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Comparison of haematological changes associated with coccidiosis in commercial layer chickens at different production stages in Zaria, Nigeria

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Abstract

Avian coccidiosis remains an economically important disease affecting the poultry industry worldwide. The dearth of information on haematological changes resulting from field cases of coccidiosis in commercial layer chickens necessitated this research. One hundred and twenty commercial layer chickens sampled from different farms in Zaria, diagnosed of coccidiosis at early, mid/peak and late production stages were used for this study. Results showed a significant ($p < 0.05$) decline in almost all haematological parameters (except for elevated MCV) of commercial layer chickens which were diagnosed of coccidiosis at the different production stages when compared to corresponding values of other apparently healthy layers that served as controls. The mean corpuscular volume (MCV) values in the layers diagnosed of coccidiosis at all production stages were significantly higher than the values obtained from the corresponding apparently healthy ones that served as control, whereas the MCHC showed significant decreases across all layer chickens diagnosed of coccidiosis at different production stages when compared to the corresponding values of the apparently healthy control layers. Thus, the erythrocytic indices of the layer chickens with coccidiosis showed the RBCs were macrocytic and hypochromic. On the basis of mean PCV, layer chickens at early production stage (PCV: 24.51 ± 3.17%) were most severely affected by coccidiosis, following closely by the layers at mid/peak production stage (PCV: 24.66 ± 1.64%). The highest mean WBC (19.15 ± 2.99 x10^9/L) was recorded in layers with coccidiosis at mid/peak production stage, which showed they were better in mounting inflammatory response when compared to the mean values of layers at early (14.92 ± 2.85 x10^9/L) and late (17.99 ± 2.70 x10^9/L) stages of production. In conclusion, coccidiosis in commercial layer chickens caused significant haematological alterations which could necessitate dietary supplementation to prevent occurrence of anaemia and decline in egg production.

Keywords: Coccidiosis, Haematological changes, Layer chickens, Production stages

Introduction

Coccidiosis is the commonest and most economically important protozoan disease of poultry resulting in great economic losses worldwide (Mohammed & Sunday, 2015; Latif *et al*., 2016). Confinement rearing and high-density flocks of commercial poultry have increased the exposure to the disease usually caused by the intracellular protozoan parasite of the genus *Eimeria*, parasitizing the gastrointestinal tract of birds of all age groups (Peek & Landman, 2011; Oyegbemi & Adejinmi, 2012). Coccidiosis is usually characterized by dysentery, enteritis, bloody diarrhea, emaciation, lower feed conversion rate, delayed sexual maturity, drooping wings, poor growth and low egg production with attendant high mortality and morbidity rates (Awais *et al*., 2012; Ola-Fadunsin & Ademola, 2013; Habibi *et al*., 2016).

Globally, the annual loss inflicted by coccidiosis to the poultry industry has been estimated to be more than 14 billion USD (N5.7 trillion) as reported by Blake *et al*. (2020). These losses result from reduced growth, reduced production, mortality, as well as due to the cost of treatment and preventive measures (Usman *et al*., 2011). According to McDougald & Reid (1997), breeder and layer pullets are at greater risk of infection by coccidia because most are managed on deep litter for several weeks, thus the chances of the chickens to pick-up large numbers of sporulated oocysts over time is high, and hence higher frequency of coccidia infection in layers than in broilers which could be due to stress of egg production in the former (Etuk *et al*., 2004; Jatau *et al*., 2012). Although clinico-pathological changes associated with coccidiosis in poultry were well established, there is dearth of adequate and recent published information on coccidiosis and its effects on haematological parameters of layer chickens as well as the severity of the disease in layer chickens at different stages of production.

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 Nigeria, between latitude 70º and 11º N, and longitude 70º and 44º E; the average annual rainfall of this zone ranges from 1000 to 1250 mm, and the average temperature ranges from 19 ^{o}C to 33 ^{o}C (Sawa & Buhari, 2011).

Experimental animals

A total of one hundred and five commercial layer chickens, mostly ISA Brown breed, ranging between 18 and 73 weeks were sampled from various farms reported with outbreaks of clinical coccidiosis across Zaria metropolis, Kaduna State, Nigeria. The chickens were divided into three groups based on their production stages; group A (Early production stage; 18-30 weeks old), group B (Mid/peak production stage; 31-55 weeks old) and group C (Late production stage; 56-73 weeks old) containing 35 birds each. The groups have their corresponding control (apparently healthy) counterparts, also divided into early production stage (18-30 weeks old), mid/peak production stage (31-55 weeks old) and late production stages (56-73 weeks old) containing 5 birds each.

Blood collection

Two millilitres (2 ml) of blood were collected from the brachial vein of each bird using a 5 ml sterile hypodermic syringe and a 23-gauge needle after the birds were properly restrained by an assistant. The blood collected were transferred immediately into sample bottles containing ethylene diamine tetra acetic acid (EDTA), as anticoagulant, and used for haematological evaluations. Prior to this, the blood collection site was swabbed with 70% ethanol to allow for easy access to the vein and for collection of blood.

Haematological analyses

Haematological parameters were analyzed as follows; packed cell volume (PCV) was determined using a modified microhaematocrit technique as described by Rehman *et al*. (2003), the haemoglobin concentration (Hb) was assayed colorimetrically by the cyanomethhaemoglobin method (Drabkin, 1945), red blood cell (RBC) and total white blood (TWBC) counts were counted and determined using haemocytometers as described by (Campbell & Ellis, 2007). Erythrocytic indices, such as mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were calculated from values already obtained. Differential leucocyte counts, namely; heterophils, lymphocytes, monocytes and eosinophils were counted and classified based on their morphologic features through microscopic examination of Giemsa-stained thin blood smears as described (Hawkey & Dennatt, 1989; Campbell & Ellis, 2007).

Data analyses

Data obtained were subjected to statistical package for Social Sciences (SPSS) version 26 (IBM, 2018) for analysis. Data obtained from healthy and diseased

birds were compared using Student T-test for significance. Also, One-Way Analysis of Variance (ANOVA) was used to compare means of variables of infected layers at three different stages of production. Tukey Post Hoc was used to test for significance within different production stages of infected layer chickens. Values of $p < 0.05$ were considered significant.

Results

The haematological parameters such as the packed cell volume, haemoglobin concentration, total erythrocyte count, total leukocyte count and differential leukocyte count that were recorded in all studied groups (different production stages) were summarized in Table 1. Layers of different production stages naturally diagnosed of coccidiosis had significantly ($p < 0.05$) lower mean values of PCV, RBC, Hb but significantly ($p < 0.05$) higher mean differential leucocytes count (except for heterophils) to their apparently healthy control counterparts (Table 1). Comparison of haematological parameters among infected layers at three different stages of production demonstrated that groups A and B had significantly ($p < 0.05$) lower PCV but higher Hb compared to group C. Mean WBC and differential leucocytes counts were significantly ($p < 0.05$) lower in group A compared to those in groups B and C (Table 2).

Discussion

Findings from this study revealed anaemia, evidenced by significantly low PCV, Hb and RBC count in layer chickens with natural coccidiosis, as observed in this study, when compared with the control groups as previously reported (Hirani *et al*., 2007; Ogbe *et al*., 2010; Meskerem *et al.,* 2013; Samrawit *et al.,* 2018).

Table 1: Haematological values of layers at different stages of production naturally infected by coccidiosis

Parameters	Group A		Group B		Group C	
	Control (n=5)	Infected (n=5)	Control (n=5)	Infected (n=5)	Control (n=5)	Infected (n=5)
PCV (%)	29.22 ± 1.11	24.51 ± 3.17*	30.70 ± 1.44	24.66 ± 1.64*	29.75±1.69	26.80 ± 2.82*
Hb (g/dL)	11.16 ± 0.42	7.21 ± 1.09*	11.26 ± 0.90	7.28 ± 0.77*	11.09±0.58	6.32 ± 0.98*
RBC ($x10^{12}$/L)	3.25 ± 0.27	2.46 ± 0.46*	3.39 ± 0.29	2.59 ± 0.55*	3.11 ± 0.44	2.41 ± 0.42*
MCV (fl)	90.54 ± 10.09	103.23±24.18*	91.28±11.18	98.52 ± 16.94	96.63±9.26	114.22±20.73
MCHC (g/dL)	38.22 ± 1.96	29.43 ± 2.76*	36.77 ± 3.89	29.49 ± 1.78*	37.29±0.83	23.51 ± 2.17*
WBC ($x10^9$/L)	9.53 ± 0.43	14.92 ± 2.85*	8.89 ± 0.97	19.15 ± 2.99*	9.28 ± 0.95	17.99 ± 2.70*
Heterophils ($x10^9$/L)	5.32 ± 0.38	2.96 ± 1.01*	3.81 ± 0.65	4.70 ± 1.28	4.32 ± 0.32	3.25 ± 0.70*
Lymphocytes ($x10^9$/L)	2.97 ± 0.38	11.85 ± 2.33*	2.73 ± 0.27	14.08 ± 2.33*	2.87 ± 0.15	14.37 ± 2.36*
Monocytes ($x10^9$/L)	0.20 ± 0.20	0.10 ± 0.03	0.00 ± 0.00	0.18 ± 0.04*	0.20 ± 0.20	0.14 ± 0.04
Eosinophils ($x10^9$/L)	0.00 ± 0.00	0.06 ± 0.02*	0.00 ± 0.00	0.21 ± 0.05*	0.00 ± 0.00	0.16 ± 0.05*

Mean values with asterisks* within rows differ significantly ($p < 0.05$) from their corresponding control values
Keys: A= Layers at early stage of production; B= Layers at mid/peak stage of production; C=Layers at late/end stage of production

Table 2: Comparison of mean haematological parameters of layers naturally infected with coccidiosis at different stages of production

Parameter	Group A	Group B	Group C
PCV (%)	24.51 ± 3.17^a	24.66 ± 1.64^a	26.80 ± 2.82^b
Hb (g/dL)	7.21 ± 1.09^a	7.28 ± 0.77^a	6.32 ± 0.98^b
RBC ($x10^{12}$/L)	2.46 ± 0.46	2.59 ± 0.55	2.41 ± 0.42
MCV (fl)	103.23 ± 4.09^{ab}	98.52 ± 2.86^a	114.22 ± 3.50^b
MCHC (g/dL)	29.43 ± 0.47^a	29.49 ± 0.30^a	23.51 ± 0.37^b
WBC ($x10^9$/L)	14.92 ± 2.85^a	19.15 ± 2.99^b	17.99 ± 2.70^{bc}
Heterophil ($x10^9$/L)	2.96 ± 1.01^a	4.70 ± 1.28^b	3.25 ± 0.70^a
Lymphocyte ($x10^9$/L)	11.85 ± 2.33^a	14.08 ± 2.33^b	14.37 ± 2.36^b
Monocyte ($x10^9$/L)	0.10 ± 0.03	0.18 ± 0.04	0.14 ± 0.04
Eosinophil ($x10^9$/L)	0.06 ± 0.02	0.21 ± 0.05	0.16 ± 0.05

Mean values with different superscripts ^{a,b,c} within rows are significantly different ($p < 0.05$)
Keys: A= Layers at early stage of production; B= Layers at mid/peak stage of production; C= Layers at late/end stage of production

Although Meskerem *et al*. (2013) reported a non-significant difference in Hb of chickens infected with coccidia parasites. Comparison of mean values of PCV among infected groups showed that PCV was higher in group C (late production stage), probably due to some level of resistance possessed or acquired by older birds to the infection, and some level of enhanced erythropoiesis due to high or large numbers of reticulocytes in circulation. So the increase in PCV may be a function of size and also due to higher mean MCV value in layer chickens at late production stage, which may suggest a more reticulocyte response in this group than in layer chickens at early and mid-production stages. This inference is supported by low values of RBC, haemoglobin concentration and MCHC. The fact that mean erythrocyte parameters, namely PCV, Hb, and RBC count, were lower than the reference values reported for layer chickens suggest that the natural coccidiosis in layers in the current study had led to anaemia. Blood loss from damaged intestinal mucosae, a widely reported consequence of infection with *Eimeria* species in chickens is a major cause of anaemia (Anosa *et al*., 2011; Meskerem *et al*., 2013; Sultan *et al*., 2021). Other possible causes of the anaemia in the coccidia-infected layer chickens in this study could be anorexia, resultant loss of nutritional elements from diarrhoea and malabsorption of nutrients due to intestinal lesions (Cowell, 2004; Samrawit *et al*., 2018). Although the mean MCV and MCHC values were within the reference intervals reported for this species (Meskerem *et al.,* 2013), the finding that the MCV was higher and MCHC was lower in the layer chickens naturally infected with coccidia suggests a macrocytic hypochromic anaemia and hence a regenerative anaemia as similarly reported by Patra *et al.* (2010) in broiler chickens infected with coccidia. Since mean MCV and MCHC were within the reference intervals in layer chickens at early and mid-production stages, in this study, the anaemia produced was normocytic normochromic which agrees with those of McDougald & Reid (1997) and Esievo (2017). On the other hand, the anaemia was normocytic hypochromic in the layer chickens at late stage of production, which had a mean MCV and MCHC values that were within and below the reference intervals, respectively.

In this study, the significantly higher mean values of WBC and differential leucocytes counts except for the heterophils and monocytes, in infected layers when compared with the control groups, agree with previous reports (Hirani *et al*., 2007; Patra *et al.,* 2010; Meskerem *et al*., 2013; Samrawit *et al*., 2018; Khaligh *et al.,* 2019; Ahmed El-Shazly *et al*., 2020). Low mean values of heterophil counts observed in infected layers of groups A and B in the present study agree with findings in the reports of Zulpo *et al*. (2007). While heteropaenia was observed in the present study, Wakenell (2010) and Meskerem *et al*. (2013) reported heterophilia in coccidia infected-chickens. Heteropaenia seen in the present study may be due to possible overwhelming concurrent secondary bacterial infection. The significantly elevated WBC and lymphocyte counts in infected birds across all production stages could be attributed to an enhanced antibody production. Also, chronic antigenic stimulation may result in a greatly expanded circulating lymphocyte pool because the primary functions of the lymphocytes are immunological response, humoral antibody formation and cell mediated immunity (Irizaary-Rovia, 2004). The eosinophilia observed in infected layers when compared to the corresponding eosinophil count in the control birds might probably be the result of interactions between eosinophils and mast cells, basophils and IgG and IgE to modulate the inflammatory reaction in parasitic infections as in the previous reports (Irizaary-Rovira, 2004; Meskerem *et al*., 2013; Ahmed El-Shazly *et al*., 2020; Sultan *et al*., 2021). The finding that the WBC, lymphocyte and eosinophil counts were higher in the layer chickens at mid-stage of production suggests that more damage was caused by the coccidial infection in this group of birds since the degree of leukocyte response could serve as a measure of severity of a disease at this stage of production (Coles, 1986; Esievo, 2017).

In conclusion, this research demonstrated that layer chickens at early production stage with PCV 24.51 ± 3.17% were most severely affected by coccidiosis, following closely by the layers at mid/peak production stage (PCV: 24.66 ± 1.64%). The highest mean WBC (19.15 ± 2.99 x 10^9/L) was recorded in layers with coccidiosis at mid/peak production stage, which showed they were better in mounting inflammatory response when compared to the mean values of layers at early (14.92 ± 2.85 x 10^9/L) and late (17.99 ± 2.70 x 10^9/L) stages of production. It is recommended that a robust and all-inclusive management system against coccidiosis should be accorded to layers at early stage of production for optimal production, since this is the critical stage that precedes peak of production.

Conflict of interest

The authors declare that there is no conflict of interest.

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