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COMPARATIVE STUDY OF THE HAEMATOLOGY AND BLOOD CHEMISTRY OF VELOGENIC NEWCASTLE DISEASE INFECTION IN BROILERS AND PULLETS

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ABSTRACT

This study was conducted to investigate the effects velogenic Newcastle disease virus (NDV) on the hematological and biochemical parameters in 10-weeks old broilers and pullets. Thirty broilers and pullets were each inoculated intramuscularly with 0.1ml of a velogenic Newcastle disease virus strain (KUDU-113) with a median embryo infective dose (EID_{50}) of 10^5 per ml. The red blood cell (RBC) count and haemoglobin concentration of infected broilers were significantly ($p < 0.05$) higher than those of the uninfected on day 4 post inoculation (PI) but the increase in the Packed cell volume (PCV) of infected broilers was not significantly ($p > 0.05$) different from that of the uninfected. There were no significant ($p > 0.05$) differences in the red blood cell count and haemoglobin concentration of infected and uninfected pullets. Also, there were no significant ($p > 0.05$) differences in the total white blood count (WBC), heterophil, and lymphocytes counts in both infected broilers and pullets. There were no significant ($p > 0.05$) differences in the total serum protein values between the infected and uninfected broilers on day 4 PI. However, the total serum protein values of the infected pullets was significantly ($p < 0.05$) higher than those of the uninfected pullets. The differences in globulin and albumin values of the infected and uninfected were not significant ($p > 0.05$) on day 4 PI. These results indicate that haematological changes are more severe in broilers than in pullets in velogenic NDV infection.

Keywords: Chicken – Broiler – Pullets – Newcastle disease virus – Experimental infection – Haematology - Blood chemistry

INTRODUCTION

Newcastle disease (ND) is a viral disease caused by a ND virus (NDV). The virus is an avian paramyxovirus that belongs to the *genus Avulavirus* in the family *Paramyxoviridae* [1]. It is a serious avian disease that can cause substantial economic losses and remains a major threat to the poultry industry around the world. The commonest strain of NDV in Nigeria is the velogenic, viscerotropic NDV which is the most virulent among the five pathotypes [2]. NDV is a single-stranded, negative-sense non-segmented RNA virus composed of six genes: nucleocapsid (NP), phosphoprotein (P), matrix (M), fusion (F), haemagglutinin neuraminidase (HN), and the RNA-dependent RNA polymerase (L) [3].

Changes in the haematological parameters are often used to determine various statuses of the body and stresses due to environmental, nutritional and/or pathological factors [4]. The pathology and pathogenesis of ND have been studied in chicken Studies have also been done on the effect of NDV on the haematology of breeds of chicken but there is limited information on the comparative study of the effect of the disease on the haematology of different types of chickens. The objective of this study is to compare the haematology and blood chemistry of velogenic NDV in broilers and pullets.

MATERIALS AND METHODS

Experimental Birds

Sixty broiler chicks and sixty pullets hatched on the same day were obtained at day old and used for this study. The experimental birds were not given ND vaccination. The birds were given infectious bursal disease (IBD) vaccines. The birds were housed in isolated pens and brooded on deep litter system separately under the same environmental conditions at the Poultry Disease Research Unit of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. They were given commercial poultry feed and drinking water *ad-libitum*.

Newcastle Disease Virus Inoculum

The inoculum was a local velogenic NDV which was isolated, studied and characterized by [5]. The virus which was named NDV Kudu 113 was isolated from an apparently healthy duck in Kuru, Plateau State, Nigeria.

Experimental Procedure

At ten weeks of age the pullets and broilers that were randomly divided into four groups of thirty birds each designated; uninfected pullets (UP) infected pullets (IP), uninfected broilers (UB) and infected broilers (IB). The

infected pullets and infected broilers were each intramuscularly (IM) inoculated with 0.2 ml of the viral inoculum that was reconstituted with phosphate buffered saline (PBS) to obtain a median infective dose (EID_{50}) titre of $10^{5.0}$ per ml. obtain a median infective lethal dose (EID50 ELD50) titre of 105.0 per ml. The infected pullets and infected broilers were each intramuscularly (IM) inoculated with 0.2 ml of the inoculum. The infected and uninfected groups were housed and reared on deep litter in separate pen.

Virus Isolation

Samples of the spleen, thymus, bursa of Fabricius, intestines (including contents), and brain were collected on day 3 PI from dead and humanely sacrificed birds in each infected and control groups. The samples were stored at – 20^0C until used for virus isolation.

Haematological examination

On days 0 and 4 PI, six birds in each group were randomly selected and 2ml of blood were collected from the wing veins into EDTA bottles using sterile syringes.

Packed Cell Volume (PCV)

The PCV was determined by the haematocrit method [6], using micro capillary tubes, micro haematocrit centrifuge and reader (Hawskey®, England). The microcapillary tube was nearly filled with the blood sample and sealed at one end. It was centrifuged at 3333.3g (10,000 rpm) using a microhaematocrit centrifuge for ten minutes. The PCV was later read using the microhaematocrit reader.

Hemoglobin Concentration (HbC)

The HbC was determined by the cyanomethamoglobin method [7]. About 0.02 millilitre of the blood sample was added to 5 millilitre of Drabkins haemoglobin reagent in a clean test tube and allowed to react for 20 minutes; the absorbance of the mixture was read at 540ŋm wavelength against a reagent blank using a spectrophotometer. Standards were also prepared and read. The HbC was calculated by multiplying the spectrophotometer reading with a calibrating factor obtained from the absorbance and concentration of the standard.

Total Erythrocyte Count (TEC)

The red blood cell (RBC) count was done by the haemocytometer method (Schalm *et al.*, 1975) [8] using an improved Neubauer counting chamber (Hawksley, England) and avian RBC diluting fluid [6, 9]. About 0.02 ml of blood was pipetted from the blood sample and added to 4ml of RBC avian diluting fluid in a clean tube to make a 1:200 dilution of the blood sample [6, 9].

Total Leucocyte Count (TLC)

The total WBC count was carried out by the haematocytometer method [6, 9] and avian white blood cell diluting fluid composed of aqueous phloxine, propylene, glycol, and sodium carbamate.

Differential Leukocyte Counts (DLC)

Differential Leucocyte Count was estimated by counting one hundred cells (leucocytes) under oil immersion magnification on a blood film prepared from fresh blood and fixed with methanol for 3 minutes [6]. The smears were stained with modified Wright's stain for poultry [10].

Serum chemistry

Three (3) ml of blood was collected from five birds from each group on day 4 PI through the jugular vein. The blood samples allowed to clot and sera were harvested. The serum samples were harvested into 2ml vials and stored at -20^0C.

Determination of Total Serum Protein (TSP)

The total serum protein (TSP) was determined using direct Biuret method [11]. Biuret reagent containing NaOH, potassium iodide, copper (II) sulphate and sodium – potassium tartarate and Standard containing aqueous solution of protein, equivalent to 5g/dL (50g/L) were used. The absorbance of the samples and standards were read against the blanks at 540ŋm.

The total proteins were calculated as follows:

Total protein (g/dl) = absorbance of sample/ absorbance of standard x 5

Determination of serum albumin level (SAL)

Serum albumin level (SAL) was determined using bromocresol green method [11]. Bromocresol green reagent containing bromocresol green, succinate buffer (pH 4.2), surfactants, preservatives and stabilizers, and standard containing aqueous solution equivalent to 5g/dL (50g/L) of albumin were used [11]. The absorbance of the samples and standards were read against the blanks at 630ŋm wavelength. The reading was taken immediately at 5 minutes of keeping to avoid error of further colour changes due to binding of other proteins. The serum albumin was calculated as follows:

Serum alumin (g/dl) = absorbance of sample/ absorbance of standard x 5

Calculation of Serum Globulin Fractions (SGF).

The globulin fraction was calculated as the difference between total serum proteins and serum albumin level;

Serum globulin fraction (SGF) (g/dl) = TSP - SAL

STATISTICAL ANALYSIS

Mean values and significance of the differences of the mean haematological values and blood chemistry values were analysed using student t-test within groups. The significant means were separated using t-test for Equality of means and Levene's test for Equality of Variances [12]. All tests were performed with a 5% level of significance.

RESULTS

Haematology

The haematological values are given as the mean ± standard error mean for each value (Tables 1 and 2). There was an increase in the PCV in the infected pullets and broilers on the day 4 PI. However there were no significant differences ($p > 0.05$) in these values.

Results of the RBC counts and Hbc of the infected and uninfected broiler showed that there was significant ($p < 0.05$) increase in infected broiler, but the increase in the infected pullet is not significant ($p > 0.05$) on day 4 PI (Table 1).

The WBC counts of the infected broiler on day 4 PI was higher than the uninfected but there was no significant difference ($p > 0.05$) but that of infected pullet was lower than the uninfected and the difference was not significant ($p > 0.05$) (Table 2). The heterophil count in the infected was lower than that of the uninfected in the pullet but higher than the uninfected in the broiler on day 4 PI but the difference was not significant ($p > 0.05$). The absolute lymphocyte count was higher in the infected than in the uninfected in pullets but lower in the broiler, but the differences were not also significant ($p > 0.05$). The values of absolute monocytes count in infected and uninfected pullets were the same but the value of the infected was higher than that of the uninfected in broilers but there was no significant difference ($p > 0.05$). The broilers and pullets showed decrease in absolute eosinophils count in infected however, there were no significant differences. The values of absolute basophils count in infected and uninfected pullets were 0.00 (no difference). The broiler showed decrease in the absolute basophil in the infected but there was no significant difference ($p > 0.05$).

Blood chemistry

The serum total blood protein, serum albumin and serum globulins in uninfected and infected birds are given as mean± standard error mean for each value (Table 3). The results showed that there was increase in the serum protein of the infected broiler and pullets, but the increase was not significantly ($p > 0.05$) different in the broiler but there was significant ($p < 0.05$) difference in the pullets. The serum albumin and globulin

showed increase in the infected broilers and pullets; however these increases had no significant differences ($p > 0.05$) (Tables 3).

DISCUSSION

The erythrocytic findings in both types showed that the PCV in the infected was higher than the control however this increase was not significant. The Hb concentration and rbc count was also higher in the infected than in the control, the difference was significant in the broiler and insignificant in the pullet. This could be as a result of complete withdrawal from water or shock [13, 14, 15]. This result disagreed with that of [16] who found decreased PCV in broilers infected with KUDU 113, 2 days PI.

The findings of higher WBC counts (leucocytosis) due mainly to the higher heterophil count (heterophilic) in infected broilers with NDV infection demonstrate the marked reactivity of the white cells of broiler to ND [6]. The leukocytic alterations recorded in this study correspond closely with those reported by [17] in their studies with VNDV in which they reported heterophilia and lymphopenia 72hrs PI. Leukocytosis is usually due to heterophilia and usually relates to the magnitude or severity of the inflammatory process [9]. Heterophilia is frequently observed in conjunction with tissue damage induced by inflammation or viral infection, including NDV [18, 19].

However, in pullet there were lower WBC counts (leucopaenia) with higher lymphocyte count than heterophil. This could be as a result of advanced reaction to the infection [20].

The serum protein findings showed that there were significant increases in serum protein in the infected pullet day 4 PI; the increase in broilers was not significant. There were also increase in the serum albumin and globulin in the infected pullets and broilers on day 4 PI. However these increases were not significant. This increase could be as a result of complete withdrawal from water and feed [21].

Monocyte, eosinophil, and basophil percentage values in both infected birds, in the present study showed no relevant changes. This is consistent with previous reports [17].

The above observations indicate that VND may be more severe in broilers than in pullets as reported by [22]. Higher levels of serum proteins and lymphocytosis in pullets could produce better immunity in pullets than in broilers.

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Table 1: The RBC profile of pullets and broilers Day 4 PI

Parameter	Experimenal groups	means±Standard error	
		Pullets	Broilers
Packed Cell volume (%)	Uninfected	29.63±-0.77	34.25± 1.36
	Infected	34.65±4.70	39.38±0.78
Haemoglobin concentration (g/dl)	Uninfected	0.7.76±0.37	09.30±0.37*
	Infected	09.57±1.31	11.76±0.58*
Red blood cell	Uninfected	02.40±0.10	02.81±011*
	Infected	02.63±0.33	03.43±0.21*

*p < 0.05 = means with significant difference

Table 2: Total and Absolute Differential White Blood Cell Count of Pullets and Broilers day 4 PI

Parameter	Experimental Groups	mean±Standard error	
		Pullets	Broiler
Total white blood Cell count($10^3/\mu l$)	Uninfected	17.5±1.00	14.79±2.88
	Infected	16.69±2.50	17.08±0.67
Absolute heterophil Count (%)	Uninfected Infected	43.30±3.38	42.50±3.59
		41.75±3.20	44.75±3.33
Absolute lymphocyle count (%)	Uninfected Infected	53.50± 0.87	53.25±3.12
		55.00±3.08	51.5±3.30
Absolute monocyte count (%)	Uninfected Infected	2.00±0.41	1.50±0.65
		2.00±0.71	1.75±0.49
Absolute Eosinophil Count (%)	Uninfected Infected	1.75±0.63	2.25±0.25
		1.25±0.48	1.75±0.63
Absolute basophil Count (%)	Uninfected	00.00± 0.00	0.50± 0.29
	Infected	00.00± 0.00	0.25± 0.29

Table 3: Total serum protein, Albumin and Globulin level, in Pullets and Broilers day 4 PI

Parameter	Experimental group	Mean±standard error	
		Pullets	Broilers
Serum total protein (g/dl)	Uninfected	2.38± 0.15*	2.38± 0.15
	Infected	3.20± 0.18*	3.26± 0.36
Serum albumin (g/dl)	Uninfected	1.38± 0.10	1.50± 0.21
	infected	1.88± 0.19	1.82± 0.41
Serum globulin (g/dl)	Uninfected	1.00± 0.17	0.88± 0.20
	Infected	1.40± 0.11	1.44± 0.24

*p < 0.05 = means with significant differences