 ml was administered by intra-muscular injection [17].

Clinical Observation

Starting from Day 0 post infection (pi), all the experimental birds were routinely monitored for clinical signs of fowl cholera and observations were recorded accordingly throughout the experimental period of six weeks.

Collection of Blood for Haematological Evaluations

Blood samples (0.5 ml) were collected from each of the birds in the infected and control groups via the brachial vein, using a 5-ml syringe and 23 G needle. Blood samples were first collected on day 0 and subsequently on days 2, 4, 7, 14, 21, 28, 35 and 42 pi. The blood collection was carried out in the morning (08.00 to 09.00 hour). The blood was dispensed into EDTA sample bottles and used for haematological evaluations.

Haematological Evaluations

Packed cell volume (PCV) was determined by the microhaematocrit method, haemoglobin concentration by the cyanmethhaemoglobin method, while the red blood cell (RBC) and white blood cell (WBC) counts were determined using the improved Neubauer haemocytometer method [18]. Erythrocytic indices, namely, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Campbell and Ellis [19]. Blood smears for differential WBC counts were routinely prepared, stained with Wright-Giemsa stain and examined under oil immersion magnification (X 1,000) according to [20]. Differential leukocytes counts were carried out according to [21, 22]

Bacteriological isolation and recovery of *Pasteurella multocida* Post-infection

At necropsy, swabs were aseptically taken immediately from the blood in the hearts, lungs and spleens of the birds in the infected group for the isolation of *P. multocida* on days 4, 7 and 21 pi. The heart blood and tissue samples were inoculated into Blood agar, MacConkey agar and incubated at 37^oC with 5 % carbondioxide (CO_2) for 24 hrs. The colonies suggestive of *P. multocida* were further subjected to biochemical tests, including sugar fermentation, catalase, oxidase and indole tests for final identification [23].

Statistical Analysis

Data collected during the study were summarized as means and standard errors of means and differences between groups were determined using Student t-test and values of $p < 0.05$ were considered significant using Graph Pad Prism Version 5.00 for Windows, Graph pad Software, San Diego California USA.

RESULTS

Following infection with *Pasteurella multocida* serotype A:1, birds in the infected group showed clinical signs of fowl cholera as from day 5 post-infection. The clinical signs observed included drop in egg production, reduced feed and water consumption, ruffled feathers, watery greenish-yellow faeces and weakness, loss of weight, laboured breathing, paleness and cyanosis of wattles and combs. The infected

group had 20% mortality while all the birds in the control group remained apparently healthy throughout the study.

Effect of *Pasteurella multocida* Infection on the Haematological parameters

Mean packed cell volume

The mean packed cell volume in the infected and control groups were as shown in Fig. 1. There was a slight decrease in the packed cell volume (PCV) in the infected group on days 2 and 4 post-infection (pi). Thereafter, PCV in the infected group from day 7 pi rose to reach a peak level (37.80 ± 0.94 %) on day 14 pi that was significantly ($P < 0.05$) higher than the corresponding value in the control (30.90 ± 0.33 %).

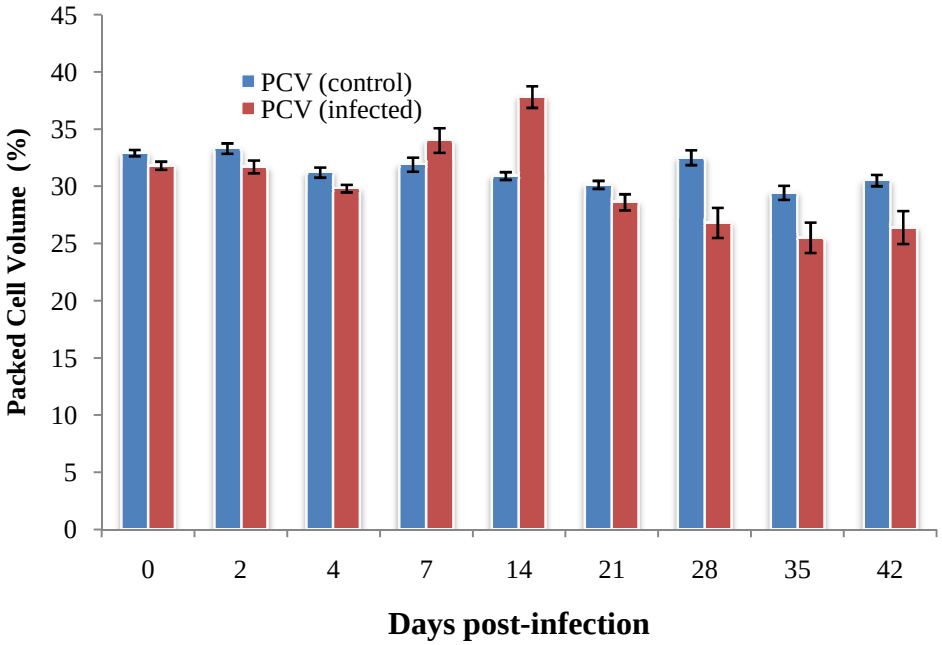

Figure 1: Mean (± SEM) packed cell volume of *Pasteurella multocida* infected and control groups of layers.

Mean Red Blood Cell Count

The mean red blood cell count (Fig. 2) in the infected and control groups remained comparable on day 2 pi but by day 4 pi it significantly ($P < 0.05$) increased in the infected group. Thereafter, it decreased to reach a lowest level ($1.57 \pm 0.11 \times 10^{12}$L) on day 28 pi in the infected group which was significantly ($P<0.05$) lower than that ($2.30 \pm 0.04 \times 10^{12}$L) in the control up to the period when the study was terminated on day 42 pi.

Mean haemoglobin concentration

The mean haemoglobin concentration in the infected and control groups were as shown in Fig. 3. Following the infection, mean haemoglobin concentration gradually decreased progressively in the infected group from day7 pi to reach a lowest level (6.30± 0.49 g/dL) on day 35 pi that was significantly ($P < 0.05$) lower than the corresponding value (9.70 ± 0.33) in the control group. At the end of the study (day 42 pi), the value of haemoglobin concentration was still significantly lower in the infected than control group.

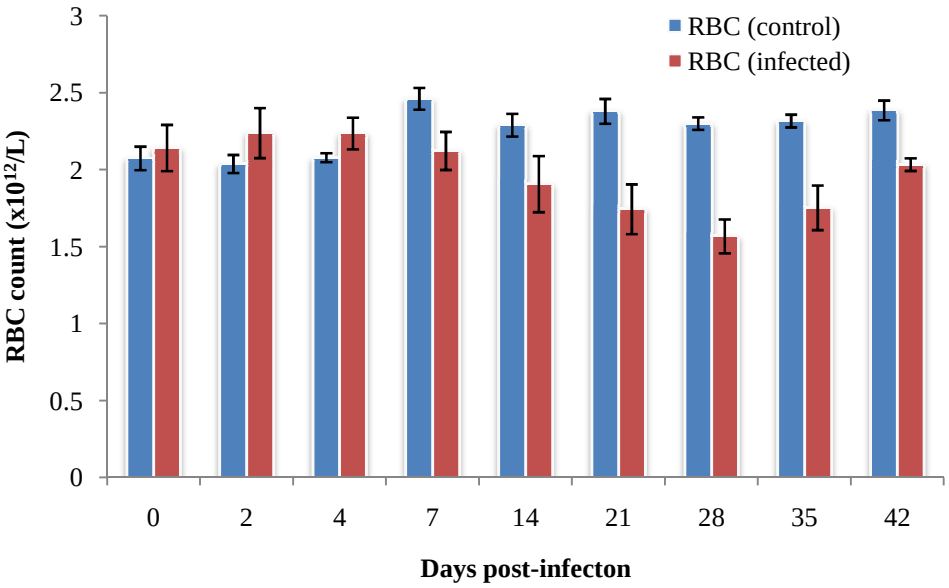

Figure 2: Mean (± SEM) red blood cell counts of *Pasteurella multocida* infected and control groups of layers.

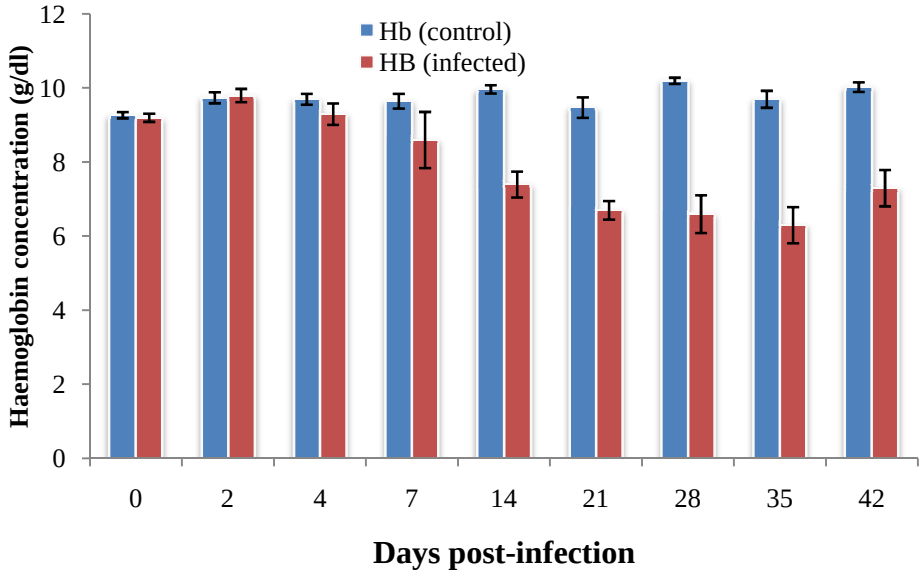

Figure 3: Mean (± SEM) haemoglobin concentration of *Pasteurella multocida* infected and control groups of layers.

Mean corpuscular volume

The values of mean corpuscular volume in the *Pasteurella multocida*-infected and control layers are shown in Fig. 4.The mean value of the MCV of birds in the infected group (1.33 ± 5.70 fl) was significantly ($p < 0.05$) lower than that of the control group (146.0 ± 4.23 fl) on day 2 pi, and afterwards, there were no significant differences ($p > 0.05$) between the MCV of the till the end of the experiment.

Mean corpuscular haemoglobin concentration

The values of the mean corpuscular haemoglobin concentrations (MCHC) of the *P.multocida*-infected and control group of layers are as shown in Fig. 5. There were no significant ($p > 0.05$) differences between the means of MCHC of the two groups all through the experimental period.

Mean total white blood cell count

The mean total white blood cell count in the *P. multocida*-infected and control groups of layers are shown in Fig. 6. The mean total white blood cell count increased progressively in the infected group from day 4 to reach a peak value (78.40 ± 8.10 x 10^9/L) on day 14 pi that was significantly ($P < 0.05$) higher than that in the control group (21.70 ±1.00 x 10^9/L) after which the mean TWBC count remained high in the infected group up to day 42 pi when compared with that of the control group.

Mean heterophil count

The mean heterophil count in *P. multocida*-infected and control groups of layers are shown in Fig. 7. There were no significant differences ($p > 0.05$) in the mean heterophil counts of the two groups from day 0 to 4 pi, but from day 7 to 28 pi, the heterophil count of the infected group was significantly ($P < 0.05$) higher than of the control. However, on days 35 and 42 pi, the heterophil counts of the infected group became significantly ($P < 0.05$) lower than that of the control.

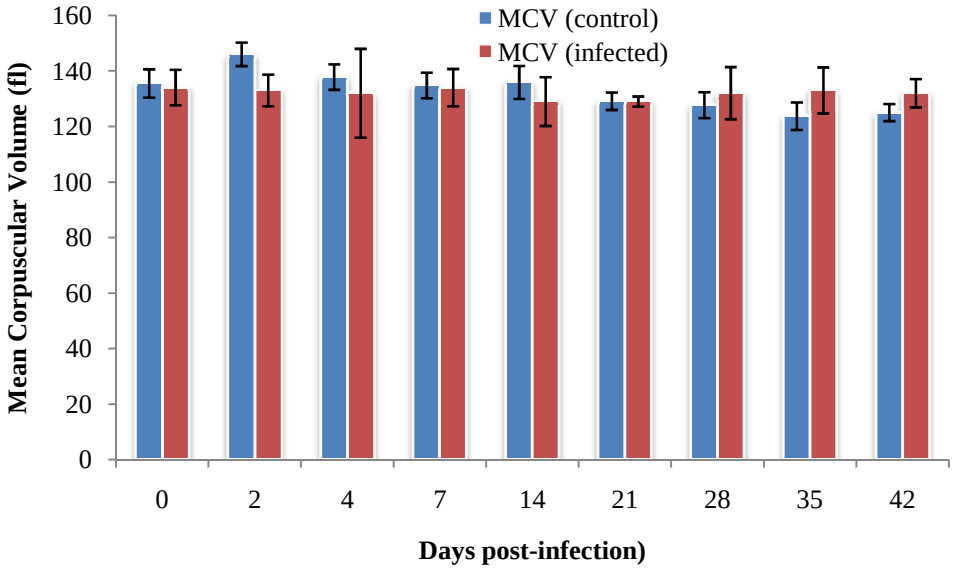

Figure 4: Mean (± SEM) corpuscular volume (MCV) of *Pasteurella multocida* infected and control groups of layers.

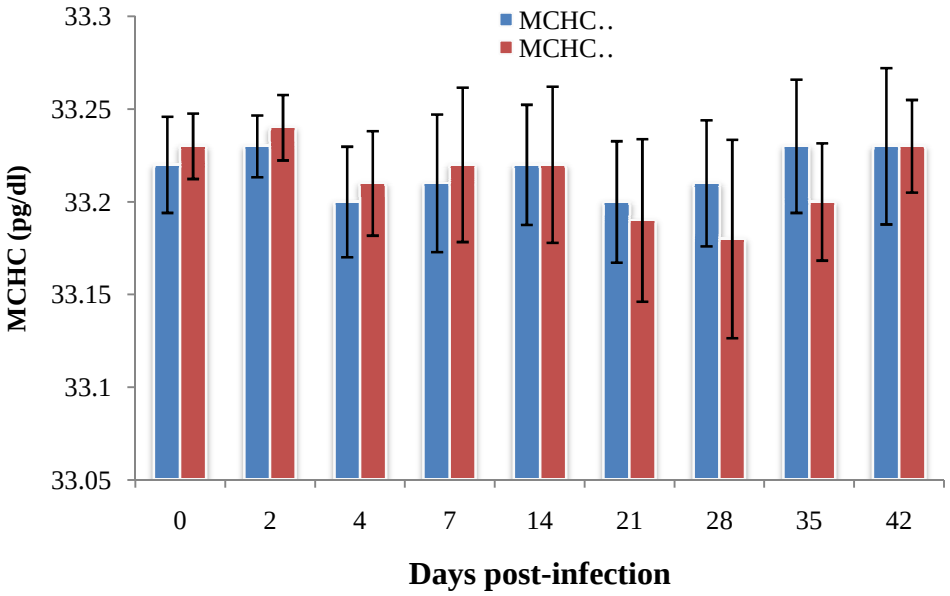

Figure 5: Mean (± SEM) mean corpuscular haemoglobin concentrations (MCHC) of *Pasteurella multocida* infected and control groups of layers.

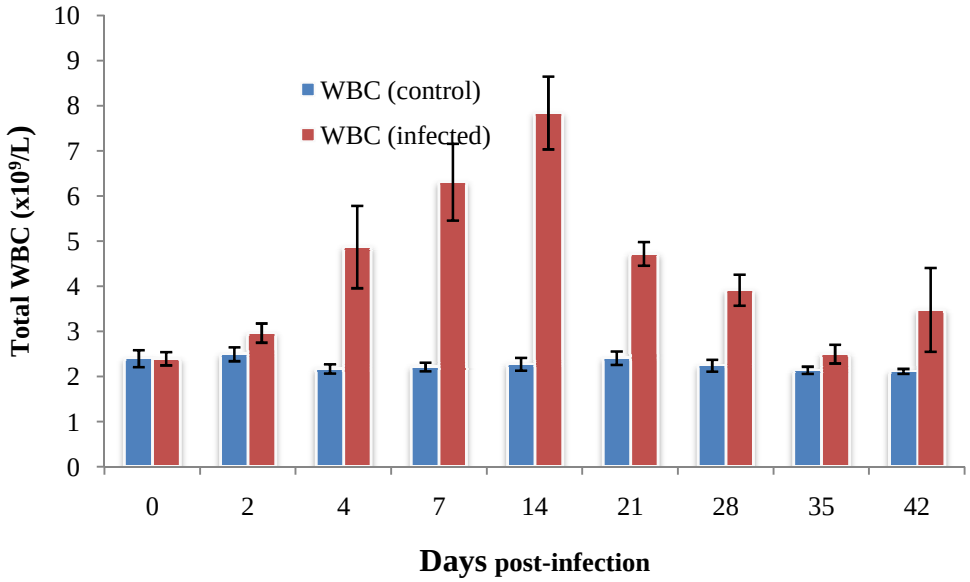

Figure 6: Mean (± SEM) total white blood cell counts in *Pasteurella multocida* infected and control groups of layers.

Mean lymphocyte count

The mean lymphocyte count in the *P.multocida*-infected and control groups of layers are shown in Fig. 8. There were no significant differences ($p > 0.05$) in the lymphocyte counts between the two groups on days 0, 2 and 4 pi, although there was a relative increase in the lymphocyte counts of both groups on day 4 pi. From day 7 to 42 pi, the lymphocyte counts of the infected group was significantly ($p < 0.05$) higher than that of the control.

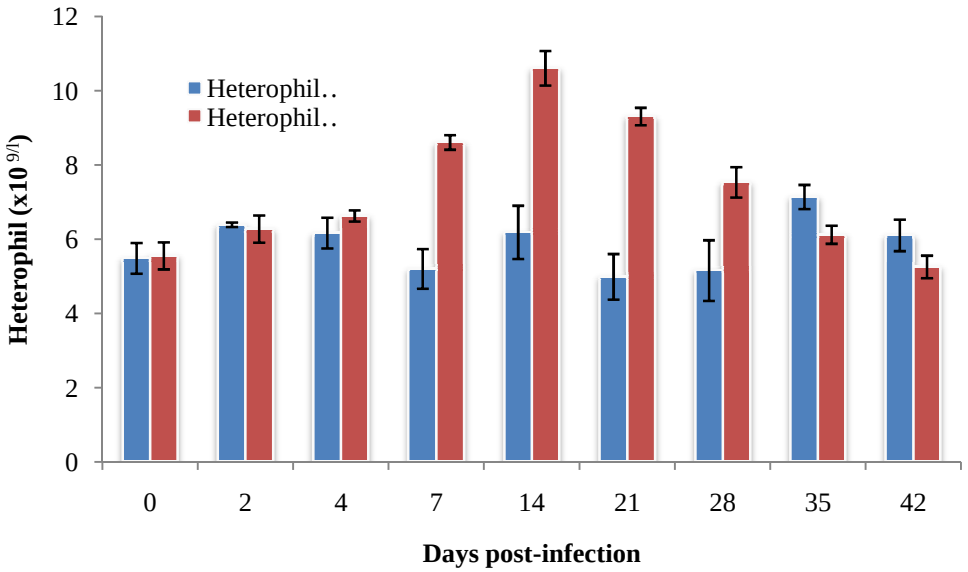

Figure 7. Mean (± SEM) heterophil counts in *Pasteurella multocida* infected and control groups of layers.

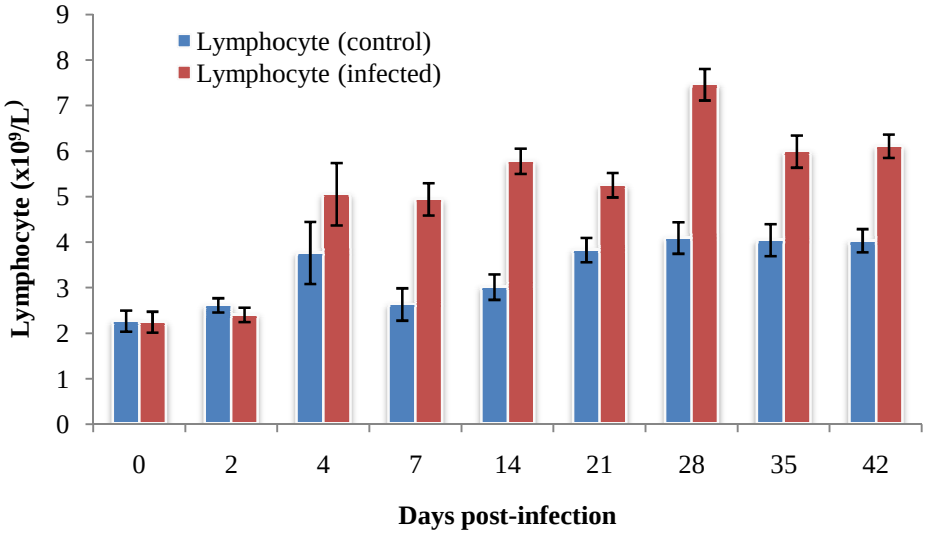

Figure 8: Mean (± SEM) lymphocyte counts in *Pasteurella multocida* infected and control groups of layers.

DISCUSSION

The clinical signs that were observed in the *Pasteurella multocida*-infected layers during the experiment included anorexia, weakness, loss of weight, greenish-yellowish diarrhoea, drop in egg production, pale wattles and combs which are in agreement with previous reports [24, 25]. The changes noticed in the haematology of *Pasteurella multocida*-infected layers in this study which were significant ($p < 0.05$) increase in packed cell volume (PCV) on day 14 pi and decrease on day 21 pi, significant ($p < 0.05$) decrease in haemoglobin concentration (Hbc) on day 7 pi, and significant ($p < 0.05$) increase in red blood cell count (RBC) on day 4 pi and decrease on day 28 pi correspond to previous reports of normocytic normochromic anaemia in fowl cholera disease [26] which was attributed to impaired erythropioesis due to poor absorption of iron, copper, proteins, cobalt and pyridoxine as consequence of the disease. The anaemia may also be partly due to the reported production of haemolytic endotoxins by *P. multocida* [27].

In this study the fact that the mean MCV and MCHC, on the days in which abnormal mean values for PCV, Hb concentration and RBC count were recorded were within the reference values suggests that the anaemia caused by infection with *P. multocida* in layers was normocytic normochromic.

The leukogram of the *P.multocida*-infected layers showed significant increase ($p < 0.05$) in the TWC count in the early period of the infection. The significant increase ($p < 0.05$) in total white blood cell, heterophil and lymphocyte counts in the infected group from days 7 to 28 pi in this study could be due to inflammatory reaction as a result of antigenic stimulation caused by the *P. multocida* [28] and/or its endotoxin while a progressive and significant decrease ($p < 0.05$) in the mean heterophils count between days 35 and 42 pi could be as a result of shift of cells (heterophils) from the circulating pool into the marginal pool and then into the body tissues by diapedesis [29] in order to phagocytise the offending organism. The significant increase in the mean lymphocytes count observed from days 7 to 42 pi indicates lymphocytosis which means that *P.multocida* stimulates lymphocytosis which is in agreement with the reports of Mitchell and Johns [30]. Another possible reason for lymphocytosis in this study could be that the birds were recovering from infection with *P. multocida* as lymphocytosis had been reported in the recovery stage of most infectious diseases.

CONCLUSION

It was concluded from this study that the experimental infection with *Pasteurella multocida* in layers as done in this study was associated with normocytic, normochromic anaemia, leukocytosis, heterophilia and lymphocytosis.

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