Original Research Article

Changes in some haematological parameters of two breeds of chicken inoculated with *Eimeria* oocyst

ABSTRACT

Coccidian parasites, especially those in the genus *Eimeria*, are intestinal parasites common in poultry. They cause coccidiosis in infected birds. The effect of the parasites on the haematology of infected chickens was studied using broilers and white-leghorns. A total of 30 day-old chicks, fifteen each from the two breeds, were procured for the experiment. They were grown to three weeks old, and confirmed to be free from any intestinal parasite, before inoculating 10 birds each, randomly selected from the two breeds, with *Eimeria* oocyst. The packed cell volume (PCV), thrombocyte number, leukocyte number and proportion of leukocyte types were determined from blood samples obtained from the birds within ten days after inoculation and confirmation of infection. Infected broilers (B) and white-leghorns (WL) both had packed cell volumes that were lower than the control (C) (B. 31.8±2.2, WL. 32.8±1.8, C. 34.3±2.6). However, estimates of the effect sizes suggested that coccidiosis accounted for only about 5% of the observed variance in the PCV of broilers and 2% in white-leghorns. The disease significantly affected the thrombocyte number of infected broilers only (B. 7.0±0.5, WL. 10.4±1.1, C. 10.0±0.8). A remarkable difference was found in the leukocyte numbers of infected birds (B. 13.4±2.2, WL. 21.4±3.2, C. 14.9±2.0). However, the infection increased the proportion of circulating monocytes from 1% in control to 5% in the infected birds regardless of their breed. The observation that broilers are prone to parasite induced thrombocytopaenia, supported by the wide disparity in the leukocyte numbers of the two breeds of chicken during an infection, suggest that white-leghorns are better adapted to withstand the effects of coccidiosis when compared with broilers reared under the same environmental conditions.

Keywords: Haematology, Eimeria, Broilers, White-leghorns

1. INTRODUCTION

Coccidiosis of poultry is caused by protozoan parasites of the genus *Eimeria*. The parasites invade the epithelial tissues of birds' intestines leading to the destruction of host cells due to the disruption of the cellular organization, cytoplasm blubbing and release of cell organelles [1]. Large numbers of *Eimeria* cause damage to the bird's intestinal lining, resulting in leakage of proteins, including plasma into the bowel. This disrupts digestive processes or nutrient absorption, dehydration, anaemia and increased susceptibility to other disease agents [2].

Eimeria species are ubiquitous. About seven species are widely recognized as the causative agents of coccidiosis in Nigeria, of which *E. tenella*, *E. necatrix, E. maxima* and *E. brunette* are highly pathogenic; *E. acervulina* and *E. mitis* are less pathogenic, whilst *E.*

praecox is regarded as the least pathogenic [3]. Infections are usually not caused by a single species of *Eimeria*. Instead, the disease can be considered to come as a result of a mixture of *Eimeria* species. In reality, the parasites develop in different gut regions and depending on the magnitude of the infection; they can cause mild-to-severe lesions [4]. Chickens become infected when they swallow food or water contaminated with sporulated oocysts. Inside the intestine of the host, the sporozoites are released from oocysts they enter the cell, round up, grow and become first-generation schizonts. This divides many times producing either a few or many offspring known as first-generation merozoites. The number produced depends on the species of *Eimeria* involved. Each merozoite may enter another intestinal cell to become second-generation schizonts and later develop to second-generation merozoites. This cycle may be repeated several times. Because of this cyclic multiplication, many intestinal cells are destroyed [5, 6]. Some second-generation merozoites enter new epithelial cells to begin gametogony. Microgametocytes bud to form many slender, biflagellated microgametes that leave their host cell and enter cell containing macrogametes, where fertilization occurs [5]. Oocysts are produced. They rupture from the intestinal cell and are passed to the external environment with faeces [6]. Oocysts appear in faeces within six days of infection and are passed for several days because not all second-generation merozoites re-enter host cells simultaneously [5].

According to the poultry management systems practiced in Nigeria, chickens may be grouped into two major breeds. The intensively managed breeds, usually kept in confined spaces, and the extensively managed breeds that move about freely in the environment. Coccidiosis is mostly reported as a problem for farmers keeping the intensively managed breeds of chicken. This is probably because outbreaks are common to birds kept in confinements and less common in non-confined birds [3]. The extensively managed breeds may have developed some form of resistance to the disease. The study aimed to determine the effects of *Eimeria* species on the haematology of an intensively managed chicken and an extensively managed chicken with emphasis on the packed cell volume, thrombocyte number, leukocyte number and leukocyte types. Also, an argument was provided, stating the reasons why one breed of chicken may be considered better adapted to withstand the effects of the coccidian parasites, from a haematological perspective.

2. MATERIAL AND METHODS

2.1 Breeding Site

The study was conducted in the Department of Parasitology and Entomology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka (6.2459^{o}N, 7.1199^{o}E). Brooding of the chicks was done in a 1.35×0.9×1.2m cage. Inoculated birds were isolated in 0.45×0.45×0.6m cages screened with window nets to prevent mechanical transmitters (such as flies) to make contact with their droppings.

2.2 Study Design

This study is an experimental work in which the haematological parameters of the birds were measured after inoculating them with oocyst of *Eimeria* species. Statistically, it is a randomized posttest only control group design.

2.3 Procurement of Experimental Birds

The experiment was carried out using commercial broilers (Ross 308 strain) and white-leghorns. Broilers are white chickens that are generally reared in confinement. They were obtained from Aggrited Farm in Ibadan (Nigeria). The white-leghorns are semi-intensive or extensively managed breeds of chicken. They were sourced from the local market (Eke-Awka Market) in Awka.

2.4 Brooding of the Chicks

Thirty day-old chicks (i.e. 15 broilers and 15 white-leghorns) were used for the experiment. After procurement, the chicks were kept in a deep litter cage. Glucose (25g/ml of water) and Multivitamins (25g/ml of water) were administered to the birds (through their drinker) on arrival. Kerosene lantern was used to provide warmth to the animals. Warmth was provided for about twenty-four hours on the day of arrival. This was reduced to twelve hours daily (i.e. every evening) for two weeks. After two weeks the lantern was removed. The birds were fed *ad-libitum* with compounded feed (starters mash) and boiled water throughout the study. Brooding lasted for three weeks to enable the birds acclimatize with the environment.

2.5 Procurement of Coccidian Parasites

Coccidian parasites were sourced from fresh chicken droppings and litter samples obtained from various poultry farms with known cases of coccidiosis in Awka.

2.6 Isolation and Purification of the *Eimeria* Oocyst

Eimeria oocysts were isolated from the chicken droppings and litter samples. Oocysts were purified following a modified protocol of Eckert 1995 [7]. The droppings/litter samples were transferred to a five-litre (5L) plastic bucket. After that, 2 litres of tap water was added, and with the aid of hand gloves, the mixture was homogenized. The homogenate was filtered through a 250µm-pore size sieve, transferred to a litre (1L) beaker, and then allowed to sediment for twelve hours. After sedimentation, the supernatant was discarded, and the sediment was re-suspended in saturated saline solution, centrifuged at 1300rpm for ten (10) minutes. After centrifugation, the suspended oocysts were collected, confirmed microscopically and washed with tap water by centrifugation at 1300rpm for ten (10) minutes. Finally, purified oocysts were incubated for sporulation in 2 percent potassium dichromate solution at room temperature for forty-eight (48) hours. Sporulated oocysts were stored at 4ºC for future use. Post-mortem examinations carried out on the carcasses of some chicks inoculated with the purified oocyst revealed that the inoculum contained multiple *Eimeria* species.

2.7 Pre-inoculation test for coccidiosis and other intestinal parasites

Before inoculation, stool samples from all the thirty birds were examined to confirm they were free from coccidiosis and other intestinal parasites. This was done using direct wet-mount technique, and Willis floatation technique described by the World Health Organization [8]. Approximately 0.5g of stool sample from each bird was placed in separate test-tubes. The tubes were filled with 2.5ml saturated Sodium chloride (Willis solution). The mixtures were emulsified using applicator sticks and more Willis solution was added to fill the tubes to the top. Cover-slips were placed on top of each test-tube, left for 10 minutes and after the cover-slips were placed on microscope slides and examined using ×10 objective lens.

2.8 Inoculation of the Birds

On the twenty-first day of rearing the birds, twenty birds (ie, 10 broilers and 10 white-leghorns) were randomly selected from the flock and transferred to separate cages (the isolation cages). These birds were inoculated orally with 1ml of solution containing the sporulated oocysts. The remaining ten birds (ie, 5 broilers and 5 white-leghorns) were kept as control in the deep litter cage.

2.9 Monitoring and Confirmation of Infection

The Inoculated birds had their stool samples monitored daily, between the third and sixth day after inoculation, for the presence of *Eimeria* oocyst using direct wet-mount technique and Willis floatation technique described in 2.7. These methods were also used for post-inoculation check for possible contamination of the inoculum by other intestinal parasites. Stool samples from birds kept as control were also monitored to ensure the animals were free from coccidiosis and other intestinal parasites during the experiment.

2.10 Collection of Blood Samples

Blood samples were obtained from the wing veins of the birds on or before the tenth day post-inoculation, only after infection has been confirmed (in infected birds). Birds with severe symptoms of weakness had their blood sample collected slightly earlier than the others. Each bird was placed on its side with the ventral surface toward the operator. The wing lying

uppermost was turned back and 21 gauge needles were used to puncture the brachial vein where it crosses the elbow. About 2ml of blood sample was collected from each bird in a container with EDTA (Anticoagulant).

2.11 Estimating the Packed Cell Volume

Micro-haematocrit capillary tubes were filled to three-quarters with the blood samples. One capillary tube was used for one animal sample. One end of the tubes was sealed. All sealed tubes were centrifuged at 3000rpm. The packed cell volume was read off a micro-haematocrit scale.

2.12 Estimating the Thrombocyte number

Thrombocyte number was estimated within two hours of sample collection. Well mixed anti-coagulated blood samples of the birds (20µL) were dispensed into test-tubes containing Ammonium oxalate (0.38ml of 1%w/v). These mixtures were used to fill a haemocytometer (one after the other) and left undisturbed for 3 minutes. The thrombocytes were counted using ×40 objective lens of the microscope. Thrombocyte number (per cubic millimetre) was estimated using the formular:

$$Thrombocyte\ (\frac{10^3}{mm}) = \frac{N \times 20 \times 1}{0.02}$$

Where N is the number of cells counted.

2.13 Estimating Leukocyte number

The diluting fluid used for this procedure (Turk's solution) was prepared by adding 2ml of Glacial acetic acid, 1ml of 1% Gentian violet and distilled water to make up the volume to 100ml. The diluting fluid (0.38ml) was dispensed into small test-tubes using a micro-pipette. A volume of 20µl of blood was transferred into the tubes containing the diluting fluid (one test-tube for one animal sample). This gave a 1 in 20 dilution. The diluted blood was used to fill the haemocytometer (one sample at a time). This was left for 3 minutes. The number of leukocytes was counted using ×10 objective. The leukocyte number (per cubic millimetre) was estimated using the formula below:

$$Leukocyte\ (\frac{10^3}{mm}) = \frac{N \times 20}{4 \times 0.01}$$

Were N is the number of cells counted.

2.14 Estimating the Proportion of Leukocyte types

A drop of blood from each animal sample was used to make thin blood films. The films were allowed to air-dry, fixed in absolute methanol for 2-3 minutes and stained with Geimsa stain (1:10 dilution) for 30minutes. The thin blood films were examined using oil immersion objective. For each slide, 100 leukocytes were counted and the number of each type (Heterophils, Lymphocytes, Eosinophil, Monocytes and Basophils) was recorded. The proportion of each type of leukocytes was reported in percentages.

2.15 Data Analysis

Data analysis was done using Statistical Package for Social Sciences (SPSS) version 23. All charts were plotted with Microsoft excel version 2010. Descriptive statistics including the mean and standard errors of the mean was calculated for every haematology parameter. The proportion of leukocyte types was summarized in pie charts. The t-test for independent samples was used to test for significant differences between the experimental and control groups at 0.05 significant levels. The effect size was estimated using the formula:

$$E = \sqrt{\frac{t^2}{t^2 + df}}$$

Where, 't' is the calculated t-test value and 'df' is the degrees of freedom.

3. RESULTS

All the thirty (30) birds were tested for the presence of *Eimeria* parasites as well as other intestinal parasites and were confirmed to be free from any parasites before they were inoculated. Out of all the twenty (20) birds that were inoculated with sporulated oocysts of *Eimeria* species, only two (2) broilers developed acute coccidiosis, showing symptoms like weakness, loss of appetite and diarrhea mixed with blood. One died on day 7 and the other on day 9 post-inoculation. The remaining eighteen (18) birds showed no symptoms even though they all tested positive to the parasite. No evidence of contamination of the inoculum, by other intestinal parasites, was found. The control group remained free from coccidiosis and other intestinal parasites throughout the study (Table 1).

Table 1. Infection status of the birds before and after inoculating with *Eimeria* oocyst.

Stage	Broilers			White-leghorns			Control		
	No. tested	+ve for coccidia	+ve for other parasites	No. tested	+ve for coccidia	+ve for other parasites	No. tested	+ve for coccidia	+ve for other parasites
Pre-inoculation	10	0	0	10	0	0	10	0	0
Post-inoculation	10	10	0	10	10	0	10	0	0

[a]*'No.' is the short form for number. '+ve' stands for positive. The control is a pool of five broilers and five white-leghorns that were not inoculated.*

The infected birds' packed cell volume (PCV) was observed to be lower than the control (un-infected birds). Broilers had the lowest mean PCV (t= 0.69, df= 10, *P*=.51, E^2= 0.05), while the white-leghorns had a mean PCV that was higher than the broilers but lower than the control group (t= 0.42, df= 10, *P*=.68, E^2=0.02). The effect size (E), converted to percentage, shows that coccidiosis explained 5 percent of the variations in the packed cell volume of broilers and 2 percent of the variation in the PCV of white-leghorns.

The mean thrombocyte number in infected broilers was lower than the control (t=3.42, df=9, *P*=.007, E^2=0.57), and the effect size also suggests that coccidiosis accounts for 57 percent of the observed difference. However, in infected white-leghorns the mean thrombocyte number was slightly higher than the control (t=-0.31, df= 10, *P*=.76, E^2=0.01).

The infected broilers had a leukocyte number that was lower than the control group (t=0.52, df=10, *P*=.61, E^2=0.03), infected white-leghorns had a mean leukocyte number that was higher than the control group (t=-1.81, df=10, *P*=.10, E^2=0.49). The effect size suggests that coccidiosis explains a 49 percent increase in leukocyte number in white-leghorns. Table 2 summarizes the above observations.

Table 2. Mean packed cell volume, thrombocyte and leukocyte numbers of infected birds and control.

Haematology Parameters	Broilers (Inf.)	White-leghorns (Inf.)	Control
Packed cell volume	31.8±2.2	32.8±1.8	34.3±2.6
Thrombocyte number	7.0±0.5	10.4±1.1	10.0±0.8
Leukocyte number	13.4±2.2	21.4±3.2	14.9±2.0

[b]*'Inf.' is the short form for infected. The values in the table represent the mean and standard error of the mean. The unit for the mean packed cell volume is in percentages. Mean thrombocyte and leukocyte numbers are in cubic millimeter (ie, ×10^3). The control is a pool of five broilers and five white-leghorns that were not inoculated.*

Two-third of all leukocyte types counted, regardless of the breed and infection status of the birds, comprised of lymphocytes and the heterophils followed this. In infected birds (broilers and white-leghorns), 5 in every 100 leukocytes counted were monocytes. This contrasts with the control group (un-infected birds) where 1 in every 100 leukocyte counted was a monocyte. Thus, there was an increase in the number of monocytes in infected birds (Fig.1-3).

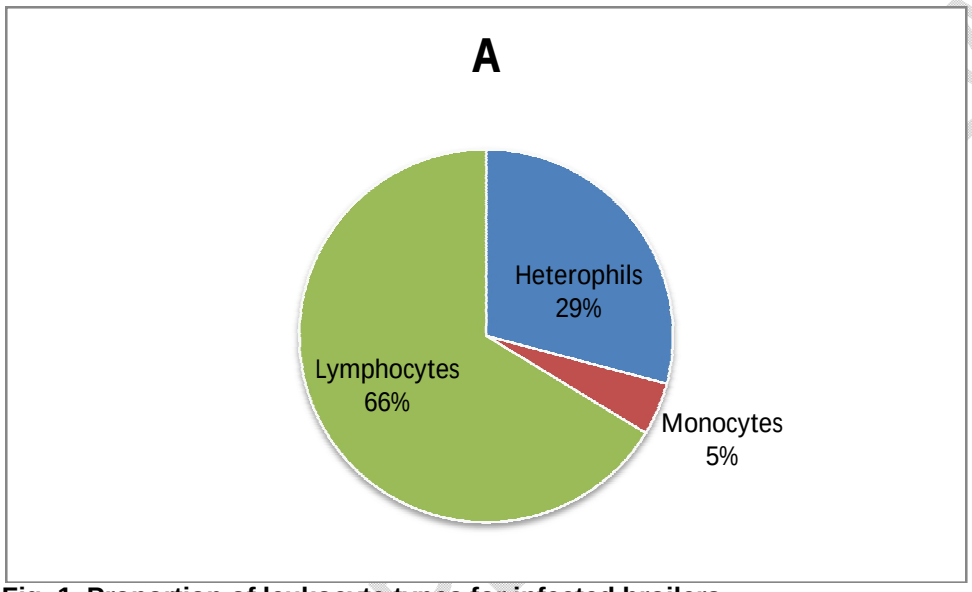

Fig. 1. Proportion of leukocyte types for infected broilers.

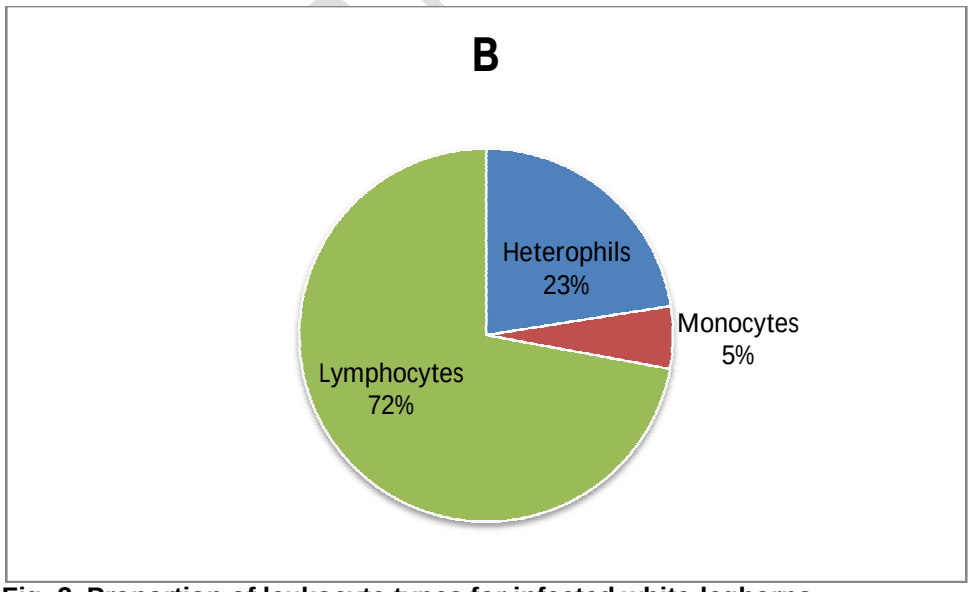

Fig. 2. Proportion of leukocyte types for infected white-leghorns

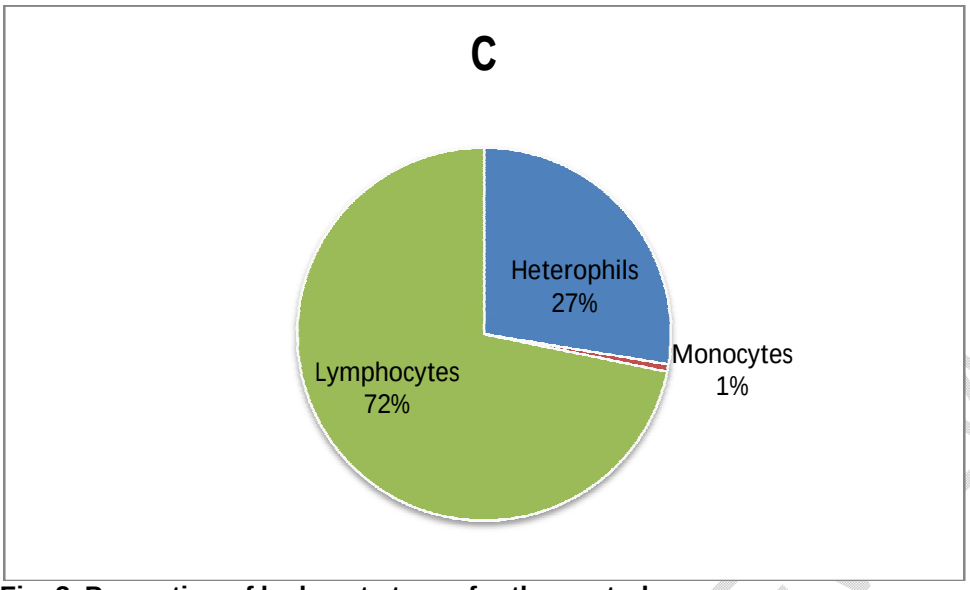

Fig. 3. Proportion of leukocyte types for the control.

4. DISCUSSION

The normal range for the packed cell volume (PCV) in birds is 35-55 percent. When the value of the PCV is less than 35 percent, it is an indication of anaemia [9]. From our result, the PCV value for the un-infected birds is the closest to the normal range while that of the infected birds are far below the normal range. Adamu [10] and Melkamu [11] also reported a PCV value below the normal range. However, estimates of the effect sizes, in the present study, showed that the coccidian parasites accounted for only about 5 percent of the observed variance in the PCV of broilers and 2 percent in white-leghorns. A period of ten days (from inoculation to blood sample collection) was probably insufficient for any significant change to be observed in the packed cell volume. This means that more time is needed to fully estimate the effect of coccidiosis on this parameter.

Like their mammalian counterpart, avian thrombocytes play a primary role in haemostasis (arrest of bleeding). When a blood vessel is damaged, the cells stick together to form a plug that seal the wound. A normal thrombocyte count ranges from 10-15 per 1000 erythrocytes for most birds [9]. Only the broilers were observed with a thrombocyte number below this normal range in the present study and coccidiosis explained up to 57 percent of this variance. This condition, known as thrombocytopaenia, usually indicates excessive demand for thrombocytes [9].

Both the broilers and white-leghorns showed an increase in the proportion of circulating monocytes, however, there was a remarkable difference in their leukocyte numbers. While the leukocyte number of infected white-leghorns was much higher than the un-infected birds, the leukocyte number of the infected broilers was slightly lower than the un-infected birds. Coccidiosis stimulated the host's immune system to produce more leukocytes (mainly the monocyte type) especially in infected white-leghorns where it accounts for about 49 percent of the variance in the leukocyte number of this breed. Previous researchers have reported leukocytosis and monocytosis in broilers [10, 12]. Avian monocyte, a fraction of the total leukocytes types, is known to destroy foreign organisms and defend the host against infectious agents [13]. This leukocyte type has been found in certain disease conditions such as avian chlamydiosis, mycotic, and bacterial granulomas that produce monocyte chemotactic agents [9].

From a haematological point of view, white-leghorns (extensively managed breeds) seem to be better adapted to withstand the effects of coccidian parasites when compared with broilers (intensively managed breeds) reared under the same environmental conditions. During coccidiosis, the developing parasites disrupt the blood vessels that service the intestinal epithelium, leading to haemorrhage. In response to the blood loss, there is a demand for circulating thrombocytes which tries to arrest the bleeding. The present study has demonstrated that infected broilers are prone to parasite induced thrombocytopenia, which might have resulted from excessive demand of thrombocytes. Depletion in the number of these cells affects haemostasis, leading to anaemia. On the contrary, no significant effect was observed on the thrombocyte number of infected white-leghorns. This strongly suggests that the later breed of chicken has adapted to withstand the excessive demand of thrombocytes, and possibly anaemia, caused by coccidian parasites. This study shows that coccidiosis stimulates the host's defense system to produce more leukocytes (especially monocytes) to fight the invading parasites. A wide disparity exists in the leukocyte numbers of infected broilers and infected white-leghorns. This observation can be used to support the idea that white-leghorns have a better chance of containing an infection than broilers. The longer life-span of extensively managed breeds as well as their long-term exposure to a wide range of environmental conditions possibly led to the development of adaptations that mitigates the effects of common poultry diseases such as coccidiosis.

It is worth noting that estimating leukocyte numbers using Turk's solution (as described in the methods) was challenging. This difficulty arose because the solution does not lyse the erythrocytes completely. Natt and Herrick noted that the nuclei of avian erythrocytes are not destroyed in the usual procedure for mammalian cell enumeration [14]. Thus, we recommend the diluting fluid proposed by Natt-Herrick during leukocyte enumeration.

5. CONCLUSION

Broilers (intensively managed breeds) and white-leghorns (extensively managed breeds) can be infected by *Eimeria* species. This infection results in observable changes in the haematology of infected chickens. Examination of blood samples collected from the birds, within ten days after inoculating them with the parasites and confirming the infection, showed a packed cell volume (PCV) below the normal range in infected chickens. However, estimates of effect sizes suggested that coccidiosis accounted for only about 5 percent of the observed variance in the PCV of broilers and 2 percent in white-leghorns. The disease significantly affected the thrombocyte number of infected broilers only. Although the infection increased the proportion of circulating monocytes in both broilers and white-leghorns, a remarkable difference was found in their leukocyte numbers. The proposition that broilers are prone to parasite induced thrombocytopaenia, backed by the wide disparity in the leukocyte numbers of the two breeds of chicken during an infection, strongly suggest that white-leghorns are better adapted to withstand the effects of coccidiosis when compared with broilers reared under the same environmental conditions.

ETHICAL APPROVAL

All authors hereby declare that principles of laboratory animal care were followed, as well as specific national laws where applicable. All experiments have been examined and approved by Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC). Ref. NAU/AREC/2023/00013.

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