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ORIGINAL ARTICLE

White-Leghorn Chickens Are Less Susceptible To The Haemo-Pathological Effects Of Avian Coccidiosis Compared To Commercial Broilers

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SUMMARY

The present study was conducted to investigate the differences in susceptibility of the white-leghorn breed of chicken to the haemo-pathological effects of avian coccidiosis when compared to commercial broilers. An experiment was performed with twenty-day-old chicks (10 white-leghorns and 10 broilers) which were grown for 3-weeks before infecting them. The chicks were first confirmed to be free from intestinal parasites then 5 birds each, randomly selected from the two breeds, were orally inoculated with *Eimeria* oocyst. Thrombocyte numbers and packed cell volumes (PCVs) were measured to indicate thrombocytopaenia and anaemia respectively. This was determined from blood obtained from the chicks on the 10th day post-inoculation. An estimate of the effect-size revealed that coccidiosis produced a negligible effect (a 5% decrease) on the thrombocyte number of white-leghorns. However, no effect was observed on the PCV of the same breed. On the contrary, a 47% decrease in the thrombocyte number and a 29% decrease in the PCV of broilers can be attributed to coccidiosis. The extensive system of breeding white-leghorns satisfied the conditions necessary for natural selection of favourable traits to occur in the breed. This might explain the difference in susceptibility to the haemo-pathological effects caused by *Eimeria* parasites because commercial broilers that are managed intensively, do not survive long enough, thereby disrupting natural selection. The difference implies that an apparently healthy flock of white-leghorn may be a reservoir of the infection, which could be transmitted to susceptible breeds.

Key words: White-leghorn, broiler, haemo-pathological, coccidiosis, susceptibility.

INTRODUCTION Of the twelve varieties of leghorn chickens, the single comb white-leghorn is the most popular and is also the leading egg producer of the world (Encyclopedia Britannica, 2024). This breed is prolific, with hens laying from 280-320 eggs annually. Because they are excellent foragers, they are often managed extensively (Agrifarming, 2023). Commercial broilers, on the other hand, are chickens that are bred to be fast growing for the purpose of meat production. After hatching, this breed is moved from the hatcheries to the farms as day-olds where they are managed until they attain the requisite weight for slaughter. Globally, over 70% of broiler chickens are kept intensively (CIWF, 2019).

Avian coccidiosis is an enteric disease caused by intracellular protozoan parasites of the genus *Eimeria* which are of economic importance in the poultry industry. The destruction of intestinal mucosa during acute invasion and egress of the sporozoites and merozoites of *Eimeria* species is mainly responsible for the pathogenesis of avian coccidiosis (Bould *et al.,* 2009). A well-known sign of the disease in infected birds is anaemia. However, a recent study on the haematology of chicks inoculated with *Eimeria* oocysts has shown that these parasites are capable of causing thrombocytopaenia in infected chicks (Ekezie *et al.,* 2023). Therefore, the haemo-pathological effects of coccidiosis may be defined by the thrombocytopaenia and anaemia caused by parasites of the genus *Eimeria* in susceptible birds.

Previous studies have shown that poultry breeds differ in their ability to resist coccidiosis. For instance, the fayoumi line was shown to be the most resistant of five outbred lines of chicken (Pinard-Van Der Laan *et al.,* 1998). While some poultry breeds resist coccidiosis, commercial broilers are highly affected by the disease. About 96 percent of the economic losses in the broiler industry have been attributed to cocidiosis (Bera, *et al.,* 2010). This study was conducted to investigate the differences in susceptibility of the white-leghorn breed of chicken to the haemo-pathological effects of avian coccidiosis when compared to commercial broiler breed. A hypothesis was proposed on the difference in susceptibility, and also the implication for the difference in susceptibility between the two breeds of chicken was discussed.

MATERIALS AND METHODS

Study Site

This experiment 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 built in the department. Inoculated birds were isolated in 0.45×0.45×0.6m cages screened with wire mesh to prevent mechanical transmitters (of the parasites) from making contact with the droppings of the infected chicks.

Experimental Design

The experiment was conducted as a randomized posttest only control group design. It was made of two experimental groups and two control groups. Experimental groups were made of five (5) white-leghorn chickens and five (5) commercial broilers (Ross 308 strain). The control groups were made

of five (5) white-leghorn chickens and five (5) commercial broilers.

Procurement of the Birds

White-leghorn breed used for the experiment were procured from the local market (Eke-Awka Market) in Awka, Anambra State (6.2074807ºN, 7.0684160ºE). Commercial broilers (Ross 308 strain) were procured from Agrited Nigeria Ltd. Ibadan, Oyo State (7.279850ºN, 3.859405ºE).

Brooding of the Chicks

After procurement, the chicks were kept in a deep litter cage. Glucose (25g/ml of water) and Multivitamins (Multivita-extra®, 25g/ml of water) were administered to the birds (through their drinker) on arrival. Kerosene lantern was used to provide warmth to the birds. 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 (Top-feeds®, starters mash) and cooled boiled water throughout the study. Brooding lasted for three weeks.

Isolation and Purification of the *Eimeria* Oocyst

Eimeria oocysts were isolated from chicken droppings obtained from a commercial poultry farm with a known case of avian coccidiosis. Oocysts were purified following a modified protocol of Eckert 1995 (Zaida *et al*., 2020). The droppings were transferred to a five-litre (5L) plastic bucket into which two-litres of tap water was added and the mixture homogenized with the aid of handgloves. The homogenate was filtered through a 250µm-pore size sieve, transferred to a one 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 oocyst were collected, confirmed microscopically and washed with tap water by centrifugation at 1300rpm for ten (10) minutes. Finally, purified oocysts were incubated in 2 percent potassium dichromate solution at room temperature for forty-eight (48) hours to allow for sporulation. 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.

Pre-inoculation Test for Avian Coccidiosis and other Intestinal Parasites

Before inoculation, samples of droppings from all the twenty 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 (WHO, 2003). Approximately 0.5g of the droppings 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 of the Willis solution was added to fill the tubes to the brim. 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.

Inoculating the Birds On the twenty-first day of rearing the birds, ten birds (i.e. 5 white-leghorns and 5 broilers) were

randomly selected from the flock and transferred to the isolation cages. The isolated birds constituted the experimental groups. These birds were inoculated orally with 1ml of solution containing the sporulated *Eimeria* oocysts. The remaining ten birds (i.e. 5 white-leghorns and 5 broilers) were kept as control in the deep litter cage.

Monitoring and Confirmation of Infection

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

Collecting Blood Samples

After the infection was established in inoculated birds, blood samples were obtained from them on the tenth day post-inoculation. Each bird was placed on its side with the ventral surface toward the operator. The wing lying uppermost was turned back to locate the brachial vein. This area was first sterilized with ethyl-alcohol and a 21 gauge hypodermic needle was 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).

Measuring the Thrombocyte Number (to indicate thrombocytopaenia)

Thrombocyte number was estimated within two hours of blood 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:

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

Where, N is the number of cells counted.

Measuring the Packed Cell Volume (to indicate anaemia)

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

Estimating the Relative Effect-Sizes

The proportion of total variability in the thrombocyte number and packed cell volume which coccidiosis accounts for (i.e. the effect-sizes), were estimated for white-leghorns and broilers. This was achieved using the eta squared formula for calculating effect-sizes given below (Avwokeni, 2013), where *t* is the calculated t-test value and *df* is the degrees of freedom. This index was used to compare the relative strength of the haemo-pathological effects caused by *Eimeria* spp in white-leghorns with commercial broilers.

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

E^2 can be converted to percentages by multiplying this value by 100.

Data Analysis

Data was analyzed with Statistical Package for Social Sciences (SPSS) version 23. The mean (and standard error) thrombocyte number and packed cell volume was calculated for the four groups. T-test for independent samples was used to test for significant differences between the experimental and control groups at $P< 0.05$ level of significance.

RESULTS

All twenty birds were screened for intestinal parasites (including *Eimeria spp*) before inoculating them with the coccidian parasite. None of the birds were infected prior to inoculation (TABLE I). Both broilers and white-leghorns tested positive to *Eimeria spp* after they were inoculated with the parasites. No evidence of contamination by other intestinal parasites was found post-inoculation. The control groups remained free from intestinal parasites during the study.

TABLE I. Infection status of the birds before and after inoculating with *Eimeria* oocyst

Stage	**Wl. (Exp)**			**Wl. (Cont)**			**Br. (Exp)**			**Br. (Cont)**		
	No. Exam	**+ve *Eimeria***	**+ve other parasites**	**No. Exam**	**+ve *Eimeria***	**+ve other parasites**	**No. Exam**	**+ve *Eimeria***	**+ve other parasites**	**No. Exam**	**+ve *Eimeria***	**+ve other parasites**
Pre-inoculation	5	0	0	5	0	0	5	0	0	5	0	0
Post-inoculation	5	5	0	5	0	0	5	5	0	5	0	0

Wl. and Br. are short forms for white-leghorns and broilers respectively. Exp. and Cont. represents experimental groups and control groups respectively. No.= number, +ve= positive.

In TABLE II, the mean values for the thrombocyte number and packed cell volume of infected and un-infected white-leghorns was presented. A decrease in the thrombocyte number was observed (t= -0.54, df =6, *P*= .61, E^2= 0.05). However, the effect-size (E) converted to percentage showed that 5% of the total variance in the thrombocyte number of white-leghorns can be attributed to coccidiosis. The effect on the packed cell volume shown in the table was not caused by coccidiosis. *Eimeria* species are not known to increase avian PCV. Therefore, the observation (t= 1.08, df =6, *P*= .32, E^2= 0.16) resulted from extraneous variables.

TABLE II. Effect of coccidiosis on the thrombocyte number and packed cell volume of white-leghorns

	Value for Exp. group	**Value for Cont. group**	**Direction of effect**	***P*-value**	**Effect-size (E^2×100)**
Thrombocyte number	10.4±1.1	11.3±1.2	-ve	.61	5%
PCV	32.8±1.8	29.3±2.9	+ve	.32	16%*

The asterisk (*) was used to indicate that the effect was not produced by coccidiosis. The value in the table represents the mean and standard errors of the mean. The unit for the thrombocyte number is in cubic millimetres while that of PCV was given in percentage. Direction of effect was gotten from the sign of the calculated t-test value or as the difference between the experimental (Exp) and control groups (cont) (i.e. Exp.> Cont. = +ve; Exp. < Cont. = -ve).

In TABLE III, the mean values for the thrombocyte number and packed cell volume (PCV) in infected and un-infected broilers was presented. The infection led to a decrease in the thrombocyte number (t= -2.49, df =7, *P*= .04, E^2= 0.47) as well as the packed cell volume (t= -1.7, df= 7, *P*= .13, E^2= 0.29) of broilers. When converted to percentages, the effect-size (E) showed that 47% of the total variance observed in thrombocyte number and 29% of the total variance observed in packed cell volume can be attributed to coccidiosis.

TABLE III. Effect of coccidiosis on the thrombocyte number and packed cell volume of broilers

	Value for Exp. group	**Value for Cont. group**	**Direction of effect**	***P*-value**	**Effect-size (E^2×100)**
Thrombocyte number	7.0±0.5	9.0±0.7	-ve	.04	47%
PCV	31.8±2.2	38.0±3.1	-ve	.13	29%

The value in the table represents the mean and standard errors of the mean. The unit for the thrombocyte number is in cubic millimetres while that of PCV was given in percentage. Direction of effect was gotten from the sign of the calculated t-test value or as the difference between the experimental (Exp) and control groups (cont) (i.e. Exp.> Cont. = +ve; Exp. < Cont. = -ve).

DISCUSSION

A Comparison of the estimated effect-sizes (attributed to coccidiosis) revealed that the effect of *Eimeria* species on the thrombocyte number of infected white-leghorns was negligible ($P > .05$). However, no effect (due to coccidiosis) was observed on the packed cell volume of the same breed. On the contrary, the parasites significantly affected the thrombocyte number ($P < .05$) of commercial broilers leading to a 47% decrease in the platelet count. There was also a 29% decrease in the packed cell volume of the same breed. Ekezie *et al.* (2023) argued that white-leghorn chickens were better adapted to withstand the effects of coccidian parasites when compared to broilers reared under the same environmental conditions. Their argument was based on the premise that broilers are prone to parasite induced thrombocytopaenia, while white-leghorns can withstand the excessive loss of thrombocytes resulting from the infection.

An interesting inference that could be made from our results is that a positive association exists between the two parameters that were measured. In TABLE II, no effect was found on the packed cell volume of infected birds when the effect on the thrombocyte number was negligible. However, the packed cell volume decreased when there was a significant decrease in the thrombocyte number of the infected birds (see TABLE III). This relationship suggests that thrombocytopaenia is responsible for anaemia that occurs in avian coccidiosis.

Hypothesis on the observed difference in susceptibility to coccidiosis

We propose that white-leghorns were once as susceptible to the haemo-pathological effects of coccidiosis as commercial broilers. However, susceptible white-leghorns that recover naturally from an onslaught of coccidiosis are the ones with mutations (or a mutation) in the gene(s) that control thrombocytopoiesis, which enabled them to make more thrombocytes than the average bird. While majority of infected birds die due to anaemia that results from thrombocytopaenia, these birds (with mutant genes) survive long enough for the immune system to eliminate the parasites. These traits that mitigate the effects of coccidiosis were selected by nature leading to an increase in the population of white-leghorns that are less susceptible to the haemo-pathological effects of coccidiosis. As a result of continued selection over many generations, the average thrombocyte count of white-leghorns increased and their immune system became efficient in recognizing and eliminating *Eimeria* parasites that invades the host.

The above hypothesis explained the difference in mean thrombocyte count found between white-leghorns and broilers that were kept as control in the current study (compare the mean thrombocyte number for the control groups in TABLE II and III). It also explained the remarkable difference in the total leukocyte count (TLC), of the two breeds infected with the parasites, as observed by Ekezie *et al.* (2023). In their experiment, coccidiosis led to a 49-percent increase in the mean TLC of infected white-leghorns. Meanwhile, the mean TLC of infected broilers (in the same experiment) was not very different from that of the birds kept as control (i.e. un-infected birds).

The extensive system of breeding white-leghorns meets the conditions necessary for natural selection of the favourable traits (i.e. survival of the species and reproduction).White-leghorn

chickens that are managed extensively have a longer lifespan when compared with broiler chickens managed intensively. Because the leghorn chickens are productive layers, they live for 4-6 years (Smith, 2020). Unlike the extensive system, the intensive system of breeding commercial broilers disrupts natural selection. Broilers only live for several weeks (5-7 weeks) before they are slaughtered (CIWF, 2019). Even broiler breeders are slaughtered at the end of their egg production cycle which lasts for 60-65 weeks (CIWF, 2019). Therefore, commercial broilers do not survive long enough (even those that may have the favourable traits) for natural selection of these traits to occur in the population. This disruption of natural selection could explain the difference in susceptibility observed between white-leghorns and broilers.

Implications for the difference in susceptibility to coccidiosis

The difference in susceptibility between white-leghorn and broiler chickens, demonstrated in the present study, implies that the former breed can act as a reservoir of *Eimeria* parasites. As reservoir hosts, a flock of white-leghorn will maintain the parasites in the environment where they are bred without showing signs of infection. Farmers that keep multiple breed of chickens, including white-leghorns, may inadvertently transmit these parasites to the susceptible birds.

CONCLUSION

The estimated effect-sizes attributable to coccidiosis suggested that an infection with *Eimeria* spp is less likely to result in thrombocytopaenia and anaemia in white-leghorn breed of chicken as opposed to commercial broilers that are more likely to develop these conditions. Natural selection of favourable traits, which occurred in the population of white-leghorns, could explain this difference in susceptibility. This is because commercial broilers do not survive long enough for the same event to occur among them. A consequence of this difference in susceptibility is that a flock of white-leghorn can maintain the parasites in the environment where they are bred making transmission to susceptible breeds possible.

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